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Solid-state carbon NMR characterization of the polymorphs of roxifiban

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Abstract

Roxifiban, an experimental antithrombotic prodrug, exists as crystalline forms I and II. A quantitative solid-state nuclear magnetic resonance (NMR) method was developed to characterize the two polymorphs of roxifiban. The differences in the NMR spectra of the polymorphs were utilized in analyses of physical blends of the pure crystalline forms to establish a calibration curve. A detection limit of 9% form II in form I was determined from analysis of a 10% form II blend. Solid-state NMR was a valuable technique to quantify the polymorphic purity of roxifiban where other techniques such as differential scanning calorimetry (DSC) could not be used for this purpose.

Keywords: Solid-state nuclear magnetic resonance; ^{13}C CP/MAS NMR; Polymorph; Roxifiban

1. Introduction

The objective of these solid-state carbon NMR studies was to characterize the two polymorphs of roxifiban, which is a cardiovascular prodrug which was being developed for potential use in antithrombotic therapy. Roxifiban {(*R*)-methyl 3-[[[3-[4-(aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine monoacetate} is the prodrug of a potent glycoprotein IIb/IIIa receptor antagonist. Irrespective of the stimulus of platelet aggregation, fibrinogen binding to the glycoprotein IIb/IIIa receptor is the common convergent pathway prior to aggregation. By acting as an antagonist to the glycoprotein IIb/IIIa receptor, the free acid resulting from the hydrolysis of roxifiban blocks the binding of fibrinogen to the receptor thereby inhibiting platelet aggregation and providing a mechanism for antithrombotic therapy.

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Preformulation studies routinely include the important process of characterizing polymorphs or the confirmation of the absence of polymorphs of a drug substance. Polymorphs have the same chemical structure but different single crystal structures and are of critical concern in pharmaceutical development. Thorough polymorph characterization studies are required to identify the role the crystalline form may have in solid-state chemical and physical stability, solubility, dissolution rate, and bioavailability [1]. Techniques typically employed to identify and characterize polymorphs are X-ray powder diffraction (XRPD), melting point, differential scanning calorimetry (DSC), and solubility versus temperature experiments used to generate a Van't Hoff plot. Roxifiban exists as two different crystalline forms which were indistinguishable by DSC, demonstrated similar aqueous solubility values at room temperature, and differed by only two characteristic peaks by XRPD as previously reported [2]. Synchrotron X-ray powder diffraction techniques determined that the two extra peaks found in the low-angle region of form II are the consequence of the fact that the *b*-axis, and hence the volume of the single crystal unit cell, is approximately double that of form I [3]. Solid-state carbon NMR has also been used extensively to qualitatively characterize a variety of drug substances [4–6] and it has been shown that by utilizing observed peak splitting, quantitative polymorphic composition measurements are achievable [7]. Using conventional ^{13}C cross-polarization (CP)/magic angle spinning (MAS) NMR [8], a quantitative solid-state carbon NMR method was developed to determine the polymorphic purity of roxifiban crystalline forms I and II.

2. Experimental

2.1. Instrumentation

Solid-state ^{13}C nuclear magnetic resonance (NMR) spectra were acquired on a Varian Unity 200 spectrometer operating at 50 MHz for ^{13}C and 200 MHz for ^{1}H using the CP/MAS technique. Approximately 200 mg of each polymorph was used in the acquisition of their respective spectra. All measurements were made at ambient temperature. Chemical shifts are reported on the TMS scale using hexamethylbenzene as a secondary reference. Solid-state resonance assignments were made using the interrupted decoupling pulse sequence in combination with solution-state ^{13}C experiments performed on a Varian Unity 400 high-resolution NMR spectrometer.

A positive assignment of the origin of signal multiplicities in the spectra required additional ^{13}C CP/MAS NMR experiments to be performed at a lower field strength. This was done on a 100 MHz spectrometer with a ^{13}C resonance frequency of 25.2 MHz [2].

2.2. Reagents and materials

Roxifiban (empirical formula: $C_{23}H_{33}N_5O_{8;}$ mw: 507.54, Fig. 1) forms I and II were prepared by Bristol-Myers Squibb Process Research Facilities South and were used as received.

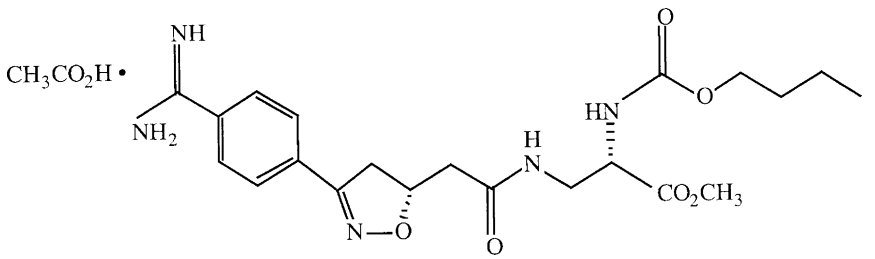

Fig. 1. The chemical structure of roxifiban.

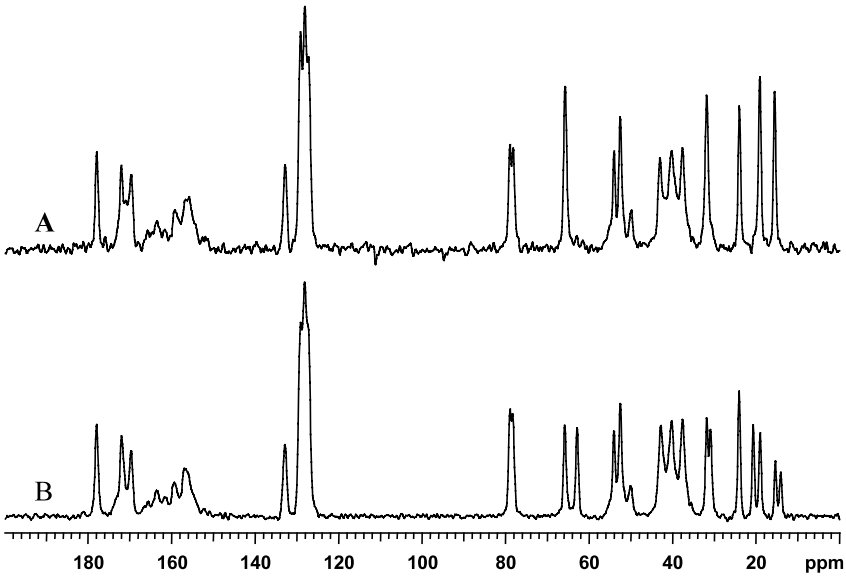

Fig. 2. ^{13}C CP/MAS NMR spectra of roxifiban, (A) form II and (B) form I.

2.3. Sample preparation

Two lots of roxifiban that were found to be pure form I and II by both solid-state NMR and X-ray powder diffraction were used to prepare physical blends to be used as calibration standards. Samples were prepared at form I: form II ratios of 100:0, 75:25, 50:50, 25:75, and 0:100. The blends were prepared by geometric dilution and such that the entire sample was filled into a 5 mm supersonic rotor thereby eliminating artifacts associated with sampling only a portion of the blend.

3. Results and discussion

In analysis by solid-state carbon NMR, observed chemical shifts are influenced by molecular packing and other solid-state effects, the method can be quite useful in developing an understanding of structural differences between related materials. The solid-state NMR differences were exploited to gain a handle on the quantitative composition with the blends providing insight to the linearity of the technique. The solid-state ^{13}C CP/MAS NMR spectra confirmed the existence of two polymorphs. Form I had a lower-symmetry structure as evidenced by the occupation of the *n*-butyl group of roxifiban in one of two crystallographically-inequivalent positions based on the three split methylene signals at 63 and 66, 31 and 32, and 19 and 21 ppm and the split methyl signal at 14 and 16 ppm (Fig. 2). The *n*-butyl group of form II of roxifiban resides in a single defined structural location based on the three methylene signals at 66, 32, and 19 ppm and the methyl signal at 16 ppm. The 66/63 and 21/19 ppm signals were identified as the two regions of the spectra that provided the greatest resolution between the split signals observed in form I. Pure form I exhibited peaks at 66, 63, 21 and 19 ppm while pure form II had peaks at 66 and 19 ppm only. The solid-state NMR spectra peak areas at 66 and 19 ppm remained relatively constant throughout the blends whereas the peak areas at 63 and 21 increased proportionally as the concentration of form I increased (Fig. 3). The mole fraction of form I within the blend is best described as follows;

$$\text{Mole Fraction Form I} = \frac{(\text{Area at 63 ppm})}{(\text{Area at 66 ppm})} \quad (1)$$

alternatively;

$$\text{Mole Fraction Form I} = \frac{(\text{Area at 21 ppm})}{(\text{Area at 19 ppm})} \quad (2)$$

The theoretical mole fraction and measured mole fraction based on the two equations for form I in the blends is summarized in Table 1 and is followed by the statistical data analysis for each equation. Employing a data analysis that forced the fit through the origin, the slope, intercept and correlation coefficient were in excellent agreement with theoretical values. The data analysis shows that the cross-polarizations and relaxation times for form I and form II were equivalent and that the cross-polarizations and relaxation times for the split peaks of form I were equivalent based on the fact the calibration curve was linear and the slope was equal to 1. The blends provided a standard curve for analysis of unknowns (Fig. 4). All results were reported as the average value obtained from Eqs. (1) and (2).

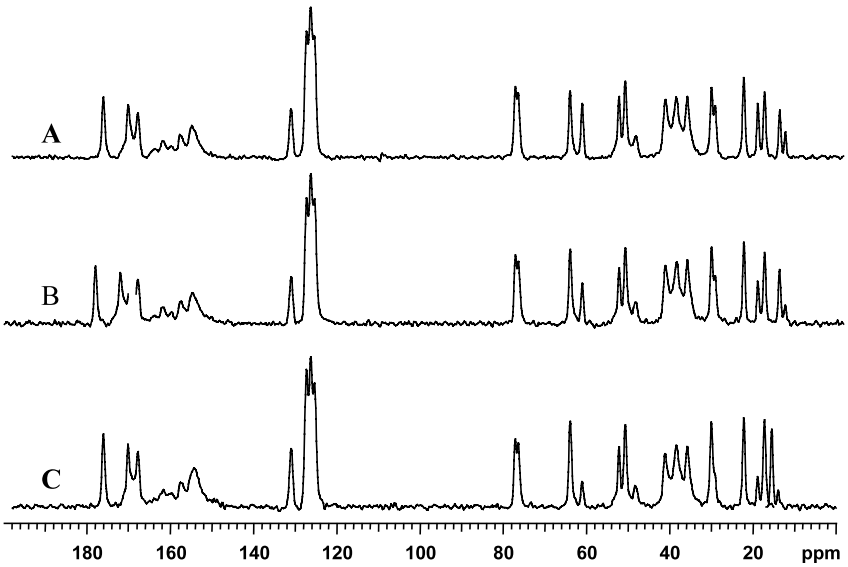

Fig. 3. ^{13}C CP/MAS NMR spectra of the blends of roxifiban at (A) 75%, (B) 50%, and (C) 25% form I in form II.

Table 1
Theoretical and experimental mole fraction form I

Theoretical	Eq. (1) (63/66 ratio)	Eq. (2) (21/19 ratio)	Average value
0.00	0.00	0.00	0.00
0.25	0.25	0.31	0.28
0.50	0.46	0.55	0.51
0.75	0.74	0.72	0.73
1.00	0.93	1.03	0.98
Data analysis			
Slope	0.95	1.03	
Intercept	0.0	0.0	
Correlation coefficient	0.997	0.989	

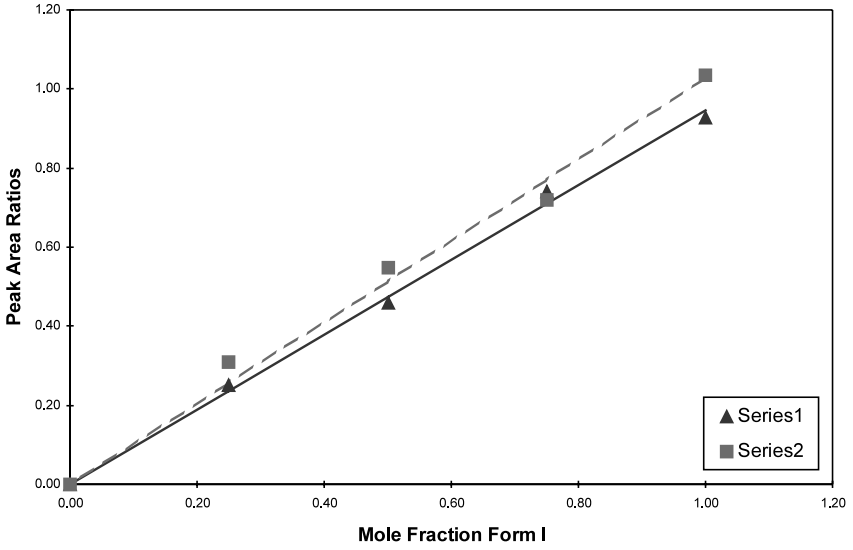

Fig. 4. ^{13}C CP/MAS NMR signal ratio as a function of the mole fraction of roxifiban form I in form II. The characteristic ratios were determined at 63/66 and 21/19 ppm.

To estimate the detection limit, a physical blend of form I and form II was prepared at a 90:10 (form I:form II ratio). The results of the analysis of the blend in triplicate were $87.1 \pm 3.1\%$ form I, which would indicate a detection limit (DL) of form II in form I of 9% with the DL defined as three times the intrasample standard deviation of the solid-state NMR assay.

4. Conclusion

The solubility and the XRPD patterns of the two forms had minor differences and extensive synchrotron X-ray powder diffraction studies were required to solve the single crystal structures. The ^{13}C CP/MAS NMR studies were successful in providing a quantitative analysis to determine the polymorphic purity of roxifiban which could be used further for evaluation of the drug substance.

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