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Nanosized bicalutamide and its molecular structure in solvents

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ABSTRACT

Nanosized bicalutamide particles have been obtained by anti-solvent precipitation after screened DMSO and EtOH as co-solvents. The produced nanoparticles have been characterized by scanning electron microscopy (SEM), Fourier transform infrared spectrophotometry (FTIR), X-ray diffraction (XRD) and a dissolution test. The mean particle size of bicalutamide is about 450 nm with a narrow distribution. The results of the dissolution test show that dissolution rate of the produced nanoparticles are higher than that of the raw material. Besides, DFT calculations of the bicalutamide conformers have firstly been presented. It is found that the calculated geometry structure of lower-energy conformer is very similar to the experimental structure existing within the crystal lattice. The solvent effects have been taken into account based on the polarizable continuum model (PCM). The computed results appear that the introduction of dielectric medium has obvious effect on the molecular geometry of bicalutamide.

1. Introduction

Bicalutamide (molecular structure as shown in Fig. 1) is an antineoplastic hormonal agent primarily used in the treatment of prostate cancer. It is a pure, non-steroidal anti-androgen with affinity for androgen receptors. Bicalutamide, approved by the FDA in 1995, is thought to prevent the growth of prostate cancer by blocking the action of androgens on the cancer cells. Bicalutamide belongs to class II (low solubility, high permeability) of the biopharmaceutics classification systems (BCS) (Kanfer, 2000). The low solubility/dissolution rate of bicalutamide is still a key factor limiting its oral bioavailability. If its dissolution rate can be enhanced, bioavailability following oral administration of bicalutamide could be significantly improved. According to the Noyes–Whitney equation, the administration of a drug in a reduced particle size is a promising way to improve drug bioavailability of poorly soluble substances (Noyes and Whitney, 1987).

Many techniques have been utilized to reduce the particle size, such as mechanical milling (Rasenack et al., 2003; Horn and Rieger, 2001), supercritical fluids process (Steckel et al., 1997; Domingo et al., 1997; Chattopadhyay and Gupta, 2001), spray drying (Vehring, 2008), microemulsion (Trotta et al., 2003; Debuigne et al., 2001) and liquid precipitation (Zhong et al., 2005). Mechanica-milling, is a common way for micronizing the particles. However, these techniques show several disadvantages. The process is extremely inefficient due to the high energy input, and provides poor control of particle size, morphology and surface properties. Moreover, the unavoidable milling element abrasion contaminates the product and is difficult to separate. Supercritical fluids process, microemulsion technique and spray drying, attractive methods for the size reduction, can provide particles with narrow size distribution. However, these techniques have the limitation of low yields and high machine expenditure.

In contrast with techniques hereinabove, liquid precipitation technique has good prospect because of its lowcost, convenience in processing and need of common equipment. Anti-solvent precipitation is a physical method to prepare ultrafine/nanosized particles, which is based on the change of the supersaturation caused by mixing solution and anti-solvent. This technique presents numerous advantages, in which it is a straightforward method, rapid and easy to perform, and often enables the production of small nanoparticles with narrow unimodal distribution. This technique has been used to prepare nanosized hydrophobic drugs such as cefuroxime axetil (Zhang and Shen, 2006) and beclomethasone dipropionate (Wang et al., 2007).

From the structural studies of bicalutamide, it presents a flexible molecule during crystallization, namely conformational polymorphism. Pioneering work from Vega et al. (2007) laid the foundation of the acetechture of conformational flexible in bicalutamide molecule in terms of two crystalline polymorphs. These two crystalline forms have the same chemical composition but different internal crystal structures due to different molecular conformation and, therefore, possess different physico-chemical properties (Vippagunta and Brittain, 2001). The polymorphism in drugs has

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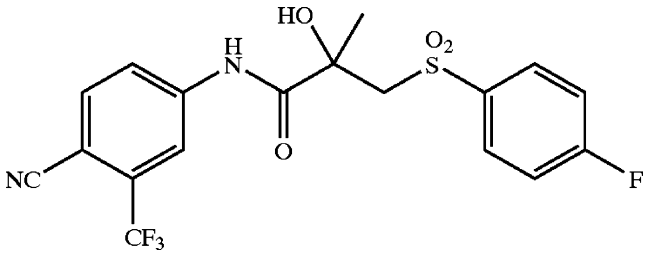

Fig. 1. Structure of bicalutamide molecule.

become very important in order to control the stability, solubility, bioavailability and to explore different behavior and activity of drugs.

Various processes during drug nanoparticles preparation significantly influence the final crystalline form of the drug. Despite the rigorous conditions to which these particles are subjected during size reduction, the resulting powders are generally confronting polymorphic conversion. The degree of polymorphic conversion depends on the relative stability of the phases in question, and on the type and degree of grinding processing applied. Especially in anti-solvent precipitation process, the properties of drug molecules are significantly influenced by solvent molecules. Therefore, it is necessary to investigate the behavior of drug molecules in different solvents.

Solvent effects on the thermodynamics and kinetics of chemical and biological phenomena attract continuously increasing interest. Recent advance in quantum calculation techniques are making ab initio calculations possible on larger size systems, thus ab initio procedures could provide reliable treatments of solute–solvent interactions, which makes it possible to describe chemical processes in solutions using theoretical methods (Wong et al., 1992; Foresman et al., 1996; Barone and Cossi, 1998).

In this work, the first objective was to prepare nanosized bicalutamide particles under controlled anti-solvent precipitation to enhance the dissolution rate. Additionally, the effects of the operation parameters on bicalutamide nanoparticles formation were also discussed by utilizing multiple analytical techniques. Secondly, we reported the application of DFT methods to explore the conformational structure of bicalutamide and investigated the solvent effects on molecular geometry of bicalutamide using polarizable continuum model (PCM) method (Cossi et al., 1996, 1998; Aguilar et al., 1993).

2. Experiments

2.1. Materials

The bulk bicalutamide was purchased from Beijing Guolian Chenhui Pharmaceutical Technology Co., Ltd. Because the bicalutamide molecule contains one asymmetric carbon atom, which allows for the existence of both single enantiomers and a racemate, the commercial crude bicalutamide used in this work is racemic or a mixture of enantiomers.

2.2. Method

In the typical experiment, about 0.60 g raw bicalutamide was dissolved in 10 ml of a mixture of ethanol (EtOH) and dimethylsulfoxide (DMSO) at the concentration of 6% (w/v). The drug solution was poured rapidly into the antisolvent, i.e., 200 ml deionized water by means of stirring. The system became turbid immediately, indicating the onset of precipitation. After precipitation, the bicalutamide suspension was filtered and washed with distilled water in order to gradually remove the residual solvent. Following the aforementioned procedure, the solid separated was vacuum dried at 60 °C, and then the nanosized drug powder was obtained.

2.3. Characterization

The nanoparticle surface appearance and shape were analyzed by SEM (JSM-6360LV, JEOL, Japan). Samples were prepared by finely spreading concentrated nanoparticles over slabs and then coated with a fine gold layer in an argon atmosphere and observed by scanning electron microscope.

The volume particle size was determined using a laser diffractometer (Zetasizer, Malvern Instruments Ltd., UK). Each blank nanoparticles batch was appropriately diluted with the water in the presence of dispersant (Lutrol F127, 0.1%, w/w). Mean size was measured three times for each batch.

FT-IR spectra were recorded with a Bruker IFS66 spectrometer in the range 500–4000 cm^{-1} using a resolution of 2 cm^{-1} and 32 scans. Samples were diluted with KBr mixing powder at 1% and pressed to obtain self-supporting disks.

X-ray diffraction analysis was performed using an X-ray diffractometer (XRD-6000, Shimadzu Inc., Japan) to detect any changes in the physical characteristics and crystallinity of the raw and formed bicalutamide. The measuring unit consists of a rotating anode in transmission technique and with the following specifications: Cu Kα1 radiation generated at 30 mA and 40 kV. The scanning speed is 5°/min from 5° to 40° with a step size of 0.02°.

Dissolution studies were performed assuring sink-conditions according to the paddle method (USP) using a dissolution apparatus (D-800LS, Tianjin, China). The stirring speed used was 50 rpm, and the temperature was maintained at 37.0 ± 0.5 °C. The dissolution medium (1000 ml) was an aqueous medium containing the surfactant sodium lauryl sulphate (SLS) at 1% (w/w), which was deaerated in a 10 l suction flask under vacuum for hours. Surface tension was lowered by SLS in an attempt to mimic in vivo conditions. Quantification of the dissolved amount of drug was conducted by a spectrophotometer at 270 nm (UV-3000, Shimadzu, Japan).

3. Computational methods

All quantum calculations were carried out using the B3LYP flavor of density functional theory (DFT). Geometry optimizations were performed at the B3LYP/6-311+G** levels both in gas phase and in solutions. Consequently, polarizable continuum model (PCM) is used for evaluating the bulk solvent effects, in which one divides the problem into a solute part (bicalutamide) lying inside a cavity and a solvent part (in our case, EtOH and DMSO) represented as a structureless material, characterized by its dielectric constant as well as other parameters (Preat et al., 2006). For gas-phase geometry vibrational frequencies were calculated analytically to ensure it to be a true local minimun (no imaginary frequencies). The effect of solute–solvent interaction was taken into account by PCM method.

All calculations were performed using Gaussian 03 package on the Intel Pentium IV PC.

4. Results and discussion

In fact, screening suitable solvent (S) and anti-solvent (AS) is a key process for getting nanosized drug particles. In this work, water was served as anti-solvent, DMSO and EtOH were compared as initial solvent to obtain nanoparticles without addition of any other surfactants. DMSO is fairly non-selective in its solubilization properties, dissolving a wide range of polar and nonpolar organic compounds. Ethanol is also a good concern from both low toxicity

and easily removing point of view. Additionally, according to the ICH solvent toxicity, both of them belong to class III which presents very low risks to human health.

However, at a temperature of 25 °C, the solubility of bicalutamide raw material (form I) in DMSO is 0.9297 g/ml, whereas the corresponding value in EtOH is 0.0077 g/ml. Also, the powder obtained from EtOH presented uniform but large particles, while that of DMSO could enhance the recovery rate due to its relatively high solubility. Given the integrated properties of them, the co-solvents system was taken into consideration, which literally was mixture of EtOH and DMSO.

The concentration of the drug depends on its solubility properties. During the precipitation process, operation from highly supersaturated conditions leads to the formation of many small particles rather than a few large ones. Nevertheless, the viscosity for drug solution with the increasing of concentration should be concerned carefully, due to its hindering of diffusion between S and AS and non-uniform supersaturation. To avoid the side effect, we adjusted the volume ratio of DMSO and EtOH so as to balance the solvent solvency and solution viscosity. The particle size changed along with volume ration in co-solvent system (see Fig. 2). When the volume ratio between EtOH and DMSO changed from 1:1 to 5:1, the average particle sized decreased with the increase of EtOH, from 6.5 μm to 450 nm, however, a special conversion appeared when EtOH increased from 5:1 to 8:1, the particle size increased from 450 nm to 1 μm. Hence, it could be assume that the ratio of 5:1 should be suitable.

The SEM images and the particle size distribution of the commercial crude and nanosized bicalutamide prepared from anti-solvent precipitation are shown in Fig. 3. It can be seen that the mean particle size of prepared bicalutamide was about 450 nm with narrow particle size distribution (PSD), while the crude drug performed about 150 μm with wide PSD. Evidently, the particle sized of prepared bicalutamide is significantly smaller and more uniform than that of crude drug, which should be more beneficial for enhancing bioavailability.

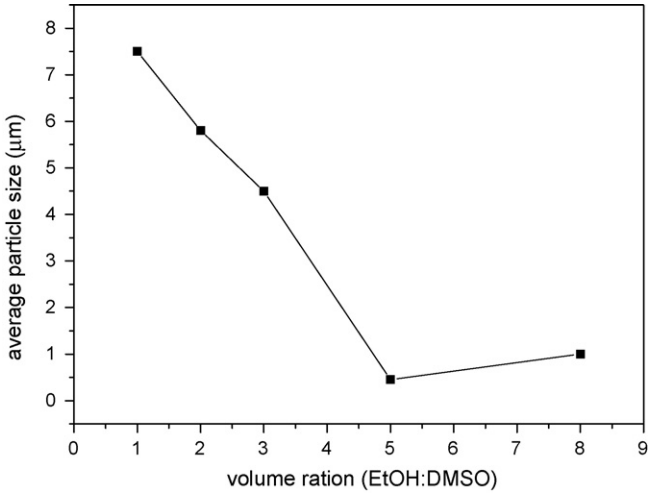

Fig. 2. Particle size changed with co-solvent volume ratio.

The crystalline and the molecular structure of the crude and nanosized bicalutamide were studied by means of XRD and FT-IR. The resultant profiles are shown in Figs. 4 and 5. XRD patterns and IR spectra between crude and nanosized bicalutamide are identical, which suggested that there are no crystallinity and chemical structure changes in bicalutamide molecule during the anti-solvent precipitation process.

Aqueous dissolution rates have been correlated to the in vivo performance of drug delivery systems. The in vitro release profiles of commercial crude bicalutamide and nanosized bicalutamide were compared in Fig. 6. The dissolution rate of the nanosized bica-

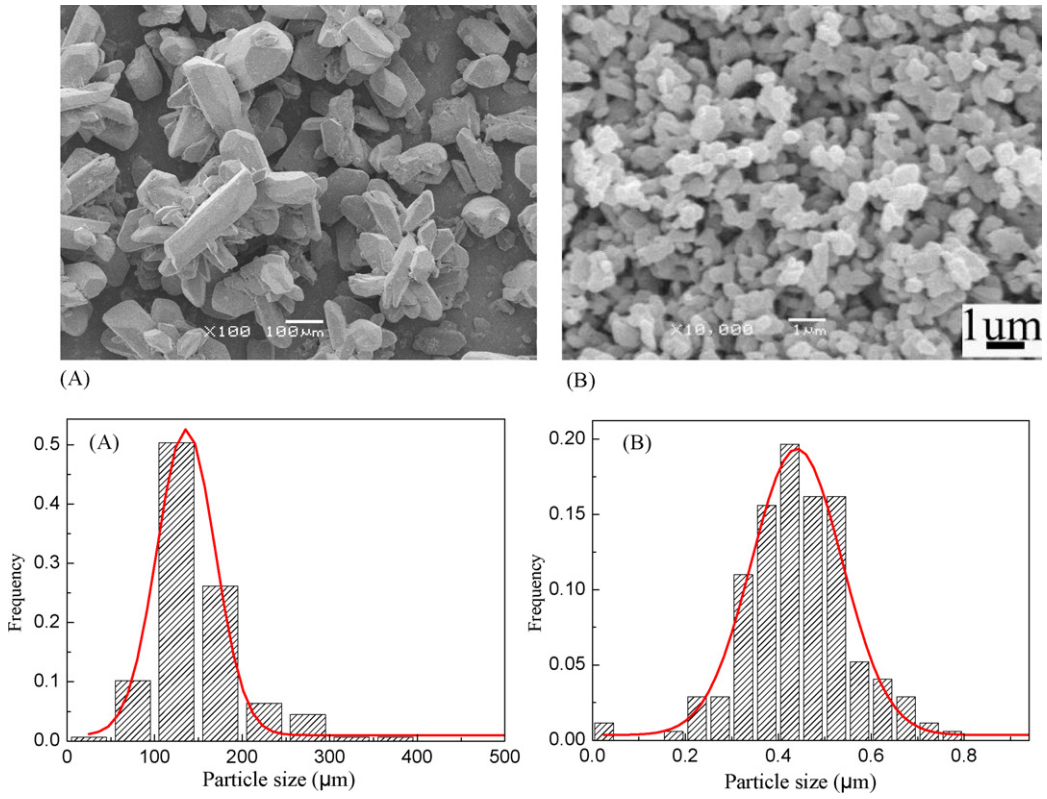

Fig. 3. SEM images and particle size distributions of bicalutamide (A) commercial crude bicalutamide and (B) nanosized bicalutamide.

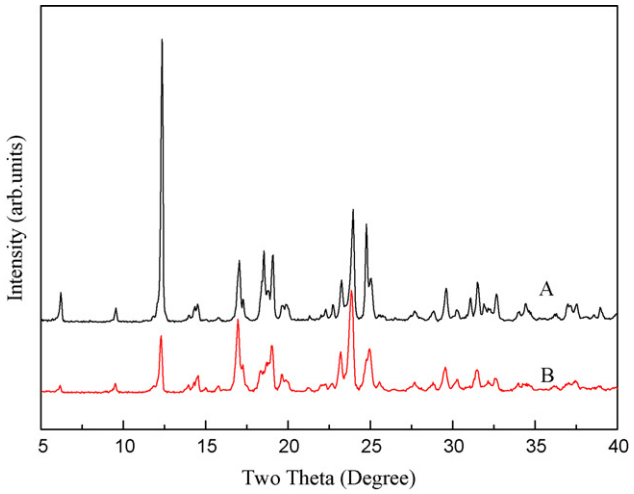

Fig. 4. XRD patterns of bicalutamide (A) commercial crude bicalutamide and (B) nanosized bicalutamide.

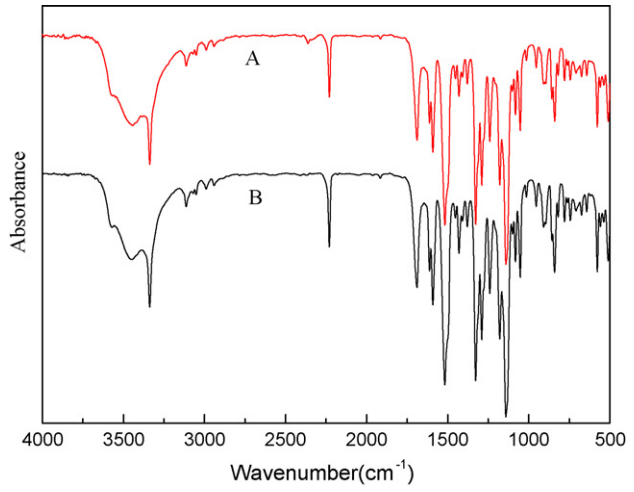

Fig. 5. FT-IR spectra of bicalutamide (A) commercial crude bicalutamide and (B) nanosized bicalutamide.

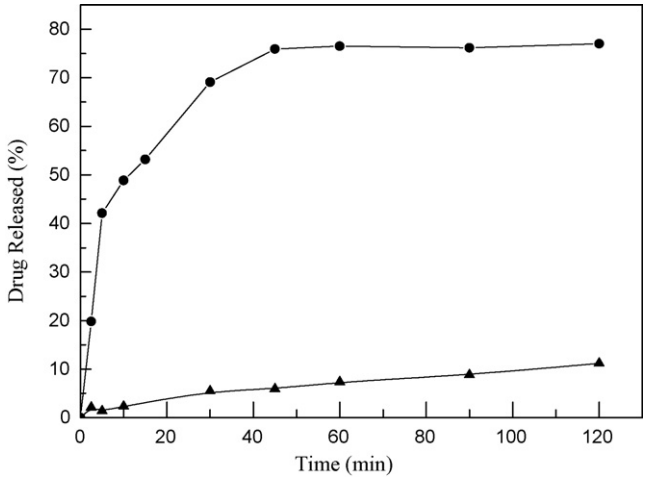

Fig. 6. Dissolution profiles of (▲) commercial crude bicalutamide (●) nanosized bicalutamide.

lutamide was increased to 70% after 30 min, while only 7% crude material dissolved at that time. After 120 min, nearly 80% of the nanosized bicalutamide were dissolved, but the crude material was dissolved only about 10% during the same period. The dramatic increase in drug dissolution rate can be explained by reduced particle size, and enhanced specific surface area, which may further lead to higher drug potential in the gastrointestinal tract and finally result in an improvement in oral bioavailability.

It has been reported there are two crystalline forms (forms I and II) of bicalutamide, form I is more stable than form II, the main difference is provided by C_2–S_{11}–C_{14}–C_{15} torsion angle which assumes a value of −88.3° and 72.5° in forms I and II, respectively (Vega et al., 2007). Form I is already applied for medical use, while form II is being explored for making medicament (Jenkins and Liversidge, 2006). Both commercial raw materials and our prepared nanoparticles are form I.

In order to explore the conformations and possibility of intermolecular interactions, the molecular structure is studied by quantum mechanical calculation. The geometry of bicalutamide molecule was fully optimized using DFT at B3LYP/6-311+G** level starting form a random structure with no symmetry constraint. The optimized geometry and atom numbering of conformer A is shown in Fig. 7 as well as the key geometrical features of the optimized molecule named conformer A are described in Table 1 along with the experimental data from the crystal structure. Compared the results, it is observed that the conformer A is similar to the crystalline form I, the calculated bond length and bond angles are very close to the crystallographic data, the average difference between the experimental and calculated geometries are

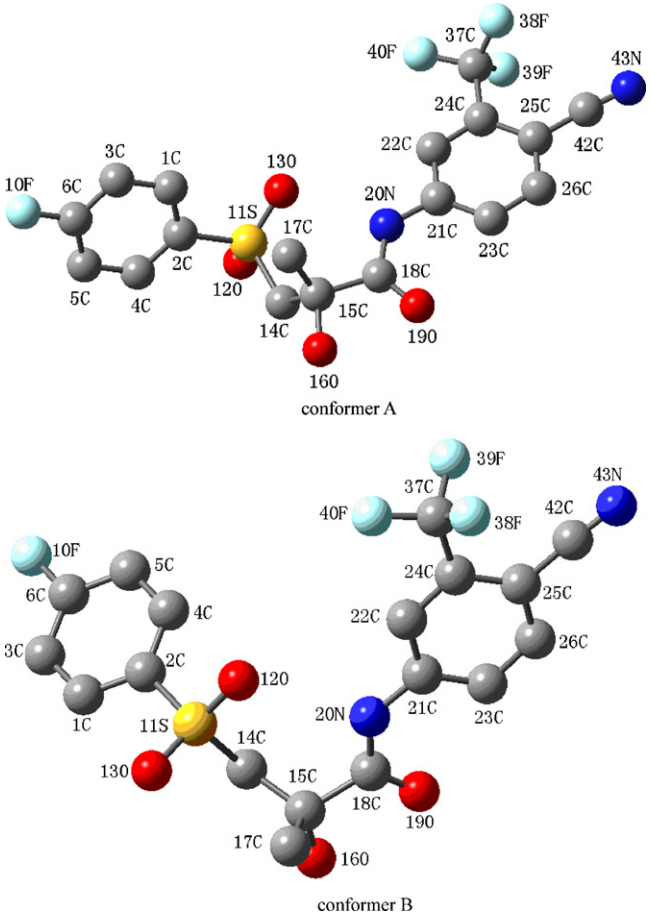

Fig. 7. Optimized structure of bicalutamide in gas phase determined at the B3LYP/6-311+G** level.

Table 1
Comparison between calculated and experimental geometries of bicalutamide.

	Conformer A gas (ε_r = 1.0)	B3LYP6-311+G**		DMSO (ε_r = 46.7)	XRD	
		Conformer B gas (ε_r = 1.0)	EtOH (ε_r = 24.55)		Form I	Form II
C_2–S_{11}	1.798	1.796	1.795	1.792	1.761	1.753
C_{14}–C_{15}	1.554	1.558	1.555	1.551	1.494	1.498
S_{11}–C_{14}	1.838	1.832	1.837	1.833	1.793	1.800
C_{15}–O_{16}	1.416	1.416	1.419	1.418	1.442	1.454
C_{15}–C_{17}	1.535	1.537	1.535	1.535	1.513	1.509
C_{15}–C_{18}	1.563	1.563	1.563	1.561	1.548	1.545
C_{18}–O_{19}	1.223	1.223	1.225	1.225	1.212	1.216
C_{18}–N_{20}	1.358	1.360	1.358	1.359	1.350	1.351
N_{20}–C_{21}	1.406	1.405	1.403	1.402	1.413	1.406
S_{11}–O_{12}	1.464	1.464	1.470	1.472	1.433	1.427
S_{11}–O_{13}	1.487	1.483	1.485	1.487	1.446	1.423
S_{11}–C_{14}–C_{15}	122.1	118.5	121.7	121.5	116.4	121.0
O_{16}–C_{15}–C_{17}	108.7	109.2	108.7	108.6	110.2	108.4
C_{14}–C_{15}–C_{18}	112.6	113.4	112.7	112.5	110.4	111.8
O_{19}–C_{18}–N_{20}	125.0	124.8	124.9	124.9	125.1	124.6
C_{15}–C_{18}–N_{20}	116.8	117.0	116.8	116.5	114.9	115.2
C_{18}–N_{20}–C_{21}	128.1	128.1	128.6	128.4	126.8	127.8
O_{12}–S_{11}–O_{13}	120.0	119.7	118.5	118.1	118.3	119.6
C_2–S_{11}–C_{14}	106.9	103.5	107.9	107.2	108.3	106.1
C_2–S_{11}–C_{14}–C_{15}	−87.5	169.8	−84.0	−82.7	−88.3	72.5
C_{15}–C_{18}–N_{20}–C_{21}	−178.7	177.6	−179.0	−178.2	−175.1	178.2
S_{11}–C_{14}–C_{15}–C_{18}	−83.1	−89.2	−84.6	−85.0	−64.2	−65.6

Table 2
Calculated energies and dipole moments.

	Conformer A	Conformer B	EtOH	DMSO
E_{total}/Hartree	−1902.7132	−1902.7110	−1902.7425	−1902.7435
ΔE (kJ mol^{-1})		5.776	−76.927	−79.553
μ (D)	9.352	7.708	11.928	12.034

0.028 Å and 1.8°, respectively. Concerning the more important dihedral angles, in general the calculated values are similar to the experimental ones. For example, crystallographic study reveals that C_2–S_{11}–C_{14}–C_{15} torsion angle, which mainly leads to polymorphism in bicalutamide, is −88.3°, the calculated value of this torsion angle is −87.5°, which matches the experimental one very well. In order to investigate the conformers of bicalutamide, the molecular structure starting from the previous crystal structure form II was also optimized, and the results are listed in Fig. 7 and Table 1. In general, good agreement between calculated and experimental values of crystal form II can be found with small differences in bond lengths and bond angles. However, value of C_2–S_{11}–C_{14}–C_{15} torsion angle calculated to 169.8°, the experimentally observed is 72.5°. These discrepancies can be explained by the molecular physical state. DFT calculations base on the molecule completely isolated for the energy minimization while the experimental geometry is in its crystalline state where the intermolecular interactions are included.

A noteworthy point is that, in the gas phase, conformer A is more stable than conformer B by 5.776 kJ/mol lower in energy, and possesses a larger dipole moment, which illustrate the calculated results are consistent with the experimental (Vega et al., 2007).

Because our prepared experiment occurs in solutions, it was considered important to gain some information on geometry structure in a solvated environment. Thus, for lower-energy conformer A, the gas phase equilibrium structure was completely re-optimized using the PCM at DFT-B3LYP/6-311+G** level in ethanol and DMSO solutions. As shown in Table 1, solvation compacted the molecule, compared with gas phase structure, the geometry parameters in the dielectric continuum corresponding to the DMSO solution (ε_r = 46.7) leads to small changes by 0.06 Å in bond lengths and 1.9° in bond angles as well as notable difference by 4.8° in torsion angle, which means the introduction of a solvent reaction field has obvious effect on the geometry of bicalutamide.

The calculated total molecular energies (E_T), solvent energies (ΔE) and ground-state dipole moments of bicalutamide in vacuo and in solutions by B3LYP/6-311+G** are listed in Table 2. With the increase of solvent dielectric constant, the total molecular energies decrease while solvent energies and dipole moments enhance. Solvent effects improve the charge delocalized in the molecules, therefore, induce the dipole moments raised, furthermore, solvent energies show correlation with the dielectric constant or dipole moment. Ground-state dipole moment is an important factor in measuring solvent effect, a large ground-state dipole moment give rise to a strong solvent polarity effects (Masternak et al., 2005). Meanwhile, the ground state dipole moment caused the reorientation of solvent molecules to produce a large reaction field, in turn results in a shift between the energies of the ground and excited state, which induce the shift of UV absorption wavelength. From Table 2, we can see the dipole moments of bicalutamide varies from 9.352 to 12.034 D and the solvent energy decreases 79.553 kJ/mol in going from the ε_r = 1 to ε_r = 46.7, which also demonstrate that the solvent effects on bicalutamide are notable.

5. Conclusion

Nanosized bicalutamide particles have been prepared by liquid anti-solvent precipitation method using EtOH and DMSO as co-solvents without any surfactants. Optimized volume ratio of EtOH:DMSO is at 5:1. Determined by multiple analytical techniques, the produced bicalutamide powder achieved the mean particle size of 450 nm with the same chemical structure as the raw material. The dissolution of nanosized bicalutamide is significantly enhanced compared with raw material. In a word, the anti-solvent precipitation process offers a direct and rapid pathway to obtain pharmaceutical nanoparticles.

DFT calculations of bicalutamide molecular structure and conformers have been reported. The results from these calculations

show the structure of lower-energy conformer has been in a good agreement with crystallographic data. It is suggested that DFT should be very useful tool for investigation on the conformational polymorphs. The solvent effects on bicalutamide molecule have also been explored with PCM method. The results suggest that the effects of polar solvents on the molecular structure of bicalutamide are remarkable.

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