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Original Research Article

Physics, chemistry, and Hirshfeld surface analyses of gamma-irradiated thalidomide to evaluate behavior under sterilization doses

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ABSTRACT

Thalidomide was indicated as a sedative and antiemetic and prescribed for pregnant women. Its tragic teratogenic effects culminated in withdrawal from the market. Since the discovery of its anti-angiogenic and anti-inflammatory actions, thalidomide has been used in the treatment of leprosy and multiple myeloma, which justify studies of its stability. We investigated the effects of irradiation of thalidomide up to 100 kGy (fourfold the usual sterilizing dose for pharmaceutics). The β polymorph of thalidomide was obtained in an isothermal experiment at 270 °C. All samples underwent gamma irradiation for specific times. At different doses, decomposition of the pharmaceutical was not observed up to 100 kGy. The observed effect was angle turning between the phthalimide and glutarimide rings modulated by repulsion towards the carbonyl group, leading to a stable energetic configuration, as measured by the equilibrium in the torsion angle after irradiation. The thalidomide molecule has a center of symmetry, so a full turn starting from 57.3° will lead to an identical molecule. Further irradiation will start the process again. Samples irradiated at 30 and 100 kGy have more compact unit cells and a lower volume, which leads to an increase in the intermolecular hydrogen interaction within the unit cell, resulting in higher thermal stability for polymorph α.

1. Introduction

To ensure adequate conditions of use, sterility is a crucial attribute to any pharmaceutical material, main component, excipient, or formulation. In general, sterilized materials should have microbial survivor probability of $< 10^{-6}$. This criterion is the basis of the sterility assurance level.

There are several sterilization procedures, and each has advantages and disadvantages [1,2]. There is no suitable procedure for general use. Physical removal of microorganisms by membrane filtration does not require heat. Dry heat or even moist heat promotes microbiological reduction at high temperature, but results in considerable degradation of temperature-sensitive materials or devices. Sterilization using ethylene oxide is highly effective but can leave a toxic residue in porous materials such as implants. Electron-beam radiation can be used to prevent temperature effects and toxic residues in the final material, but is limited by poor penetration in bulky materials.

Gamma irradiation has advantages over other conventional sterilization methods in solids: high penetration, uniform efficacy, low isothermal stability, and absence of toxic residues. The main advantage is that irradiation can be used as the final sterilization procedure in starting materials and final products. In this way, the usual 25kGy dose can ensure sterilized pharmaceutical materials [2,3]. Due to the potential sensitivity of pharmaceuticals, validation procedures with lower doses are usually accepted as long as reliable and adequate reduction of the biologic burden can be ensured. In this way, the risk of undesired effects over pharmaceuticals, formulations, or devices submitted to the sterilization process is minimized [4].

Thalidomide ((RS)-2-(2,6-dioxopiperidin-3-il)-1H-isoindol-1,3 (2H)-dione) was synthesized by Chemie Grünenthal in West Germany in 1954. It was introduced to the West German market in 1956 as an antiemetic for pregnant women. In the 1960s, the teratogenic effects of this drug were recognized. Fetal malformation due to the S-isomer of thalidomide resulted in restricted use of thalidomide and increased surveillance by regulatory agencies [5].

Since then, thalidomide has been recognized as having anti-angiogenic and anti-inflammatory properties. It has been used to treat leprosy and multiple myeloma. Hence, stability studies of thalidomide under radioactive stress aimed at sterilization of the drug are warranted [5].

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2. Materials and methods

A sample of thalidomide from a validated production batch was obtained during the shelf-life of this pharmaceutical. All analyses were conducted within the validity period of the batch.

2.1. Powder X-ray diffraction (PXRD)

PXRD data were collected in an XRD-7000 diffractometer (Shimadzu, Kyoto, Japan) at room temperature under 40 kV, 30 mA, using CuK_α ($\lambda = 1.54056$ Å) equipped with polycapillary focusing optics under parallel geometry coupled with a graphite monochromator. The sample was spun at 60 rpm, and scanned over an angular range of 4–60° (2θ) with a step size of 0.01° (2θ) and a time constant of 2s/step. All fitting procedures were obtained using FullProf Suite [6,7]. Crystalexplorer v 17 was used to calculate the Hirshfeld surface [8].

2.2. Single-crystal X-ray diffraction (SCXRD)

SCXRD data were collected in a Gemini A Ultra X-ray Diffraction system (Agilent Technologies, Santa Clara, CA, USA) at room temperature using a MoK_α ($\lambda = 0.71073$ Å) tube as the X-ray source, equipped with a graphite monochromator and a charge-coupled device plate detector. Data collection and refinement details are given in Table 1.

2.3. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA)

TGA and DTA experiments were carried out on a DTG60H system (Shimadzu) in a dynamic N_2 atmosphere (50 mL/min) using alumina pans containing ≈2.0 mg of sample. Experiments were conducted at a heating rate of 10 °C/min from 25 °C to 400 °C.

2.4. Differential scanning calorimetry (DSC)

DSC experiments were undertaken on a DSC60 system (Shimadzu). The equipment cell was calibrated with indium (melting point, 156.6 °C; heat of fusion, $\Delta H_{fus} = 28.54$ J/g) and lead (melting point, 327.5 °C). Aluminum pans containing ≈1 mg of sample were used under a dynamic N_2 atmosphere (50 mL/min) and a heating rate of 10 °C/min from 25 °C to 300 °C. Thalidomide can exist as two polymorphs, α and β, and the latter shows different thermal behavior. Therefore, an isothermal experiment was carried out at 270 °C to obtain a pure material for comparison, as needed.

Table 1
Single crystal refinement data for polymorph α, space group, Hall symbol, lattice parameters *a*, *b* and *c* (Å), ß angle (θ), volume, number of formulae unit per unit cell, X-ray density, wavelength, experimental angular range (θ), crystal absorption coefficient, crystal shape and dimensions, number of reflections considered for cell parameters calculation, and independent reflections used for single crystal fitting.

Crystal data	Values
$C_{13}H_{10}N_2O_4$	Thalidomide
Space group	Monoclinic *P*21/*n*
Hall symbol	-P 2yn
a ± σ (Å)	8.2440 ± 0.0007
b ± σ (Å)	10.0899 ± 0.0009
c ± σ (Å)	14.8991 ± 0.0001
β ± σ (degrees)	102.636 ± 0.008
Volume ($Å^3$)	1209.31 ± 0.02
Z	4
X-ray density (Dx)	1.418 mg/m^3
Wavelength (Mo/Kα)	0.71073 Å
Experimental angular range (θ)	3.2–26.8°
Crystal absorption coefficient (μ)	0.11 mm^{-1}
Crystal shape and dimensions	Prism, 0.24 mm × 0.24 mm × 0.80 mm
Number of reflections considered for cell parameters calculation	1449
Independent reflections used for single crystal fitting	2884

2.5. Ultraviolet spectroscopy

Ultraviolet spectroscopy was undertaken at 200–400 nm for thalidomide at 10 μg/mL in ethanol on a spectrophotometer (1800; Shimadzu). Origin v9.1 was used to adjust data.

2.6. Raman spectroscopy

Raman spectroscopy of solid thalidomide was done on a confocal micro-Raman spectrometer (Senterra; Bruker, Billerica, MA, USA) with an excitation laser set at 785 nm. The measurement conditions were as follows: integration time of 5 s; spectral resolution of 3–5 cm^{-1}; and spectral range of 2000–100 cm^{-1}. The laser was focused with a 4× dry objective lens, with the laser power set to 25 mW. Origin v9.1 was used to adjust data.

2.7. Gamma irradiation

Experiments involving gamma irradiation were done at Comissão Nacional de Energia Nuclear-Centro de Desenvolvimento da Tecnologia Nuclear (Belo Horizonte, MG, Brasil). The radiation system (IR-214; MDS Nordion, Ottawa, Canada) was equipped with a dry cobalt-60 source. The source had a maximum activity of 2200 TBq (60,000 Ci). The specific irradiation times were calculated, and then all samples were exposed to doses of 2, 5, 10, 15, 25, 30 or 100 kGy.

2.8. Attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR)

FTIR analysis was performed at room temperature on a Spectrum 1000 spectrophotometer (PerkinElmer, United States) equipped with an attenuated total reflectance (ATR) accessory. The sample was pressed into a zinc selenide crystal, and 32 scans were averaged. For single FTIR without ATR, the samples were measured in KBr pressed pellets in the wavenumber range between 400 and 3400 cm^{-1} at room temperature, with a resolution of 4 cm^{-1}.

2.9. Statistical analyses

Data are the mean ± standard deviation. All fitting procedures took into account three independent measurements with statistical analyses conducted using Origin v9.1.

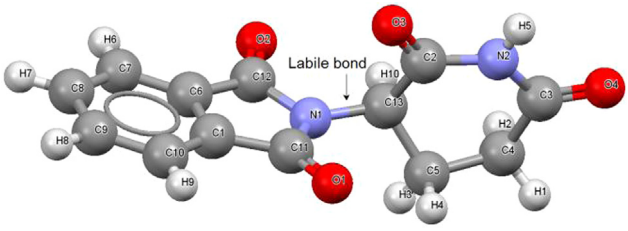

Fig. 1. Thalidomide molecule showing the labile bond between phthalimide and glutarimide rings.