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NMR characterization of a novel bile acid sequestrant, DMP 504

Susan A. Lerke *, Gregory Nemeth, Ernest Schubert [1], Paul K. Hovsepian

DuPont Pharmaceuticals Company, Experimental Station, P.O. Box 80353, Wilmington, DE 19880-0353, USA

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Abstract

DMP 504, a potential bile acid sequestrant for the treatment of hypercholesterolemia, is a highly insoluble, cross-linked polymer which does not lend itself to ordinary means of characterization used for drug substances in the pharmaceutical industry. Therefore, alternative characterization techniques have been sought. As part of an effort into extensive characterization of DMP 504 drug substance, nuclear magnetic resonance (NMR) was employed to provide insight into details of the DMP 504 polymer structure. The primary motivation for determining the structure of the polymer chain is to relate the DMP 504 structure to its performance properties as a bile acid sequestrant. Characterization of the polymer chain and understanding of the structural basis of its properties is essential in optimizing and controlling the manufacture of reproducible drug substance. NMR has proven a versatile tool for the description of polymer structure and dynamics because of the wide range of nuclear interactions affecting the NMR signal. This allows the design of experiments to elicit information about specific polymer interactions or properties. The methods of sample preparation utilized to obtain NMR spectra of the insoluble polymer, as well as a discussion and comparison of results for the characterization of DMP 504 obtained using several different NMR techniques will be presented.

Keywords: NMR spectroscopy; Bile acid sequestrant; Hydrogel; Polymer; DMP 504

1. Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool often used in the elucidation of structure of small molecules and polymers [1]. ^{13}C-NMR is useful in providing insight into details of cross-linked polymer structures because of its spectral dispersion and the relatively narrow line widths.

DMP 504 is a highly cross-linked, insoluble polymer prepared from units of 1,10-dibromodecane and 1,6-diaminohexane. This bile acid sequestrant has been used in clinical trials for its utility as a hypercholesterolemic agent [2]. The structure shown in Fig. 1 is representative of the polymer. Although the polymer is cross-linked,

* Corresponding author. Tel.: +1-302-6958641; fax: +1-302-6959802.

E-mail address: susan.a.lerke@dupontpharma.com (S.A. Lerke).

[1] Present address: Bristol Myers Squibb, 5 Research Pkwy., Wallingford, CT 06492, USA.

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and therefore insoluble [3], it swells with addition of solvent. The swollen polymer behaves as a very concentrated solution and allows NMR spectra to be obtained. In this paper, we present results of analyses of DMP 504 using high-resolution solution-state and solid state ^{13}C-NMR and demonstrate the ability of NMR to characterize polymer structure.

2. Experimental

DMP 504 drug substance was manufactured at the DuPont Pharmaceuticals Company.

Sample preparation for high-resolution solution-state ^{13}C-NMR on various lots of DMP 504 was carried out as follows. An accurately weighed amount of DMP 504 is placed in a 10-mm NMR tube. Dioxane is added to the NMR tube, which is then capped and agitated vigorously. The resulting mixture is allowed to settle before addition of D_2O. Again, the tube is quickly capped, agitated vigorously and then allowed to stand. Following a second addition of D_2O, the mixture is capped, agitated and allowed to stand prior to measurements being made.

The following procedure was employed in the study of swelling agent ratio for high-resolution solution-state ^{13}C-NMR. To a known weight of polymer, a known weight of internal standard (1,4-diazabicyclo[2,2,2]octane) was added and the sample was solubilized by swelling with D_2O. A second sample was prepared similarly by swelling with D_2O then dioxane-D8 and a third sample was prepared by swelling first with dioxane and then with D_2O. ^{13}C-NMR spectra of all preparations were recorded under quantitative conditions, and the numbers of NCH_2 carbons in the primary, secondary and tertiary environments in the solubilized polymer were calculated.

The ^{13}C-NMR solution spectra were obtained using a VXR-400S (Varian Instruments, Palo Alto, CA) with proton-decoupling. Scans (2048) were used for signal averaging with a 60° excitation pulse, an acquisition time of 0.5 s and a 5-s relaxation delay or a Varian VXR-400S spectrometer under the following conditions — sfrq 100.577 Mhz, 10-mm probe, 80° flip angle, acquisition time 2.003 s, delay 18.622 s, sweep width 31948.9 Hz, number of transients ca. 2800, Waltz decoupling, decoupler gated off during delay, probe temperature ca. 20°C, line broadening 5 Hz.

Solid state ^{13}C magic-angle spinning (MAS) NMR spectra were acquired on a Varian VXR-200S NMR spectrometer with a ^{13}C frequency of 50.29 MHz. The sample was spun at approximately 8 kHz at the magic angle and proton decoupling was applied during the acquisition time.

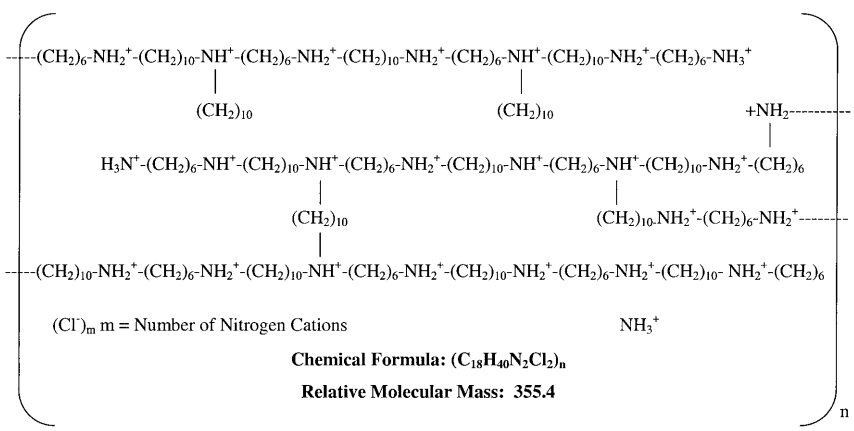


Fig. 1. Chemical structure of DMP 504.

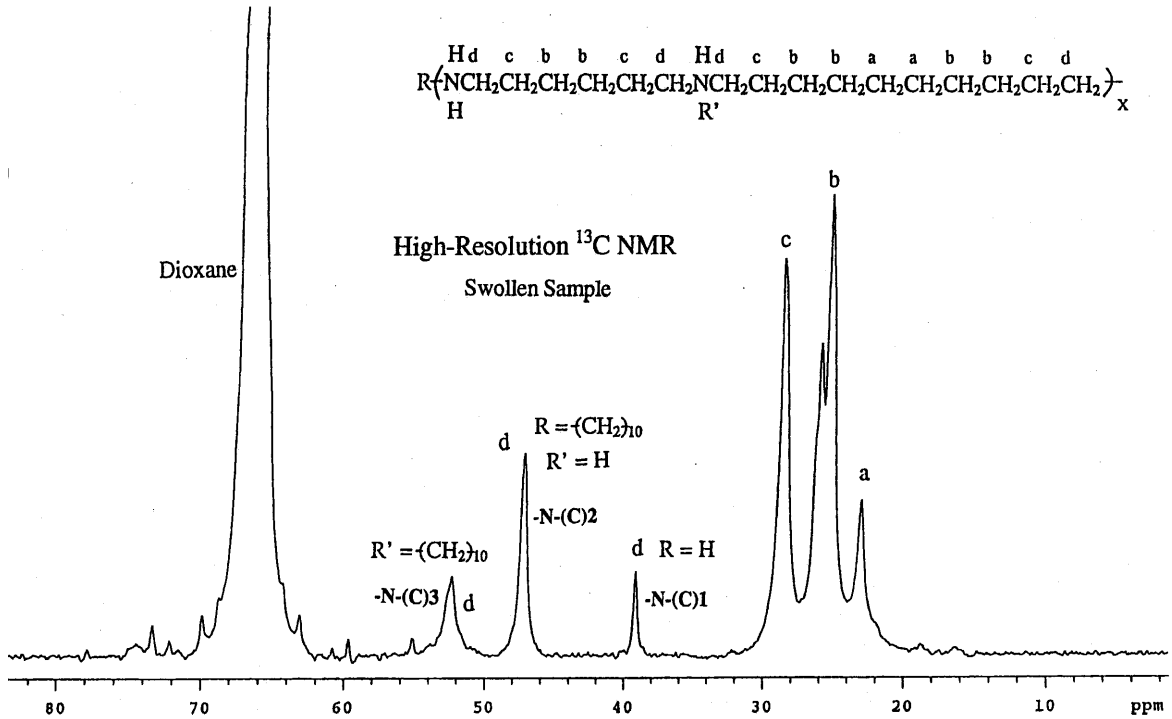


Fig. 2. High-resolution ^{13}C-NMR spectrum of a DMP 504 sample swollen with D_2O/dioxane.

3. Results and discussion

Although the ^{1}H isotope has a high natural abundance, high-resolution ^{1}H NMR has been found to be of little use when working with cross-linked polymers due to overlapping of broad peaks [4]. Indeed ^{1}H NMR spectra obtained on a few polymer samples resulted in signals, which were not clearly separable and the spectra provided no useful information [5].

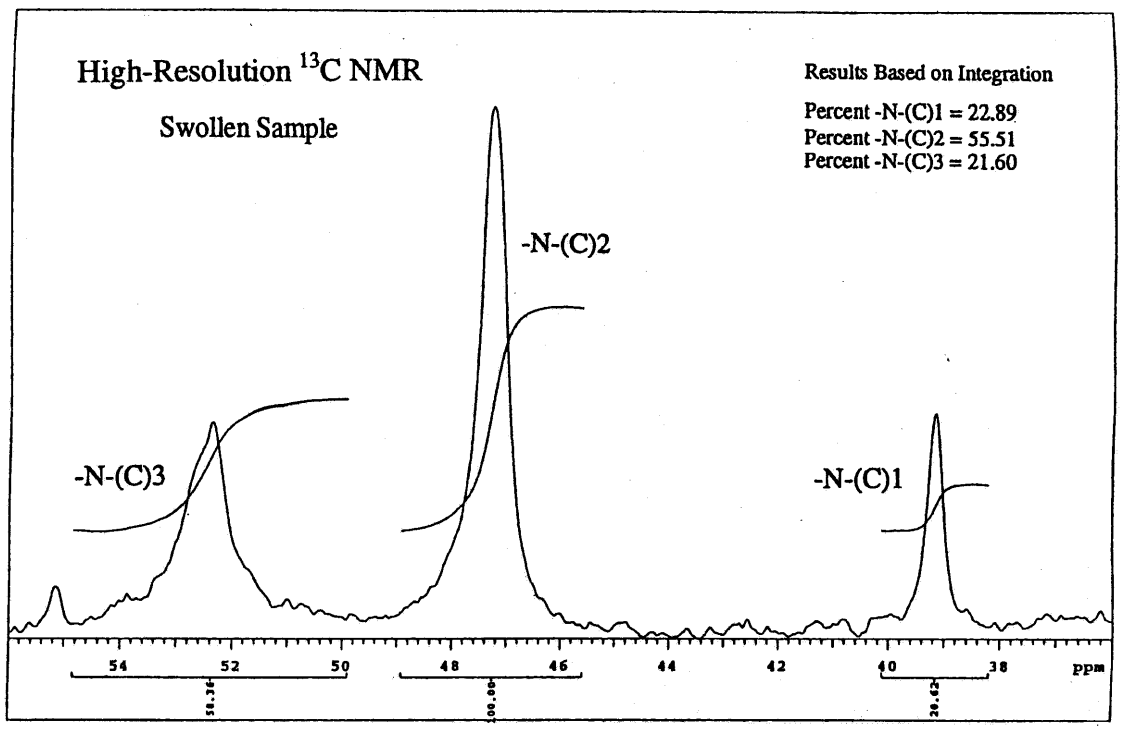
Although the ^{13}C isotope is less abundant than the ^{1}H isotope, the signals achieved with ^{13}C-NMR are not nearly as broad and resolved peak spectra are obtained. A typical ^{13}C-NMR spectrum of DMP 504 swollen in D_2O/dioxane is shown in Fig. 2. The signals at 39, 47 and 52 ppm correspond to carbon atoms immediately adjacent to primary, secondary and tertiary amines, respectively. The very large peak at 66 ppm is attributed to dioxane. The peaks between 20 and 30 ppm correspond to the remaining polymer carbon atoms as shown in Fig. 2.

High-resolution solution-state ^{13}C-NMR spectroscopy was utilized as a tool to determine the relative quantities of primary, secondary and tertiary amines in the 504 polymer. The integrals can be normalized to represent numbers of nitrogen atoms by dividing the integral for each peak by the number of carbon atoms associated with that amine. The relative percentages of amines can then be calculated as shown in Fig. 3. A small percentage ($< 2\%$) of quaternary amine sites (as estimated from swollen-state solution NMR work) are included in the calculation of the results for the tertiary amines since we were not able to obtain the spectral resolution desired to clearly distinguish them from the tertiary amines.

For the DMP 504 lots measured, 22% of the nitrogens in the polymer are primary and represent end groups, 54% of the nitrogens are secondary and thus, part of the linear polymeric chain and 24% of the nitrogens are tertiary, associated with branching or crosslinking within the polymer structure. As seen in Table 1, excellent

reproducibility is observed when spectra were obtained of replicate samples of the same lot of drug substance obtained on different days and reasonable agreement with results obtained by other groups using a similar technique [6].

The DMP 504 polymer was swollen using a dioxane/D_2O mixture. It appears that D_2O prevents the polar or hydrophilic regions of the polymer from forming internal hydrogen bonds while the more hydrophobic dioxane solvates the nonpolar parts of the polymer unit. Swelling the polymer in this way allows solution-state ^{13}C-NMR spectra to be obtained since the polymer acts as a very concentrated solution. Summarized in Table 2 are relative percents, respectively, of primary, secondary and tertiary amines in samples of a single lot of bile acid sequestrant as a function of the swelling agents and swelling agent to



Peak ID	Integrals		No. of Assoc. Carbons		Normalized Integrals		Sum of Normalized Integrals		Relative % Amines
-N-(C)1	20.62	/	1	=	20.62	/	90.07	x 100 =	22.89
-N-(C)2	100.00	/	2	=	50.00	/	90.07	x 100 =	55.51
-N-(C)3	58.36	/	3	=	19.45	/	90.07	x 100 =	21.60

Fig. 3. Relative percentages of amines in DMP 504 calculated from integration of high-resolution ^{13}C-NMR spectrum of DMP 504.

Table 1
Precision of relative percent amines lot Q9214-065

Lot Q9214-065	–N–(C)1 (%)	–N–(C)2 (%)	–N–(C)3 (%)
Sample 1, day 1	21.8	52.3	25.8
Sample 2, day 2	22.1	52.7	25.2
Sample 3, day 3	22.0	53.2	24.8
Mean	22.0	52.7	25.3
R.S.D. (%)	0.7	0.9	2.0

sample weight ratio. The results show a significant increase in the measured amount of tertiary amines when the polymer is swollen in D_2O with increasing amounts of dioxane-d8. This is consistent with the assumption that the mobility, and hence the sharpness of the NMR resonance, of the relatively hydrophobic tertiary amine sites is largely affected by the non-polar dioxane-d8 solvent while the polar D_2O solvent is responsible for 'solubilizing' the more hydrophilic groups. The results of this study show that a D_2O/Dioxane/sample weight ratio of 2/4/1 yields results that are comparable to the results obtained by solid state ^{13}C-NMR. Additional amount of dioxane gave results that were not significantly different than those obtained using the 2/4/1 method, although it does increase the sample's 'fluidity'. The limitation in this approach is that those portions of the polymer molecule that do not undergo hydrogen bridge dissociation under these conditions (e.g. quaternary NCH_2 centers) are not readily observed.

Information about the extent of swelling of the polymer using different relative amounts of D_2O and dioxane as well as methods of sample preparation is limited to an approximation due to the assumptions which need to be made. The molecular weight is not known, a repeating unit structure is assumed and the integral contributions of the various areas have to be normalized. With this in mind, it is estimated that as little as one half of the original polymer weight has been solubilized for detection by liquid state ^{13}C-NMR. This suggests that this method results in only an approximation of the polymer composition.

While the development of a solvent pair (dioxane/water) capable of swelling the polymer yielded high-resolution ^{13}C-NMR spectra, the question remained as to whether the entire polymer sample is represented in the spectrum so obtained. The solid-state MAS NMR technique offers an advantage in that it does not suffer from this problem [7]. The resolution demonstrated in a ^{13}C MAS NMR study of DMP 504, however, fell far short of what would be necessary to reliably quantitate the amine percentages, see Fig. 4.

The best approach found for the analysis of DMP 504 employs a combination of solution- and solid-state NMR techniques. After preparing a swollen sample of DMP 504 as is done for solution-state NMR, the excess solvent mixture is vacuum filtered off. Although the solid, which remains appears to be totally dry, it is actually DMP 504 swollen with both water and dioxane. Even under a variety of stress conditions, including the centrifugal forces associated with MAS as is used for resolution enhancement in solid-state NMR spectroscopy, the polymer did not relinquish solvent.

Fig. 4 shows spectra obtained using solid-state, solid-state/swollen sample and high-resolution solution-state/swollen sample ^{13}C-NMR. As can be seen in that figure, MAS-Bloch decay ^{13}C-NMR

Table 2
NMR swelling agent ratio analysis

D_2O/dioxane/sample weight ratio	Primary amines (%)	Secondary amines (%)	Tertiary amines (%)
14.1/…/1.0	28.9	56.4	14.7
2.1/3.9/1.0	22.7	53.6	23.7
2.3/5.5/1.0	23.4	52.5	24.1
Solid state NMR	23	51	26

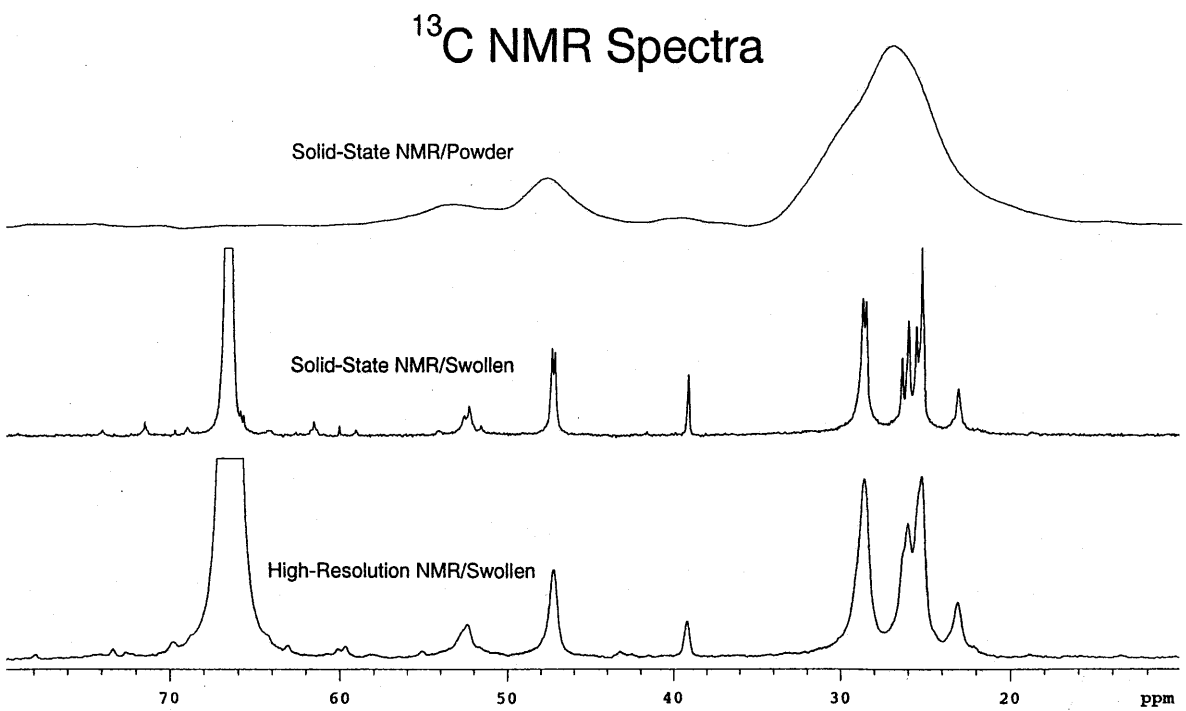


Fig. 4. Comparison of spectra of DMP 504 obtained using solid-state and high-resolution ^{13}C-NMR.

Table 3
Relative percent amines based on ^{13}C-NMR of swollen samples

Type of NMR	Lot	–N–(C)1 (%)	–N–(C)2 (%)	–N–(C)3 (%)
High-resolution	Q9214-67	20.7	54.1	25.2
Solid state		19.9	50.1	30.0
High-resolution	Q9214-73	21.7	53.7	24.6
Solid state		18.0	45.8	36.2
High-resolution	Q9214-76	22.0	53.8	24.2
Solid state		19.3	53.2	27.5

experiments on swollen DMP 504 samples are found to give the best spectral resolution demonstrated to date for the quantitation of amines present in these materials. While this experiment clearly provides superior data over the high resolution ^{13}C method, it requires considerably more time to run. Table 3 shows that reasonable agreement is achieved between the results of high-resolution solution-state NMR analysis and swollen solid-state NMR.

4. Conclusions

While higher resolution spectra were achievable with the swollen solid-state technique, instrument

availability and time favor use of solution-state NMR in some environments, where rapid turnaround is essential and lot-to-lot comparisons are sought. Both techniques provide information representative of the entire polymer and allow estimation of relative amounts of primary, secondary and tertiary amines in the polymer. NMR proved a useful technique where traditional methods could not be used to demonstrate reproducibly synthesized drug substance consistent with the proposed structure.

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