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(54) Title: POLYMORPHIC FORMS OF ST-246 AND METHODS OF PREPARATION

(57) Abrégé/Abstract:
Polymorph forms of 4 -trifluoromethyl -N- (3, 3a, 4, 4a, 5, 5a, 6, 6a- octahydro-1, 3 -dioxo-4, 6 -ethenocycloprop [f] isoindol -2 (1H) -yl) - benzamide are disclosed as well as their methods of synthesis and pharmaceutical compositions.

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(54) Title: POLYMORPHIC FORMS ST-246 AND METHODS OF PREPARATION

(57) Abstract: Polymorph forms of 4 -trifluoromethyl -N- (3, 3a, 4, 4a, 5, 5a, 6, 6a- octahydro-1, 3 -dioxo-4, 6 -ethenocycloprop [f] isoindol -2 (1H) -yl) - benzamide are disclosed as well as their methods of synthesis and pharmaceutical compositions.

Polymorphic Forms of ST-246 and Methods of Preparation

CROSS REFERENCE TO RELATED APPLICATIONS

[00001] This application claims priority to and benefit of U.S. Provisional Application No. 61/316,747, filed March 23, 2010 and U.S. Provisional Application No. 61/373,031, filed August 12, 2010.

FIELD OF THE INVENTION

[00002] The present invention relates to particular crystalline forms of a pharmaceutical compound, 4-trifluoromethyl-N-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl)-benzamide, named ST-246, to processes for their preparation, pharmaceutical composition comprising different crystalline forms and its use in therapy..

[00003] --

BACKGROUND OF THE INVENTION

[00004] Throughout this application, various publications are referenced within the text.

[00005] Following the eradication of smallpox (Fenner et al., The epidemiology of smallpox. In: Smallpox and its eradication. Switzerland: World Health Organization; 1988) and the subsequent cessation of routine childhood vaccinations for smallpox, the number of people susceptible to infection with variola virus (VARV), the etiologic agent that causes smallpox, has dramatically increased worldwide. In addition, encroachment into wildlife habitats, the trade of exotic pets, and the trade of bush meat increase the risk for zoonotic infection with other orthopoxviruses, such as monkeypox virus (MPXV), for which vaccination against smallpox provides some cross protection (Jezek et al., Human monkey pox. In: Melnick JL ed. Monographs in virology. Vol. 17. Basel, Switzerland: S Karger AG. 1988:81-102).

[00006] Given that a large proportion of the worldwide population is susceptible to smallpox, the emergence of MPXV in the United States in 2003, and the continued concern over the intentional release of VARV, there is renewed interest in the development of safer smallpox and other orthopoxvirus vaccines and antiviral therapeutics.

[00007] One recently discovered antiviral compound is ST-246, a specific and potent inhibitor of an orthopoxvirus protein critical for virus maturation. Several studies evaluating ST-246 for activity against orthopoxviruses have demonstrated excellent in vitro and in vivo efficacy (Quenelle et al. 2007. Efficacy of delayed treatment with ST-246 given orally against

systemic orthopoxvirus infections in mice. Antimicrobial Agents and Chemotherapy Feb; 51(2):689-95, Smee et al, 2008. Progress in the discovery of compounds inhibiting orthopoxviruses in animal models. Antiviral Chemistry and Chemotherapy 19(3):115-24). When evaluated in *vitro* against vaccinia virus (VV), cowpox virus (CV), ectromelia virus (ECTV), monkeypox, camelpox, and variola viruses, ST-246 inhibited virus replication by 50% (50% effective concentration [EC_{50}]) at a concentration of ≤0.07 μM. With animal models using lethal infections with ECTV, VV, or CV, ST-246 was reported to be nontoxic and highly effective in preventing or reducing mortality even when treatments were delayed up to 72 h post-viral inoculation (Quenelle et al., 2007. Efficacy of delayed treatment with ST-246 given orally against systemic orthopoxvirus infections in mice. Antimicrobial Agents and Chemotherapy Feb; 51(2):689-95, Smee et al. 2008. Progress in the discovery of compounds inhibiting orthopoxviruses in animal models. Antiviral Chemistry and Chemotherapy 19(3):115-24). ST-246 was also evaluated with the nonlethal mouse tail lesion model using intravenous VV. When ST-246 was administered orally twice a day at 15 or 50 mg/kg of body weight for 5 days, the tail lesions were significantly reduced (Smee et al., 2008. Progress in the discovery of compounds inhibiting orthopoxviruses in animal models. Antiviral Chemistry and Chemotherapy 19(3):115-24). Most recently, an infant was given ST-246 as an FDA-authorized emergency treatment for eczema vaccinatum which developed after exposure to the parent's predeployment military smallpox immunization (Vora et al., 2008, Severe eczema vaccinatum in a household contact of a smallpox vaccine. Clinical Infectious Disease 15; 46(10):1555-61).

[00008] ST-246 was disclosed in WO 2008/130348, WO 2004/112718 and WO 2008/079159 as one of the tetracyclic acylhydrazide compounds for treatment or prophylaxis of viral infections and diseases associated herewith, particularly those viral infections and associated diseases caused by the orthopoxvirus. These publications disclose a process for the preparation of ST-246 but do not disclose what polymorphic form is made. Nonetheless, the disclosed process yields ST-246 hemihydrate, the polymorphic Form V as discussed herein below.

[00009] The process of making a monohydrate of ST-246 was disclosed in CN 101445478A. The data shown in this publication corresponds to polymorphic Form III according to the present classification of polymorphs of ST-246.

[000010] It has now been unexpectedly discovered that ST-246 can exist in many different polymorphic forms. A particular crystalline form of a compound may have physical properties that differ from those of other polymorphic forms and such properties may influence markedly the physico-chemical and pharmaceutical processing of the compound, particularly when the compound is prepared or used on a commercial scale. Such differences may alter the mechanical handling properties of the compound (such as the flow characteristics of the solid material) and the compression characteristics of the compound. Further, the discovery of new polymorphic forms of such pharmaceutically important compound as ST-246, provides a new opportunity to improve the performance characteristics of a pharmaceutical end product and enlarges the repertoire of materials that a formulation scientist has available for designing, for example, a pharmaceutical dosage form of a drug with targeted release profile or other desired physico-chemical properties.

[000011] Further, given that new polymorphic forms of a drug substance may display different melting point, hygroscopicity, stability, solubility and/or dissolution rate, crystallinity, crystal properties, bioavailability, toxicity and formulation handling characteristics, which are among the numerous properties that need to be considered in preparing medicament that can be effectively administered. Furthermore, regulatory agencies require a definitive knowledge, characterization and control of the polymorphic form of the active component in solid pharmaceutical dosage forms. Thus, there is a need in the art for crystallization and characterization of new polymorphic forms of ST-246.

SUMMARY OF THE INVENTION

[000012] The present invention provides a polymorph Form I of ST-246 which shows an X-ray powder diffraction pattern having characteristic peaks of about 7.63,10.04,11.47,14.73,15.21,15.47,16.06,16.67,16.98,18.93,19.96,20.52,20.79,22.80,25.16,26.53,27.20,27.60,29.60,30.23,30.49,30.68,31.14,33.65,34.33,35.29,35.56,36.30,37.36,38.42,38.66 degrees.

[000013] The present invention also provides a polymorph Form II of ST-246 which shows a X-ray powder diffraction pattern having characteristic according to Figure 2.

[000014] The present invention further provides a polymorph Form III of ST-246 which shows an X-ray powder diffraction pattern having characteristic peaks of about 6.71,9.05,12.49,13.03,13.79,14.87,15.72,16.26,16.74,18.10,18.43,19.94,21.04,21.51,23.15,23.51,25.32,26.24,26.87,27.32,27.72,28.55,29.08,29.50,29.84,31.27,33.48, 35.36,39.56 degrees.

[000015] The present invention also provides a polymorph Form IV of ST-246 which shows an X-ray powder diffraction pattern having characteristic as shown in Figure 4.

[000016] The present invention further provides a polymorph Form VI ST-246 which shows an X-ray powder diffraction pattern having characteristic peaks as shown in Figure 6.

[000017] The present invention also provides pharmaceutical compositions comprising each of the ST-246 polymorphs Forms I-VI and further comprising one or more pharmaceutically acceptable carriers, excipients, diluents, additives, fillers, lubricants or binders.

[000018] The present invention further provides methods of treating orthopoxvirus infections or eczema vaccinatum comprising administering to a subject amimal or human in need thereof a therapeutically effective amount of each of the ST-246 polymorphs Forms I-VI.

[000019] The present invention also provides methods for the synthesis of each of the ST-246 polymorphs Forms I-VI.

[000020] The present invention also provides a dosage unit form for oral administration, wherein ST-246 has a D90% particle size diameter of up to about 300 microns. In some embodiments, ST-246, polymorph I, II, III, IV and VI has a D90% particle size diameter of about 5 microns, in other embodiments, the D90% particle size diameter is about 16.6 microns, in yet another embodiment, a D90% particle diameter is about 26.6 microns and in yet another embodiment, the D90% particle diameter is about 75 microns.

[000021] In another aspect of the invention, a unit dosage form for oral administration comprising 200 mg of ST-246, ,wherein ST-246 is selected from a group consisting of ST-246 polymorph Form II, ST-246 polymorph Form III, ST-246 polymorph Form IV and ST-246 polymorph Form VI and further comprising 33.15 mg of lactose monohydrate; 42.90 mg of croscarmellose sodium; 1.95 mg of colloidal silicon dioxide;13.65 mg of hypromellose, 7.8 mg of sodium lauryl sulfate; 1.95 mg of magnesium stearate; and a quantity of microcrystalline cellulose up to 88.60 mg such that the total weight of the dosage form, including any impurities, water and residual solvents, is 390 mg.

BRIEF DESCRIPTION OF THE DRAWINGS

[000022] Figure 1 shows an X-ray powder diffraction (XRPD) pattern of Form I.

[000023] Figure 2 shows three X-ray powder diffraction (XRPD) patterns of Form II (from three different samples).

[000024] Figure 3 shows an X-ray powder diffraction (XRPD) pattern of Form III.

[000025] Figure 4 shows two X-ray powder diffraction (XRPD) patterns of Form IV (from two different samples).

[000026] Figure 5 shows an X-ray powder diffraction (XRPD) pattern of Form V.

[000027] Figure 6 shows two an X-ray powder diffraction (XRPD) patterns of Form VI (from two different samples).

[000028] Figure 7 depicts Fourier transform infra red (FTIR) spectrum of Form I.

[000029] Figure 8 depicts Fourier transform infra red (FTIR) spectrum of Form III.

[000030] Figure 9 depicts Fourier transform infra red (FTIR) spectrum of Form V.

[000031] Figures 10, 11, 12 and 13, depict magnified view of FTIR Spectra of Form I (upper panel), Form V (middle panel) and Form III (lower panel).

[000032] Figure 14 depicts XRPD Pattern of Micronized (upper pattern) and unmicronized (lower pattern) Form I.

[000033] Figure 15 depicts XRPD Pattern of micronized (top pattern) and unmicronized (middle and lower patterns from 2 different samples) Form III.

[000034] Figure 16 depicts effect of particle size on dissolution of 200 mg ST-246 Form I capsules with 3% HDTMA wherein the dissolution conditions are 900ml, 0.05M Phosphate buffer, pH 7.5, USP 2 at 75 RPM, 37°C and the capsule is made from Form I APIs with particle size D90 of about 5.5 microns.

[000035] Figure 17 depicts effect of particle size on dissolution of 200 mg ST-246 Form I capsules with 3% HDTMA wherein the dissolution conditions are 900ml, 0.05M Phosphate buffer, pH 7.5, USP 2 at 75 RPM, 37°C and the capsule is made from Form I APIs with particle size D90 of about 16.73 microns.

[000036] Figure 18 depicts effect of particle size on dissolution of 200 mg ST-246 Form I capsules with 3% HDTMA wherein the dissolution conditions are 900ml, 0.05M Phosphate buffer, pH 7.5, USP 2 at 75 RPM, 37°C and the capsule is made from Form I APIs with particle size D90 of about 26.55 microns.

[000037] Figure 19 depicts effect of particle size on dissolution of 200 mg ST-246 Form I capsules with 3% HDTMA wherein the dissolution conditions are 900ml, 0.05M Phosphate buffer, pH 7.5, USP 2 at 75 RPM, 37°C and the capsule is made from Form I APIs with particle size D90 is about 75 microns.

[000038] Figure 20 depicts effect of particle size on dissolution of 200 mg ST-246 Form I capsules with 3% HDTMA wherein the dissolution conditions are 900ml, 0.05M Phosphate buffer, pH 7.5, USP 2 at 75 RPM, 37°C and the capsule is made from Form I APIs with particle size D90 of about 254 microns.

[000039] Figure 21 depicts dissolution profile of Form I.

[000040] Figure 22 depicts dissolution profile of Form III.

[000041] Figure 23 depicts dissolution profile of Form V.

[000042] Figure 24 depicts the mean (SD) ST-246 plasma concentrations over time (PK population) after a single oral administration.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[000043] In accordance with this detailed description, the following abbreviations and definitions apply. It must be noted that as used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[000044] The term "polymorphic form, polymorph, polymorph form, crystalline form, physical form or crystalline polymorph" of ST-

246 in the present invention refers to a crystal modification of ST-246, which can be characterized by analytical methods such as X-ray powder diffraction pattern, (XRPD), differential scanning calorimetry (DSC), by its melting point analysis or Infrared Spectroscopy (FTIR).

[000045] The term "hydrate" as used herein means a compound of the invention or a salt thereof that further includes a stoichiometric or non-stoichiometric amount of water bound by non-covalent intermolecular forces. Hydrates are formed by the combination of one or more molecules of water with one molecule of the substances in which the water retains its molecular state as H_2O, such combination being able to form one or more hydrate. The term "hemihydrate" as used herein refers to a solid with 0.5 molecule of H_2O per molecule of the substance.

[000046] The term "pharmaceutical composition" or "pharmaceutical formulation" is intended to encompass a drug product including the active ingredient(s), pharmaceutically acceptable excipients that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing the active ingredient, active ingredient dispersion or composite, additional active ingredient(s), and pharmaceutically acceptable excipients.

[000047] The particle size distribution (PSD) of a powder, or granular material, or particles dispersed in fluid, is a list of values or a mathematical function that defines the relative amounts of particles present, sorted according to size. PSD is also known as grain size distribution. Since particle size for a

complex media is a distribution of diameters, statistics can be used to convey the results. A common method is to use d10, d50 and d90 values based on volume distribution. That is to say that 10%, 50% and 90%, respectively, of the particle size distribution is smaller than the stated diameter.

[000048] The term "dosage unit" refers to a single unit of the dosage form that is to be administered to the patient. The dosage unit will be typically formulated to include an amount of drug sufficient to achieve a therapeutic effect with a single administration of the dosage unit although where the size of the dosage form is at issue, more than one dosage unit may be necessary to achieve the desired therapeutic effect. For example, a single dosage unit of a drug is typically, one tablet, one capsule, or one tablespoon of liquid. More than one dosage unit may be necessary to administer sufficient drug to achieve a therapeutic effect where the amount of drug causes physical constraints on the size of the dosage form.

[000049] The term "half-life" is a pharmacokinetic term used to indicate the length of time necessary to eliminate 50% of the remaining amount of drug present in the body.

[000050] The term "AUC" (i.e., "area under the curve," "area under the concentration curve," or "area under the concentration-time curve") is a pharmacokinetic term used to refer a method of measurement of bioavailability or extent of absorption of a drug based on a plot of an individual or pool of individual's blood plasma concentrations sampled at frequent intervals; the AUC is directly proportional to the total amount of unaltered drug in the patient's blood plasma. For example, a linear curve for a plot of the AUC versus dose (i.e., straight ascending line) indicates that the drug is being released slowly into the blood

stream and is providing a steady amount of drug to the patient; if the AUC versus dose is a linear relationship this generally represents optimal delivery of the drug into the patient's blood stream. By contrast, a non-linear AUC versus dose curve indicates rapid release of drug such that some of the drug is not absorbed, or the drug is metabolized before entering the blood stream.

[000051] The term "C_{max}" (i.e., "maximum concentration") is a pharmacokinetic term used to indicate the peak concentration of a particular drug in the blood plasma of a patient.

[000052] The term "T_{max}" (i.e., "time of maximum concentration" or "time of C_{max}") is a pharmacokinetic term used to indicate the time at which the C_{max} is observed during the time course of a drug administration. As would be expected, a dosage form that would include an immediate release as well as a gastric retentive component would have a T_{max} that is higher than the C_{max} for an immediate release dosage form, but lower than the T_{max} for a purely gastric retentive dosage form.

[000053] It has now been surprisingly discovered that ST-246 exists in different crystalline forms denominated Form I, Form II, Form III, Form IV, Form V, and Form VI.

[000054] All forms have been fully characterized and comparability data have been generated. All the forms are as characterized hereinafter inter alia by the following methodology:

PHYSICAL EXPERIMENTAL METHODOLOGY

X-RAY POWDER DIFFRACTION (XRPD)

[000055] Diffraction patterns were collected using a Bruker D8 Discovery™ diffractometer configured with an XYZ stage, laser video microscope for positioning, and HiStar™ area detector. Collection times were nominally 60 seconds. A Cu *Ka* radiation 1.5406 tube was operated at 40kV and 40mA was used to irradiate samples. The X-ray optics consists of a Gobel mirror coupled with a pinhole collimator of 0.5 mm. Theta-theta continuous scans were employed with a sample-detector distance of 15 cm, which gives an effective 2θ range of 4-40°. Samples were mounted in low background quartz plates. A variable temperature hot stage was used to manipulate sample temperature for some experiments.

[000056] The polymorphs of ST-246 are characterized by their X-ray powder diffraction patterns (XRPD) and/or their Raman spectroscopy peaks. With respect to X-ray powder diffraction, the relative intensities of the X-ray powder diffraction peaks of a given polymorph may vary depending upon the crystal size of the polymorph used to determine the pattern. This is a phenomenon of preferred orientation. Preferred orientation is caused by the morphology of crystals. In this case, the XRPD analysis may be carried out with the sample spinning in the sample holder during XRPD analysis to reduce the preferred orientation effects. For the XRPD analysis, the pattern is given in terms of the "degree of 2θ (two theta)" angles of the peaks.

[000057] With respect to the percent value of relative intensity (*I/Io*), *Io* represents the value of the maximum peak determined by XRPD for the sample for all "degree 2θ." angles and *I* represents the value for the intensity of a peak measured at a given "degree 2θ" angle".

[000058] The angle "2θ degree" is a diffraction angle which is the angle between the incident X-rays and the diffracted X-rays. The values for the relative intensities for a given peak set forth in percent and the "degree.2θ" angles are calculated. However, there are key major peaks at given angles in these X-ray powder diffraction patterns which are unique to each given polymorph form. These peaks are present in the XRPD patterns of each of the polymorph forms having a crystal size of about 10 to 40 microns. Any of these major peaks, either alone or in any distinguishing combination, are sufficient to distinguish one of the polymorph forms from the other present polymorphic forms.

INFRARED SPECTROSCOPY (FTIR)

[000059] Infrared spectra were obtained with a Nicolet™ 510 M-O Fourier transform infrared spectrometer, equipped with a Harrick Splitpea™ attenuated total reflectance device. Spectra were acquired from 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 128 scans were collected for each analysis.

PREPARATION OF THE CRYSTALLINE FORMS

[000060] The present invention provides a method of producing polymorphic Form I of ST-246, comprising the steps of:

a) dissolving ST-246 in at least one organic solvent and an amount of water to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form I; and

c) optionally drying the formed crystals of ST-246,

wherein said organic solvent is selected from a group consisting of isopropyl alcohol (IPA), ethyl acetate, ethanol, methanol, acetone, isopropyl acetate and tetrahydrofuran (THF).

[000061] Preferably, the method further comprises adding seed crystals of polymorphic Form I ST-246 during step (b). Also preferably, the cooling step takes place over at least 15 minutes, more preferably over at least 2 hours and most preferably over at least 5 hours.

[000062] Also preferably, the organic solvent is ethyl acetate and the water content is about 40% by volume of total solvent volume, more preferably about 5% by volume of total solvent volume, more preferably about 3% by volume of total solvent volume and most preferably about 2% by volume of total solvent volume. Also preferably, the organic solvent is isopropyl alcohol and the water content is about 5% by volume of total solvent volume.

[000063] The present invention also provides a method of producing crystal polymorphic Form II of ST-246, comprising the steps of:

a) dissolving ST-246 in at least one solvent to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form II; and

c) optionally drying the formed crystals of ST-246,

wherein said solvent is selected from the group consisting of ethyl acetate, chloroform, 1-propanol, isopropyl alcohol (IPA) ethanol, acetone, acetonitrile, toluene, isopropyl acetate and dimethylformamide (DMF).

[000064] Preferably, the method further comprises adding seed crystals of polymorphic Form II ST-246 during step (b). Also preferably, the solvent does not contain water and is selected from the group consisting of ethyl acetate and chloroform.

[000065] The present invention further provides a method of producing crystal polymorphic Form II of ST-246, comprising the steps of:

a) dissolving ST-246 in ethanol and water to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form II; and

c) optionally drying the formed crystals of ST-246.

[000066] Preferably, the method further comprises adding seed crystals of polymorphic Form II ST-246 during step (b). Also preferably, the volume ratio of ethanol: water is about 1:1.

[000067] The present invention also provides a method of producing crystal polymorphic Form III of ST-246, comprising the steps of:

a) dissolving ST-246 in at least one organic solvent and water to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form IIII: and

c) optionally drying the formed crystals of ST-246,

wherein the organic solvent is selected from the group consisting of isopropyl alcohol (IPA), ethyl acetate and ethanol.

[000068] Preferably, the method further comprises adding seed crystals of polymorphic Form III ST-246 during step (b). Also preferably, the cooling step takes place over less than 15 minutes.

[000069] The present invention further provides a method of producing crystal polymorphic Form III of ST-246, comprising the steps of:

a) dissolving ST-246 in at least one organic solvent to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form III; and

c) optionally drying the formed crystals of ST-246,

wherein the organic solvent is selected from the group consisting of acetone, isopropyl alcohol (IPA), dimethylamine (DMA), pyridine, trifluoroethanol (TFE), methanol, ethanol, chloroform, acetonitrile (ACN), and tetrahydrofuran (THF).

[000070] Preferably, the method further comprises adding seed crystals of polymorphic Form III ST-246 during step (b).

[000071] The present invention also provides a method of producing crystal polymorphic Form IV of ST-246, comprising the steps of:

a) dissolving ST-246 in at least one organic solvent optionally containing water to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form IV precipitation in crystal form of ST-246; and

c) optionally drying the formed crystals of ST-246,

wherein said solvent is selected from the group consisting of: a mixture of acetonitrile and ethyl acetate, a mixture of ethanol and toluene, a mixture of water and ethyl acetate, and a mixture of trifluoroethanol and THF.

[000072] Preferably, the method further comprises adding seed crystals of polymorphic Form IV ST-246 during step (b). Also preferably, the solvent is a mixture of ACN and ethyl acetate at a volume ratio of about 1:4, a mixture of ethanol and toluene at a volume ratio of about 1:4, a mixture of water and ethyl acetate at a volume ratio of about 1:4, and a mixture of TFE and THF at a volume ratio of about 1:1.

[000073] The present invention further provides a method of producing crystal polymorphic Form IV of ST-246, comprising the steps of:

a) dissolving ST-246 in at least one solvent to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form IV; and

c) optionally drying the formed crystals of ST-246,

wherein said solvent is selected from the group consisting of 1-butanol, trifluoroethanol (TFE), chloroform, dichloromethane and toluene.

[000074] Preferably, the method further comprises adding seed crystals of polymorphic Form IV ST-246 during step (b). Also preferably, the solvent does not contain water. Also preferably the solvent is 1-butanol.

[000075] The present invention also provides a method of producing crystal polymorphic Form VI of ST-246, comprising the steps of:

a) dissolving ST-246 in at least one solvent to make a solution;

b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form VI; and

c) optionally drying the formed crystals of ST-246,

wherein said solvent is selected from the group consisting of nitromethane, methanol and chloroform.

[000076] Preferably, the method further comprises adding seed crystals of polymorphic Form VI ST-246 during step (b). Also preferably, the solvent does not contain water and is nitromethane.

[000077] The ST-246 is prepared as outlined in the Examples below. Processes for crystallization of polymorphs of the ST-246 may embrace multiple combinations of techniques and variations thereof. Crystallization of polymorphs of the ST-246 may be executed by dissolving, dispersing, or slurrying ST-246 at a suitable temperature in the solvent whereby portion of the said solvent evaporates increasing the concentration of the ST-246 in the said solution, dispersion, or slurry, cooling the said mixture, and optionally washing and/or filtering and drying the resulting crystals of the ST-246.

[000078] Crystal formation may as well involve more than one crystallization process. In certain cases, one, two or more extra crystallization steps may be advantageously performed for different reasons, such as, to increase the quality of the resulting crystal form. For instance, the polymorphs of the

present invention could also be prepared by adding a solvent to an initial starting base material of the ST-246, stirring the solution at a fixed temperature until the substances would be fully dissolved, concentrating the solution by vacuum distillation, and cooling. A first crystallization would take place and the formed crystals would be washed with a solvent, and followed by solubilization of the ST-246 with the same or different solvent to form the desired polymorph. The reaction mixture may be heated to reflux and recrystallization of the reaction mixture would occur, followed by a cooling step from reflux. The formed polymorph would optionally be filtered and allowed to dry.

[000079] By dissolving, dispersing, or slurrying the ST-246 in the solvent, one may obtain different degrees of dispersion, such as suspensions, slurries or mixtures; or preferably obtain homogeneous one-phase solutions. The term "suspension" refers to a two-phase system consisting of a finely divided solid, i.e. ST-246 in amorphous, crystalline form, or mixtures thereof, dispersed (suspended) in a liquid or dispersing medium, usually the solvent. The term "slurry" refers to a suspension formed when a quantity of powder is mixed into a liquid in which the solid is only slightly soluble (or not soluble). "Slurrying" refers to the making of a slurry.

[000080] Optionally, the solvent medium may contain additives, for example dispersing agents, surfactants or other additives, or mixtures thereof of the type normally used in the preparation of crystalline suspensions. The additives may be advantageously used in modifying the shape of crystal by increasing the leniency and decreasing the surface area.

[000081] The solvent medium containing the solid may optionally be stirred for a certain period of time, or vigorously agitated using, for example, a high shear mixer or homogenizer or a combination of these, to generate the desired particle size for the organic compound. Control of precipitation temperature and seeding may be additionally used to improve the reproducibility of the crystallization process, the particle size distribution and the dosage form of the product. As such, the crystallization can be effected without seeding with crystals of the ST-246 or preferably in the presence of crystals of the ST-246, which are introduced into the solution by seeding. Seeding can also be effected several times at various temperatures. The amount of the seed material depends on the scale of the experiment and can readily be determined by a person skilled in the art. Typically, the amount of seeding material is about 0.1 to 1 weight % of the amount of crystalline material expected from the reaction.

[000082] The time for crystallization in each crystallization step will depend on the conditions applied, the techniques employed and/or solvents used. Breaking up the large particles or aggregates of particles after crystal conversion may additionally be performed in order to obtain a desired and homogeneous particle size. Accordingly, the crystals, powder aggregates and coarse powder of the polymorphic forms of the ST-246 may be optionally milled and sorted by size after undergoing conversion. Milling or grinding refers to physically breaking up the large particles or aggregates of particles using methods and apparatus well known in the art for particle size reduction of powders. Resulting particle sizes may range from millimeters to nanometers, yielding i.e. nanocrystals, microcrystals. A preferred apparatus for milling or grinding is a fluid energy mill, or micronizer, because of its ability to produce particles

of small size within a narrow range of particle size distribution.

[000083] The invention provides as well a process wherein the obtained crystalline form is isolated by filtration or centrifugation, optionally combined with washing and drying. The starting material used for the processes of the present invention may be any crystalline or amorphous form of the ST-246, including a hydrate thereof.

[000084] In one aspect of the invention, the solvents employed in the preparation of the crystalline forms of the present invention are pharmaceutically acceptable or pharmaceutically non-acceptable solvents. Pharmaceutically non-acceptable solvents will have to be removed prior to using the polymorph into a pharmaceutical formulation.

[000085] The processes for the production of the polymorphic forms of the present invention typically include obtaining a crystalline solid material from a solution or dispersion of the ST-246 in a solvent medium, or from slurrying the ST-246, which can be initially in amorphous or crystalline form. The conditions concerning crystallization may be modified in order to improve the crystallization process or to induce precipitation, and without affecting the form of the polymorph obtained. These conditions include bringing the solution, dispersion, or slurry of the ST-246 and the solvent(s) to a desired concentration, cooling it following a defined cooling/temperature curve, adding crystal seeds, bringing the said solution, dispersion, or slurry to a desired temperature, effecting any suitable pressure, removing and/or separating any undesired material or impurities, drying the formed crystals to

obtain the polymorphs in a solid state, if such state is desired.

[000086] One way of inducing precipitation is to reduce the solubility of the ST-246. The solubility of the compound may be reduced, for example, by cooling the solution. The solubility of the ST-246 may be reduced by adding an anti-solvent. Bringing the solution, dispersion, or slurry of the ST-246 and solvents to a desired concentration does not necessarily imply an increase in the concentration of the ST-246. In certain cases, a decrease or no change in concentration of the ST-246 could be preferable. The techniques used for obtaining a desired concentration include, for instance, evaporation by atmospheric distillation, vacuum distillation, fractioned distillation, azeotropic distillation, film evaporation, heating, cooling, other techniques well known in the art and combinations thereof. An optional process for obtaining a desired concentration could as well involve the saturation of the solution of the ST-246 and solvent, for example, by adding a sufficient volume of a non-solvent to the solution to reach the saturation point.

[000087] Other suitable techniques for saturating the solution include, by way of example, the introduction of additional ST-246 to the solution and/or evaporation of a portion of the solvent from the solution. As referred to herein, a saturated solution encompasses solutions at their saturation points or exceeding their saturation points, i.e. supersaturated. A nearly saturated solution refers to solutions that are near saturation but have not reached their saturation solubility limits.

[000088] In the preferred aspect of the invention, crystallization solvent is an important factor in determining which ST-246

polymorph is formed. Water content is also important, because the different polymorphic forms have varying levels of hydration. In the mixtures of water and water miscible solvents, the amount of water can vary from about 0.1% by volume to about 95% by volume, preferably from about 10% to about 20% by volume, more preferably from about 5% to about 10% by volume and most preferably from about 5% to about 1% of water.

[000089] ST-246 Polymorphic Forms I and III are monohydrates, and thus there is a minimum threshold of water that must be present in order for ST-246 to crystallize as a monohydrate. In addition, the cooling rate and isolation temperature and amount of water may play a role in determining which ST-246 polymorphic form and/or hydrate is formed. As summarized in Table 1 below, there is a correlation between cooling rate isolation temperature, water content and generation of ST-246 Form I or ST-246 Form III. Further, the data summarized in Table 1 suggests that the solvent composition, crystallization temperature, or cooling rate, may have an impact on ST-246 polymorph Form formation. For example, as shown in Table 1, when both isopropyl alcohol (IPA) and ethyl acetate are used as the major solvent, ST-246 Form III is generated when a warm, about 35°C to about 40 °C, solution containing higher water content is cooled directly into an ice bath. In contrast, in the presence of the lower water content or when solutions were cooled to room temperature for isolation, ST-246 Form I is obtained. Polymorphic form of the material prior to final crystallization does not impact final polymorph form, as long as the material completely dissolves in the crystallization solvent.

Table 1 - Correlation between ST-246 Form formation, solvent system and isolation temperature.

Solvent System	Isolation Temperature	Form
IPA, 5% Water	Room Temperature	I
IPA, 5% Water	Ice Bath (2-5 °C)	III
IPA, 2% Water	Ice Bath	I
Ethyl acetate, 5% Water	Room Temperature	I
Ethyl acetate, 5% Water	Ice Bath (2-5 °C)	III
Ethyl acetate, 2% Water	Ice Bath (2-5 °C)	I

[000090] Removing and/or separating any undesired material or impurities may be performed by purification, filtering, washing, precipitation or similar techniques. Separation, for example, can be conducted by known solid-liquid separation techniques. The filtrations can be performed, amongst other methods, by passing the solution, dispersion, or slurry through a filter paper, sintered glass filter or other membrane material, by centrifugation, or using Buchner style filter, Rosenmund filter or plates, or frame press. Preferably, in-line filtration or safety filtration may be advantageously intercalated in the processes disclosed above, in order to increase the polymorphic purity of the resulting crystalline form.

[000091] Crystals obtained may be also dried, and such drying process may optionally be used in the different crystallization passages, if more than one crystallization passage is applied. Drying procedures include all techniques known to those skilled in the art, such as heating, applying vacuum, circulating air or gas, adding a desiccant, freeze-drying, spray-drying, evaporating, or the like, or any combination thereof.

FORM I.

[000092] In one aspect of the invention, the crystalline form of ST-246 is disclosed and is denominated as Form I of the ST-246, or in short "Form I".

[000093] One preferred parameter to reliably crystallize Form I is the use of ethyl acetate/water mixtures. Several parameters have been varied during crystallization studies with ethyl acetate/water (amount of water added, dissolving temperature, isolation temperature, cooling rate) and are summarized in Tables 1 and 2. Form I can be generated with the use of ethyl acetate/water mixture provided enough water is present to allow formation of the monohydrate. Further, Form I has been shown to be formed using THF/water mixtures, IPA/water mixtures, and both acetone and methanol have shown the ability to crystallize Form I with higher levels of water. In addition, Form I can also be generated by holding a water slurry of Forms III and V for several days or longer.

Table 2 – Crystallization Parameters

NO.	SOLVENT	DISSOLVING		TREATMENT FOR PRECIPITATION		DRYING	POLYMORPH FORM	MELTING POINT (°C)	NB REF.	RECRYSTALLIZATION/ BATCH #
		WATER %	TEMP (°C)	TEMP (°C)	TIME (HOURS)	TEMP (°C)				
4	IPA/WATER	5	55-60	RT	14	50	I	197.9	DN-383-15	383-18-A-1
5	IPA/WATER	5	55-60	RT:ICE BATH	14:2-4	50		197.8	DN-383-15	383-18-A-1-2
8	IPA/WATER	2	35-40	ICE BATH	22	50		197.7	DN-383-19	
13	IPA/WATER	5				50-55				14KM24C
14	IPA/WATER	2.85				55-60				14KM53D
15	IPA/WATER	2.85				55-60				14KM54B
16	IPA/WATER	2.85				55-60				14KM54D
	ETHYL ACETATE/WATER#	3	70-80	RT	20	75C				DN-383-1
27	ETHYL ACETATE/WATER	5	70-80	RT	5.5	50		197.3	DN-383-9	
29	ETHYL ACETATE/WATER	5	55-60	RT:ICE BATH	14:2-4	50		198	DN-383-15	383-18-B-1-2
30	ETHYL ACETATE/WATER	2	55-60	RT	14	50		197.7	DN-383-15	383-18-B-2
32	ETHYL ACETATE/WATER	2	35-40	ICE BATH	22	50		197.9	DN-383-19	
36	ETHYL ACETATE→WATER	5	50-60	RT	O/N	50-60			MAS-518-88	MAS-518-88-1
37	ETHYL ACETATE→WATER	10	50-60	RT	O/N	50-60			MAS-518-88	MAS-518-88-2
38	ETHYL ACETATE→WATER	20	50-60	RT	O/N	50-60			MAS-518-88	MAS-518-88-3
39	ETHYL ACETATE→WATER	40	50-60	RT	O/N	50-60			MAS-518-88	MAS-518-88-4
40	ETHYL ACETATE	-				55-60				14KM40C
41	ETHYL ACETATE/WATER	2				55-60				14KM40D
42	ETHYL ACETATE/WATER	3				55-60				14KM46B
43	ETHYL ACETATE/WATER	2				55-60				14KM48B
44	ETHYL ACETATE/WATER	3				55-60				14KM57E
45	ETHYL ACETATE/WATER	2				40-47				14KM75B (400g)
46	ETHYL ACETATE/WATER	5				40-45				14KM77A (200g)
47	ETHYL ACETATE/WATER	5				40-45				14KM73B (200g)
48	ETHYL ACETATE/WATER	2				40-45				14KM98B (200g)
49	ETHYL ACETATE/WATER	2				40-45				15KM16A (400g)
50	ETHYL ACETATE/WATER	2				40-45				15KM18B (400g)
62	ETHANOL/WATER (60°C)	10	60	RT:2-8	3:4	50-60			MAS-518-88	MAS-518-88-6

63	ETHANOL/WATER	3				55-60				14KM36C
64	ETHANOL/WATER	3				55-60				14KM36D
66	METHANOL/WATER	5	55-60	RT:ICE BATH	14:2-4	50		197.6	DN-383-15	383-18-C-1-2
68	METHANOL/WATER	5	35-40	ICE BATH	22	50		197.8	DN-383-19	
71	ACETON/WATER	7.5	55-60	RT:ICE BATH	14:2-4	50		199.1	DN-383-15	383-18-D-1-2
77	THF/WATER	7.5	55-60	RT:ICE BATH	14:2-4	50			DN-383-15	383-18-E-1-2
78	THF/WATER	2	55-60	RT	14	50			DN-383-15	383-18-E-2
79	THF/WATER	5	35-40	ICE BATH	22	50			DN-383-19	
80	THF/WATER	2	35-40	ICE BATH	22	50			DN-383-19	
83	ISOPROPYL ACETATE/WATER	5	55-60	RT:ICE BATH	14:2-4	50			DN-383-15	383-18-F-1-2
84	ISOPROPYL ACETATE/WATER	2	55-60	RT	14	50			DN-383-15	383-18-F-2
96	WATER SLURRY	100	45:RT	NA						
61	ETHANOL/WATER (60°C)	10	60	RT:2-8	3:4	50-60	I AND III		MAS-518-88	MAS-518-88-5
1	IPA	-	70-80	RT	72	75		198.3	DN-383-1	0374-24
7	IPA/WATER	5	35-40	ICE BATH	22	50		197.7	DN-383-19	
17	IPA/WATER	2.85				55-60				14KM23D
18	IPA/WATER	2.85				55-60				14KM38A
19	IPA/WATER	5				55-60				14KM41A
20	IPA/WATER	5				55-60				14KM49B
21	IPA/WATER	2.85				55-60				14KM60E
22	IPA/WATER	5				55-60				14KM64C
23	IPA/WATER	5				55-60				14KM64D
24	IPA/WATER	1.25				40-45				14KM73B
31	ETHYL ACETATE/WATER	5	35-40	ICE BATH	22	50	III	197.9	DN-383-19	
56	ETHYL ACETATE: HEXANE (7:4)	-	70	RT:2-8	3:4	50-60			MAS-518-88	MAS-518-88-8
72	ACETONE/WATER	2	55-60	RT	15	50		197.5	DN-383-15	383-18-D-2
88	WATER SLURRY	100	RT:50	NA				197.5	DN-383-23	ST-246W
90	WATER SLURRY	100		NA				197.5		
91	WATER SLURRY	100	RT	NA		RT		197.9	DN-383-34	0383-34
92	WATER SLURRY	100	45	NA		RT		197.8	DN-383-34	0383-34
93	WATER SLURRY	100	RT	NA		37			WW-386-20	#51
94	WATER SLURRY	100	RT	NA		37			WW-386-22	#54
95	WATER SLURRY (14KM71A)	100	60	NA						

6	IPA/WATER	2	55-60	RT	14	50	V	198.1	DN-383-15	383-18-A-2
25	ETHYL ACETATE	-	70-80	RT	72	75		197.9	DN-383-1	0374-26
55	ETHYL ACETATE: HEXANE (7:4)	-	70	RT:2-8	3:4	50-60			MAS-518-88	MAS-518-88-7
58	ETHANOL	-	70-80	RT	72	75		197.7	DN-383-1	0374-28
67	METHANOL/WATER	2	55-60	RT	15	50		197.6	DN-383-15	383-18-C-2
69	METHANOL/WATER	2	35-40	ICE BATH	22	50		197.9	DN-383-19	
61	ETHANOL/WATER (60°C)	10	60	RT:2-8	3:4	50-60	I AND III		MAS-518-88	MAS-518-88-5
1	IPA	-	70-80	RT	72	75		198.3	DN-383-1	0374-24
7	IPA/WATER	5	35-40	ICE BATH	22	50		197.7	DN-383-19	
17	IPA/WATER	2.85				55-60				14KM23D
18	IPA/WATER	2.85				55-60				14KM38A
19	IPA/WATER	5				55-60				14KM41A
20	IPA/WATER	5				55-60				14KM49B
21	IPA/WATER	2.85				55-60				14KM60E
22	IPA/WATER	5				55-60				14KM64C
23	IPA/WATER	5				40-45				14KM64D
24	IPA/WATER	1.25				50	III	197.9	DN-383-19	14KM73B
31	ETHYL ACETATE/WATER	5	35-40	ICE BATH	22	50-60			MAS-518-88	MAS-518-88-8
56	ETHYL ACETATE: HEXANE (7:4)	-	70	RT:2-8	3:4	50		197.5	DN-383-15	383-18-D-2
72	ACETON/WATER	2	55-60	RT	15			197.5	DN-383-23	ST-246W
88	WATER SLURRY	100	RT:50	NA				197.5		
90	WATER SLURRY	100		NA		RT		197.9	DN-383-34	0383-34
91	WATER SLURRY	100	RT	NA		RT		197.8	DN-383-34	0383-34
92	WATER SLURRY	100	45	NA		37			WW-386-20	#51
93	WATER SLURRY	100	RT	NA		37			WW-386-22	#54
94	WATER SLURRY	100	RT	NA						
95	WATER SLURRY (14KM71A)	100	60	NA		50	V	198.1	DN-383-15	383-18-A-2
6	IPA/WATER	2	55-60	RT	14	75		197.9	DN-383-1	0374-26
25	ETHYL ACETATE	-	70-80	RT	72	50-60			MAS-518-88	MAS-518-88-7
55	ETHYL ACETATE: HEXANE (7:4)	-	70	RT:2-8	3:4	75		197.7	DN-383-1	0374-28
58	ETHANOL	-	70-80	RT	72					

67	METHANOL/WATER	2	55-60	RT	15	50
69	METHANOL/WATER	2	35-40	ICE BATH	22	50

197.6	DN-383-15	383-18-C-2
197.9	DN-383-19	

[000094] Form I is a monohydrate crystalline form of ST-246. Examples of X-Ray Diffraction (XRPD), data for Form I are summarized in Figure 1 and are shown below:

Angle 2-Theta	d value Angstrom	Intensity Cps	Intensity %
7.63	11.58	5.92	5.5
10.04	8.80	35.5	33.3
11.47	7.71	26.8	25.1
14.73	6.01	13.8	12.9
15.21	5.82	7.67	7.2
15.47	5.72	14.0	13.1
16.06	5.51	20.4	19.1
16.67	5.31	21.5	20.1
16.98	5.22	9.21	8.6
18.93	4.68	107	100.0
19.96	4.45	29.4	27.5
20.52	4.32	12.5	11.7
20.79	4.27	48.2	45.2
22.80	3.90	79.6	74.5
25.16	3.54	4.17	3.9
26.53	3.36	14.0	13.1
27.20	3.28	8.55	8.0
27.60	3.23	9.21	8.6
29.60	3.02	10.7	10.1
30.23	2.95	48.5	45.4
30.49	2.93	69.5	65.1
30.68	2.91	25.0	23.4
31.14	2.87	7.67	7.2
33.65	2.66	104	97.3
34.33	2.61	16.9	15.8
35.29	2.54	10.1	9.4
35.56	2.52	19.5	18.3
36.30	2.47	11.8	11.1
37.36	2.41	32.9	30.8
38.42	2.34	3.51	3.3

Angle 2-Theta	d value Angstrom	Intensity Cps	Intensity %
38.66	2.33	28.7	26.9

[000095] The characteristic infrared spectrum of Form I is described below and is summarized in Figure 7.

The region from 4000 to 400 cm^{-1}

[000096] Form I has a large single peak at 3421 cm^{-1} and also have a broad absorbance underlying these peaks, from approximately 3300 to 2600 cm^{-1}. There are also two peaks at 3008 and 2956 cm^{-1}, likely due to C-H stretch. Form I has peaks at 1791, 1717 and 1671 cm^{-1}. All three forms have a peak at approximately 1560 cm^{-1}

[000097] ST-246 Form I is the desired polymorph of ST-246. It appears to be the thermodynamically most stable form, as all other get converted to Form-I.

[000098] ST-246 Form I is stable and hence can be stored at ambient conditions. Form I has not been shown to convert to another polymorphic form under several environmental and process conditions that a drug could experience during various stages of manufacturing and storage. Some of the conditions tested include storage at high temperature and high humidity, room temperature and high humidity, low humidity, up to 60°C, capsule manufacturing using wet granulation and drying, during milling or micronization process, in suspension, long term storage at room temperature. Further, Form-I is non hygroscopic and hence does not absorb moisture even at 90% relative humidity conditions. Form I is reliably manufactured by the

commercial process crystallization process with more than 99.0% purity and with impurities not more than 0.15%.

FORM II

[000099] In another aspect of the invention, the crystalline form of ST-246 is disclosed and is denominated as Form II of the ST-246, or in short "Form II".

[0000100] ST-246 Form II has been obtained in the presence of some alcohols, as well as acetone/IPA mixtures. In the preferred aspect of the invention, Form II is reliably crystallized in the presence of ethyl acetate or chloroform. Anhydrate Form II is relatively unstable and prone to conversion to Form III due to absorption of moisture.

[0000101] Form II is an anhydrate crystalline form of ST-246. Examples of X-Ray Diffraction (XRPD) are summarized in Figure 2.

FORM III

[0000102] In another aspect of the invention, the invention concerns the crystalline form of ST-246 that is denominated as Form III of the ST-246, or in short "Form III".

[0000103] As summarized in Tables 1 and 2, IPA/water mixtures, at various water levels, tend to give Form III. Further, Form III can be generated from a water slurry of Form V. Based on the data summarized in Table 1, a faster cooling rate and lower isolation temperature may tend to yield Form III.

[0000104] Form III is a monohydrate crystalline form of ST-246. Examples of a single crystal X-Ray Diffraction (XRPD)

data for Form III are shown in Figure 3 and summarized below:

Angle 2-Theta	d value Angstrom	Intensity Cps	Intensity %
6.71	13.15	102	100.0
9.05	9.76	5.23	5.1
12.49	7.08	2.77	2.7
13.03	6.79	11.2	11.0
13.79	6.42	4.61	4.5
14.87	5.95	2.56	2.5
15.72	5.63	3.79	3.7
16.26	5.45	14.8	14.5
16.74	5.29	30.3	29.7
18.10	4.90	11.4	11.2
18.43	4.81	4.51	4.4
19.94	4.45	6.46	6.3
21.04	4.22	10.0	9.8
21.51	4.13	9.64	9.4
23.15	3.84	7.28	7.1
23.51	3.78	4.10	4.0
25.32	3.51	7.28	7.1
26.24	3.39	3.79	3.7
26.87	3.32	11.2	11.0
27.32	3.26	4.31	4.2
27.72	3.22	3.69	3.6
28.55	3.12	9.12	8.9
29.08	3.07	5.84	5.7
29.50	3.03	9.84	9.6
29.84	2.99	6.66	6.5
31.27	2.86	2.46	2.4
33.48	2.67	3.59	3.5
35.36	2.54	3.38	3.3
39.56	2.28	3.49	3.4

[0000105] The characteristic infrared spectrum of the Form III is described below and summarized in Figure 8.

The region from 4000 to 2500 cm^{-1}

[0000106] Form III has a split peak at 3452 and 3397 cm^{-1}. There is also a peak at ~3008 and 2956 cm^{-1}, likely due to C-H stretch. There are also peaks at from approximately 3300 to 2600 cm^{-1}.

The region from 2000 to 1500 cm^{-1}

[0000107] Form III has a set of peaks at 1792, 1713 and 1662 cm^{-1}. All of these are likely due to C=O stretches. There is also a peak at 1560 cm^{-1}, tentatively assigned to N-H deformation.

The region from 1500 to 400 cm^{-1}

[0000108] From approximately 1500 to 400 cm^{-1}, there are a variety of less significant peaks.

[0000109] Form III (monohydrate) can be converted to Form I in competitive slurry experiments. Conversion from Form I to Form III has never been observed, suggesting that Form I is a more thermodynamically stable Form than Form III. However, Form III has an advantage over other less hydrated forms such as for example Form V in that Form III is fully hydrated and does not absorb any further amount of moisture under humid storage conditions.

FORM IV

[0000110] Examples of XRPD, single crystal X-ray data for Form IV are shown in Figure 4.

[0000111] In a preferred aspect of the invention, Form IV is formed in the presence of chlorinated solvents and some alcohols such as for example, TFE, 1 butanol, toluene, methylene chloride, chloroform, among others. Anhydrate Form IV is relatively unstable and prone to conversion to Form V, due to absorption of moisture.

FORM V

[0000112] In yet another aspect of the invention, the invention concerns the crystalline form of ST-246 that is denominated as Form V of the ST-246, or in short "Form V".

[0000113] Form V is a hemihydrate crystalline form of ST-246. Examples of XRPD data for Form V are shown below and summarized in Figure 5.

Angle 2-Theta °	d value Angstrom	Intensity Cps	Intensity %
6.39	13.81	101	100.0
6.72	13.14	9.56	9.5
8.16	10.82	1.88	1.9
9.04	9.78	3.75	3.7
9.52	9.28	6.38	6.3
10.52	8.41	4.88	2.1
12.40	7.13	5.06	5.0
12.79	6.92	7.31	7.3
13.38	6.61	4.13	4.1
14.15	6.25	12.0	11.9
14.57	6.07	11.4	11.4
15.84	5.59	15.9	15.9
16.32	5.43	10.7	10.6
16.67	5.31	25.7	25.6
17.50	5.06	21.2	21.1
18.13	4.89	9.19	9.1

Angle 2-Theta °	d value Angstrom	Intensity Cps	Intensity %
18.48	4.80	5.44	5.4
18.78	4.72	16.9	16.8
19.79	4.48	38.3	38.1
20.68	4.29	17.3	17.2
21.07	4.21	13.9	13.8
21.54	4.12	5.25	5.2
22.01	4.04	5.81	5.8
22.73	3.91	7.50	7.5
23.60	3.77	6.38	6.3
25.25	3.52	4.50	4.5
25.73	3.46	20.1	20.0
26.27	3.39	3.94	3.9
26.73	3.33	5.63	5.6
27.24	3.27	13.3	13.2
29.02	3.07	10.1	10.1
29.50	3.03	8.06	8.0
29.83	2.99	6.94	6.9
30.44	2.93	9.00	9.0
32.04	2.79	4.50	4.5
33.52	2.67	7.13	7.1
34.84	2.57	4.69	4.7
35.68	2.51	6.19	6.2
39.78	2.26	4.31	4.3

[0000114] The infrared spectrum of the Form V has also been summarized in Figure 9 and is described below. The underlined peaks are considered the most characteristics of the polymorph:

The region from 4000 to 2500 cm^{-1}

[0000115] Form V has a split peak at 3464 and 3402 cm^{-1} along with a second broad split peak at 3238 and 3206 cm^{-1}. These peaks are likely due to OH and NH stretches and appear to

allow differentiation of the three forms. Form V also has peaks at ~3008 and 2956 cm^{-1}, likely due to C-H stretch. There are further peaks at approximately 3300 to 2600 cm^{-1}.

The region from 2000 to 1500 cm^{-1}

[0000116] Form V has significantly different spectral characteristics in this region as compared to other polymorphic forms of ST-246, showing 5 peaks rather than 3, and these are at 1791, 1733, 1721, 1681 and 1667 cm^{-1}. All of these are likely due to C=O stretches. All three forms have a peak at approximately 1560 cm^{-1}, tentatively assigned to N-H deformation. Form V has peaks at1519 and 1497 cm^{-1}.

The region from 1500 to 400 cm^{-1}

[0000117] From approximately 1500 to 400 cm^{-1}, the infrared spectra of the three forms show only slight differences, and this region is probably not useful for differentiating the three forms of ST-246 discussed here.

[0000118] Form V (hemi-hydrate) was made during early GMP syntheses of ST-246 and is disclosed in WO 2008/130348, WO 2004/112718 and WO 2008/079159. The disadvantage of this polymorph is that it is not fully hydrated. This form readily absorbs moisture when placed in a humid environment, and has been shown to convert to Form I in competitive slurry experiments.

[0000119] Form V tends to form when insufficient water is present to generate the monohydrate form. Form V is formed from ethyl acetate/hexane mixtures when the starting ST-246 used for crystallization does not contain water. If the starting ST-246 contains enough water, Form III can be

formed. Form V has also been generated by methanol/water and IPA/water mixtures containing low levels of water.

FORM VI

[0000120] In one aspect of the invention, the invention concerns the crystalline form of ST-246 that is denominated as Form VI of the ST-246, or in short "Form VI".

[0000121] Form VI is an monohydrate crystalline form of ST-246. Examples of XRPD are summarized in Figure 6. In the preferred aspect of the invention, Form VI may be formed, as an example, in the presence of nitromethane or chloroform/methanol as crystallization solvents.

[0000122] In preparing polymorph Forms I, II, III, IV, and V substantially free of other polymorph forms, crystallization from a mixture of different forms is generally utilized. However, the crystallization technique with regard to producing each of these polymorph forms substantially free of other polymorph forms is different and described below.

[0000123] More specifically, the present invention provides isolated Form I that is at least about 70% pure (i.e. free of other forms), preferably at least about 80% pure, preferably at least about 90% pure, preferably at least about 95% pure, more preferably at least about 99% pure, and most preferably at least about 99.9% pure.

[0000124] The present invention provides for isolated Form II which is at least about 70% pure (i.e. free of other forms), preferably at least about 80% pure, preferably at least about 90% pure, more preferably at least about 99% pure, and most preferably at least about 99.9% pure. Further, the

present invention provides for isolated Form III which is at least about 70% pure, preferably at least about 80% pure, preferably at least about 90% pure, preferably at least about 95% pure, more preferably at least about 99% pure, and most preferably at least about 99.9% pure.

[0000125] Also, the present invention provides for isolated Form IV which is at least about 70% pure (i.e. free of other forms), preferably at least about 80% pure, preferably at least about 90% pure, preferably at least about 95% pure, more preferably at least about 99% pure, and most preferably at least about 99.9% pure.

[0000126] Further, the present invention provides for isolated Form VI which is at least about 70% pure (i.e. free of other forms) , preferably at least about 80% pure, preferably at least about 90% pure, preferably at least about 95% pure, more preferably at least about 99% pure, and most preferably at least about 99.9% pure.

[0000127] The crystals, powder aggregates and coarse powder of the polymorphic forms of the ST-246 may be optionally milled and sorted by size after undergoing conversion. Milling or grinding refers to physically breaking up the large particles or aggregates of particles using methods and apparatus well known in the art for particle size reduction of powders. Resulting particle sizes may range from millimeters to nanometers, yielding i.e. nanocrystals, microcrystals.

[0000128] The polymorph of the invention may be milled using known milling procedures such as wet milling to obtain a particle size appropriate for tablet formation and for other

formulation types. Finely divided (nanoparticulate) preparations of the polymorph of the invention may be prepared by processes known in the art, for example see international patent application WO 02/00196 (SmithKline Beecham).

[0000129] In one aspect, the particle size of each of polymorphic Forms I-IV and VI of ST-246 in the present invention has D_{90} of the volume mean diameter of the particles within the range of about 0.01-200 □m, preferably about 15-50 □m, and most preferably about 0.01-15 □m. Such particles are better in chemical and physical stability, good material flow characteristics, improving the uniformity of dosage forms and thus suitable for bulk preparation and formulation advantages.

FORMULATIONS AND ADMINISTRATION

[0000130] Formulations of polymorphic forms of ST-246 may be prepared by processes known in pharmaceutics art. The following examples (infra) are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

[0000131] The polymorphic salts of the present invention can be administered in a variety of oral and parenteral dosage forms. Oral dosage forms can be tablets, coated tablets, hard and soft gelatin capsules, solutions, emulsions, syrups, or suspensions. Parenteral administration includes intravenous, intramuscular, intracutaneous, subcutaneous, intraduodenal, or intraperitoneal administration.

Additionally, the salts of the present invention can be administered by transdermal (which may include a penetration enhancement agent), buccal, nasal and suppository routes.

[0000132] For preparing pharmaceutical compositions from the compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, hard and soft gelatin capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, lubricants, suspending agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

[0000133] In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

[0000134] Suitable excipients for tablets, coated tablets, and hard gelatin capsules are, for example, microcrystalline cellulose, lactose, corn starch and derivatives thereof, magnesium carbonate, magnesium stearate, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, talc, and fatty acids or their salts, e.g., stearic acid. If desired, the tablets or capsules may be enteric-coated or sustained release formulations. Suitable excipients for soft gelatin capsules are, for example, vegetable oils, waxes, fats, semi-solid and liquid polyols. Liquid form preparations include solutions, suspensions, retention enemas, and

emulsions. For parenteral injection, liquid preparations can be formulated in solution in water or water/polyethylene glycol solution.

[0000135] Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing, and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

[0000136] Compositions also may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, preservatives, wetting agents, emulsifiers, salts for adjustment of the osmotic pressure, masking agents, antioxidants and the like.

[0000137] The compounds of the present invention can be administered intravenously in physiological saline solution (e.g., buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used in the present compositions.

[0000138] Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. For preparing suppositories suitable excipients include natural and hardened oils, waxes, fatty acid glycerides, semi-liquid or

liquid polyols. The molten homogenous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify. Suitable pharmaceutical carriers, excipients and their formulations are described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pa.

[0000139] The dosage can vary within wide limits and will, of course, be adjusted in each particular case to the individual requirements of the patient and the severity of the condition being treated. A typical preparation will contain from about 5% to about 95% active compound (w/w). For oral administration, a daily dosage of between about 0.01 and about 100 mg/kg body weight per day should be appropriate in monotherapy and/or in combination therapy. A preferred daily dosage is between about 0.1 and about 300 mg/kg body weight, more preferred 1 and about 100 mg/kg body weight and most preferred 1.0 and about 50 mg/kg body weight per day.

[0000140] Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstance is reached. The daily dosage can be administered as a single dosage or in divided dosages, typically between 1 and 5 dosages per day.

[0000141] The pharmaceutical preparations are preferably in unit dosage forms. In such form, the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete

quantities of preparation, such as packeted tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

[0000142] Appropriate doses will be readily appreciated by those skilled in the art. It will be appreciated that the amount of a polymorph of the invention required for use in treatment will vary with the nature of the condition being treated and the age and the condition of the patient and will be ultimately at the discretion of the attendant physician or veterinarian. The polymorph of the invention may be used in combination with other antibacterial drugs such as penicillin, cephalosporin, sulfonamide or erythromycin.

[0000143] The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier or excipient comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations by any convenient route.

[0000144] When administration is sequential, either the polymorph of the invention or the second therapeutic agent may be administered first. When administration is simultaneous, the combination may be administered either in the same or different pharmaceutical composition.

[0000145] Using the routes and methods of administration and dosage amounts described hereinabove and the dosage forms described herein below, the individual polymorph forms, such as Form I, Form II, Form III, Form IV, Form V and Form VI, and mixtures of polymorph forms of the present invention can be used for the prevention and treatment of various diseases and conditions in humans. By way of example and not of limitation, in the case of orthopoxvirus infections and associated diseases, this is accomplished by administering to a patient in need of said treatment who is suffering from orthopoxvirus infections a composition containing one of the above polymorph forms, such as Form I, Form II, Form III, Form IV, Form V and Form VI, substantially free of other polymorph forms or mixtures of polymorphs and an inert carrier or diluent, said composition being administered in an effective amount to prevent or treat said viral infection.

[0000146] In accordance with this invention, ST-246, either as a polymorph form substantially free of other polymorph forms or as a mixture of polymorph forms, is administered in an effective amount to prevent or treat orthopoxviral infection. Any effective amount of such polymorph form substantially free of other polymorph forms or mixtures of polymorph forms needed to prevent or treat such viral infection can be utilized in this composition. In general, in the case oral dosage forms, dosages of from about 0.5 mg/kg to about 5.0 mg/kg of body weight per day are used. However the amount of such polymorph form, such as Form I, Form II, Form III, Form IV, Form V and Form VI, substantially free of other polymorph forms or mixtures of polymorph forms in the oral unit dose to be administered

will depend to a large extent on the condition of viral infection, and the weight of the patient and of course be subject to the physician's judgment. In one aspect of the invention, Form I is the preferred ST-246 polymorph form for administration.

[0000147] In accordance with this invention, the oral unit dosage form containing the given polymorph form substantially free of other polymorph forms or mixtures of polymorph forms can be preferably administered at a dosage of from about 30 mg to 800 mg per day, more preferably from about 50 mg to about 600 mg per day, administered once to three times during the day or as needed.

[0000148] In some aspect of the invention, the polymorph of the present invention, preferably hydrate form of ST-246, may also be used in combination with: (1) a vaccine ; (2) Cidofovir, an injectable antiviral medication which is acyclic nucleoside phosphonate, and is therefore independent of phosphorylation by viral enzymes, to treat eczema vaccinatum (EV), a life-threatening complication of vaccinia virus infection, and other related disorders; and/or (3) CMX001 (hexadecyloxypropyl-cidofovir), a mimic of a naturally occurring lipid, lysolecithin, formed by linking a lipid, 3-hexadecyloxy-1-propanol, to the phosphonate group of cidofovir.

[0000149] The invention also provides pharmaceutical packs or kits comprising one or more containers filled with one or more crystalline polymorph of ST-246, including Form I, Form II, Form III, Form IV, Form V and Form VI. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the

manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In a certain aspect of the invention, the kit contains more than one crystalline polymorph of ST-246.

Example 1 - Preparation of polymorphic Form I:

[0000150] More specifically, to prepare ST-246 monohydrate, Form I, cycloheptatriene is reacted with maleic anhydride in the presence of toluene to yield the major product, endo isomer. The exo isomer is further produced from about 7% to 0.6% by crystallization from toluene/heptane. Further, hydrazine in the anhydrous or hydrate form is reacted with MethyI 4-(trifluoromethyl) benzoate in the presence of isopropanol to yield (4-(trifluoromethyl)-benzhydrazide. The product is then crystallized from isopropanol.

[0000151] The next step of the synthesis involves condensing endo-tricyclo[3.2.2.0]non-8-endo-6,7-dicarboxylic anhydride and (4-(trifluoromethyl)-benzhydrazide) in isopropanol. The product is isolated by crystallization from isopropanol and the slurry is further heated to reflux and held. The resulting solution is cooled and sampled for reaction completion. After analysis shows reaction completion, carbon and celite are charged and the batch is heated to reflux and held. After cooling, the batch is filtered to remove these solid materials, followed by a filter chase with IPA. The batch is cooled and held while slurry is formed. The batch is further cooled and held. Contents are centrifuged and the wet cake containing synthesis product is washed with heptane. The wet cake is dried and is referred to as partially hydrated form of ST-246 (SG3).

[0000152] The SG3 is charged followed by ethyl acetate. The mixture is heated and held to ensure dissolution of SG3. A polish filtration is performed on the batch and an extraneous material check confirms that the filtration was successful. Ethyl acetate is used to charge the filter. After heating the batch to reflux, Endotoxin reduced (ER) water is charged. The batch is seeded and the final ER water is charged. The batch is held at reflux and a slurry check is performed.

[0000153] Further, the batch is cooled, at which time a sample of the slurry is obtained for verification of correct polymorph. The batch is cooled further and is held until final isolation on the centrifuge. The final API is dried, milled using a Fitz Mill as described in WO 02/00196. Form I can be prepared by crystallization of ST-246 from a variety of solvents and solvent combinations as further summarized in Tables 2 and 3:

Table 3 - Solvent screening study results.

Solvent A	Solvent B	Ratio (volA:volB)	Saturation Temp (°C)	Overheat Temp (°C)	Growth Temp (°C)	Crystal Form
1-Propanol	None		30	35	25	II
Ethanol	Water	1:1	40	45	35	II
Acetone	IPA	4:1, 1:1, 1:4	40	45	35	II
ACN	Ethyl acetate	1:1	40	45	35	II
Ethanol	Toluene	4:1	40	45	35	II
Ethanol	Water	1:1	50	55	50	II
Acetone	IPA	4:1, 1:1, 1:4	50	55	50	II
ACN	Ethyl acetate	4:1, 1:1	50	55	50	II
Methanol	CHCl3	1:4	50	55	50	II

Solvent A	Solvent B	Ratio (volA:volB)	Saturation Temp (°C)	Overheat Temp (°C)	Growth Temp (°C)	Crystal Form
Ethanol	Toluene	4:1, 1:1, 1:4	50	55	50	II
Ethanol	Water	1:1	30	35	30	II
Acetone	IPA	4:1, 1:1, 1:4	30	35	30	II
Water	Ethyl acetate	1:1	30	35	30	II
DMF	ACN	4:1	30	35	30	II
Ethanol	Toluene	4:1	30	35	30	II
Ethyl acetate	None	1 gram scale				II
IPA	None		30	35	25	III
DMF	None		30	35	25	III
DMA	None		30	35	25	III
Pyridine	None		30	35	25	III
Isopropyl ether	None		30	35	25	III
THF	None		30	35	25	III
CH2Cl2	IPA	4:1, 1:1, 1:4	40	45	35	III
Ethanol	Water	4:1, 1:4	40	45	35	III
ACN	Ethyl acetate	4:1	40	45	35	III
TFE	THF	4:1, 1:1, 1:4	40	45	35	III
DMF	ACN	4:1, 1:1	40	45	35	III
Methanol	CHCl3	4:1, 1:1	40	45	35	III
Ethanol	Toluene	1:1	40	45	35	III
CH2Cl2	IPA	4:1, 1:1, 1:4	50	55	50	III
Ethanol	Water	4:01	50	55	50	III
ACN	Ethyl acetate	1:4	50	55	50	III
TFE	THF	4:1, 1:1	50	55	50	III
DMF	ACN	4:1, 1:1, 1:4	50	55	50	III
Methanol	CHCl3	4:1, 1:1	50	55	50	III
Water	IPA	1:1, 1:4	30	35	30	III
Ethanol	Water	4:1	30	35	30	III
Water	Ethyl acetate	4:1	30	35	30	III
Trifluoroethanol	THF	4:1	30	35	30	III

Solvent A	Solvent B	Ratio (volA:volB)	Saturation Temp (°C)	Overheat Temp (°C)	Growth Temp (°C)	Crystal Form
(TFE)						
DMF	ACN	1:1, 1:4	30	35	30	III
Methanol	CHCl3	4:1, 1:1, 1:4	30	35	30	III
Ethanol	Toluene	1:1, 1:4	30	35	30	III
Trifluoroethanol (TFE)	None		30	35	25	IV
1 Butanol	None		30	35	25	IV
CH2Cl2	None		30	35	25	IV
CHCl3	None		30	35	25	IV
Toluene	None		30	35	25	IV
ACN	Ethyl acetate	1:4	40	45	35	IV
Ethanol	Toluene	1:4	40	45	35	IV
Water	Ethyl acetate	1:4	30	35	30	IV
Trifluoroethanol (TFE)	THF	1:1	30	35	30	IV
1 Butanol	None	1 gram scale				IV
Methanol	None		30	35	25	V
Ethanol	None		30	35	25	V
2 Butanol	None		30	35	25	V
Acetone	None		30	35	25	V
Methyl Ethyl Ketone	None		30	35	25	V
Ethyl acetate	None		30	35	25	V
MTBE	None		30	35	25	V
Isopropyl acetate	None		30	35	25	V
Acetonitrile (can)	None		30	35	25	V
DMF	ACN	1:4	40	45	35	V
Nitromethane	None		30	35	25	VI
Nitromethane	None	1 gram scale				VI
Methanol	CHCl3	1:4	40	45	35	VI

[0000154] The identity of ST-246 Form I obtained by the above described process was confirmed by XRPD and IR as summarized in Figures 1 and 7.

Example 2 - Preparation of polymorphic Form II

[0000155] Standards of Form II were made by re-crystallization of Forms I and V starting material from ethyl acetate and chloroform solvents. An amount of ST-246 Form I or Form V was dissolved in either ethyl acetate or chloroform and filtered through 0.45 □m membrane filters. The filtered solutions were overheated at a higher temperature to make sure all solids were dissolved and then reduced to a lower temperature and evaporated to dryness under a nitrogen purge (~ 2 psi.).

[0000156] The preferred crystallization conditions for Form II are summarized in Table 4 below:

Table 4 - Crystallization conditions for ST-246 Form II.

Starting Material	Solvent	Overheating Temperature (°C)	Evaporation Temperature (°C)	XRPD Pattern
1.5 g	Ethyl acetate	35	25	Form II
1.1 g	Chloroform	45	35	Form II

[0000157] The further examples of crystallization conditions for Form II are also summarized in Tables 1-2 above.

[0000158] The identity of ST-246 Form II obtained by the above described process was confirmed by XRPD as summarized in Figure 2.

Example 3 - Preparation of polymorphic Form III:

[0000159] Form III is produced from reslurry of anhydrous ST-246 in water. Further examples of solvents used to generate Form III are summarized in Tables 1-9 above.

[0000160] The identity of ST-246 Form III produced by the process described above was confirmed by XRPD and IR as summarized in Figures 3 and 8.

Example 4 - Preparation of polymorphic Form IV:

[0000161] A standard of Form IV was made by re-crystallization of Form I starting material from 1-butanol solvent. The starting solid material was dissolved in 1-butanol and filtered through a 0.45 um membrane filter. The filtered solution was overheated at a higher temperature to make sure all solids were dissolved and then reduced to a lower temperature and evaporated to dryness under a nitrogen purge (~ 2 psi). The preferred crystallization conditions for Form IV are summarized in Table 5. Further examples of crystallization of Form IV are summarized in Tables 1-3.

Table 5 - Crystallization conditions for ST-246 Form IV.

Starting Material	Solvent	Overheating Temperature (°C)	Evaporation Temperature (°C)	XRPD Pattern
1.5 g	1-butanol 70 ml	50	35	Form IV

[0000162] The identity of ST-246 Form IV produced by the process described above was confirmed by XRPD as summarized in Figure 4.

Example 5 - Preparation of polymorphic Form V:

[0000163] Form V (hemi-hydrate) was made during early GMP syntheses of ST-246 and is disclosed in WO 2008/079159 and WO 2008/130348. The disadvantage of this polymorph is that it is not fully hydrated. This form readily absorbs moisture when placed in a humid environment, and has been shown to convert to Form I in competitive slurry experiments

[0000164] The identity of ST-246 Form V was confirmed by XRPD and IR as summarized in Figures 5 and 9.

Example 6 - Preparation of polymorphic Form VI:

[0000165] A standard of Form VI was made by re-crystallization of Form V starting material from nitromethane solvent. The starting solid material was dissolved in nitromethane and filtered through a 0.45 um membrane filter. The filtered solution was overheated at a higher temperature to make sure all solids were dissolved and then reduced to a lower temperature and evaporated to dryness under a nitrogen purge (~ 2 psi). Form VI can be prepared by crystallization of ST-246 from a variety of solvents and solvent combinations. The preferred crystalization conditions are summarized in Table 6 below. Further examples of crystallization of Form VI are summarized in Tables 1-3.

Table 6 - Form VI Crystallization Conditions.

Starting Material	Solvent	Overheating Temperature (°C)	Evaporation Temperature (°C)	XRPD Pattern
1.2 g	Nitromethane (60 ml)	35	25 then 35	Form VI

[0000166] The identity of ST-246 Form VI obtained by a process described above was confirmed by XRPD as summarized in Figures 6.

Example 7 - Distinguishing and comparative physical characteristic of polymorphic forms of ST-246, Form I, Form III and Form V:

[0000167] It has been determined that ST-246 can exist in three predominant physical forms (Forms I, III and V). The data was obtained on the relevant physical/chemical properties and stability of the polymorphs to determine if the different solid forms impact the quality of the product. These data include the crystallographic properties of the polymorphs and physical/chemical properties of the polymorphs (e.g. solubility, dissolution, melting range) accelerated stability data.

[0000168] The X-ray diffractions patterns of Forms I, III and V are shown in Figures 1, 3 and 5, respectively. The powder X-ray pattern of Forms I, III, and V are readily distinguishable based on the unique features in their powder patterns.

Interconversion of Forms I, III and V

[0000169] Competitive and non-competitive slurry experiments were performed to determine the most stable form of ST-246. The slurry experiments were performed by exposing excess material of one or more forms of ST-246 in a small volume of water and agitating the resulting suspensions for several days at ambient temperature and/or 45°C. Similar experiments were also done at different pH values for 60 minutes at 37°C. The slurry was filtered and the solid

analyzed by powder XRPD. To avoid possible desolvation or physical change after isolation, the samples were not subjected to drying before powder X-ray analysis. Competitive and non-competitive slurry experiments in water show that Form V and Form III convert to Form I in water and Form I remains unchanged. The slurry data are summarized in Table 7 below:

Table 7 - Interconversion of Polymorphs of ST-246 in Aqueous Liquids.

Initial Forms	Solvent/Temp	Slurry Duration	Final Form
I. III & V	Water/RT	5 days	I
I. III & V	Water/RT	13 days	I
I & III	Water/RT	2 days	I
I &III	Water/RT	30 days	I
V	pH 1.2, 6.8/37°C	30 min	III
III	Water/45°C	17 days	I
I	Water/45°C	17 days	I

Micronization of Form I and III:

[0000170] ST-246 belongs to BCS class II due to its poor solubility in physiologically relevant buffers. Phase 1 clinical trial material was made using micronized Form V with particle size of d50% 4.8 µm and d90% 12µm. Hence both Forms I and III at a scale of 400gm were micronized using an airjet mill as described in WO 02/00196. On milling, both the forms yielded the desired particle size without undergoing any transformation in physical form (based on XRPD).

[0000171] Representative XRPD patterns of both micronized and unmicronized Forms I and III are summarized in Figures 14 and 15.

Drug Substance Stability

[0000172] Drug substance Forms I and III, both micronized and unmicronized, have undergone short-term stability evaluation under stress conditions. The short-term study has been completed and the data obtained at 40°C/75% RH are presented in Tables 8 and 9.

Table 8 - Three months R&D stability data of ST-246 Forms I and III.

Batch #14KM46B (Form I)				
Test	**Initial**	**40°C/75%RH Open**		
		1 Month	**2 Month**	**3 Month**
Assay (HPLC)	97.38%	Not Done	Not Done	98.53%
Related Substances	0.06% isomer, <0.05% unknown RRT=1.4	Not Done	Not Done	0.07% isomer, <0.05% unknown RRT=1.4
Moisture (by KF)	4.7%	5.1%	5.0%	5.2%
XRD	Form I	Form I	Form I	Form I
Melting Point (by DSC)	197.15°C	196.39°C	196.21°C	Not done
Batch #14KM49B (Form III)				
Assay (HPLC)	100.64%	Not Done	Not Done	99.95%
Related Substances	0.02% isomer, <0.05% unknown RRT=1.4	Not Done	Not Done	0.03% isomer, <0.05% unknown RRT=1.4
Moisture (by KF)	4.5%	5.2%	5.1%	4.9%
XRD	Form III	Form III	Form III	Form III
Melting Point (by DSC)	196.92°C	195.85°C	196.24°C	Not done

Table 9 - R&D Stability Data of ST-246 Forms I and III (Micronized lot).

Test	T=0	25°C/60%RH		40°C/75%RH	
		1 Month	2 Month	1 Month.	2 Month
Lot # 14KM75B-4724 (Form I)					
Assay (HPLC) %	97.36	101.15	99.68	102.33	99.71
Related Substances (%)					
RRT 1.08	0.11	0.07	<0.05	0.06	0.08
RRT 1.37	<0.05	<0.05	<0.05	<0.05	<0.05
RRT 1.39	<0.05	<0.05	<0.05	<0.05	<0.05
Moisture (by TGA) %	4.8	4.7	5.09	4.7	5.26
XRD	Form I	Form I	Form I	Form I	Form I
Melting Point (by DSC)	196.33°C	Not Done	196.03°C	Not Done	196.06°C
Lot # 14KM84-4724 (Form III)					
		1 Month	2 Month	1 Month	2 Month
Assay (HPLC) %	97.87	101.6	99.48	102.1	99.38
Related Substances (%)					
RRT 1.08	0.07	0.10	<0.05	0.07	0.05
RRT 1.37	<0.05	<0.05	<0.05	<0.71	<0.05
RRT 1.39	<0.05	<0.05	<0.05	<0.09	<0.05
Moisture (by TGA) %	4.8	4.7	4.78	5.2	5.32
XRD	Form III	Form III	Form III	Form III	Form III
Melting Point (by DSC)	194.44°C	Not Done	Not Done	Not Done	Not Done

[0000173] The data on both micronized and unmicronized drug substance indicates no change in physical form of both I and III with respect to Purity, Related Substances, Moisture, XRPD and DSC.

[0000174] The long-term study has also been completed and the data obtained are presented in Table 10.

Table 10 - Long-Term Stability Testing Results - Lot # SG-08B01-M (Form-I).

Interval month	Description	Water Content	HPLC Assay	HPLC Related Substances RRt ~ 1.08	HPLC Related Substances Total	SG1 & SG1 Exo Isomer	Hydrazine By HPLC	SG2 Dimer
0	conforms	4.40%	99.7%	0.06%	0.06%	na	na	na
3	conforms	4.66%	99.7%	0.06%	0.06%	na	na	na
6	conforms	4.71%	99.7%	<0.05%	<0.05%	na	na	na
9	conforms	4.47%	97.4%	<0.05%	<0.05%	na	na	na
12	conforms	4.67%	98.5%	<0.05%	<0.05%	na	na	na

18	conforms	4.79%	99.8%	0.05%	0.05%	na	na	na
24	conforms	4.81%	100.2%	0.05%	0.05%	<0.05%	<0.1 ppm	<0.01%

Static Sorption of Forms I, III and V

[0000175] Hygroscopicity testing was done on Forms I, III and V at various humidity conditions to understand sorption/desorption properties. Approximately 1 g of each form was ground with a mortar and pestle. Water content was determined by TGA. Approximately 100 mg of each powder was placed in static humidity chambers at 11 and 97 %RH at approximately 25°C for ~10 days. The only sample that exhibited a change in weight loss from Day 0 was the Form V sample stored at 97 %RH. The data is summarized in Table 11 below:

Table 11 - Hygroscopicity data of Forms I, III and V at 11%RH and 97%RH.

Sample	%RH	Day 12 wt loss	Day 0 wt loss
Form I	11	4.8% to 117.5°C	4.8% to 117°C
Form III	11	4.8% to 97.5°C	4.8% 97.3°C
Form V	11	2.2% to 109.8°C	2.2% to 97.8°C
Form I	97.6	4.7% to 119°C	4.8% to 117°C
Form III	97.6	4.8% to 100.2°C	4.8% 97.3°C
Form V	97.6	3.4% to 112.2°C	2.2% to 111°C

Example 8.–Analysis of the effect of ST-246 API particle size on Dissolution Profiles of ST-246 Hard gelatin capsules.

[0000176] The effect of the particle size of drugs on their dissolution profile has been extensively reviewed (see Fincher et al.,1968) and it had been hypothesized that a

decrease in particle size of sparingly soluble drugs results in increased dissolution rates owing to the increased surface area of the drug exposed to the solvent.

[0000177] Table 12 summarizes micronized and unmicronized ST-246 API particle size in microns, wherein micronization done for further testing the effect of particle size on the dissolution profiles of ST-246.

Table 12 - Micronized and unmicronized ST-246 API particle size in microns.

ST-246 API Lot#	Particle size of ST-246 API in microns		
	D10	D50	D90
Micronized			
SG-08G02-M	1.008	3.243	8.097
SG-08H05-M	0.918	2.517	5.987
SG-08H06-M	1.007	2.602	5.627
SG-08K07-M	0.909	2.450	5.563
SG-08L08-M	1.032	2.479	4.999
SG-08-09-M-Trial 1	1.233	2.166	4.617
SG-08-09-M-Trial 2	1.587	4.802	13.601
SG-08-09-M-Trial 3	1.723	5.846	17.698
SG-08-09-M-Trial 4	1.888	7.111	22.806
Un micronized			
SG-08-09-M unmicronized	17.4	111.8	281.2

[0000178] In order to evaluate the effect of particle of API on dissolution of ST-246 capsules, the following formulation comprising ST-246 polymorph Form I was evaluated. For these experiments, ST-246 (Form-I), 200mg capsules were prepared using drug substance with different particle size

distributions, such as d90% less than 10 µm, d90% 16 um, d90% 25um, d90% less than 254 µm and d90% less than 75 µm. The composition of the ST-246 gelatin capsules are shown in the Table 13 below.

Table 13 - Composition of capsules used for discriminating dissolution medium experiments.

ST-246 Composition Ingredient	Lot# 0801637	SJI091023-API-Trial #3	SJI091023-API-Trial #4	SG-09K10-Q-API-40um	SG-09K10-Q-API-60um	Lot# WW386-89	Lot# DN401-93
	mg/Capsule	mg/Capsule	mg/Capsule	mg/Capsule	mg/Capsule	mg/Capsule	mg/Capsule
Particle size distribution	[d90% 5.3 µm]	[d90% 16.6 µm]	[d90% 26.6µm]	d90% 40.85µm]	d90% 58.20µm]	[d90% 75 µm]	[d90% 254 µm]
ST-246 (Form-I) Monohydrate	200	200	200	200	200	200	200
Microcrystalline cellulose, NF	88.60	88.60	88.60	88.60	88.60	88.60	88.60
Lactose monohydrate, NF	33.15	33.15	33.15	33.15	33.15	33.15	33.15
Croscarmellose sodium, NF	42.90	42.90	42.90	42.90	42.90	42.90	42.90
Colloidal silicon dioxide, NF	1.95	1.95	1.95	1.95	1.95	1.95	1.95
Hydroxypropyl methylcellulose, USP	13.65	13.65	13.65	13.65	13.65	13.65	13.65
Sodium lauryl sulfate, NF	7.80	7.80	7.80	7.80	7.80	7.80	7.80
Magnesium stearate NF	1.95	1.95	1.95	1.95	1.95	1.95	1.95
Capsule weight	390	390	390	390	390	390	390

For these experiments, ST-246 (Form I) dissolution profiles are determined in an USP apparatus 2 (paddle) which runs at 75 rpm. The dissolution profiles are determined at 37°C, in a 900 ml dissolution medium, containing 0.05 M Phosphate buffer pH 7.5, containing 3% HDTMA. Cumulative drug release over time is represented as a percent of ST-246% dissolved and is plotted as a function of dissolution medium sampling time.

[0000179] As summarized in Table 14 and Figure 16-20, ST-246 (Form I) with a D90 particle size (d90%) of 5.3 microns and 16.6 microns achieved almost 100% dissolution at approximately 22 minutes, whereas ST-246 (Form I) with a D90 particle size (d90%) of 26.6 achieved almost 100% dissolution at 30 minutes. Also, ST-246 (Form I) with a D90 particle size of 40.85 and 58.2 microns achieves almost 85 to 86% dissolution in 30 minutes. Further, ST-246 (Form I) with a D90 particle size of 75 microns achieves almost 86% dissolution in 30 minutes and ST-246 (Form I) with D90 particle size of 254 microns achieves only 44% dissolution in 60 minutes. Table 14 shows dissolution profiles using an alternate dissolution method (1% HDTMA in 900 mL of 0.05 M Phosphate buffer pH 7.5 at 37°C in an USP apparatus 2 (paddle) which runs at 50 rpm) for the capsules made with ST-246 (Form I) with a D90 particle size (d90%) of 5.3 microns, 16.6 microns, and 26.6 microns.

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Table 14 - The % Dissolution of ST-246 (Form I) Capsules with API of Various Particle Sizes in 3% HDTMA in 900 ml of 0.05 M Phosphate buffer, pH 7.5, using dissolution apparatus USP 2 with a paddle speed of 75 RPM at 37°C.

Time in Minutes	%Dissolution							%RSD						
	Lot# 080163 7	SJI0910 23-API-Trial #3	SJI091 023-API-Trial #4	SG-09K10-Q-API-40um	SG-09K10-Q-API-60um	Lot# WW38 6-89	Lot# DN401-93	Lot# 080163 7	SJI09 1023-API-Trial #3	SJI09 1023-API-Trial #4	SG-09K10-Q-API-40um	SG-09K1 0-Q-API-60um	Lot# WW 386-89	Lot# DN401 -93
	d90% 5.3 µm	d90% 16.6 µm	d90% 26.6 µm	d90% 40.85µm	d90% 58.20µm	d90% 75 µm	d90% 254 µm	d90% 5.3 µm	d90% 16.6 µm	d90% 26.6 µm	d90% 40.85µm	d90 % 58.20 µm	d90% 75 µm	d90% 254 µm
15	88	85	76	70	67	69	22	5	5	13	2.8	4.8	3	2
30	98	96	91	82	80	81	31	4	1	4	2.6	3.9	5	3
45	99	98	95	86	85	87	39	3	1	3	2.5	2.8	4	3
60	101	99	97	89	88	94	44	2	2	2	2.5	3.2	3	3

Table 15 - The % Dissolution of ST-246 (Form I) Capsules with API of Various Particle Sizes in 1% HDTMA in 900 ml of 0.05 M Phosphate buffer, pH 7.5, using dissolution apparatus USP 2 with a paddle speed of 50 RPM at 37°C.

Time in Minutes	%Dissolution			%RSD		
	Lot# 0801637	SJI091023-API-Trial #3	SJI091023-API-Trial #4	Lot# 0801637	SJI091023-API-Trial #3	SJI091023-API-Trial #4
	d90% 5.3 µm	d90% 16.6 µm	d90% 26.6 µm	d90% 5.3 µm	d90% 16.6 µm	d90% 26.6 µm
15	44	55	56	22	12	11
30	75	70	71	11	9	7
45	87	77	77	3	8	7
60	94	81	81	3	8	7

[0000180] Further, ST-246, Form I, can be formulated for oral administration in capsules comprising 200 mg of ST-246. For these experiments, ST-246 (Form I) with a D90 particle size of between about 5.3 to 75 microns may be used. All inactive ingredients may be GRAS and USPLNF excipients. The manufacturing process may include wet granulation using a high shear mixer/granulator and filling into hard gelatin capsules.

[0000181] Suitable dosage forms can include capsules containing various amounts of active ingredient. The quantitative composition of exemplary dosage form containing 200mg of ST-246 monohydrate, micronized with a D90 particle size of less than about 10 microns, is summarized in Table 15 below:

Table 16 - Quantitative Composition of ST-246 Drug Product

		200 mg Strength	
Ingredient	Function	mg/Capsule	% w/w

ST-246 monohydrate[a] (micronized, D_{90} <10 microns) monohydrate, based on anhydrous basis.	Active Ingredient; white to off-white powder	200.00	51.28
Microcrystalline cellulose, NF[b]	Water Insoluble Diluent	88.60	22.72
Lactose monohydrate, NF	Water soluble Diluent	33.15	8.50
Croscarmellose sodium, NF[b]	Disintegrant	42.90	11.00
Colloidal silicon dioxide, NF	Glidant	1.95	0.50
Hypromellose , USP	Binder	13.65	3.50
Sodium lauryl sulfate, NF	Wetting Agent/ Solubilizer	7.80	2.00
Magnesium stearate NF	Lubricant	1.95	0.50
Water USP[c]	Granulating Agent		
Hard Gelatin Capsule shell, orange/black, Size 0	Encapsulation	1 capsule	-
Capsule weight, mgs		390	100

[a]The quantity of ST-246 monohydrate may be adjusted based on the drug substance assay, which is calculated to reflect the purity and water content. The amount of Lactose will be adjusted to maintain the same capsule weight.

[b]Microcrystalline cellulose and croscarmellose sodium are added as intra granular and extra granular excipients.
[c]Removed during processing.

[0000182] Other examples of compositions are summarized in Table 17.

Table 17 - Quantitative Composition of ST-246 Drug Product

		200 mg Strength
Ingredient	**Function**	**mg/Capsule**
ST-246 monohydrate[a] (micronized, D_{90} <10 microns) monohydrate, based on anhydrous basis.	Active Ingredient; white to off-white powder	200.00
Microcrystalline cellulose, NF[b]	Water Insoluble Diluent	88.60
Lactose monohydrate, NF	Water soluble Diluent	40.95
Croscarmellose sodium, NF[b]	Disintegrant	42.90
Colloidal silicon dioxide, NF	Glidant	1.95
Hypromellose , USP	Binder	13.65
Magnesium stearate NF	Lubricant	1.95
Water USP[c]	Granulating Agent	

Hard Gelatin Capsule shell, orange/black, Size 0	Encapsulation	1 capsule
Capsule weight, mgs		390

Example 9 - Inhibition of Orthopox Viral Replication:

[0000183] The ability of the Form I of ST-246 to inhibit Vaccinia virus is established by the following experimental procedure:

Preparation of Virus Stock

[0000184] Virus stocks of Vaccinia virus (NYCBH) are prepared in Vero cells infected at low multiplicity (0.01 plaque forming units (PFU)/cell) and harvested when cytopathic effects were complete (4+CPE). The samples are frozen and thawed and then sonicated to release cell-associated virus. The cell debris are removed by low-speed centrifugation, and the resulting virus suspension is stored in 1 mL aliquots at -80.degree. C. The PFU/mL of the virus suspension is quantified by standard plaque assay on Vero and BSC-40 cells.

Vaccinia CPE Assay

[0000185] To determine the amount of vaccinia virus stock required to produce complete CPE in 3 days, Vero cell monolayers are seeded on to 96-well plates and infected with 2-fold serial dilutions of the vaccinia virus stock. At 3 days post-infection, the cultures are fixed with 5% glutaraldehyde and stained with 0.1% crystal violet. Virus-induced CPE is quantified spectrophotometrically at OD.sub.570. From this analysis, a 1:800 dilution of

vaccinia virus stock is chosen for use in the HTS assay. This amount of vaccinia virus represents a multiplicity of infection of approximately 0.1 PFU/cell.

[0000186] To establish the signal-to-noise ratio (S/N) of the 96-well assay and evaluate the well-to-well and assay-to-assay variability, six independent experiments are performed. Vero cell monolayers are infected with 1:800 dilution of vaccinia virus stock.

[0000187] Each plate contains the following controls: quadruplicate virus-infected wells, quadruplicate uninfected cell wells and a dose response curve in duplicate for cidofovir (CDV) added at 300, 100, 30 and 10 DAM, or phosphonoacetic acid (PAA) added at 2100, 714, 210, and 71 M as reference standards. At day 3 post-infection, the plates are processed as described above. The results of these experiments indicate that the 96-well assay format is robust and reproducible.

Form I Testing

[0000188] ST-246, Form I is tested in the vaccinia virus CPE assay. Form I is dissolved in DMSO and diluted in medium such that the final concentration in each well is 5 pM compound and 0.5% DMSO. Form I is added robotically to the culture medium using the Biomek.RTM. FX robot system.

[0000189] Following compound addition, the cultures are infected with vaccinia virus. After 3 days, plates are processed and CPE quantified as described. ST-246 Form I of the invention inhibited vaccinia virus-induced CPE by greater than 50% at the test concentration (5 .mu.M). Form

I is further evaluated for potency (EC_{50}) in the CPE assay and cytotoxicity (CC.sub.50) in an MTT assay. The MTT assay measures mitochondrial dehydrogenase activity in dividing cells. The absorbance of the formazin at 490 nm can be measured directly from 96-well assay plates following solubilization of the formazin in 50% ethanol. The quantity of formazin product is directly proportional to the number of living cells in culture. The EC_{50} values are determined by comparing compound-treated and compound-untreated cells using a computer program. Thus, the EC_{50} value of ST-246 Form I in the CPE assay is 50 nM.

[0000190] The specificity of ST-246, Form I, for orthopox virus inhibition is reflected in the fact that they do not inhibit the replication of unrelated viruses, including Pichinde virus, Rift Valley fever virus (strain MP12), respiratory syncytial virus and cytomegalovirus.

Example 10 - ST-246, Form I in *vivo* studies:

Study design

[0000191] The study is designed as a randomized, placebo-controlled, parallel-group, longitudinal study of oral ST-246, Form I, in cynomolgus monkeys (*Macaca fascicularis*). For these experiments, 15 NHPs are infected with 5×10^7 PFU of the Zaire 79 strain of MPX by i.v. injection and are randomized into five treatment arms of three NHP each. The vehicle or ST-246, Form I, are administered at 3 mg/kg, 10 mg/kg, 30 mg/kg, and 100 mg/kg orally once per day followed by 5 ml/kg of a 30% suspension of hydrated homogenized monkey biscuits.

[0000192] Treatment starts on day 3 post-infection and has continued once daily for 14 days. The infected animals are observed at least twice each day for up to 33 days to examine them for signs of illness. Blood samples are collected from the infected animals for virological, hematological, immunological, and chemical analyses. A full necropsy is performed on animals that died during the study to collect tissues for pathological examination. To determine the extent of infection, three animals are euthanized on day 3 and their tissues are processed to determine the virus levels in tissues. The organs are freeze-thawed, and a 10% tissue homogenate is produced and analyzed by quantitative PCR. The number of MPX genomes per milliliter of blood is determined by the extraction of DNA with a Qiagen QIAmp DNA minikit and quantitative TaqMan-MGB PCR, as described previously. Monkeypox lesions are enumerated daily.

Pharmacokinetic analysis of ST-246, Form I, in cynomolgus monkeys (NHP).

[0000193] The NHP are administered ST-246 at 10, 20, and 30 mg/kg by oral gavage under fed conditions. On the dosing days, all animals are administered a primate biscuit slurry immediately prior to dose administration. The biscuit slurry contained one can (8 oz) of Ensure or other liquid diet, approximately 36 g of fluid and electrolyte replacement formula (Prang), approximately 94 g of infant formula, 1 jar (2.5 oz) of strained fruit, five monkey chow biscuits, and approximately 2 oz of water blended together to achieve a uniform consistency. The biscuit slurry is administered via oral gavage at a dose volume of 10 ml/kg. After each dose of

the test article is administered and prior to the removal of the gavage tube, the tube is flushed with 10 ml of tap water. The dosing formulations are stirred with a magnetic stir bar and stir plate prior to and throughout the administration. Individual doses are based on the most recent body weights. With the exception of the 20-mg/kg dose group, each dose group contains three male and three female NHP; the 20-mg/kg dose group contained four male and four female NHP. The NHP in the 10- and 30-mg/kg dose groups received ST-246, Form I, for 14 consecutive days, and those in the 20-mg/kg dose group received a single dose of ST-246. Blood samples are collected from the femoral artery/vein pretesting (0 h) (prior to dosing) and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h postdosing on days 1, 7, and 14 for determination of the plasma concentrations of ST-246, Form I.

Evaluation of ST-246, Form I, efficacy in cynomolgus monkey model of monkeypox.

[0000194] The study described here is randomized, placebo-controlled, parallel-group, longitudinal study of oral ST-246, Form I, in NHP infected i.v. with MPX. The animals exhibit extensive MPX infection of major tissues, as demonstrated by the level of virus in tissues. At the time of ST-246, Form I, treatment, one-third of the NHP has viral lesions. All animals receiving vehicle alone either die or require euthanasia because they are moribund during the study period of 33 days, while all animals receiving ST-246, Form I, survive. Each of the animals receiving ST-246, Form I, survive the full study.

[0000195] For these experiments, viral load and lesion development are quantified daily. All doses of ST-246, Form I, significantly decrease the amount of viral DNA present compared to that in the vehicle-treated animals beginning on 5 dpi. At the end of the 14-day treatment, the viral loads in the ST-246, Form I,-treated groups show a linear dose-response, with all treatment groups showing a significant reduction in the level of virus replication of more than 1,000-fold compared to that in the vehicle-treated group.

Example 11 - Pharmacokinetic Comparison of a Single Oral Dose of Form I versus Form V Capsules of the Anti-Orthopoxvirus Compound ST-246 in Healthy Human Volunteers

Study design

[0000196] This was a Phase I, randomized, double-blind, crossover, exploratory study to compare the pharmacokinetics (PK; AUC variables and C_{max}) of a single 400 mg (2 X 200mg) oral dose of ST-246 Form I (the test) with ST-246 Form V (the reference), and to evaluate the safety and tolerability of both Forms in fed normal healthy volunteers. Twelve of sixty-three screened individuals (males and non-pregnant females, 18 to 50 years old inclusive) were accepted into the study, and were randomized to one of the following sequences: Form I then Form V, or Form V then Form I.

[0000197] To determine the PK of ST-246, a urine and baseline (0 hour) venous blood sample were obtained on Day 1, followed by serial blood draws after medication administration. All subjects received a single, 400-mg dose (2x200 mg) of either Form I or Form V of ST-246, orally administered within 30 minutes after a standard light meal.

Post-dose (Treatment 1) blood samples for PK analyses were taken at 0.5, 1, 2, 3, 4, 8, 12, 24, 36, 48, and 72 hours. A post-dose urine sample was obtained on Day 2. A Washout Period occurred during study Days 2-10, so Treatment 2 occurred on Day 11. At this time, those subjects originally receiving Form I of ST-246, now received a single, 400 mg dose (2x200 mg) of Form V, and vice versa. Blood sampling for PK analyses following Treatment, 2, occurred as for post-Treatment 1, and urine sampling occurred on Day 14. Plasma samples were collected and stored at -70°C until analyzed for maximum drug concentration [Cmax], time to maximum drug concentration [Tmax], terminal half-life [t1/2], area under the concentration-time curve [AUC], and renal clearance [Clr]. Urine samples were immediately centrifuged at 4°C for 10 min at 2,000 x g, and evaluated for urinary excretion. ST-246 was quantified from human plasma specimens by a validated liquid chromatography and tandem mass spectrometry method using an analog of ST-246 as an internal standard.

[0000198] The study compared the pharmacokinetic (PK) profiles of ST-246 Form 1 and Form V capsules following a single oral dose administration. This objective was achieved through the collection and analysis of plasma samples for PK assessment of Form I from 12 of 12 subjects and of Form V from 11 of 12 subjects. Pharmacokinetic parameters, peak plasma concentration (Cmax,), time at which Cmax is attained post dose administration (Tmax), plasma exposure (AUC0-τ, AUC0-∞) and elimination half-life (t½) were estimated for ST-246 by applying non-compartmental analysis using WinNonlin professional edition software (Pharsight Corporation, Version 5.2).

[0000199] The pharmacokinetic parameters for ST-246, Form I and Form V, are summarized in Table 18 below:

Table 18 - Pharmacokinetic parameters for ST-246, Form I and Form V.

Form Group	Statistics	$AUC_{0-\tau}$ (hr*ng/mL)	$AUC_{0-\infty}$ (hr*ng/mL)	$AUC_{(extrap)}$ (%)	$t_{1/2}$ (hr)	C_{max} (ng/mL)	T_{max} (hr)
Form I	N	12	11	11	11	12	12
	Mean	15624.5	19922.02	17.444	27.446	1068.9	3.8
	SD	5449.188	6543.563	7.84	13.109	294.3	1.5
	CV%	34.876	32.846	44.947	47.763	27.5	39.6
	Geometric Mean	14816.26	19049.63	15.748	24.746	1026.9	3.5
	Median	14151.15	17201.75	13.214	25.12	1170	3.5
	Minimum	8053.5	13959.18	5.7	10.94	525	2
	Maximum	26596.58	31058.8	30.4	56.48	1590	8
	Missing	0	1	1	1	0	0
Form V	N	11	8	8	8	11	11
	Mean	20065.32	21982.71	15.275	29.18	1230.2	3.8
	SD	6744.974	9330.953	10.811	21.992	348.6	1.6
	CV%	33.615	42.447	70.78	75.365	28.3	41.9
	Geometric Mean	19020.83	20409.17	12.369	23.083	1185	3.6
	Median	19398.5	19465.47	12.465	16.647	1180	4
	Minimum	10398.53	11946.95	4.53	11.48	732	2
	Maximum	30974	39058.28	37.51	69.45	1940	8
	Missing	0	3	3	3	0	0

NOTE: For a given variable and drug form, geometric mean was not calculated if any of the values were 0

KEY: $AUC_{0-\infty}$ = Area under the plasma concentration-time curve from time zero to infinity; AUC_{0-t} = Area under the drug concentration-time curve from time zero to time t where t is the last time-point with a drug concentration ≥lowest obtainable quantification; $AUC_{(extrap)}$ =Area under the curve extrapolated; $t_{1/2}$ = Terminal half-life; C_{max} = maximum plasma concentration; CV% = Coefficient of variance; h = hours; N = Number of subjects; PK = Pharmacokinetics; SD = Standard deviation; T_{max} =Time to maximum plasma concentration

[0000200] The mean (SD) ST-246 plasma concentrations over time (PK population) are shown in Figure 24 after a single oral adimistration.

CLAIMS

1. A polymorph Form I of 4-trifluoromethyl-N-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl)-benzamide (ST-246) which shows an X-ray powder diffraction pattern having characteristic peaks at a reflection angle 2θ of about 7.63, 10.04, 11.47, 14.73, 15.21, 15.47, 16.06, 16.67, 16.98, 18.93, 19.96, 20.52, 20.79, 22.80, 25.16, 26.53, 27.20, 27.60, 29.60, 30.23, 30.49, 30.68, 31.14, 33.65, 34.33, 35.29, 35.56, 36.30, 37.36, 38.42, 38.66 degrees.

2. A polymorph according to claim 1 that is isolated and at least 70% free of other forms.

3. A polymorph according to claim 1 that is isolated and at least 80% free of other forms.

4. A polymorph according to claim 1 that is isolated and at least 90% free of other forms.

5. A polymorph according to claim 1 that is isolated and at least 95% free of other forms.

6. A polymorph according to claim 1 that is isolated and at least 99% free of other forms.

7. A pharmaceutical composition comprising the polymorph of any one of claims 1-6 and further comprising one or more pharmaceutically-acceptable carriers, excipients, diluents, additives, fillers, lubricants, or binders.

8. The pharmaceutical composition of claim 7, wherein the

composition is formulated for oral administration.

9. Use of a therapeutically effective amount of the polymorph of any one of claims 1-6 to treat infections caused by Orthopoxvirus in a human or animal.

10. Use of a therapeutically effective amount of the polymorph of any one of claims 1-6 to treat eczema vaccinatum in a human or animal.

11. A method of producing a crystal polymorphic Form I of 4-trifluoromethyl-N-3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl)-benzamide (ST-246) which shows an X-ray powder diffraction pattern having characteristic peaks at a reflection angle 2θ of about 7.63, 10.04, 11.47, 14.73, 15.21, 15.47, 16.06, 16.67, 16.98, 18.93, 19.96, 20.52, 20.79, 22.80, 25.16, 26.53, 27.20, 27.60, 29.60, 30.23, 30.49, 30.68, 31.14, 33.65, 34.33, 35.29, 35.56, 36.30, 37.36, 38.42, 38.66 degrees, the method comprising the steps of:

(a) dissolving ST-246 in at least one organic solvent and an amount of water to make a solution;

(b) cooling said solution to a temperature that causes the preferential crystallization of said ST-246 polymorphic Form I; and

(c) optionally drying the formed crystals of ST-246,

wherein said organic solvent is isopropyl alcohol (IPA), ethyl acetate, ethanol, methanol, acetone, isopropyl acetate, or tetrahydrofuran (THF).

12. The method of claim 11, further comprising adding seed crystals of polymorphic Form I ST-246 during step (b).

13. The method of claim 11, wherein said organic solvent is methanol or THF.

14. The method of claim 11, wherein said cooling step takes place over at least 15 minutes.

15. The method of claim 11, wherein said cooling step takes place over at least 2 hours.

16. The method of claim 11, wherein said cooling step takes place over at least 5 hours.

17. The method of claim 11, wherein said organic solvent is ethyl acetate.

18. The method of claim 11, wherein said organic solvent is isopropyl alcohol.

19. The method of claim 11, wherein said organic solvent is ethyl acetate and the water content is about 40% by volume of total solvent volume.

20. The method of claim 11, wherein said organic solvent is ethyl acetate and the water content is about 5% by volume of total solvent volume.

21. The method of claim 11, wherein said organic solvent is ethyl acetate and the water content is about 3% by volume of total solvent volume.

22. The method of claim 11, wherein said organic solvent is ethyl acetate and the water content is about 2% by volume of

total solvent volume.

23. The method of claim 11, wherein said organic solvent is isopropyl alcohol and the water content is about 5% by volume of total solvent volume.

24. A dosage unit form comprising the pharmaceutical composition of claim 8, wherein the ST-246 has a D90% particle size diameter of up to 300 microns.

25. The dosage unit form according to claim 24, wherein said ST-246 has a D90% particle size diameter of about 5 microns.

26. The dosage unit form according to claim 24, wherein said ST-246 has a D90% particle size diameter of about 16.6 microns.

27. The dosage unit form according to claim 24, wherein said ST-246 has a D90% particle size diameter of about 26.6 microns.

28. The dosage unit form according to claim 24, wherein said ST-246 has D90% particle size diameter of about 75 microns.

29. The dosage unit form according to claim 24, which is a capsule or a tablet.

30. The dosage unit form according to claim 24, comprising 200 mg of ST-246 having a D90% particle size less than 10 microns.

31. A unit dosage form for oral administration comprising:

(a) about 200 mg of polymorph Form I of 4-trifluoromethyl-N-(3,3a,4,4a,5,5a,6,6a-octahydro-1,3-dioxo-4,6-ethenocycloprop[f]isoindol-2(1H)-yl)-benzamide (ST-246), which shows an X-ray powder diffraction pattern having characteristic peaks at a reflection angle 2θ of about 7.63, 10.04, 11.47, 14.73, 15.21, 15.47, 16.06, 16.67, 16.98, 18.93, 19.96, 20.52, 20.79, 22.80, 25.16, 26.53, 27.20, 27.60, 29.60, 30.23, 30.49, 30.68, 31.14, 33.65, 34.33, 35.29, 35.56, 36.30, 37.36, 38.42, 38.66 degrees;

(b) about 33.15 mg of lactose monohydrate;

(c) about 42.90 mg of croscarmellose sodium;

(d) about 1.95 mg of colloidal silicon dioxide;

(e) about 13.65 mg of hydroxypropyl methylcellulose;

(f) about 7.8 mg of sodium lauryl sulfate;

(g) about 1.95 mg of magnesium stearate; and

(h) a quantity of microcrystalline cellulose up to 88.60 mg such that the total weight of the dosage form is about 390 mg.

32. Use of a polymorph according to any one of claims 1-6 in the manufacture of a medicament for treating infections caused by Orthopoxvirus in a human or animal.

33. Use of a polymorph according to any one of claims 1-6 in the manufacture of a medicament for treating eczema vaccinatum in a human or animal.

FIG. 1

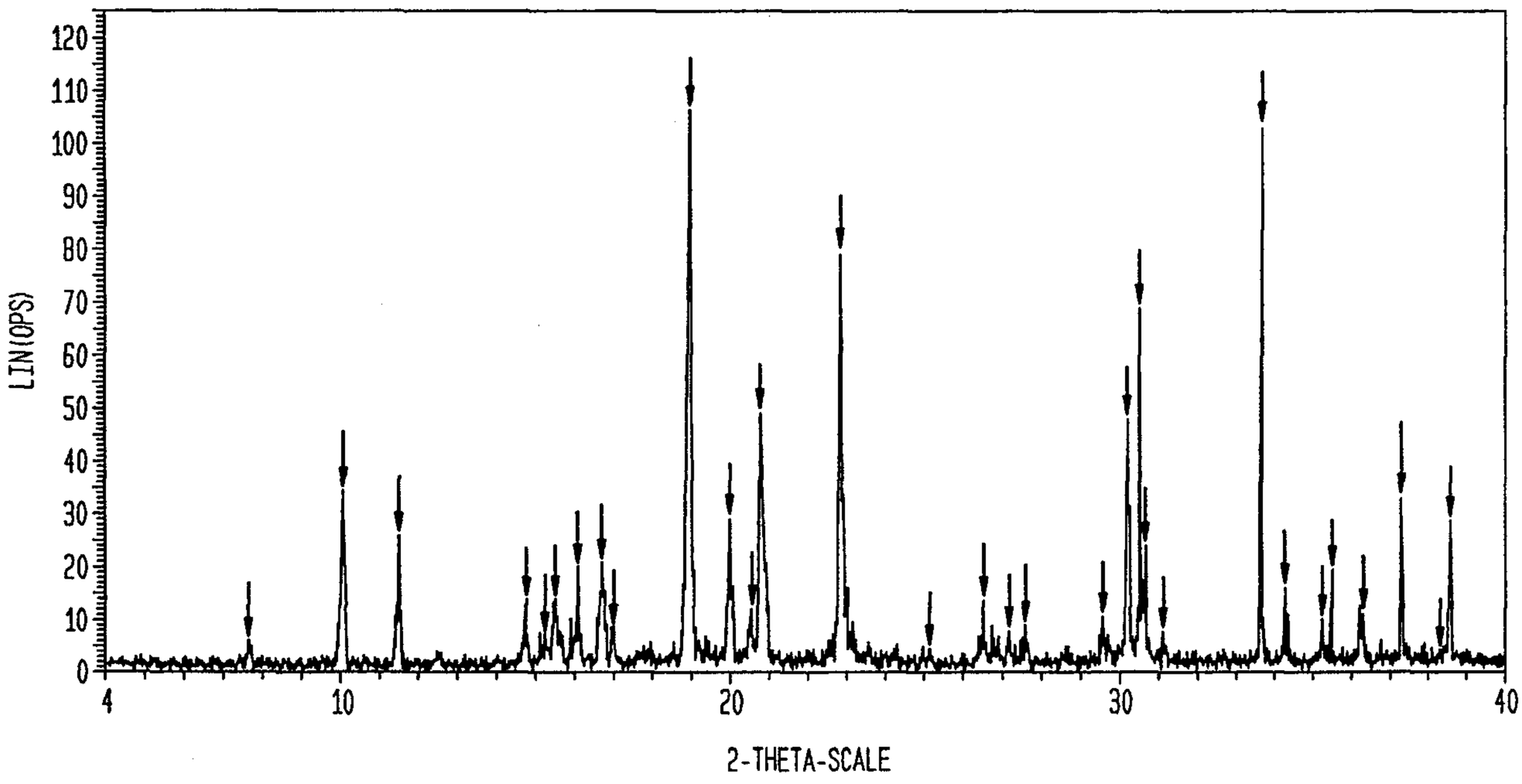

120
110
100
90
80
70
60
50
40
30
20
10
0
LIN(OPS)
4
10
20
30
40
2-THETA-SCALE

FIG. 2

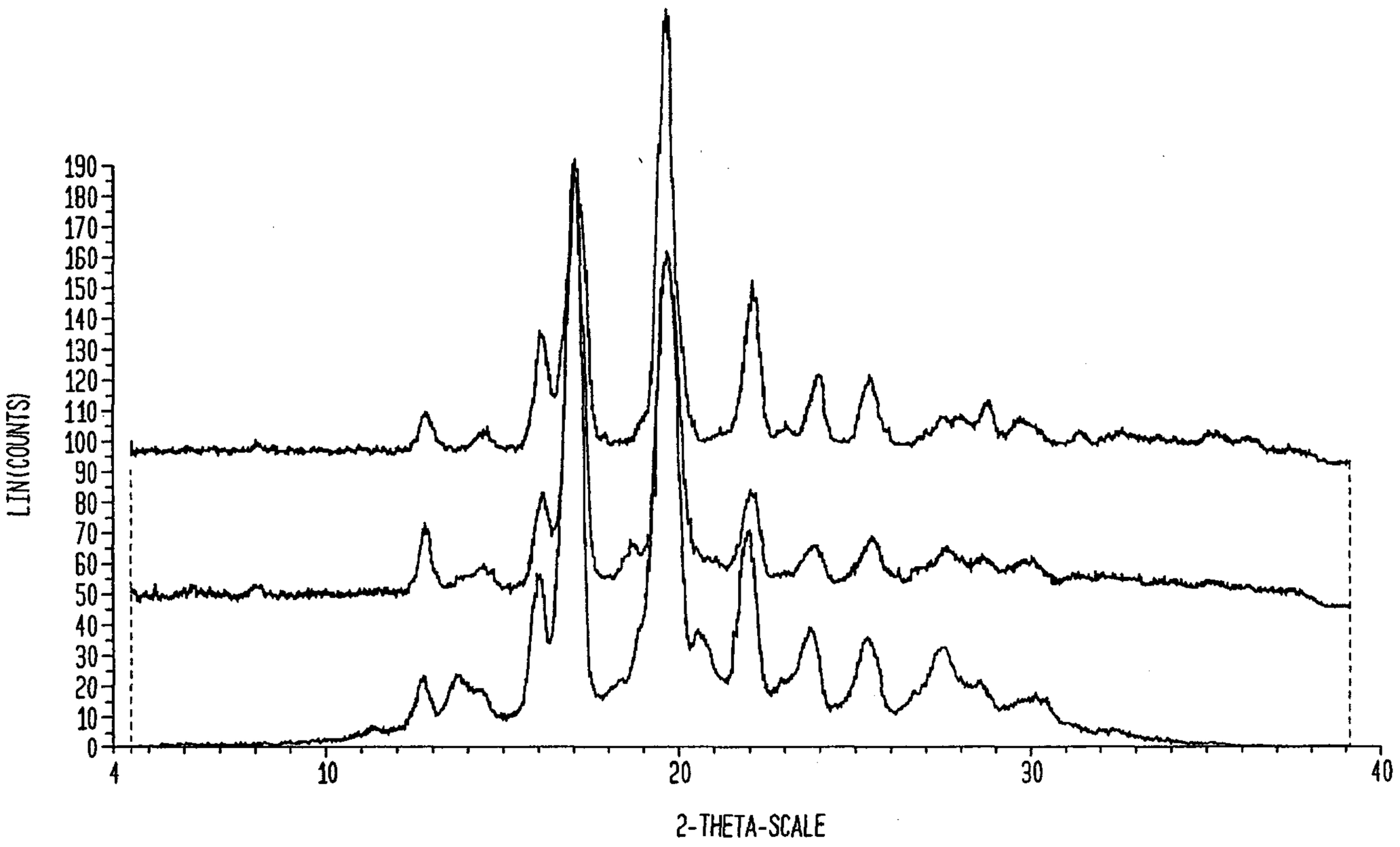

LIN(COUNTS)
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
4
10
20
30
40
2-THETA-SCALE

FIG. 3

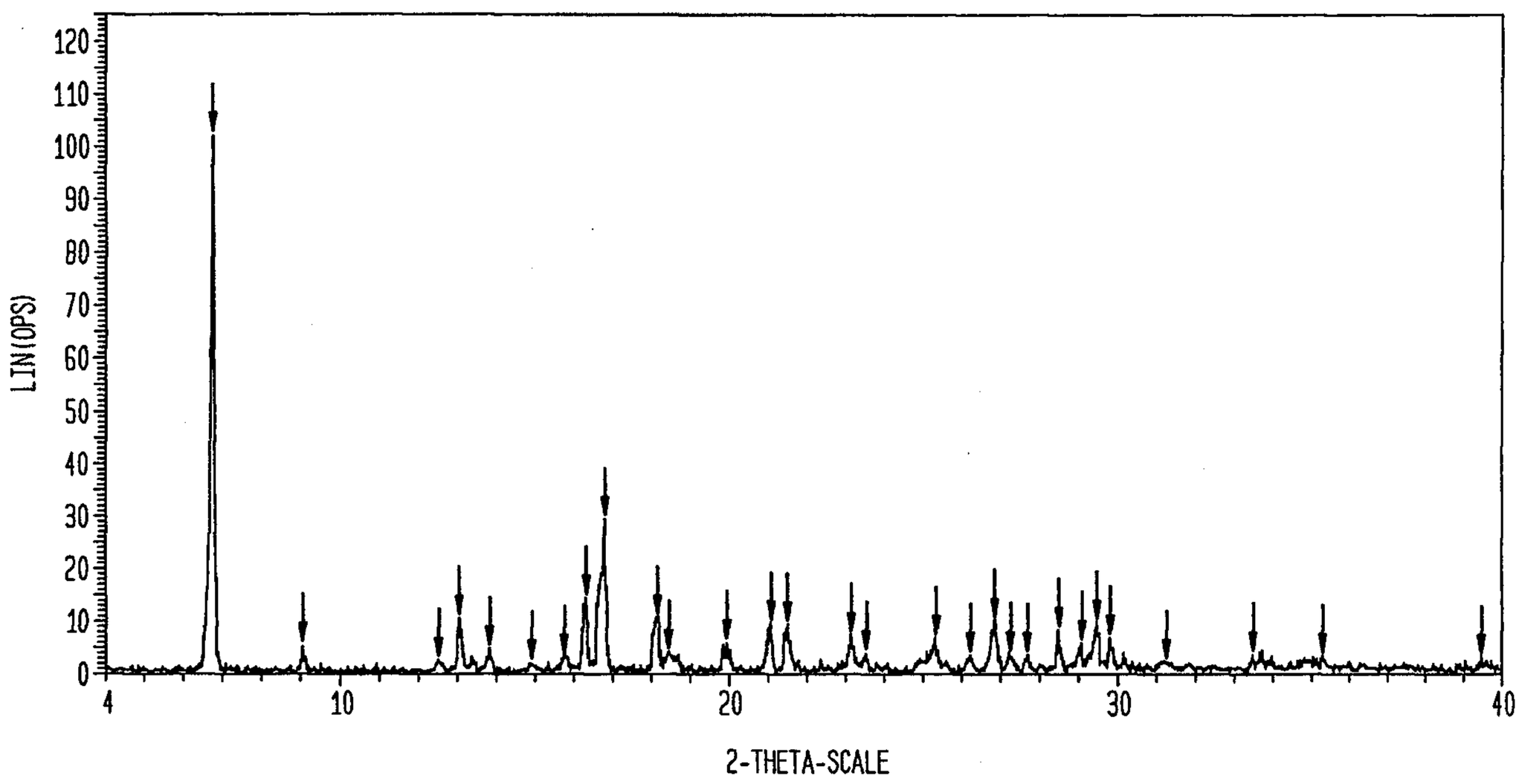

120
110
100
90
80
70
60
50
40
30
20
10
0
LIN(OPS)
4
10
20
30
40
2-THETA-SCALE

FIG. 4

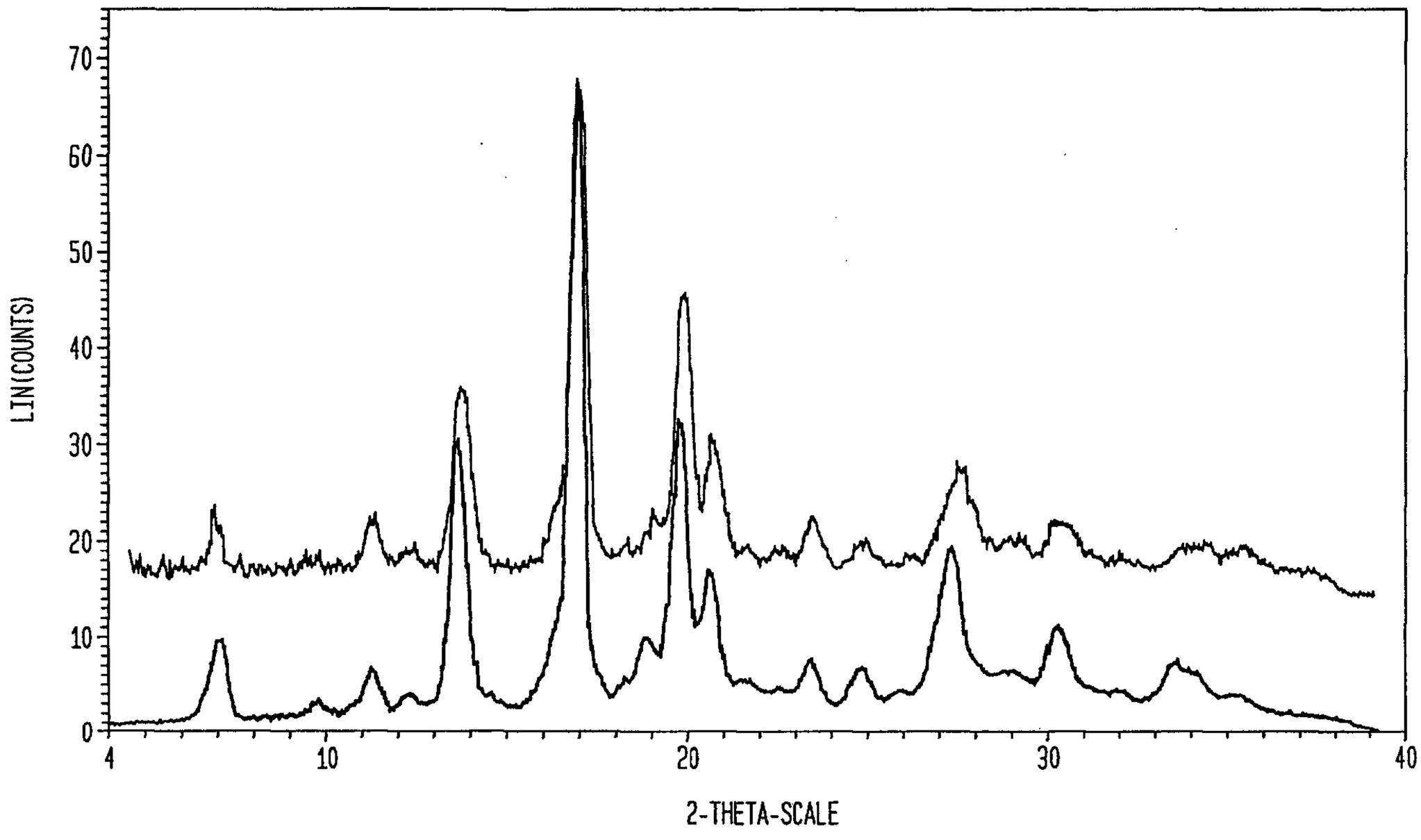


FIG. 5

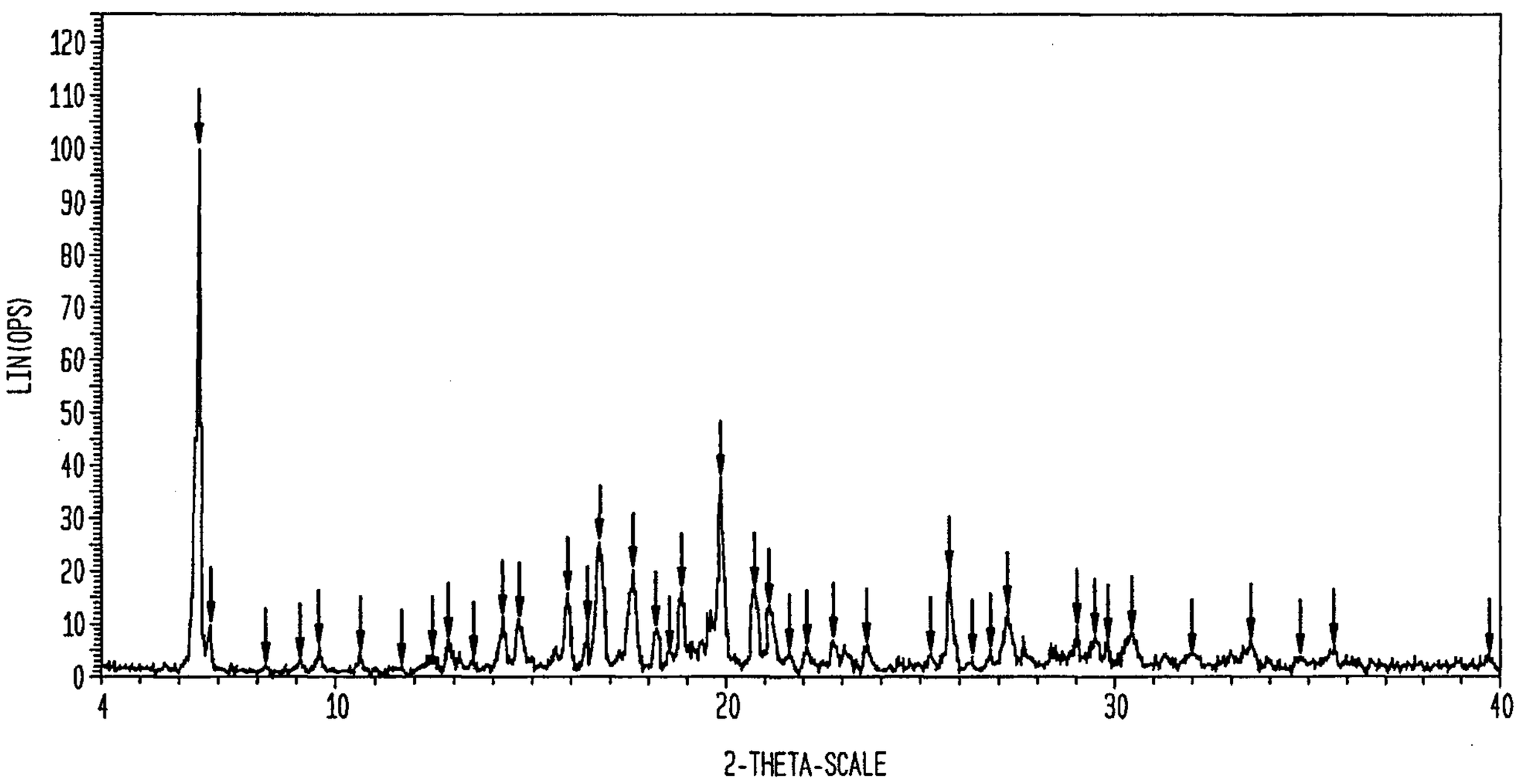

LIN(OPS)
120
110
100
90
80
70
60
50
40
30
20
10
0
4
10
20
30
40
2-THETA-SCALE

FIG. 6

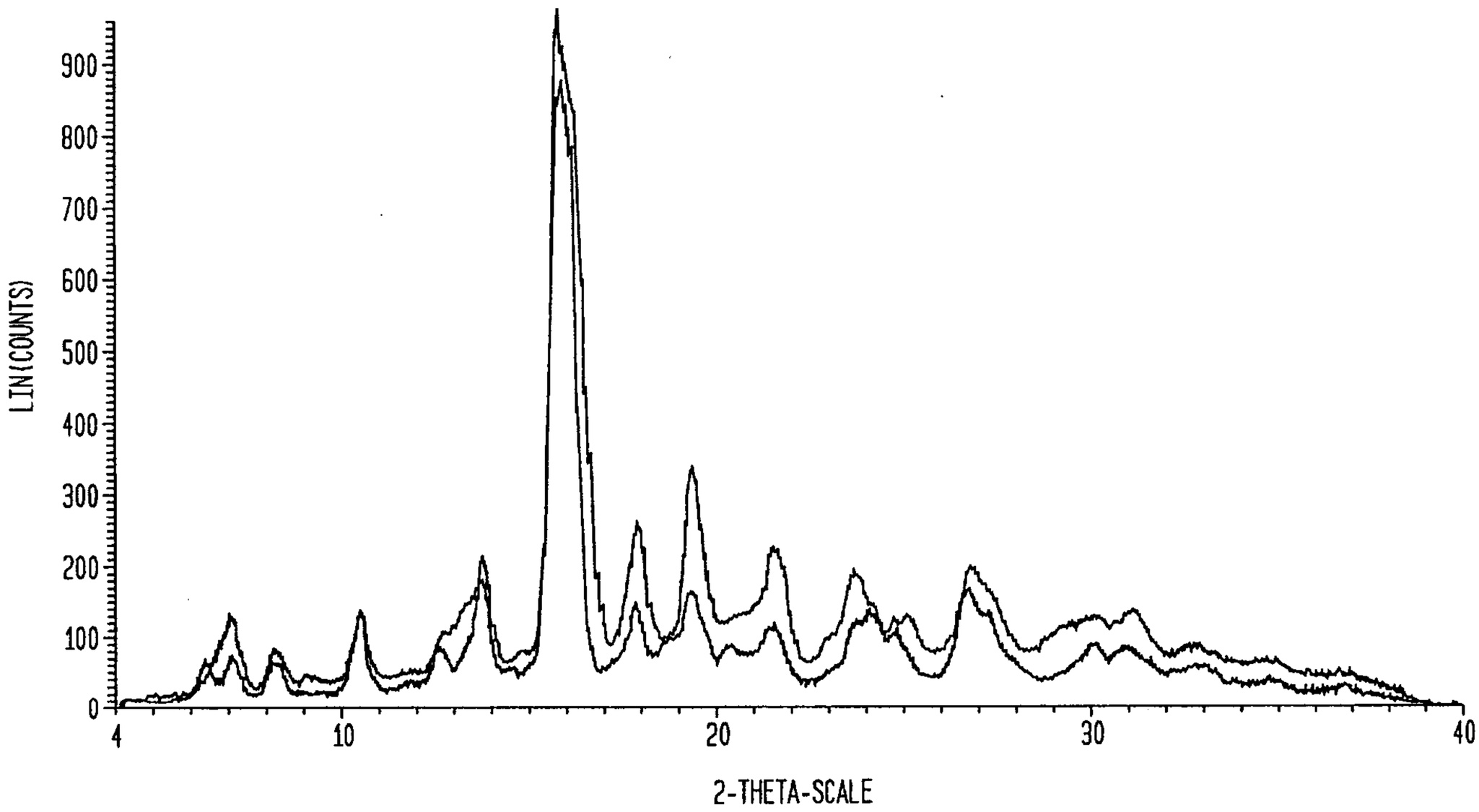

LIN(COUNTS)
900
800
700
600
500
400
300
200
100
0
4
10
20
30
40
2-THETA-SCALE

FIG. 7

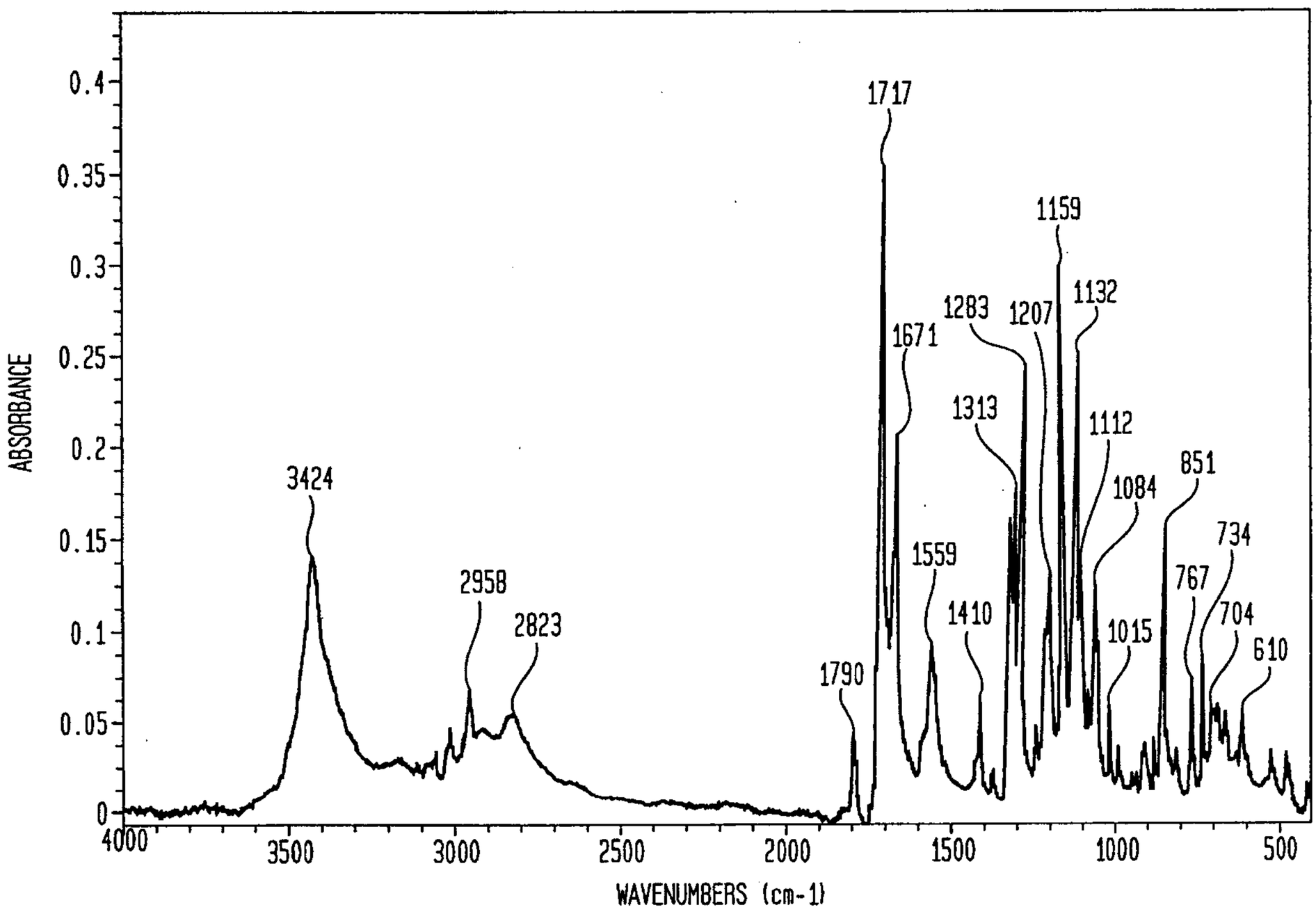

ABSORBANCE
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
4000
3500
3000
2500
2000
1500
1000
500
WAVENUMBERS (cm-1)
3424
2958
2823
1790
1717
1671
1559
1410
1313
1283
1207
1159
1132
1112
1084
1015
851
767
734
704
610

FIG. 8

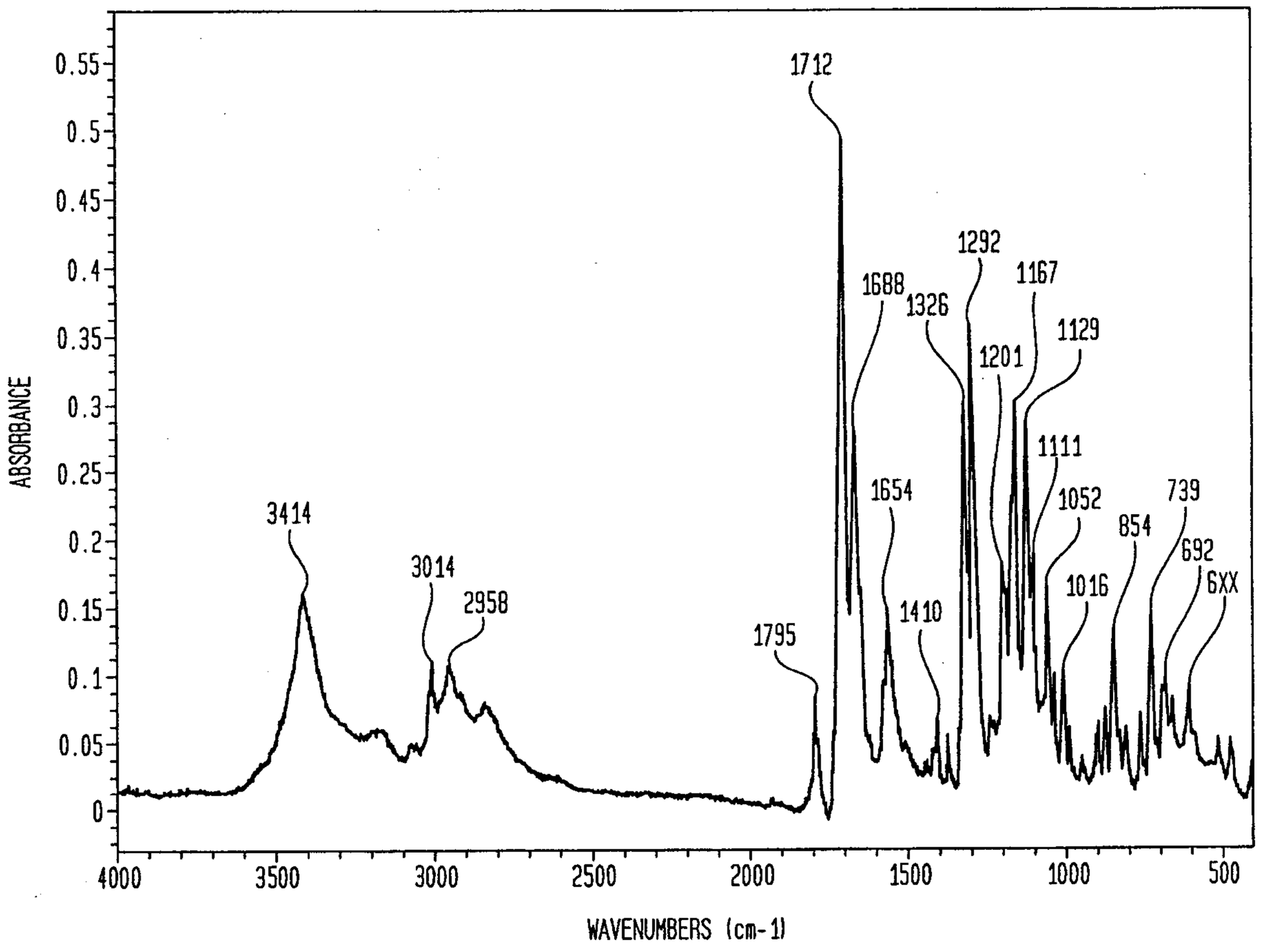

ABSORBANCE
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
4000
3500
3000
2500
2000
1500
1000
500
WAVENUMBERS (cm-1)
3414
3014
2958
1795
1712
1688
1654
1410
1326
1292
1201
1167
1129
1111
1052
1016
854
739
692
6XX

FIG. 9

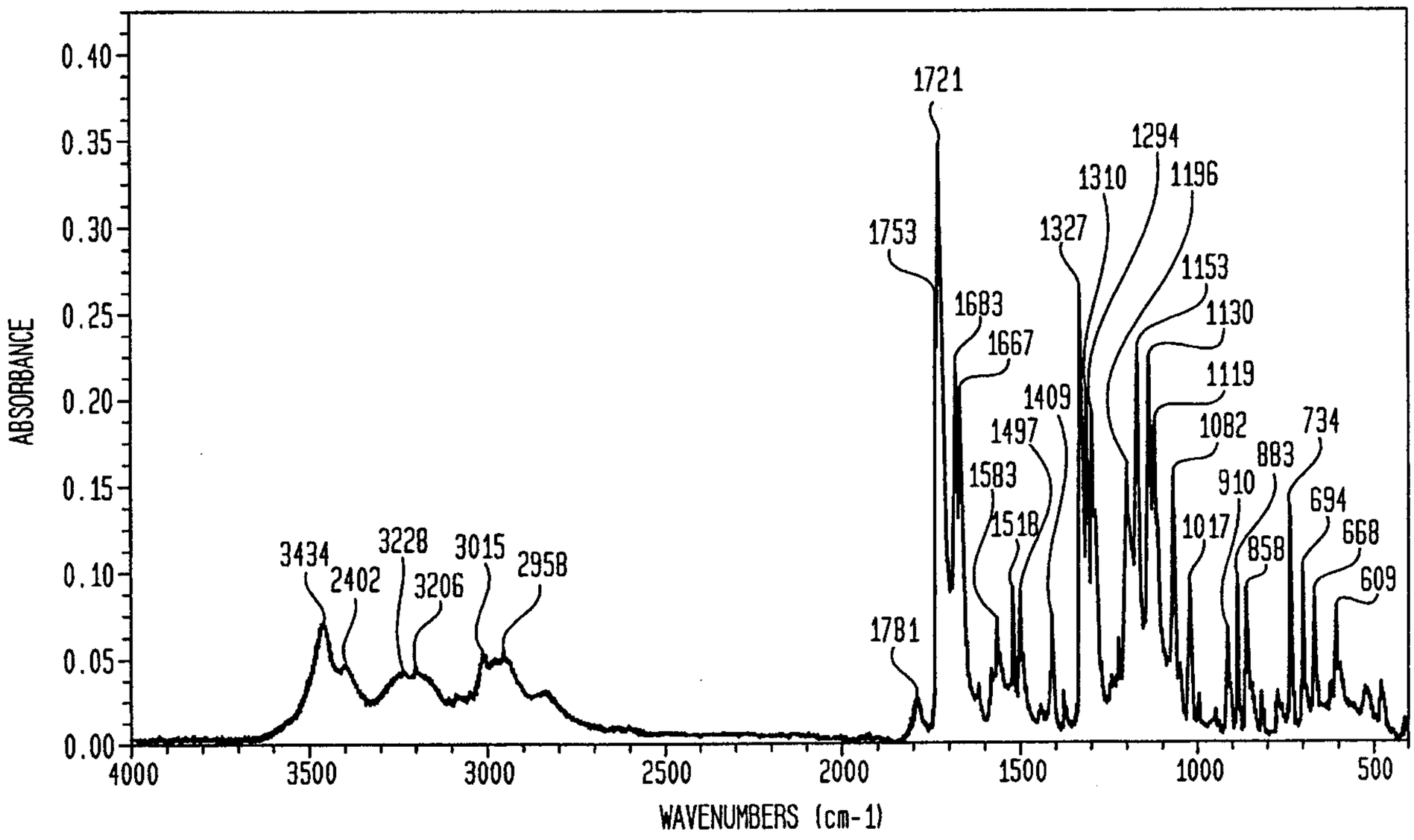

ABSORBANCE
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
4000
3500
3000
2500
2000
1500
1000
500
WAVENUMBERS (cm-1)
3434
2402
3228
3206
3015
2958
1781
1721
1753
1683
1667
1583
1518
1497
1409
1327
1310
1294
1196
1153
1130
1119
1082
1017
910
883
858
734
694
668
609

FIG. 10

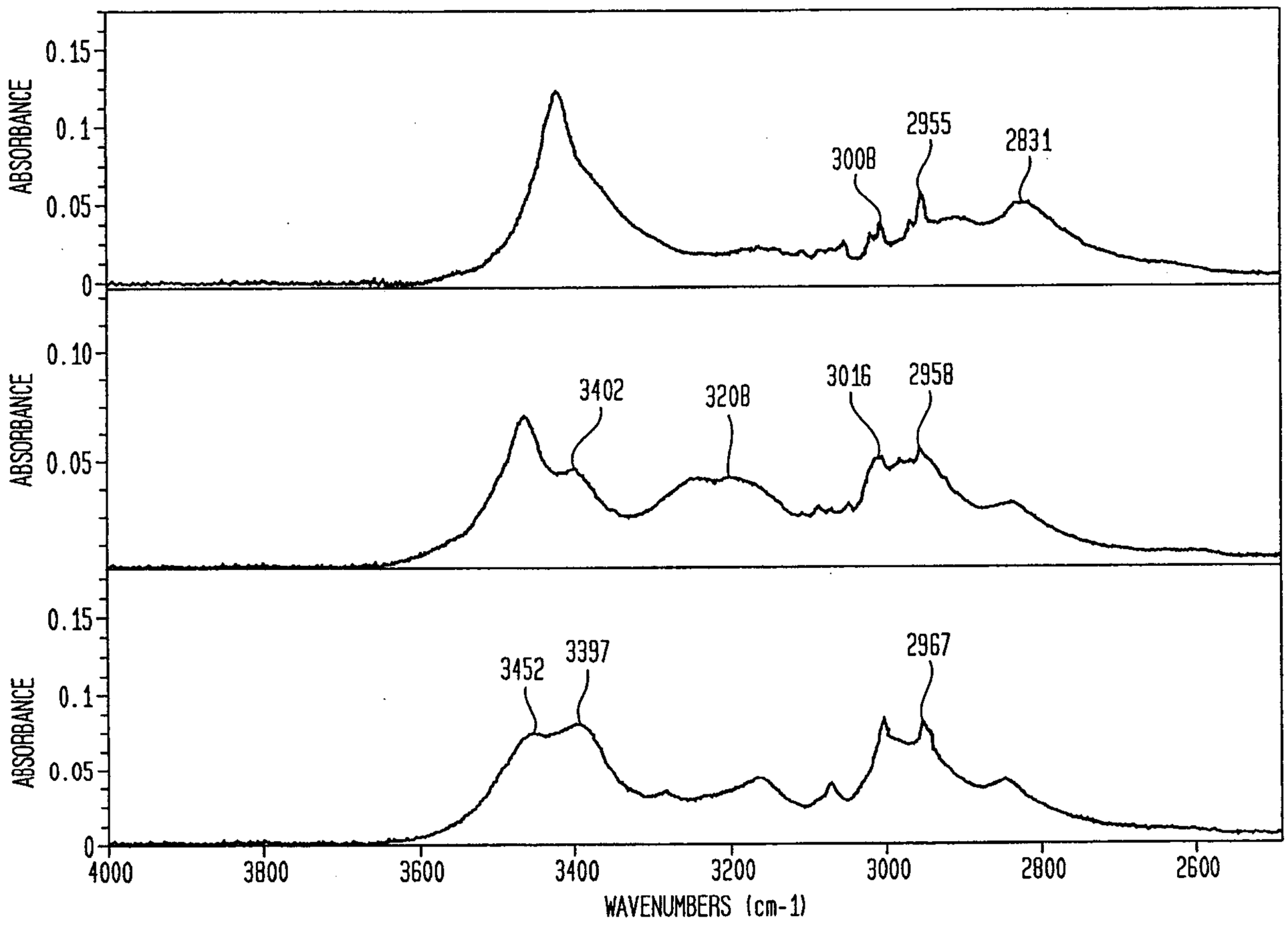

ABSORBANCE
0.15
0.1
0.05
0
3008
2955
2831
ABSORBANCE
0.10
0.05
3402
3208
3016
2958
ABSORBANCE
0.15
0.1
0.05
0
3452
3397
2967
4000
3800
3600
3400
3200
3000
2800
2600
WAVENUMBERS (cm-1)

FIG. 11

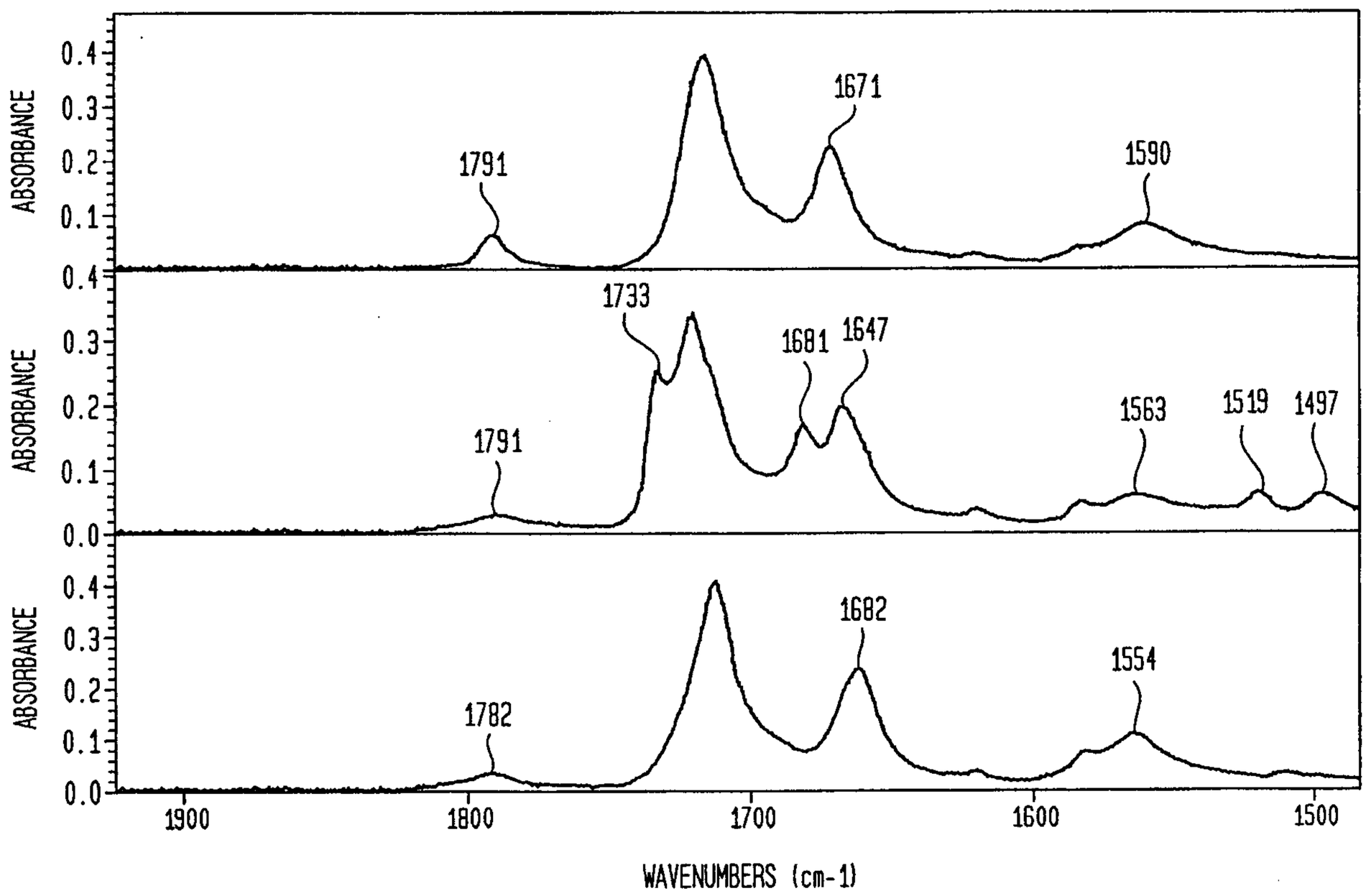

ABSORBANCE
0.4
0.3
0.2
0.1
0.0
1791
1671
1590
1733
1681
1647
1563
1519
1497
1682
1554
1782
1900
1800
1700
1600
1500
WAVENUMBERS (cm-1)

FIG. 12

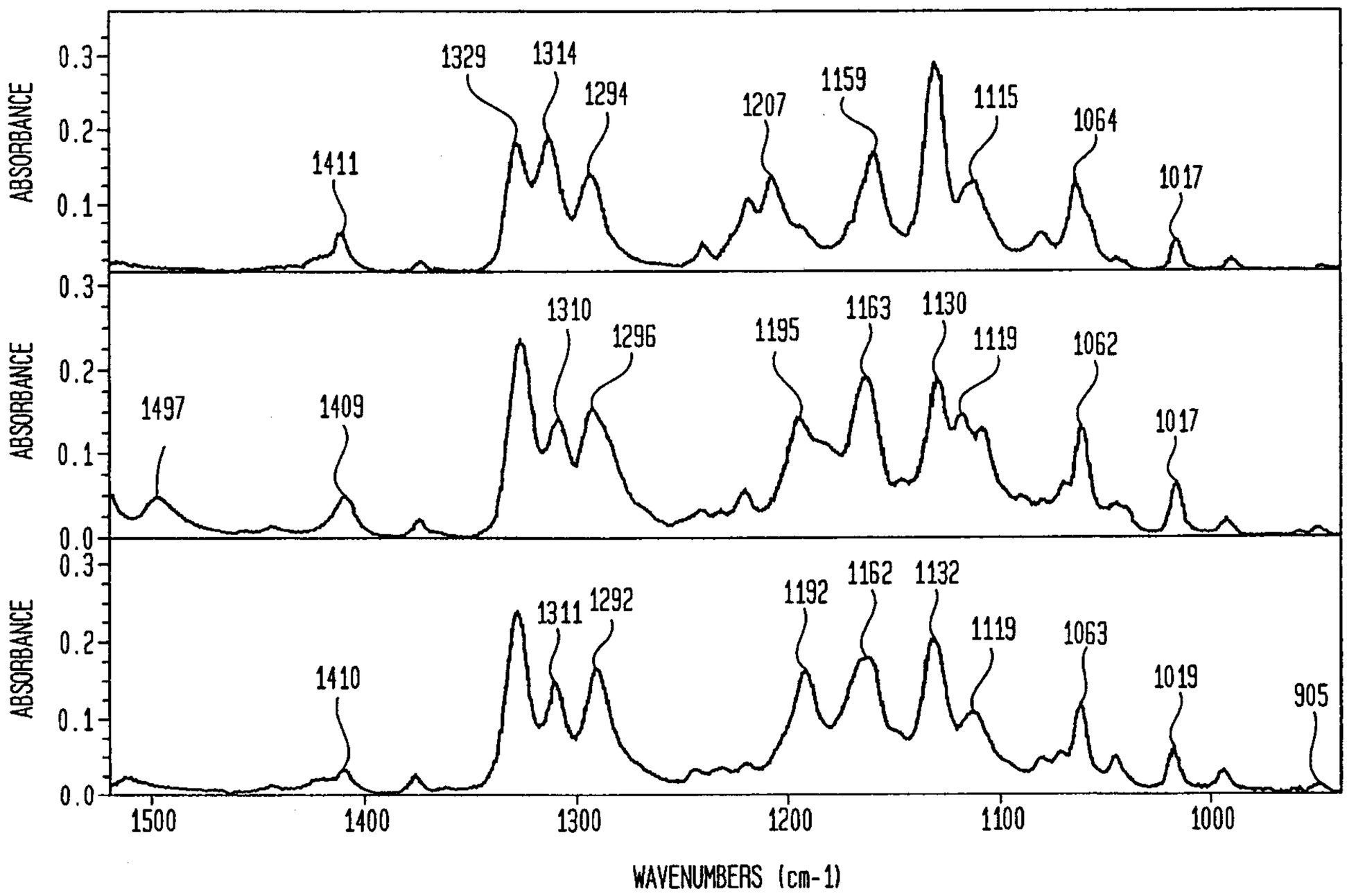

ABSORBANCE
0.3
0.2
0.1
0.0
1411
1329
1314
1294
1207
1159
1115
1064
1017
1497
1409
1310
1296
1195
1163
1130
1119
1062
1017
1410
1311
1292
1192
1162
1132
1119
1063
1019
905
1500
1400
1300
1200
1100
1000
WAVENUMBERS (cm-1)

FIG. 13

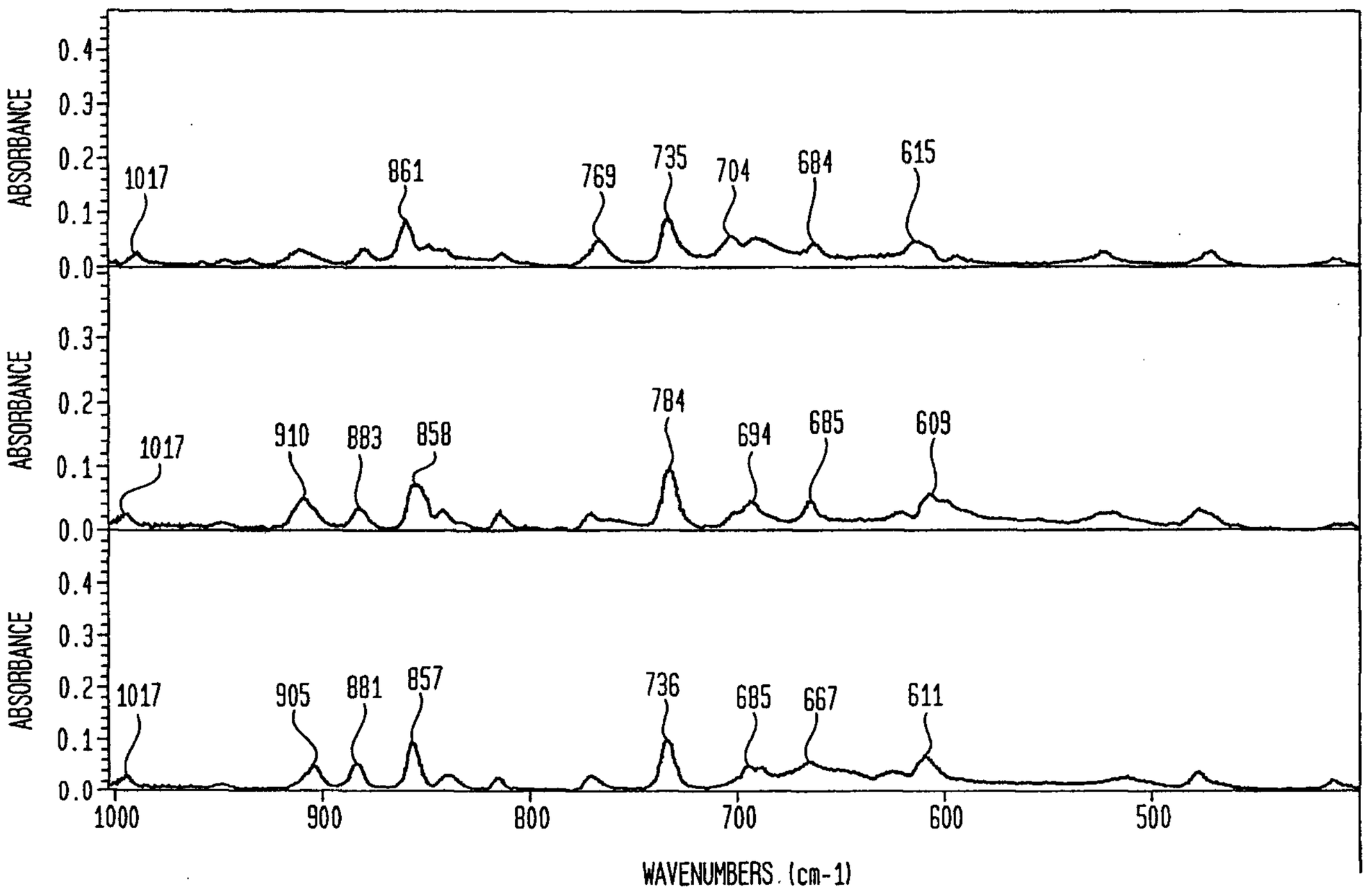

ABSORBANCE
0.4
0.3
0.2
0.1
0.0
1017
861
769
735
704
684
615
ABSORBANCE
0.3
0.2
0.1
0.0
1017
910
883
858
784
694
685
609
ABSORBANCE
0.4
0.3
0.2
0.1
0.0
1017
905
881
857
736
685
667
611
1000
900
800
700
600
500
WAVENUMBERS (cm-1)

FIG. 14

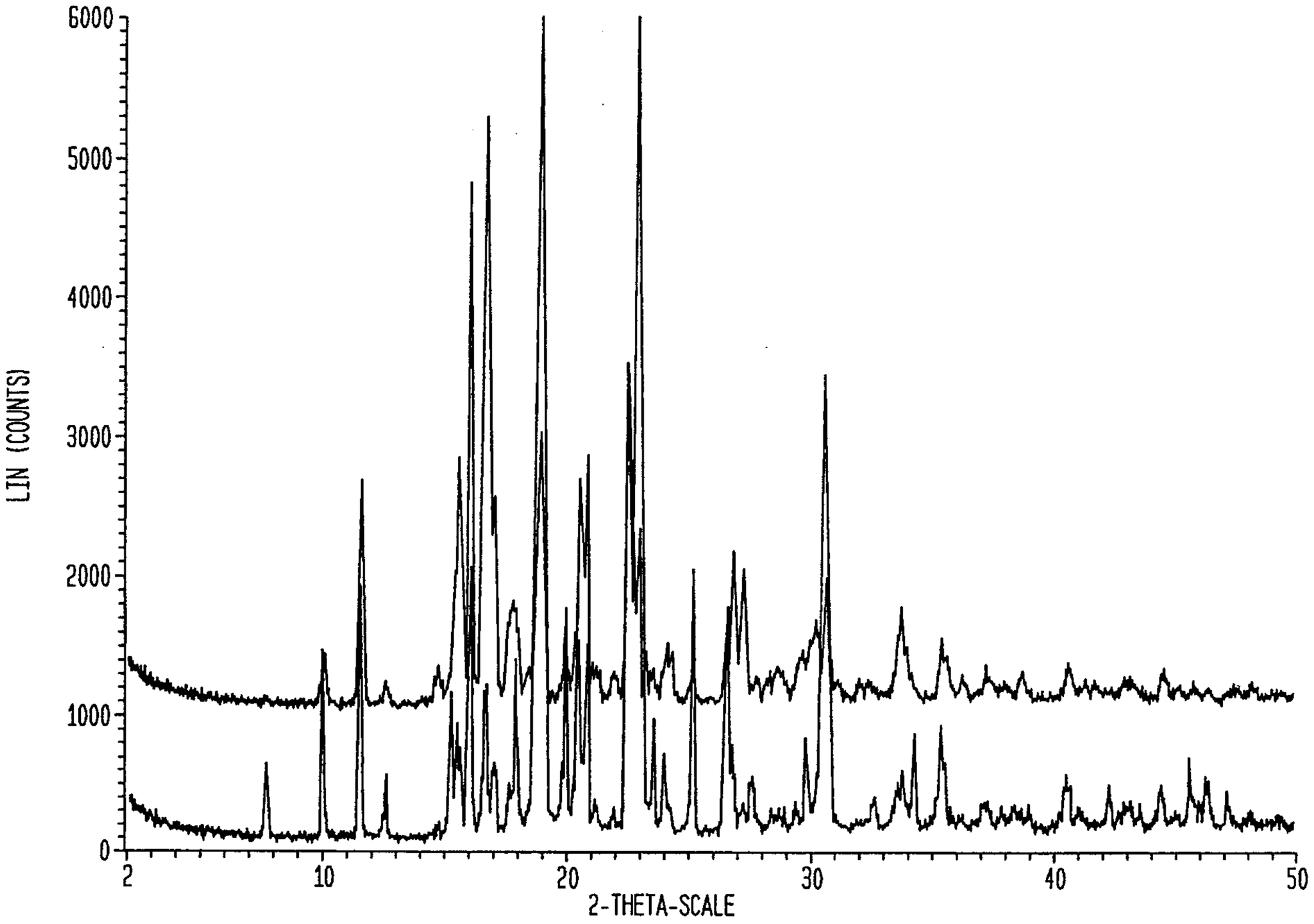

6000
5000
4000
3000
2000
1000
0
LIN (COUNTS)
2
10
20
30
40
50
2-THETA-SCALE

FIG. 15

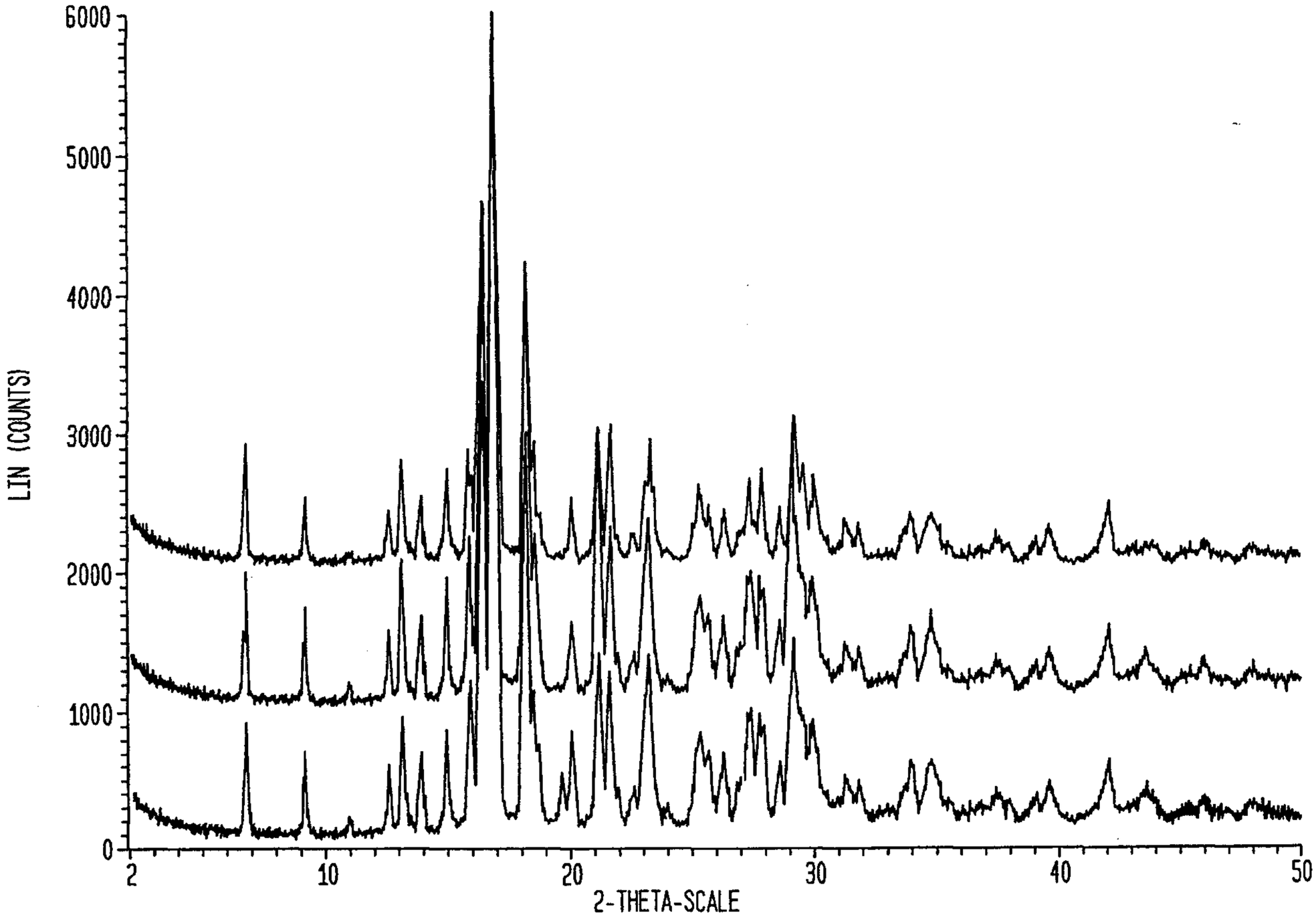

6000
5000
4000
3000
2000
1000
0
LIN (COUNTS)
2
10
20
30
40
50
2-THETA-SCALE

FIG. 16

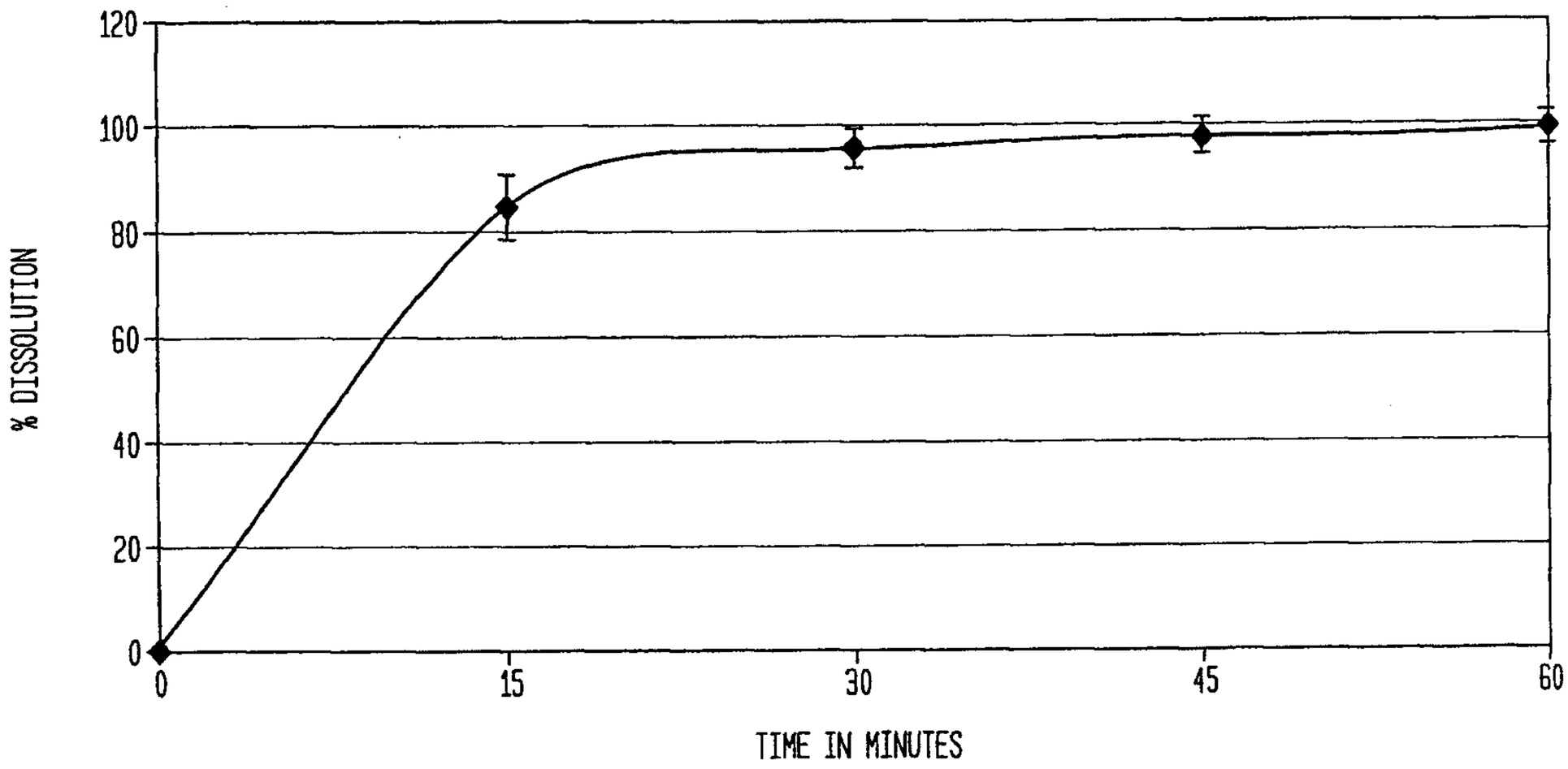


FIG. 17

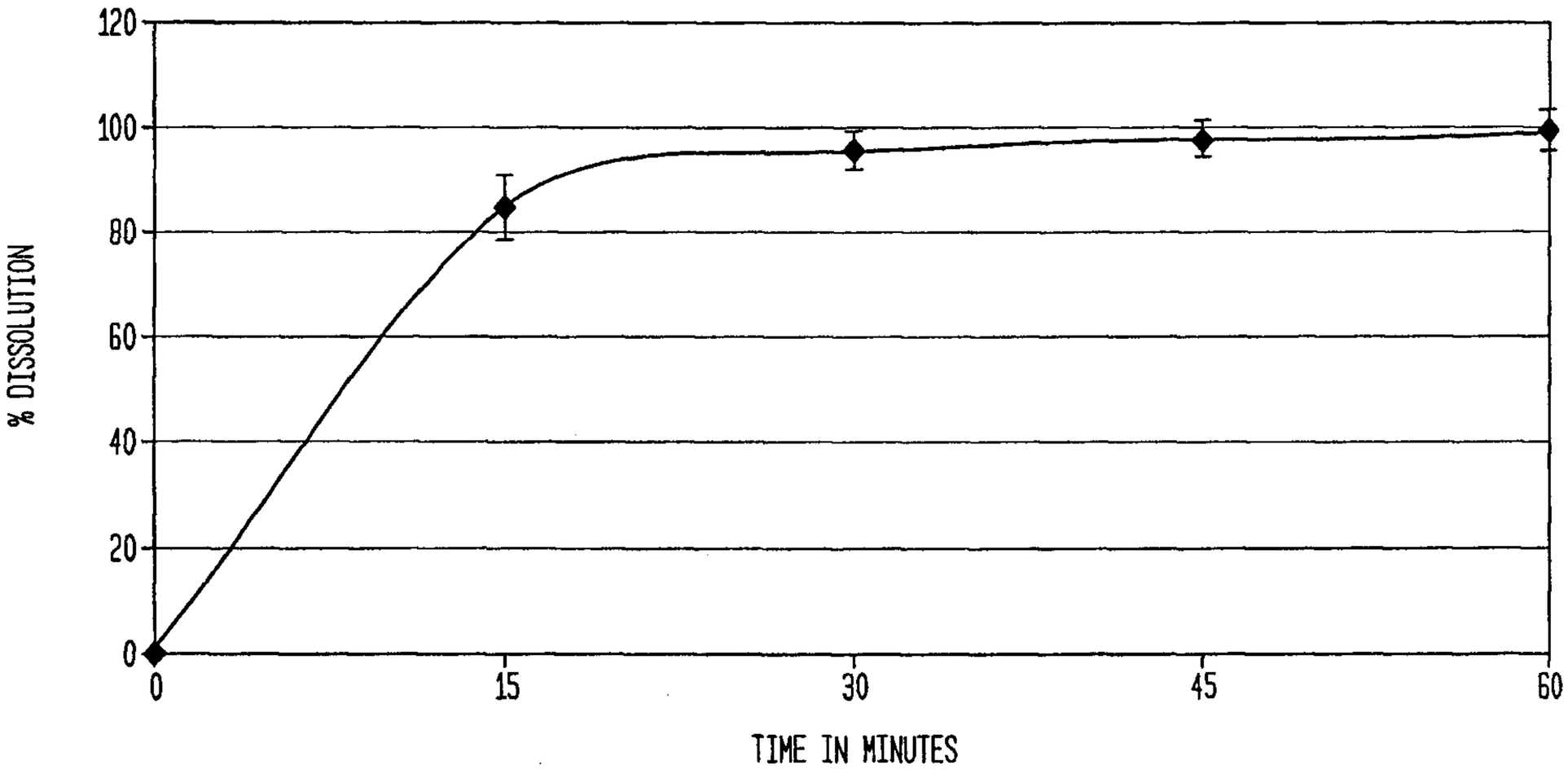


FIG. 18

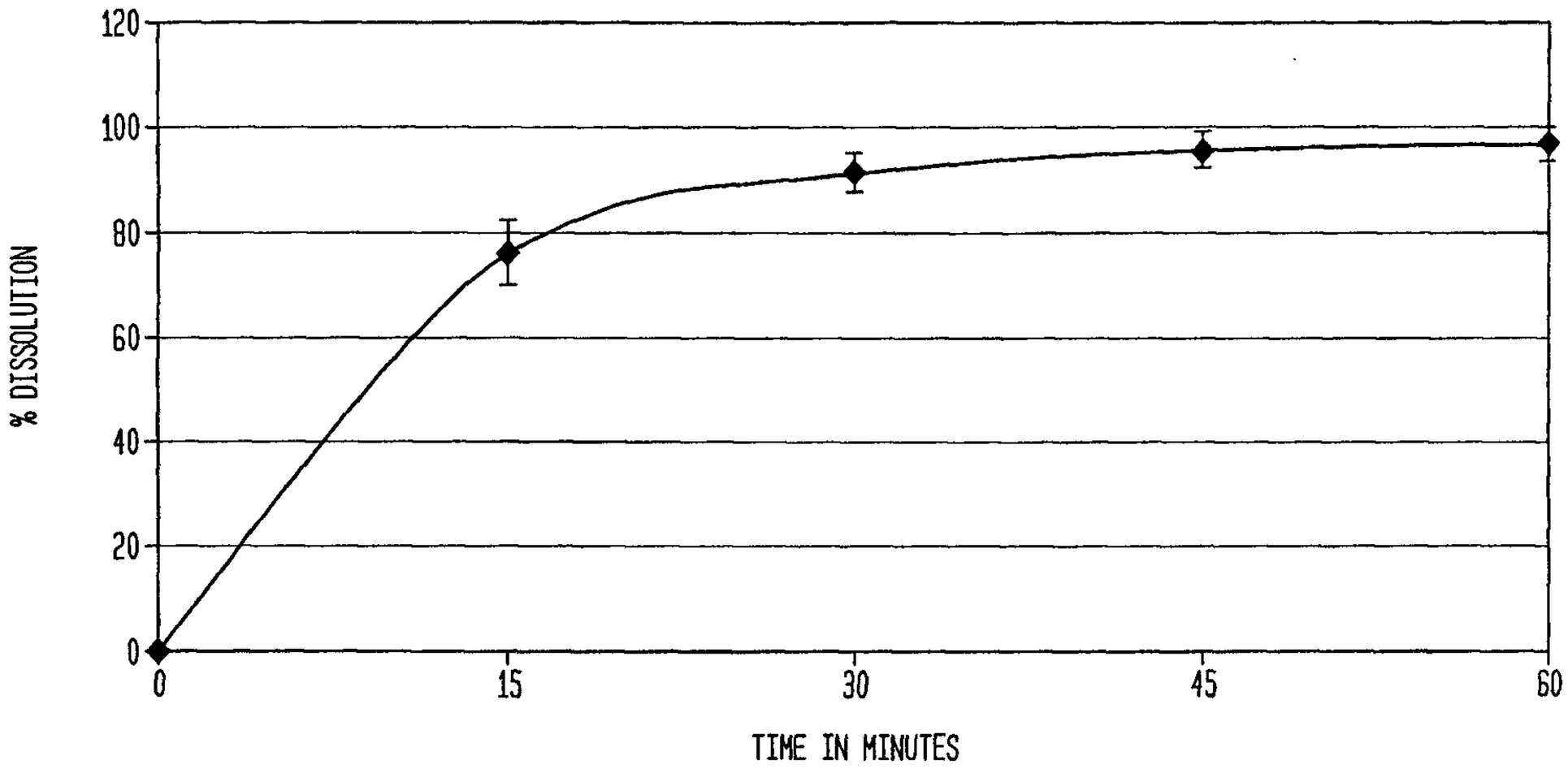


FIG. 19

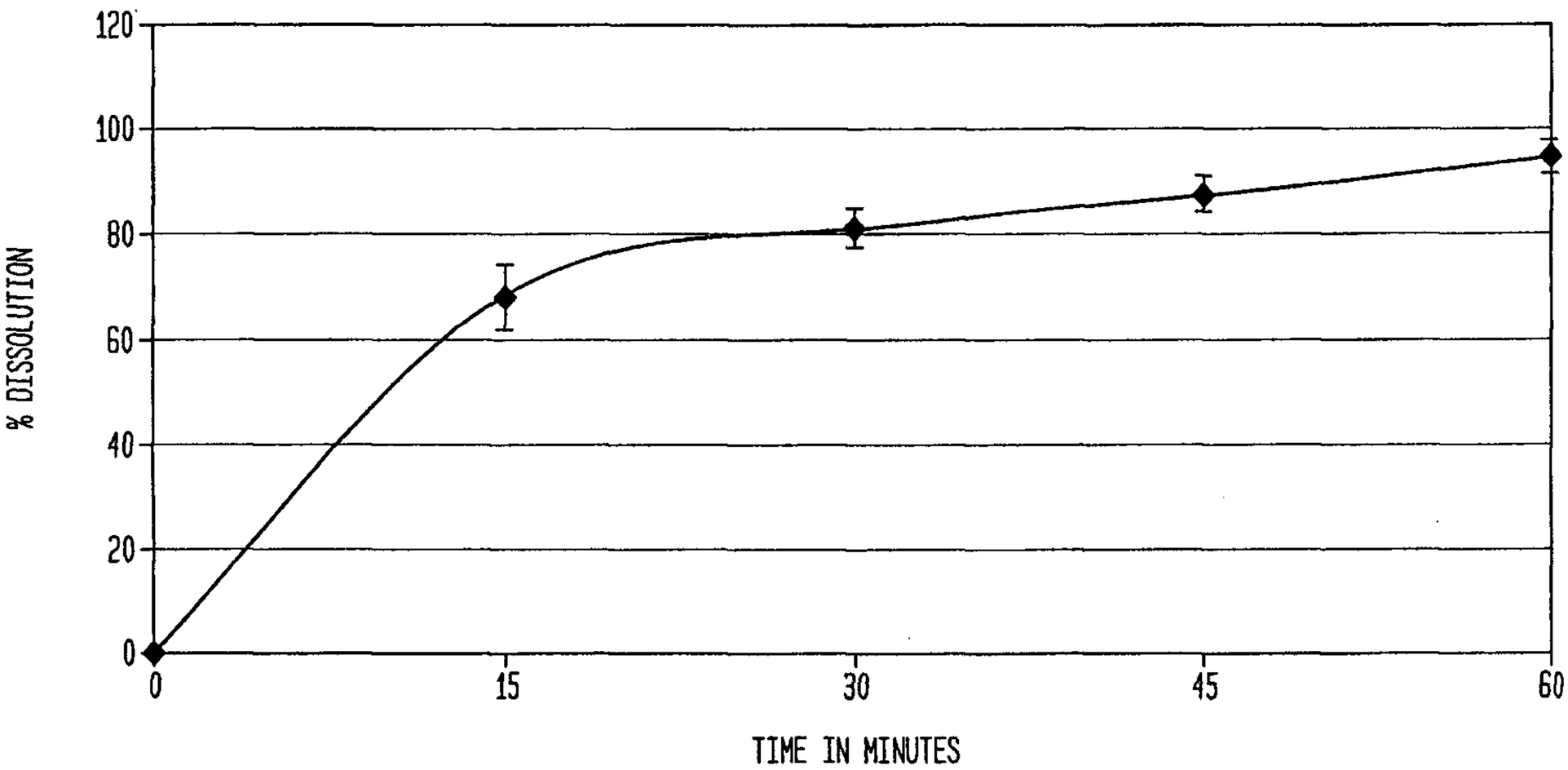


FIG. 20

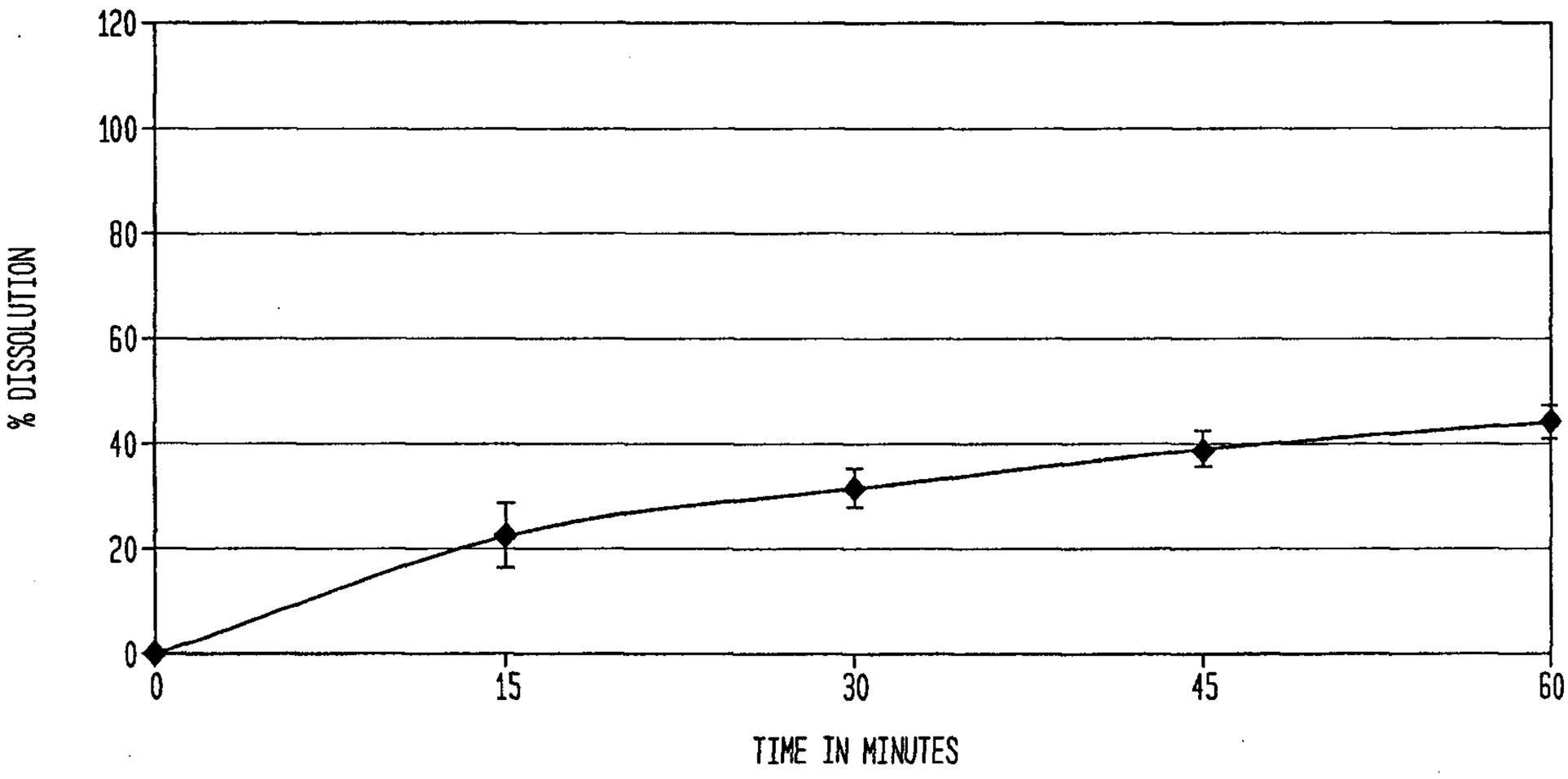


FIG. 21

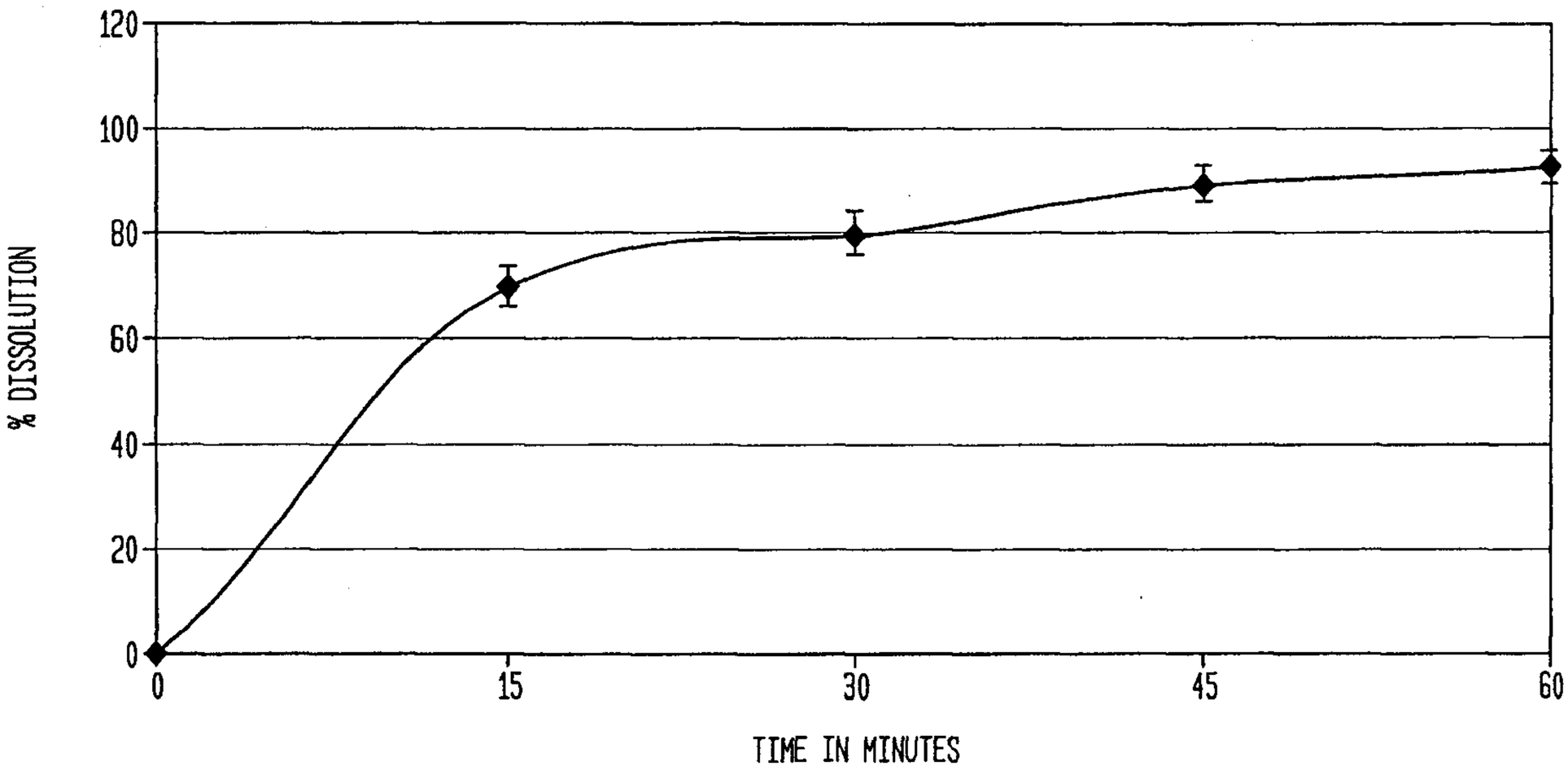


FIG. 22

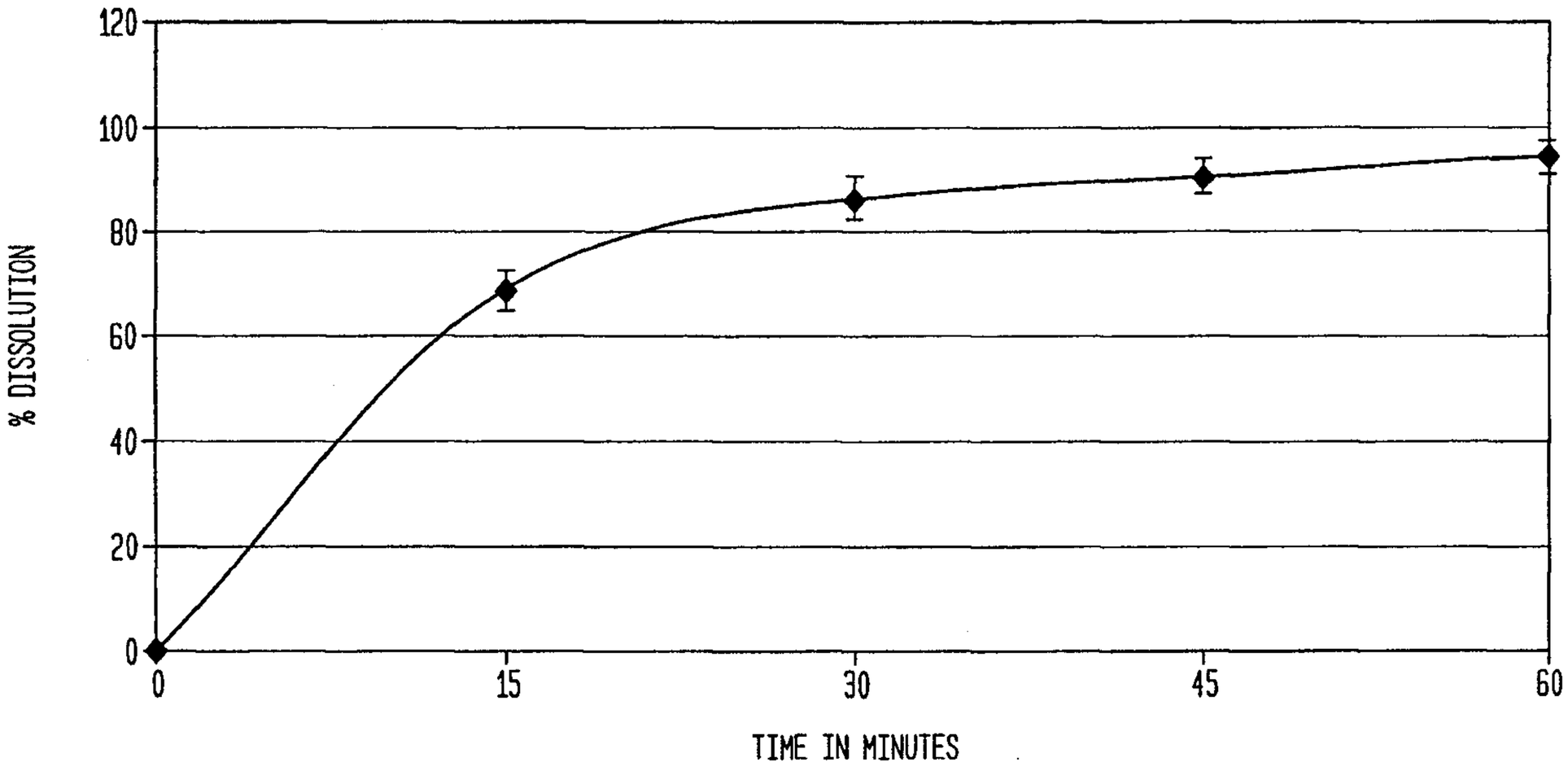


FIG. 23

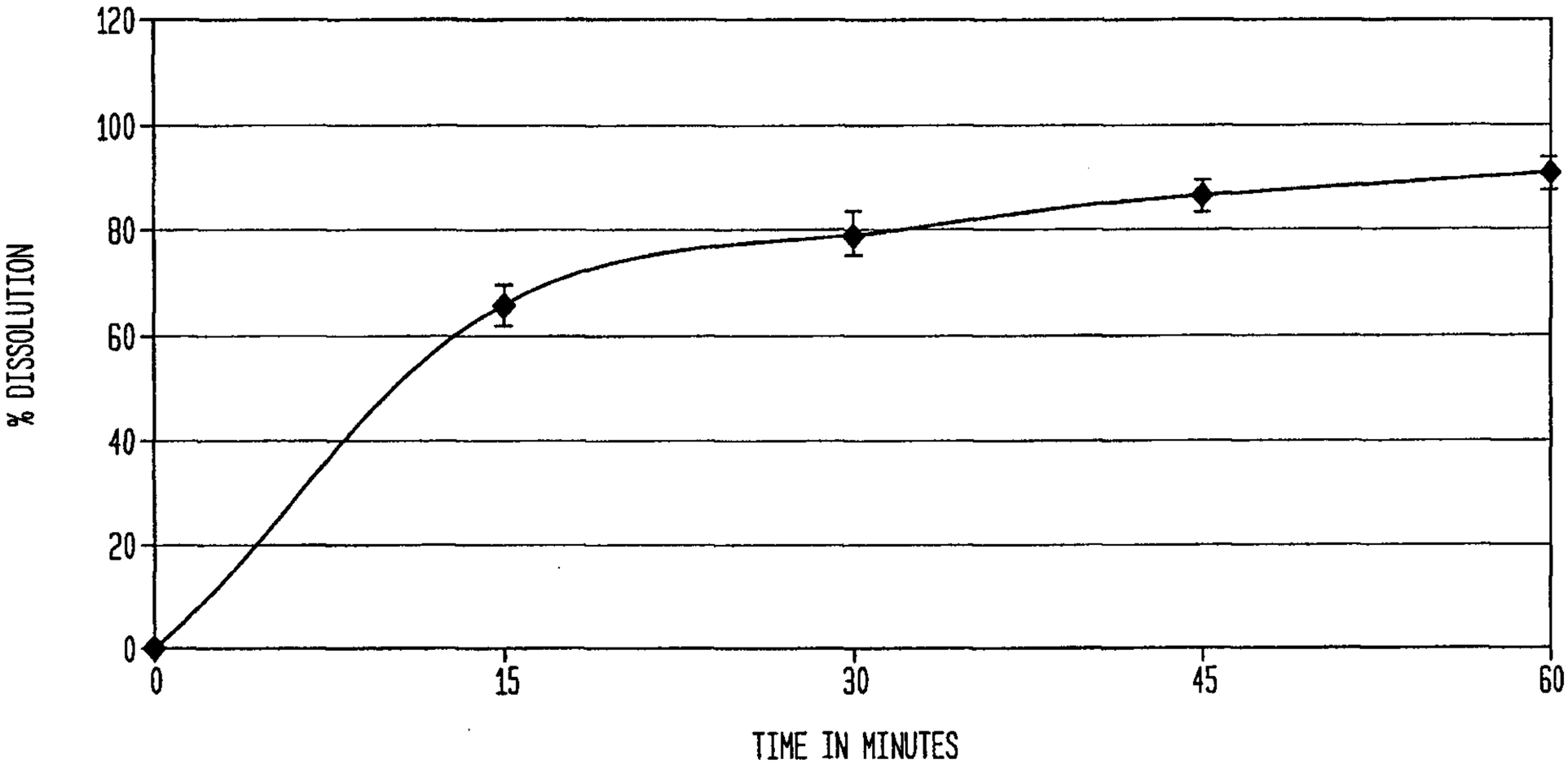
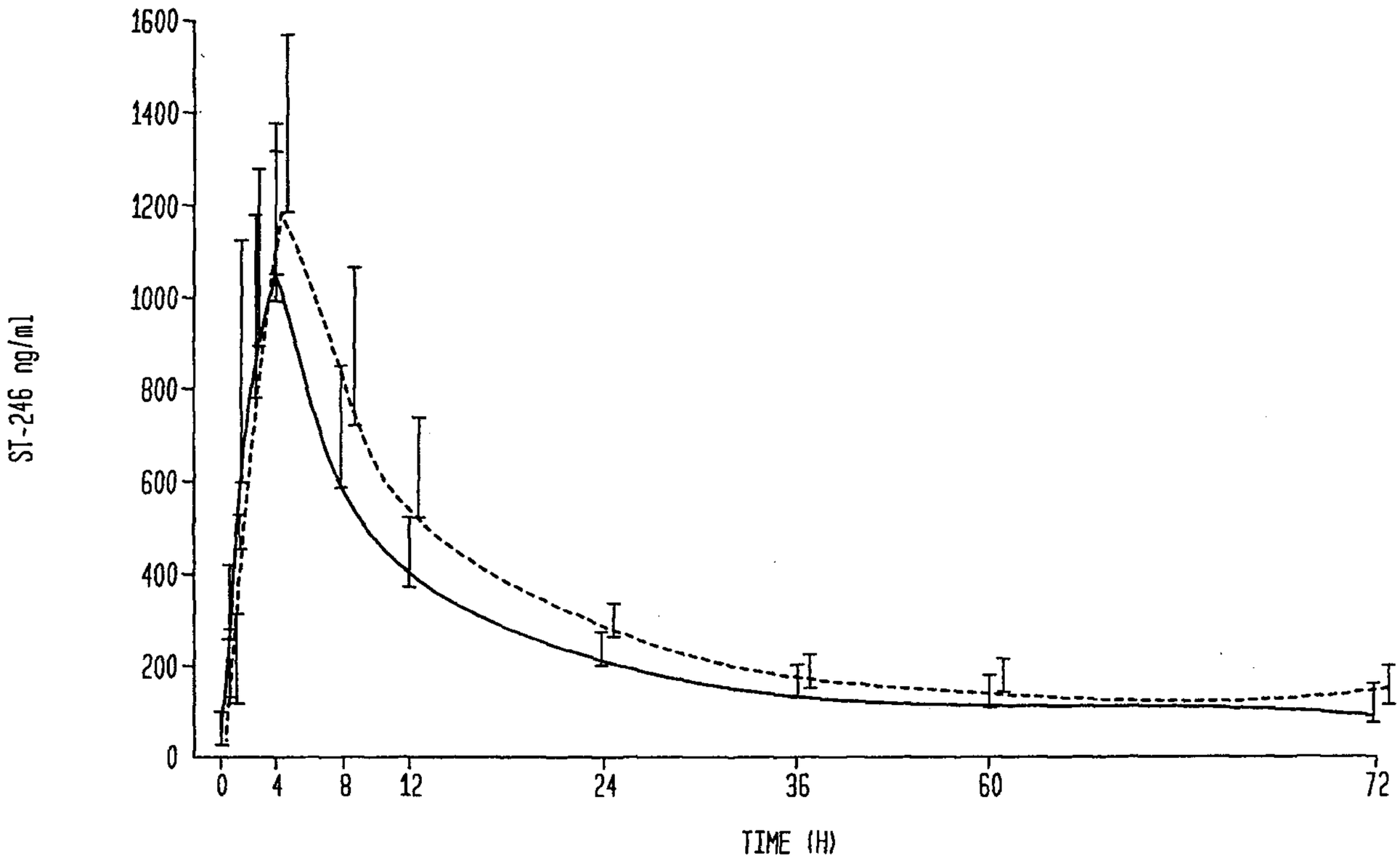


FIG. 24


1600
1400
1200
1000
800
600
400
200
0
ST-246 ng/ml
0
4
8
12
24
36
60
72
TIME (H)