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Application of inverse-NMR techniques to polyamide-4,6

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Summary

It is shown that 2D proton-carbon correlation NMR spectra can be obtained for small amounts of polymers, typically in the range of 10 mg. This is accomplished by applying the so-called inverse-NMR technique which makes use of proton detection rather than carbon-13 detection.
Several examples of direct and long-range correlations are shown applied to polyamide-4,6.

Introduction

Two- and three-dimensional NMR are already firmly established techniques for the structure elucidation of large biopolymers (1-2). The advantages of improved resolution are combined with the possibilities for the detection of specific interactions. In the field of synthetic polymers and copolymers, research has long been focussed on the determination of polymer microstructure (e.g. tacticity) by 1D ^{13}C solution NMR, and it was not until 1983 before the first application of a 2D experiment was published (3). Since then, these experiments have become increasingly popular (4,5). It was pointed out by Bovey that with the application of these multidimensional techniques, ^{1}H-NMR regained some of its importance it had always kept for the study of biopolymers (6).

Very recently, he showed this by performing a 3D NMR experiment, in this case 3D NOE/J-resolved applied to a copolymer of vinylidenecyanide and vinylacetate, which gave the proton-proton coupling constants (7).

However, if the proton-carbon correlation is the desired information, until recently the less abundant and less receptive ^{13}C-nucleus had to be detected. In the case of polymers, this meant that good solubility was a prerequisite (typically 10-30 % w/w, depending on the 2D NMR experiment) in order to reach a concentration necessary for a reasonable measurement time. This would imply that a number of interesting, but less soluble, polymers can only studied by ^{13}C CP-MAS NMR. Fortunately, developments in the research on biopolymers have led to a new technique, the so-called inverse-NMR (8).

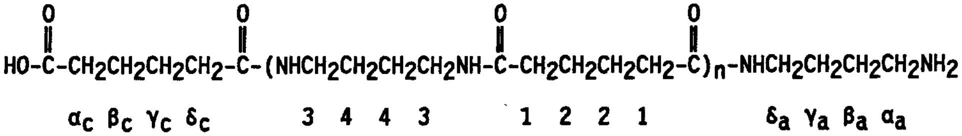
Magnetization is transferred back to the proton which is subsequently detected. The main advantage of this technique lies in the improved sensitivity which, in the case of ^{13}C, can be increased by a factor 31.6, thus allowing one to measure when only sample amounts in the mg-range are soluble (or available). We have explored the possibilities of a number of these inverse techniques for the correlation of proton and carbon resonances of polyamide-4,6.

Experimental

All NMR spectra have been recorded on a Bruker AM-400 spectrometer, equipped with an Aspect 3000 computer and array processor. For the inverse-NMR measurements a $^1H/^{13}C$ 5 mm-dual probe was used.
Polyamide-4,6 (STANYL, trademark of DSM) has been synthesized according to literature data (9). Deuteriated formic acid (DCOOD) was used as a solvent, and a concentration of 12 mg/0.5 ml DCOOD was used. The Bruker pulse programs BIRD.AUR, INVREL2D.AUR and INVDR2LP.AUR were applied. Typically, 128 scans were accumulated with a recycle delay of 1 sec for each of the 128 experiments, resulting in a 256 x 1K data matrix (after Fourier transformation), convering 1200 Hz in the F2 dimension and 2000 Hz in the F1 dimension.
T1-noise has been subtracted from the 2D-spectra.

Results and discussion

Recently, we have studied extensively the assignments for both the end-group and main-chain resonances of polyamide-4,6 via conventional 2D NMR techniques, e.g. H-H and C-H COSY, relayed coherence transfer and COLOC (10). Results are listed in the table.



$$HO\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_2CH_2CH_2CH_2\text{-}\overset{\overset{O}{\|}}{C}\text{-}(NHCH_2CH_2CH_2CH_2NH\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_2CH_2CH_2CH_2\text{-}\overset{\overset{O}{\|}}{C})_n\text{-}NHCH_2CH_2CH_2CH_2NH_2$$

PA-4,6

Table 1. Assignments for the ^1H and ^{13}C NMR resonances of polyamide-4,6 in DCOOD

	1	2	3	4	α_a	β_a	γ_a	δ_a	α_c	β_c	γ_c	δ_c
^1H	2.37	1.66	3.27	1.57	3.23	1.79	1.65	3.31	2.43	1.65	1.65	2.37
^{13}C	35.46	25.19	39.65	25.95	40.33	24.33	25.59	39.19	33.33	23.88	25.10	35.50

We have repeated these proton-carbon correlation experiments for a low molecular mass oligomer of PA-4,6, and for a high molecular mass polymer, but now applying the inverse NMR methods. In both cases, only 12 mg was dissolved in 0.5 ml DCOOD, as compared to 400 mg in 3 ml in the previous study (10), an effective decrease in concentration by a factor of 6. For the one-bone proton-carbon correlation, the ^1H-detected heteronuclear multiple-quantum coherence experiment of Bax and Subramanian was used (11). The BIRD pulse-sandwich, which preceeds the actual correlation pulse-sequence, serves to suppress the unwanted 'center-signals', caused by the protons connected to ^{12}C instead of ^{13}C. Although, in principle, phase-cycling already serves this purpose, the actual result then critically depends on the stability of both the spectrometer and the building (e.g. floor vibrations can have a large influence (12)).
In figure 1 the result of the BIRD-experiment is shown.
The experiment was done without X-nucleus decoupling, therefore all resonances are split to a doublet by a $^1J_{CH}$-coupling of 130-140 Hz. As can be seen in figure 1, besides the main chain resonances also some of the endgroup resonances can be detected. In figure 2, the results of the inverse relay experiment (with BIRD suppression) are shown (13).

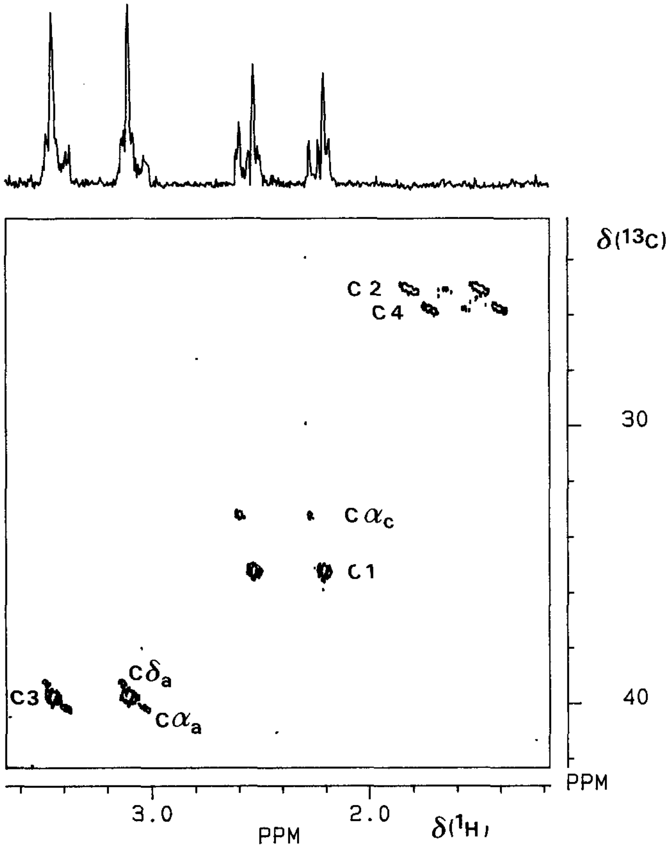


Figure 1. Inverse ^1H-^{13}C correlation experiment applied to 12 mg of an oligomer of polyamide-4,6 dissolved in 0.5 ml DCOOD

Apart from the one-bond correlations, two new correlations appear between C-3 and H-4, and between C-1 and H-2. The correlations between C-2 and H-1, and C-4 and H-3, are not observed.

Because the one-bond proton-carbon coupling constant for C-2 and C-4 is slightly smaller (128 Hz) than that for C-3 (140 Hz) and C-1 (131 Hz), we performed a second experiment optimised for a coupling constant of 128 Hz. However, this did not improve the result. Apparently, the overall magnetization transfer efficiency is not equal for all proton-carbon combinations. Even though not all correlations are observed, the applicapability of these inverse experiments to the polyamide-4,6 oligomer is evident.

Next, we turned our attention to measurements for the high molecular mass polymamide-4,6. It was pointed out by Bax (11) that the BIRD pulse-sandwich cannot be effectively used for polymers because the sensitivity of the experiment is reduced by negative interference with NOEs between ^{13}C-bound protons and the neighbouring ^{12}CHs. Indeed, for the high molecular mass polyamide-4,6 the BIRD- and the RELAY-experiment did not lead to any observable correlations. Therefore, for this polymer a different experiment was selected (14) which makes use of ^1H-^{13}C long-range coupling constants to establish correlations, and which does not use the BIRD-sequence (Bruker pulse-program INVDR2LP.AU). In figure 3 the resulting 2D-NMR spectrum is shown. All one-bond correlations are effectively suppressed by the J-filter

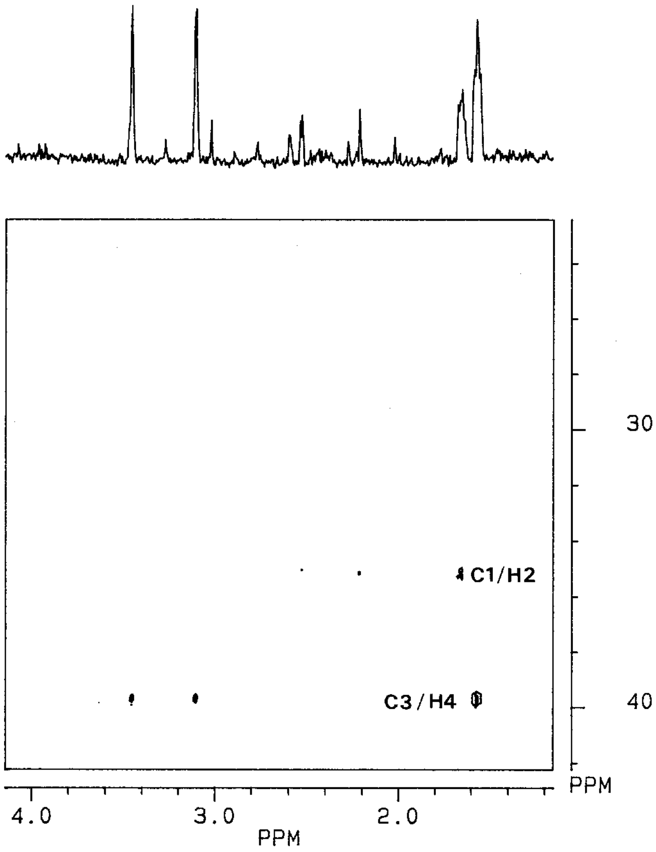


Figure 2. Inverse relayed ^{1}H-^{13}C correlation experiment applied to 12 mg of an oligomer of polyamide-4,6 dissolved in 0.5 ml DCOOD

present in the pulse-sequence. Of all the possible long-range correlations, only C-4/H-3 and C-2/H-1 are visible.
This is to be expected since only C-2 and C-4 show a sizable long-range coupling of, respectively, 4.3 and 3.6 Hz in a ^{13}C-spectrum.
On the other hand, the long-range coupling constant for C-1 and C-3 is smaller than 1 Hz, which makes it difficult to observe long-range correlations with these carbons in this particular experiment (because of the necessary length of the magnetization tranfer time, > 250 ms, relaxation becomes a competing effect).
During the course of this study it was found that the same experiment had been used for the first time to determine the tacticity of polymethylmethacrylate (52 mg in 0.7 ml $CDCl_3$) (15).
In conclusion, the aforementioned examples show that it is possible to obtain proton-carbon correlation 2D NMR spectra of small amounts of polymers with relatively simple NMR equipment. If the spectrometer is equipped with the possibilities to generate spin-lock pulses, an alternative pulse-sequence for the BIRD-sequence is available that is applicable to polymers as well. This sequence should, according to Wüthrich, give satisfactory results even with relatively unstable spectrometers (16).

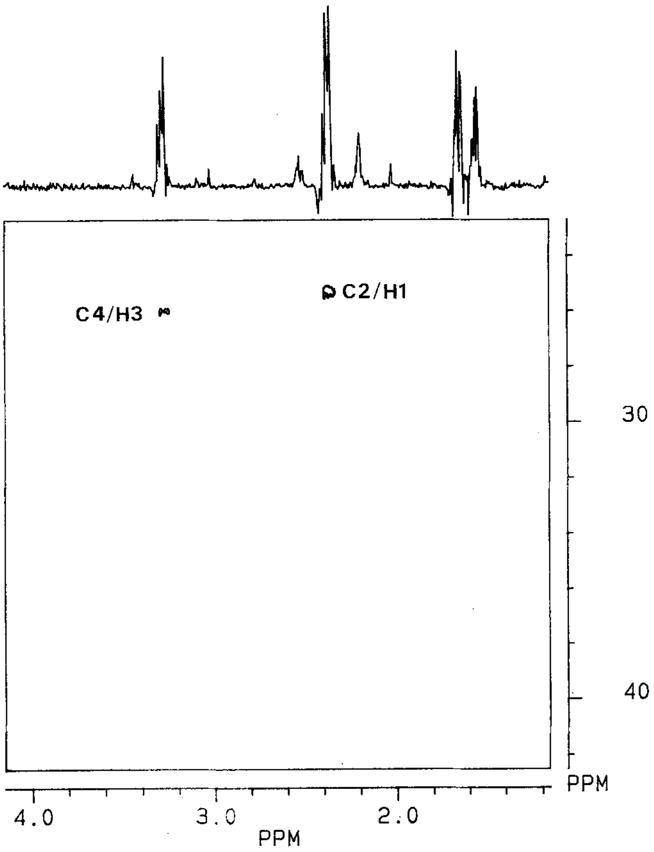


Figure 3. Inverse long-range ^1H-^{13}C correlation experiment applied to 12.4 mg of high molecular mass polyamide-4,6 dissolved in 0.5 ml DCOOD

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