Combining Nuclear Magnetic Resonance Spectroscopy and Density Functional Theory Calculations to Characterize Carvedilol Polymorphs

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ABSTRACT: The experiments of carvedilol form II, form III and hydrate by ^{13}C and ^{15}N cross-polarization magic-angle spinning (CP MAS) are reported. The GIPAW (gauge-including projector-augmented wave) method from DFT (density functional theory) calculations was used to simulate ^{13}C and ^{15}N chemical shifts. A very good agreement was found for the comparison between the global results of experimental and calculated nuclear magnetic resonance (NMR) chemical shifts for carvedilol polymorphs. This work aims a comprehensive understanding of carvedilol crystalline forms employing solution and solid-state NMR as well as DFT calculations. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

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INTRODUCTION

Drugs can exist in different crystal structures, a phenomenon known as polymorphism. Polymorphism can alter drug's physicochemical properties as solubility, stability, density, and melting point, which are essential to ensure effectiveness, safety, and quality of a product. Thus, the knowledge of the solid-state properties is very important in the pharmaceutical field.[1–3]

Carvedilol(RS)-1-(carbazol-4-yloxy)-3-[[2-(omethoxyphenoxy) ethyl]amino]-2-propanol (CAR) is a nonselective beta blocker that is used against several heart diseases as hypertension and systolic dysfunction after myocardial infarction.[4,5] It is administrated as a racemic compound, although the S-enantiomer is responsible for the beta-blocker activity.[6] It is the only beta blocker agent with the carbazole moiety in its structure (Fig. 1). CAR is practically insoluble in water and its solubility is pH dependent, which limits not only its bioavailability, but also a pharmaceutical formulation in the desired manner.[7,8] Concerning carvedilol polymorphism, three anhydrous forms were described in the literature: forms I,[9] II,[10] and III,[11] and one hydrate form.[12] CAR II is the unique that was characterized by ^{13}C solid-state nuclear magnetic resonance (SSNMR) spectroscopy.[13]

The identification and structural characterization of polymorphs can be performed using a combination of infrared/Raman spectroscopy, thermal methods, and X-ray diffraction techniques.[14] SSNMR, in particular cross-polarization (CP), at magic angle spinning (CP MAS) is also a powerful technique to study and characterize amorphous and crystalline pharmaceutical solids.[15–17] It is a valuable source of structural information, mainly because of the sensitivity chemical shifts to the structure of molecular crystals.[14,18] This is especially important for solid-state forms that can not be crystallized and studied by single-crystal X-ray techniques. In the case of organic molecules that present nitrogen in its structure, CP MAS uses the magnetization of a highly sensitive nucleus that is transferred by CP to the $^{13}C/^{15}N$ nuclei, allowing acquisition of $^{13}C/^{15}N$ NMR spectra usually in a couple of hours. The shape of the CP MAS NMR spectrum (number, position, and intensity of the lines) of a solid depends on the chemical environment of each carbon or nitrogen atom in the sample and therefore represents the fingerprint of this compound, which can, in principle, be used to discriminate among polymorphs and solvates, ionic salt complexes, or cocrystals.[19–21]

It has been shown in many research areas that much more information on structural and physicochemical characteristics of materials can be obtained if the experimental data are complemented by the prediction of properties obtained through calculations, which have been used to aid in data interpretation NMR for more than a decade.[22] Recently, a theory for calculations of NMR parameters in periodic systems was presented and this theory uses the GIPAW (gauge-including projector-augmented wave) method for the chemical shielding tensors in crystalline solids. It was developed for calculation of density functional theory approximations made to electrons from heavy nuclei (in the form of pseudopotential atoms).[23,24]

In this work, we present a powerful combination between experimental and theoretical techniques to understand solution and solid NMR spectra and to solve ambiguous NMR chemical

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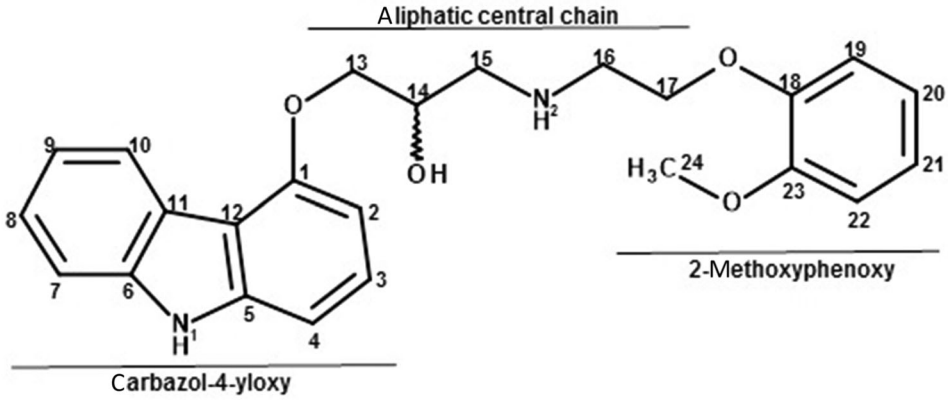

Figure 1. Molecular structure of carvedilol.

shifts assignments of ^{13}C and ^{15}N nuclei not described before, for the carvedilol form II, form III and hydrate, which can serve as a basis for further studies of structure, molecular mobility, and interactions mapping. Moreover, this is the first time that carvedilol form III and hydrate are characterized by solid-state NMR.

EXPERIMENTAL

Sample Preparation

A commercial sample of carvedilol form II (98% of purity; SIGMA) was used without further purification. Crystallizations of carvedilol form II, form III and hydrate were performed under different conditions using methanol and ethyl acetate as solvents, respectively.[11]

Powder X-Ray Diffraction

Powder X-ray diffraction (PXRD) was obtained on a D8 diffractometer Bruker AXS using Cu Kα radiation ($\lambda = 1.5418$ Å) with a graphite monochromator. The patterns were recorded at a tube voltage of 40 Kv and a tube current of 40 mA, between 5° and 40° in 2θ, with a step size of 0.02° and a scan rate of 3 s. The program MERCURY (Version 2.4) was used for the calculation of the theoretical powder patterns from single-crystal data.

Differential Scanning Calorimetry and Thermogravimetric Analysis

Calorimetric measurements and TG analysis were performed using a NETZSCH model STA 449 F3 equipment. For all experiments, the samples were weighed (~5 mg) in a hermetic aluminum pan, nitrogen flow rate of 50 mL/min, heating rate of 10°C/min, and temperature range from 25°C to 200°C.

Solution-State NMR

Deuterated solvent (DMSO-d_6) was purchased from Cambridge Isotope Laboratories, Inc. The purity of the samples was confirmed through ^1H and ^{13}C solution NMR. The samples (30 mg) were dissolved in DMSO-d_6. Samples were referenced to DMSO-d_6 (^1H at 2.50 ppm vs. TMS and ^{13}C at 39.51 ppm vs. TMS). ^1H and ^{13}C solution-state NMR spectra of carvedilol form II, form III and hydrate were collected on a 7.05T Bruker DRX300 spectrometer operating at 300.17 MHz (^1H) and 75.48 MHz (^{13}C). The ^1H spectra were recorded with 32 scans, 2 s recycle delay, 2 s acquisition time, 0.281 Hz digital FID resolution, 32K time domain with 6010 Hz spectral width. The ^{13}C spectra were recorded with Waltz 16 ^1H broadband decoupling, 1024 scans, 2 s relaxation delay, 1 s acquisition time, 0.755 Hz digital FID resolution, 4K time domain, and 18,797 Hz spectral width. All two-dimensional experiments were performed on a 14.1 T Bruker DRX600 spectrometer operating at 600.13 MHz (^1H) and 150.90 MHz (^{13}C) using the pulse sequences from the Bruker Software Library. TOCSY and COSY spectra were recorded at spectral width of 6 KHz in both F2 and F1 domain; 2K × 256 data points were acquired with eight scans per increment and the recycle delay of 2 s. Data processing was performed on a 2K × 1K data matrix. ^1H–^{13}C HSQC and ^1H–^{13}C HMQC spectra were measured over 2K complex points in F2 and 128 increments in F1, collecting eight scans per increment with a relaxation delay of 1.5 s. The spectral widths were 8 and 24 kHz in F2 and F1 dimensions, respectively. Data processing was performed on a 2K × 1K data matrix. Assignment was carried out using the interactive program SPARKY (v3.106, T. D. Goddard and D. G. Kneller, University of California, San Francisco, California).

Solid-State NMR

^{13}C and ^{15}N CP MAS NMR spectra of Carvedilol form II, form III and hydrate were collected on a 9.4 T WB Bruker Avance III 400 spectrometer operating at Larmor frequencies of 100.3 MHz (for ^{13}C) and 40.6 MHz (for ^{15}N); 3.2 mm triple-resonance MAS probe was employed. Samples were spun at 10 KHz in ZrO_2 rotors and recorded at room temperature. High-resolution spectra were obtained using CP MAS method. Samples were referenced to glycine (C=O at 176.03 ppm vs. TMS and NH_3 at −347.54 ppm vs. CH_3NO_2). Acquisitions were performed using CP.ramp.100 pulse sequence with 4.5 μs proton 90° pulse and recycle delay of 5 s. All spectra were recorded by using 1 ms (^{13}C) and 4 ms (^{15}N) contact times and 512 scans. Data were processed using the software Topspin (v2.0; Bruker BioSpin GmbH, Germany).

Density Functional Theory Calculations

All the *ab initio* calculations were performed using the codes available in the Quantum-Espresso package,[25] which employs DFT[26,27] theory, periodic boundary conditions, and plane wave basis sets. The Kohn–Sham orbitals were expanded in a plane wave basis set with a maximum kinetic energy cutoff of 60 and

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