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(54) Title: CRYSTALLINE MINOCYCLINE THERMO-RESISTANT OBTAINED BY RECRYSTALLIZATION WITH CARBON DIOXIDE

(57) Abstract: The present invention relates to a novel form of minocycline chlorohydrate characterized in that- it is crystalline and it presents characteristic powder X-ray diffraction pattern and infrared spectrum. This new polymorph constitutes the most stable form of minocycline with a melting point much higher than that of other known crystalline forms of minocycline. The process of production of this new minocycline polymorphic form consists in the dissolution of carbon dioxide in solutions or suspensions of minocycline chlorohydrate with organic solvents, under certain conditions of pressure and temperature. The present invention places minocycline in the restrict group of antibiotics with a melting temperature higher than the sterilization temperature, which enhances new applications and allows the optimization of production at industrial

DESCRIPTION

Crystalline Minocycline Thermo-resistant obtained by recrystallization with carbon dioxide

Field of the invention

This invention falls within the scope of new antibiotic crystalline forms and processes leading to its recrystallization. Particularly, it presents a novel crystalline form of minocycline with enhanced properties and describes the processes for its production with the use of anti-solvent effect caused by carbon dioxide.

Background of the invention

Antimicrobial drugs have been losing their effectiveness due to the adaptive evolution of microorganisms. The exaggerated use of antibiotics in the therapy has been pointed out as the leading cause of the emergence of resistant pathogenic agents and antibiotic multiresistance, compromising the fight against community- and hospital-acquired infections. The multiresistant species constitute the major concern in the field of surgical interventions, namely when a foreign body is implanted. Antibiotics are generally less effective treating infections along the surfaces of bone and artificial devices (due to the poor vascularization of bone tissue and the development of bacteria in the form of biofilms on implanted biomaterials surfaces), leading to multidrug-resistant bacterial infections.

This problem can be significantly attenuated by materials impregnated with antibiotics for controlled and sustained drug release.

However, for production of these materials, it is convenient to expose antibiotics to high temperature (either for polymerization, extrusion or sterilization) . Under this scope, the few heat-resistant (above 200 °C) antibiotics available restrict the formulations and antibiotic mixtures ("antibiotic cocktails"), susceptible to be incorporated in prostheses and implants, limiting therefore the antimicrobial effectiveness of the products. The existence of a crystalline minocycline thermo-resistant form which may be included in formulations used in the controlled and sustained delivery of this antibiotic, can be determinant in the therapy of associated infections to prostheses and implant placements, for example.

Minocycline is a broad-spectrum antibiotic long-acting semi-synthetic derivative of the antibiotic tetracycline, which inhibits the bacterial protein synthesis preventing the binding of aminoacyl-tRNA to the 30S bacterial ribosomal subunit. Among the several antibiotics for clinical use, minocycline (oral or intravenous administration) presents a broader spectrum when compared with other compounds from the tetracycline family. Minocycline is particularly indicated in the treatment of bone infections (among other applications) , concurring as well to a beneficial effect in the tissue regeneration processes. This antibiotic shows a relevant potential against bacterial multiresistant strains, namely methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumanii,* accountable for a high number of community- and hospital-acquired infections. Minocycline also shows anti-inflammatory and anti-apoptotic effects, exhibiting neuroprotective properties in different neurodegenerative diseases. Minocycline is the most lipo-soluble of its class, effectively crossing the blood-brain

barrier. The increased lipophilicity enhances minocycline penetration into various tissues when compared with other tetracyclines; therefore it is particularly suitable for material interfaces and subcutaneous implants, since it is not easily washed out to cause a burst effect. Instead, it is likely to stay active within the materials interface - a critical region for infection. This invention is a technological contribution to place minocycline in the restrict group of thermo-resistant antibiotics, complementing the weakness of other heat-resistant antibiotics, and leading thus to antibiotic-impregnated materials more active in treating infections.

Beyond the improved stability of minocycline mentioned before, this invention also improves the control of its crystalline form. The minocycline crystalline form is rather difficult to control, as evidenced by the thermogram of the commercial product (Sigma Aldrich, Germany) not recrystallized with carbon dioxide ($C0_2$) shown in Figure 1. For example, the mentioned commercial minocycline from Sigma Aldrich is a mixture of crystalline forms with the amorphous form. It is well-known that the mixture of polymorphs with amorphous forms is unstable and it is little reproducible because amorphous substances can react with excipients, moisture or convert into crystalline forms causing the product properties to be unpredictable and unstable. The changes in physicochemical properties (like chemical reactivity, solubility, dissolution rate, melting temperature, electrical and optical properties) compromise the bioavailbility of a bioactive compound of therapeutic utility, and consequently its efficacy. Since each crystalline form presents different properties (as: the amorphous form), the actual formulations are heterogeneous and relatively unpredictable. During drugs synthesis

process, polymorphic transformations may occur in the recrystallization stage, which defines the physicochemical properties of these bioactive compounds. The present invention enables the achievement of a crystalline minocycline thermo-resistant produced by a recrystallization with carbon dioxide. This novel crystalline form is pure and, in opposition to the commercially available formulations, it is not a mixture of crystalline forms with the amorphous form. On the other hand, the processes of recrystallization described in this invention constitute techniques that allow controlling effectively the crystalline minocycline form.

Most processes used to produce minocycline powders (such as the common technique to produce small particles of controlled size designated as spray-drying) are unable to control its crystalline form. To overcome this limitation, some inventors developed a crystallization process in order to recrystallize minocycline base into a crystalline structure of controlled form (patents PT 103661 A .2008-08-25; WO 2008102161 A2 .2008-08-28 ; US 20100286417 Al. 2010-11-11) . However, the developed process (WO2008102161) is laborious, because it involves several steps for the micronization, recrystallization and purification from organic solvents, and it is unable to produce the thermo-resistant polymorph of minocycline described in this invention, that is induced by carbon dioxide.

The production of this novel polymorph is based on the anti-solvent effect caused by carbon dioxide when the latter is added to solutions or suspensions of minocycline in organic solvents. The $C0_2$ anti-solvent effect has been used by many authors to micronize substances including its amorphization . Nevertheless, so far, the $C0_2$ anti-solvent

effect was not used to control the crystalline minocycline form.

The process for the production of crystalline minocycline chlorohydrate referred in this invention, presents as advantages the production of a novel thermo-resistant polymorph and the potential of a more effective control of the crystalline form.

Summary of the Invention

The present invention relates to a novel form of minocycline chlorohydrate and to a process for its production. This novel form of minocycline is characterized in that it is crystalline, with characteristic powder X-ray diffraction pattern and infrared spectrum, and a melting temperature of 247 °C. The production process involves the mixing of carbon dioxide with solutions or suspensions of minocycline chlorohydrate in organic solvents, under certain conditions of pressure, temperature and composition. The recrystallization method is based on the carbon dioxide anti-solvent effect. Briefly, the minocycline recrystallizes in solution or in suspension when the carbon dioxide composition in the mixture reaches the necessary composition to induce an anti-solvent effect. This new minocycline polymorph is characterized in that it has a melting temperature (247 °C), which is about 50 °C to 130 °C higher than that of other known crystalline forms of minocycline. The high melting temperature enables to subject the novel crystalline minocycline chlorohydrate to heat sources, directly or embedded in other materials, either for sterilization effects (example: autoclaving) extrusion or polymerization.

The microbiological studies performed against several reference and multiresistant bacterial strains, testified the antimicrobial activity preservation of the novel minocycline chlorohydrate polymorph, relatively to that of the product not processed with carbon dioxide.

Detailed description of the invention

This invention relates to a novel form of crystalline minocycline resistant to very high temperatures. The new polymorph is characterized in that it has a melting temperature of 247 °C (±1°C), which is 50 °C to 130 °C higher than melting and degradation temperatures of other known crystalline forms of minocycline. Given these characteristic thermophysical properties, the new polymorph related to this invention can be embedded in composite materials, prostheses, bone cements, implants and controlled drug delivery formulations, as well as in sterile pharmaceutical formulations or compositions (namely when concerning damp heat sterilizations).

The process of minocycline chlorohydrate production, which is also related to the present invention, is characterized in that it has the mixture of carbon dioxide with organic solvents and minocycline chlorohydrates under certain conditions of pressure, temperature and composition above the corresponding saturation conditions of minocycline in the mixture (determined by phase equilibria).

The novel form of crystalline minocycline thermo-resistant is also characterized in that it presents an X-ray diffraction pattern having peaks at 5.5, 9.6, 11.0, 13.5, 15.6, 15.8, 16.5, 16.8, 17.4, 18.3, 19.0, 19.2, 20.8, 22.0, 22.2 e 23.3 +0.2° 2Θ, as given in Figure 3. It is further characterized in that it presents an infrared spectrum having peaks at 1664, 1617, 1583, 1510, 1460, 1405, 1343,

1291, 1214, 1193, 1131, 1094, 1044, 1002, 973, 957, 876, 831, 717, 755, 670, 576 and 544 ± 4 cm^{-1}, as given in Figure 4.

The production of the novel minocycline chlorohydrate polymorph is based on the anti-solvent effect of carbon dioxide, after the gas mixing with solutions or suspensions of minocycline in organic solvents.

Carbon dioxide, which is miscible with most organic solvents, changes its properties after the process of dissolution, particularly the ability of solvating other molecules. This anti-solvent effect of carbon dioxide has been studied by many authors, mostly towards the micronization of active substances, but not to control its crystalline form.

Thereby, the process of producing crystalline thermo-resistant minocycline is characterized in that it comprises the mixture with carbon dioxide of a solution (or suspension) of minocycline chlorohydrate in an organic solvent.

Using ethanol as an example, Figure 2 describes the thermodynamic fundament (phase equilibria) that supports the process mentioned in this invention.

As carbon dioxide dissolves in the organic solvent, pressure increases following the liquid-vapor equilibrium line (Figure 2). When liquid carbon dioxide composition reaches approximately 0.6 molar fraction, a precipitate of minocycline chlorohydrate related to this invention is formed. The saturation composition of minocycline, to which corresponds the beginning of precipitation, is shown in Figure 2, for the particular case of a minocycline chlorohydrate solution, with an initial composition of 0.5% (mass percentage) in ethanol. Different initial compositions in minocycline, or the use of other organic

solvents, require different carbon dioxide compositions in order to reach saturation.

In the absence of thermodynamic data of this nature, the necessary conditions to produce the polymorph to which this invention is related would be hardly achievable. This reason may explain the production of minocycline chlorohydrate only as amorphous, by other authors who also used methods involving the minocycline precipitation by carbon dioxide.

There are essentially two alternative ways for the mixing process which characterizes the production of minocycline chlorohydrate thermo-resistant . A) adding carbon dioxide to a precipitator containing the solution (or the suspension) ; B) adding the solution (or the suspension) to a precipitator containing carbon dioxide.

Example 1 illustrates the first alternative. In this exemplification, carbon dioxide is fed to a reactor containing minocycline chlorohydrate dissolved in an organic solvent. Example 2 describes a change-over of this method, which compasses the processing of a minocycline chlorohydrate suspension rather than a solution. In this last example, the minocycline chlorohydrate (purchased by Sigma Aldrich, Germany) was processed. The minocycline chlorohydrate is a mixture of several crystalline forms, as indicated by the corresponding thermogram shown in Figure 1. The suspension consists in a supersaturated solution of the initial product. In this case, the anti-solvent effect of carbon dioxide causes precipitation of the most stable crystalline form of dissolved chlorohydrate fraction - the thermo-resistant polymorph. Over time, the suspension is totally converted into a single polymorph - the most stable - which is the object of this invention. The use of suspensions, when compared with solutions, enables the

achievement of relatively high yields of minocycline per unit of organic solvent and carbon dioxide.

Alternatively, and as the processing form B, the minocycline solution may be fed to a reactor containing pressurized carbon dioxide.

Carbon dioxide contained in the precipitator must be under such pressure and temperature conditions, so that $C0_2$ final composition in the mixture equals or exceeds the necessary value to cause the minocycline precipitation by anti-solvent effect. This process may be performed in a continuous or a semi-continuous mode. In the semi-continuous mode, the solution is fed into the precipitator in conditions that ensure the minocycline chlorohydrate precipitation and, simultaneously, the dissolution of the organic solvent in the $C0_2$-rich liquid phase. In this case, $C0_2$ feed flow rate must be enough to dissolve the flow-rate of the solvent contained in the solution. The solutes are loaded in the $C0_2$ pressurized precipitator, being separated from the out stream through a filter. This processed is exemplified in Example 3.

In the continuous mode operation, complete solvent dissolution in the $C0_2$ rich-liquid phase is not required. In this case, the liquid solution is continuously mixed with the $\mathbf{CO_2}$ in a static mixer, aiming to cause only the precipitation of the crystalline chlorohydrate minocycline. Here, both $C0_2$ and the organic solvent are separated from the chlorohydrate through *spray-drying* of the suspension previously formed on account of the two flows mixing. Minocycline chlorohydrate particles generated like this can be separated in cyclones or electrostatic precipitators, as Example 4 exemplifies .

The minocycline chlorohydrate polymorph, which this invention relates to, was also characterized in terms of

its antibacterial activity. Microbiological studies were carried out against reference bacterial strains and multidrug resistant strains. The obtained results testified the antimicrobial activity preservation of the novel minocycline chlorohydrate polymorph, relatively to that of the product not processed with carbon dioxide.

Description of the figures

Figure 1 shows the comparison between the minocycline thermo-resistant thermogram produced by recrystallization with carbon dioxide (curve A) and the pre-processed minocycline thermogram (curve B). The variable t refers to temperature values in °C, ΔΛ refers to the variation of enthalpy in arbitrary units and "endo" refers to the direction of the endothermic reaction.

Figure 2 illustrates the experimental observation of minocycline chlorohydrate precipitation in ethanol solutions, through pressure-composition diagram of ethanol-liquid phase carbon dioxide mixture, at different temperatures. Points corresponding to pressure and composition, where precipitation occurs, are marked by X. Lines were obtained using the equation of state described by Li *et al.* 2005.

Figure 3 represents the XRPD (X-Ray Powder Diffraction) pattern of minocycline thermo-resistant produced by recrystallization with carbon dioxide.

Figure 4 represents the infrared spectra of minocycline thermo-resistant produced by recrystallization with carbon dioxide.

Exemplification of the invention

Example 1

This example describes the production of minocycline chlorohydrate thermo-resistant polymorph after adding carbon dioxide to minocycline chlorohydrate solutions in ethanol .

1 g of minocycline chlorohydrate solution in ethanol (concentration of 5 mg/g) was added to a stainless steel reactor equipped with glass viewing windows. Carbon dioxide was added to the solution by means of a compressor. Dissolution of carbon dioxide in the solution induced the precipitation of minocycline chlorohydrate in the form of a thermo-resistant polymorph. The precipitation operation of minocycline chlorohydrate thermo-resistant polymorph occurred at different pressures, which were dependent on the reactor temperature, as described in Figure 2. The production of the precipitate was verified when the molar fraction of $C0_2$ (in the liquid phase) reached the value of 0.6, approximately, at all temperatures, namely at 35, 40 and 45 °C.

Example 2

This example describes the recrystallization of a mixture of crystalline forms of minocycline chlorohydrate (which characteristic thermogram is evidenced in Figure 1, curve B) into the thermo-resistant polymorph. This process consists in the suspension of minocycline chlorohydrates in a $C0_2$-rich liquid phase containing an organic solvent. Minocycline chlorohydrate (0.1 g) and ethanol (0.1 g) were added to a stainless steel reactor with a volume of 8 cm^3, under stirring. The reactor was pressurized up to 20 MPa and 50 °C. The resulting suspension was kept under these conditions for 2 hours, while stirred with a magnetic

stirrer at 200 rpm. Ethanol was removed together with $C0_2$ during de slow depressurization of the precipitator at 50 °C. The minocycline thermo-resistant polymorph was collected from the inside of the reactor.

Example 3

This example demonstrates the production of minocycline chlorohydrate thermo-resistant polymorph through a method that operates in a semi-continuous mode. This process consists in the feeding of a solution of minocycline chlorohydrate in ethanol (5 mg/g) to a precipitator pressurized with $C0_2$. The mentioned solution was atomized inside the precipitator by means of a nozzle. This atomization was assisted by a $C0_2$ flow rate, which was co-depressurized along with the liquid through a nozzle (with a internal diameter and length of 150 μm and 250 μm, respectively) .

The solution flow rate was Ig/min. The $C0_2$ flow rate was 14g/min. Adding the solution to the precipitator pressurized with $C0_2$, causes the precipitation of the solutes as a result of the anti-solvent effect. Herein, the solvent remains in the $C0_2$-rich liquid phase, while the solutes precipitate and are separated from the $C0_2$-rich liquid phase by a filter placed at the precipitator exit. The experiment procedure is carried out by means of the continuous injection of solution and $C0_2$, which circulate through the precipitator (where precipitates are accumulated) . Pressure is kept through the discharge of the $C0_2$-rich liquid phase, which is regulated by a "back-pressure" type controller. Approximately 100 g of solution were processed, for each tested pressure value. Then, the precipitator was depressurized and the minocycline chlorohydrate was collected from the filter and from the

precipitator walls. In the three pressure values which were tested (10 MPa, 13 MPa and 20 MPa) the minocycline thermo-resistant polymorph was recovered. In all cases, the precipitator temperature was 50 °C.

Example 4

This example describes the production of minocycline chlorohydrate thermo-resistant polymorph through a method that operates in a continuous mode. Herein, a solution of minocycline chlorohydrate in ethanol (5 mg/g) is mixed to the carbon dioxide (in the conditions at which precipitation of the minocycline chlorohydrate thermo-resistant occurs), in a static mixer. In this example, the following conditions concerning the mixture were selected: pressure of 15 MPa, temperature of 45 °C, $C0_2$ flow rate of 20 g/min and solution flow rate of 1 g/min) . The mixer consisted in a tube with 5 mm of internal diameter and 10 cm of length (unfilled) . The mixture caused the precipitation of minocycline in the thermo-resistant chlorohydrate that stayed in suspension. This suspension was then subjected to atomization through a nozzle for the solvent drying and CO_2 removal by *spray-drying. In* this example, the orifice nozzle presented a 150 μm of internal diameter and 500 μm of length. Drying operation took place in a precipitator at atmospheric pressure. Drying was aided by a current of hot air; in this example, nitrogen was used (30 L per min, approximately) at 70 °C. Precipitates were recovered in a cyclone and in an electrostatic precipitator.

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Date: December 17th, 2012

CLAIMS

1. Crystalline minocycline chlorohydrate characterized in that it presents an X-ray diffraction pattern having peaks at 5.5, 9.6, 11.0, 13.5, 15.6, 15.8, 16.5, 16.8, 17.4, 18.3, 19.0, 19.2, 20.8, 22.0, 22.2 and 23.3 ±0.2° 2Θ.

2. Minocycline chlorohydrate, according to the previous claim, characterized in that it has an infrared spectrum having peaks at 1664, 1617, 1583, 1510, 1460, 1405, 1343, 1291, 1214, 1193, 1131, 1094, 1044, 1002, 973, 957, 876, 831, 717, 755, 670, 576 and 544 ± 4 cm^{-1}.

3. Minocycline chlorohydrate, according to the previous claims, characterized in that it has a melting temperature of 247 °C.

4. Minocycline chlorohydrate, according to the previous claims, characterized in that it is essentially a single polymorph.

5. Process of production of the minocycline chlorohydrate, defined in claims 1-4, characterized in that it comprises a mixture of carbon dioxide with organic solvents and minocycline chlorohydrates under pressure, temperature and composition conditions that exceeds the correspondent conditions to minocycline saturation in the mixture, determined by the phase equilibria.

6. Use of minocycline chlorohydrate defined in claims 1-4, characterized in that the minocycline chlorohydrate is incorporated into the preparation of composite materials, prostheses, bone cements, implants, and controlled drug delivery formulations.

7. Use of minocycline chlorohydrate, according to claim 6, characterized in that the minocycline chlorohydrate is incorporated into the preparation of sterile pharmaceutical compositions or formulations, namely the ones sterilized by damp heat.

Date: December 17th, 2012

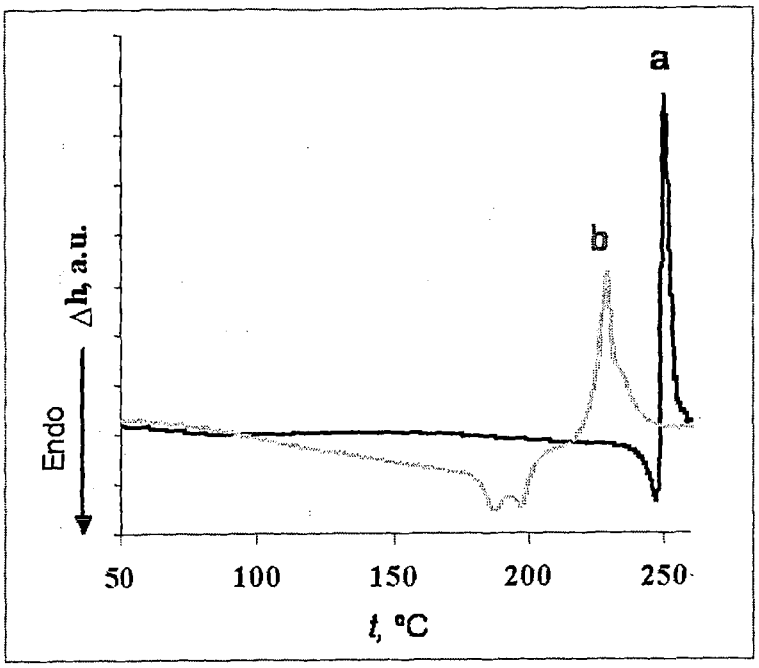

Figure 1

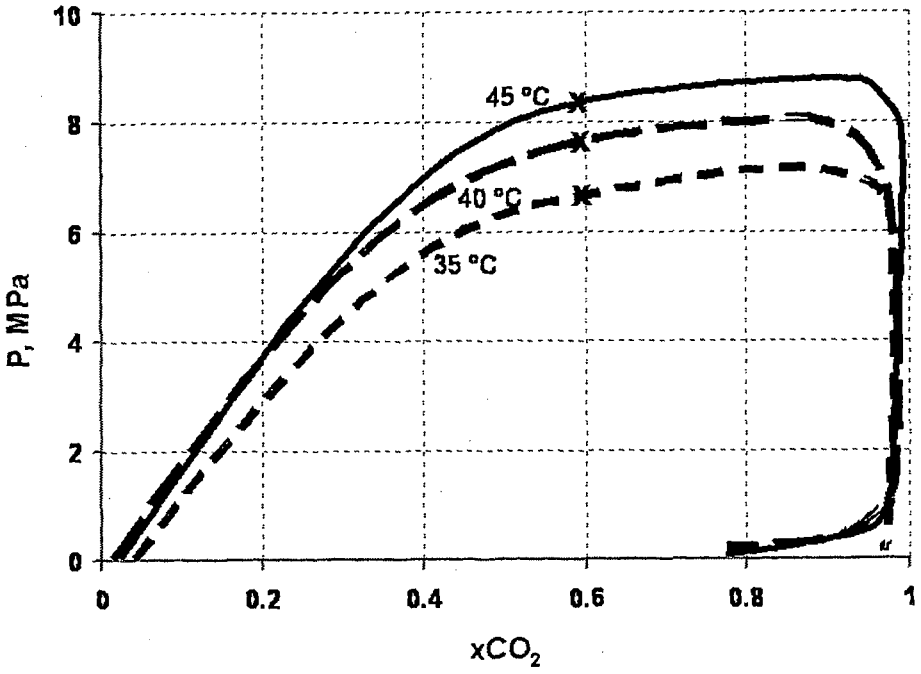

Figure 2

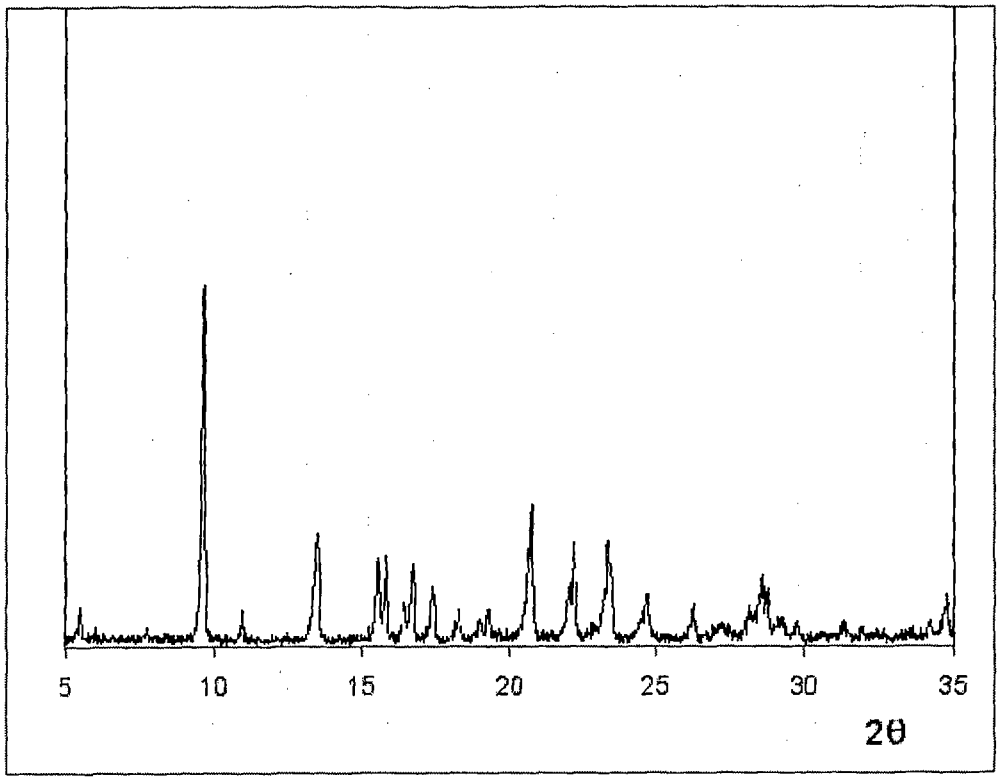

Figure 3

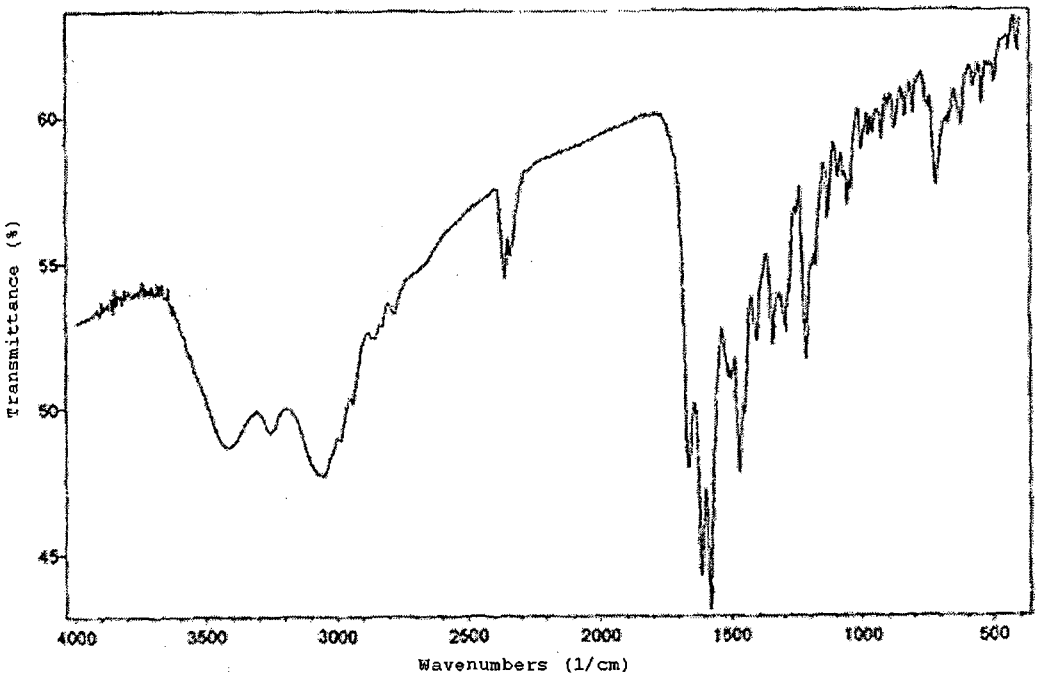

Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/PT2012/000051

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07C237/26 A61K31/65 A61P31/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 038 315 A (TOBKES MARTIN) 26 July 1977 (1977-07-26) claims; example 4 -----	1-7
A	CUIXIANG SUN ET AL: "A Robust Platform for the Synthesis of New Tetracycline Antibiotics", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, ACS PUBLICATIONS, US, vol. 130, 31 December 2008 (2008-12-31), pages 17913-17927, XP002604988, ISSN: 0002-7863, DOI: 10.1021/JA806629E [retrieved on 2008-12-03] scheme 3; compound 9 ----- -/-	1-7

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2013	20/03/2013

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INTERNATIONAL SEARCH REPORT

International application No
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