to obtain a better understanding of synthetic reactions or biosynthetic pathways.

More generally, when in a spectrum two overlapping signals exist, as they correspond to two different forms of the same site (by isotopic exchange or other phenomena), two-dimensional *J* spectroscopy will be able to differentiate between them, as far as their induced chemical shift variation is in agreement with the experimental digital resolution. Furthermore, quantitative analysis will be made easier and will lead to a better conception of the phenomena involved. Of course, it will be advantageous to use the two-dimensional technique with higher field spectroscopy in order to overcome most of the problems related to strongly coupled spin systems.

Acknowledgment. We are grateful to Le Centre Grenoblois de Rêsonance Magnêtique Nuclêaire for giving us free access to the high-field spectrometer.

References and Notes

(1) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976,** *64,* 2229.
(2) Bodenhausen, G.; Freeman, R.; Niedermeyer, R.; Turner, D. L. *J. Magn. Reson.* **1976,** *24,* 291. *Ibid.* **1977,** *26,* 133.
(3) Hall, L. D.; Sukumar, S.; Sullivan, G. R. *J. Chem. Soc., Chem. Commun.* **1979,** 292. Hall, L. D.; Sukumar, S. *J. Am. Chem. Soc.* **1979,** *101,* 3120. *Carbohydr. Res.* **1979,** *74,* C1–C4.
(4) Bain, A. D.; Bell, R. A.; Everett, J. R.; Hughes, D. W. *Can. J. Chem.* **1980,** *58,* 1947.
(5) Koch, H. J.; Stuart, R. S. *Carbohydr. Res.* **1977,** *59,* C1–C6.
(6) Hestrin, S. *Methods Carbohydr. Chem.* **1963,** *3.*
(7) Chumpitazi-Hermoza, B.; Gagnaire, D. Y.; Taravel, F. R., unpublished results.
(8) Dutton, G. G. S.; Mackie, K. L.; Savage, A. F.; Stephenson, M. D. *Carbohydr. Res.* **1978,** *66,* 125.
(9) Goldstein, I. J.; Hay, G. W.; Lewis, B. A.; Smith, F. *Methods Carbohydr. Chem.* **1965,** *5.*
(10) Bobbit, T. F.; Nordin, J. H.; Gagnaire, D.; Vincendon, M. *Carbohydr. Res.* **1980,** *81,* 177.
(11) Gagnaire, D.; Horton, D.; Taravel, F. R. *Carbohydr. Res.* **1973,** *27,* 363.
(12) Bebault, G. M.; Choy, Y. M.; Dutton, G. G. S.; Funnell, N.; Stephen, A. M.; Yang, M. T. *J. Bacteriol.* **1973,** *113,* 1345.
(13) Van Derveen, J. M. *J. Org. Chem.* **1963,** *28,* 564.
(14) de Bruyn, A.; Anteunis, M.; de Gussem, R.; Dutton, G. G. S. *Carbohydr. Res.* **1976,** *47,* 158.
(15) Chumpitazi-Hermoza, B.; Gagnaire, D. Y.; Taravel, F. R. *Biopolymers* **1978,** *17,* 2361.
(16) Bock, K.; Gagnaire, D.; Vignon, M.; Vincendon, M.; unpublished results.
(17) Doddrell, D.; Glushko, V.; Allerhand, A. *J. Chem. Phys.* **1972,** *56,* 3683.

NMR Study of Nylon 66 in Solution (^{1}H, ^{13}C, and ^{15}N NMR Using Adiabatic *J* Cross Polarization)

Brenda S. Holmes* and William B. Moniz

Chemistry Division, Naval Research Laboratory, Washington, D.C. 20375

Raymond C. Ferguson

Central Research and Development Department,[†] *E. I. du Pont de Nemours, Wilmington, Delaware 19898.* *Received March 9, 1981*

ABSTRACT: We have utilized ^{15}N NMR in conjunction with ^{1}H and ^{13}C NMR chemical shifts to study nylon 66 in acid solutions. Using the adiabatic *J* cross-polarization (AJCP) technique, we obtained signal enhancements which resulted in ~100-fold time savings and eliminated the need to match exactly the radio-frequency amplitudes of the ^{1}H and ^{15}N during polarization transfer. The effects of solvent acidity, variation of polymer concentration, and variation of degree of polymerization (DP) on nylon 66 in solution have been studied. ^{15}N chemical shifts were more responsive to variation of the experimental parameters than the corresponding proton and ^{13}C data. ^{15}N data exhibited deshielding with increasing solvent acidity, deshielding with decreasing polymer concentration, but little variation with respect to degree of polymerization below 25% (w/v). At higher nylon concentrations, however, the DP of the polymer apparently affects the extent of protonation and/or hydrogen bonding, which causes an increase in the shielding at the ^{15}N nucleus of the amide group. Using the ^{15}N NMR data in conjunction with the ^{1}H and ^{13}C data of the nylon 66, we have confirmed the site of protonation/hydrogen bonding as the oxygen of the amide carbonyl in the presence of excess acid.

Introduction

In our previous paper we studied the ^{15}N nuclear magnetic resonance (NMR) chemical shifts of polyamides in solution with respect to solvent acidity, concentration, and temperature.[1] We found that the ^{15}N chemical shifts of nylon 66 in trifluoroacetic acid (CF_3COOH, TFA) were most sensitive to changes in concentration. Nylon 66 in 1,1,1,3,3,3-hexafluoro-2-propanol ($(CF_3)_2CHOH$, HFIP) solutions had ^{15}N chemical shifts least sensitive to concentration changes. These two systems were chosen for this study using ^{1}H, ^{13}C, and ^{15}N NMR techniques in order to determine if protonation and/or hydrogen bonding varies with changes in concentration. We also studied two different molecular weights of nylon 66 to determine if the changes were dependent on degree of polymerization.

Schilling and Kricheldorf[2] have shown that ^{13}C NMR chemical shifts of carbonyl carbons of polyamides and peptides vary with solvent acidity, as do ^{15}N chemical shifts of polyamides;[1,3] the ^{15}N shifts, however, are more sensitive to sequence variation than ^{13}C chemical shifts in polyamides and peptides.[3] The small magnetogyric ratio (γ) and low natural abundance, in addition to long T_1 values of ^{15}N nuclei, have made natural-abundance ^{15}N NMR more tedious than ^{13}C NMR.

The *J* cross-polarization (JCP) technique,[5] however, was used to increase the sensitivity of ^{15}N NMR in the previous study of polyamides in solution. In the present study, we used the adiabatic JCP (AJCP) technique[6] to examine the ^{15}N NMR chemical shifts of the nylon 66/TFA and nylon 66/HFIP solutions.

Experimental Section

Samples. Two different molecular weights of nylon 66 were provided by du Pont. The number-average molecular weights,

---

[†] Contribution No. 2883.

0024-9297/82/2215-0129$01.25/0 © 1982 American Chemical Society

determined by titration and viscosity analyses, respectively, were ~3700 for the low molecular weight sample (high amine content) and ~15 000 for the standard nylon 66. Solutions of 6, 12, 25, and 45% (w/v) of each nylon were prepared in TFA. In addition, a 35% (w/v) solution of standard nylon 66 in TFA was used. Solution concentrations for the standard nylon in HFIP were 6, 12, 25, and 30% (w/v).

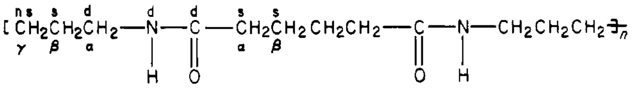
^1H NMR. The ^1H chemical shift measurements were made on a JEOL FX60-Q spectrometer at 59.75 MHz. Experimental conditions were as follows: spinning sample in 10-mm tube; 27 °C; 90° pulse width, 27 μs; acquisition time, 2.04 s; 32 accumulations at 1-s intervals; 8K data points; external deuterium lock. Chemical shifts were measured against external tetramethylsilane (Me_4Si) contained in another 10-mm tube.

^{13}C NMR. The ^{13}C chemical shift measurements were made at 67.89 MHz at Florida State University. They were also done at 15.00 MHz on a JEOL FX60-Q spectrometer. The chemical shifts were referenced to dioxane-d_8 in the TFA solutions (5% (v/v) dioxane-d_8 in TFA); the shifts for the HFIP solutions were referenced to the methylene carbon γ to the NH group (γ-NH) (see Results and Discussion).

^{15}N NMR. The ^{15}N chemical shift measurements were made at 10.13 MHz on a home-built spectrometer with external deuterium lock. The adiabatic J cross-polarization (AJCP)[6] method, in which the spin magnetization is transferred from the high-γ nucleus (^1H) to the low-γ nucleus (^{15}N) by slowly crossing the rotating frame energy levels, was utilized in this experiment. The need to match the radio-frequency amplitudes of the two nuclei is eliminated by this technique, yet the expected signal enhancements are achieved. The amplitude of the ^1H radio-frequency field was decreased from $\omega_{1H}/2\pi = 950$ to $\omega_{1H}/2\pi = 650$ Hz, while the ^{15}N radio-frequency field amplitude was held at 800 Hz. This provided for a chemical shift coverage of 800 Hz (8 ppm) for ^1H and 600 Hz (59 ppm) for ^{15}N. Other experimental conditions were as follows: spinning sample in 10-mm tube; 25 °C; 50-ms cross-polarization time; 1000 accumulations at 2-s intervals; broad-band proton decoupling; 1200-Hz sweep width/quadrature detection; 2K data points; 2-Hz exponential line broadening. A signal-to-noise ratio (S/N) of 3–4 was typical. Chemical shifts were measured against an external standard of ^{15}N-enriched acetamide in water (saturated solution). These values were referenced to external 15 M aqueous acidified $^{15}NH_4NO_3$ (0 ppm).

Results and Discussion

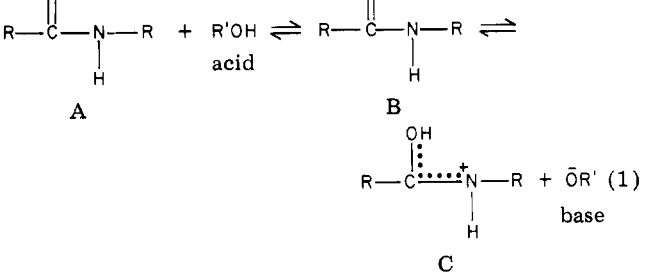
Nylon 66 has the following general formula and the groups are identified as indicated below (omitting end groups, which were not detected in this work):



$$\left[\underset{\gamma}{\overset{ns}{C}}H_2\underset{\beta}{\overset{s}{C}}H_2\underset{\alpha}{\overset{d}{C}}H_2-\underset{H}{\overset{d}{N}}-\underset{O}{\overset{d}{C}}-\underset{\alpha}{\overset{s}{C}}H_2\underset{\beta}{\overset{s}{C}}H_2CH_2CH_2-\underset{O}{C}-\underset{H}{N}-CH_2CH_2CH_2\right]_n$$

The subscripts refer to the positions of the methylene groups relative to the NH and CO groups of the polyamide. The superscripts refer to the changes observed in this study for the chemical shifts of the ^{13}C and ^{15}N nuclei with decreasing nylon concentration, where d = deshielding, s = shielding, and ns = no significant shift change.

It has been postulated[3,4] that hydrogen bonding and/or protonation by strong acids occurs at the amide carbonyl group in the following manner.



(1)

Decreasing the nylon (A) concentration should shift the equilibrium to the right in eq 1, resulting in an increase in the concentrations of forms B and C since hydrogen bonding and/or protonation is more likely to occur in the presence of excess acid. On the other hand, with a decrease in the relative amount of acid, the equilibrium should shift toward the left.

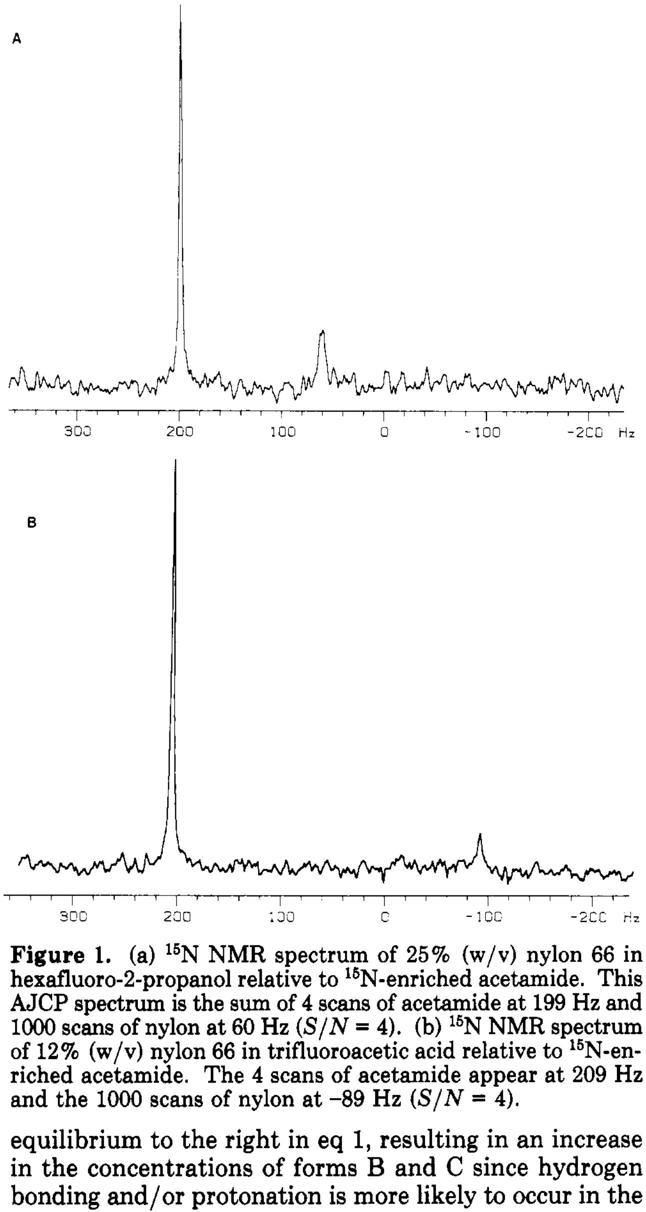


Figure 1. (a) ^{15}N NMR spectrum of 25% (w/v) nylon 66 in hexafluoro-2-propanol relative to ^{15}N-enriched acetamide. This AJCP spectrum is the sum of 4 scans of acetamide at 199 Hz and 1000 scans of nylon at 60 Hz ($S/N = 4$). (b) ^{15}N NMR spectrum of 12% (w/v) nylon 66 in trifluoroacetic acid relative to ^{15}N-enriched acetamide. The 4 scans of acetamide appear at 209 Hz and the 1000 scans of nylon at -89 Hz ($S/N = 4$).

Upon protonation of the oxygen, the C–N π-bond character increases, and the positive charge is delocalized over the two nuclei. This delocalization should lead to a deshielding effect on the carbon and nitrogen nuclei of the amide group. Since protonation is more likely in a stronger acid, the amide group should be more deshielded in TFA ($pK_a = 0.23$) than in HFIP ($pK_a = 9.3$) solutions (Figure 1).

The ^{15}N, ^{13}C, and ^1H chemical shift data of the nylon solutions are listed in Tables I–III. With decreasing nylon concentration, the nitrogen and carbon nuclei of the amide group as well as the α-NH carbon are deshielded. This effect is in agreement with the increased π-bond character of the C–N bond. The delocalization effect is also felt by the α-NH carbon. The increased shielding at the α-CO, β-CO, and β-NH carbons as nylon concentration decreases is also consistent with the protonation and/or hydrogen bonding at the carbonyl oxygen.

In the solvents trifluoroacetic acid and hexafluoro-2-propanol, the carbon nuclei not bound to fluorine nuclei were deshielded with decreasing nylon concentration while the protons involved in hydrogen bonding and/or protonation were shielded. The changes in chemical shifts

Table I
^{15}N Chemical Shifts of Nylon 66 in Acid Solutions

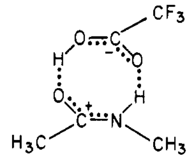
solvent	concn, % (w/v)	$\delta_{^{15}N}$ [a] standard	$\delta_{^{15}N}$ [a] high amine
TFA	45	ns [b]	115.0
TFA	35	114.3	
TFA	25	116.8	117.1
TFA	12	118.7	118.9
TFA	6	119.0	118.6
HFIP	30	102.8	
HFIP	25	103.2	
HFIP	12	104.2	
HFIP	6	104.6	

[a] Relative to $\delta_{^{15}NH_4NO_3} = 0$; ^{15}N shifts accurate to ±0.2 ppm. [b] No signal.

for all the observed nuclei in the solvents and the nylons were more pronounced in the more acidic TFA as expected.

The ^{15}N chemical shifts (δ) were more sensitive to changes in concentration than the ^{13}C shifts for the same solutions. The $\Delta\delta$ for the standard nylon 66 in TFA was 4.7 ppm for ^{15}N over the concentration range but was only 0.9 ppm for the carbonyl carbon of the amide group. The ^{15}N shift data were also more sensitive to the acidity of the solvents, having a $\Delta\delta = 4.7$ ppm in TFA and a $\Delta\delta = 1.8$ ppm in HFIP over the concentration range; the corresponding carbonyl carbon data varied 0.9 ppm in TFA and 0.8 ppm in HFIP.

The greatest change in the ^{15}N shifts in the TFA solutions occurs between 25 and 45% (w/v), where the ratio of amide/acid goes from ~1/6 to ~1/3. In our previous study,[1] we found a similar upfield shift in the ^{15}N data between 25 and 35% (w/v). The upfield shift (decreasing δ) of the ^{15}N signal would be consistent with the formation of a complex between the amide and TFA, as proposed by Stewart et al.[7] for small amides in TFA, as the amide/acid ratio approaches 1/1.



Formation of such a complex would increase the shielding at the nitrogen and increase the proton exchange rate at the NH site. In the HFIP solutions, however, the am-

Table II
^{13}C Chemical Shifts of Nylon 66 in Acid Solutions

solvent	concn, % (w/v)	type of nylon 66	$\delta_{^{13}C}$ methylene groups β-CO	γ-NH	β-NH	α-CO	α-NH	C=O	solvent CF$_3$	COOH	CHOH
TFA [a]	45	high amine	25.5	26.5	28.8	35.0	41.8	178.2	116.2	160.6	
TFA	25	high amine	25.3	26.5	28.6	34.4	42.5	179.1	115.9	161.0	
TFA	12	high amine	25.2	26.5	28.6	34.2	42.8	179.3	115.7	161.3	
TFA	6	high amine	25.2	26.5	28.5	34.1	43.0	179.4	116.6	161.7	
TFA	45	standard	25.7	26.6	28.7	34.9	41.8	178.4	116.2	160.8	
TFA	35	standard	25.2	26.6	28.6	34.6	42.3	178.8	116.1	161.1	
TFA	25	standard	25.5	26.5	28.6	34.4	42.6	179.1	115.9	161.1	
TFA	12	standard	25.2	26.5	28.4	34.2	42.8	179.3	115.7	161.3	
TFA	6	standard	25.2	26.5	28.3	34.2	43.0	179.3	115.6	161.6	
HFIP [b]	30	standard	26.9	27.9	30.5	37.6	41.7	177.8	124.0		71.3
HFIP	25	standard	26.9	27.9	30.5	37.6	41.8	178.0	123.9		71.4
HFIP	12	standard	26.9	27.9	30.5	37.7	42.0	178.5	123.7		71.8
HFIP	6	standard	26.9	27.9	30.4	37.7	42.1	178.6	123.6		72.0

[a] $\delta_{^{13}C}$ relative to $\delta_{^{13}C}$ of dioxane-d_8 in TFA solutions (=66.500 ppm); ^{13}C shifts accurate to ±0.1 ppm. [b] $\delta_{^{13}C}$ relative to $\delta_{^{13}C}$ of γ-NH methylene carbon for HFIP solutions (=27.902 ppm); ^{13}C shifts accurate to ±0.1 ppm.

Table III
^{1}H Chemical Shifts of Nylon 66 in Acid Solutions

solvent	concn, % (w/v)	type of nylon 66	$\delta_{^{1}H}$ [a] methylene groups β-NH	β-CO	α-CO	α-NH	amine NH$_2$	amide NH	acid OH	H
TFA	45	high amine	1.4	ns [b]	ns	3.1	7.1	8.3	14.2	
TFA	25	high amine	1.4	1.7	2.6	3.4	6.7	8.4	12.9	
TFA	12	high amine	1.4	1.7	2.6	3.3	6.5	8.4	12.0	
TFA	6	high amine	1.3	1.7	2.5	3.3	6.5	8.4	11.6	
TFA	45	standard	1.6	ns	ns	ns	ns	ns	14.0	
TFA	35	standard	1.2	ns	ns	ns	ns	8.0	12.9	
TFA	25	standard	1.4	ns	2.5	3.3	6.7	8.3	12.8	
TFA	12	standard	1.3	1.7	2.5	3.3	ns	8.4	11.9	
TFA	6	standard	1.3	1.6	2.5	3.3	ns	8.4	11.5	
HFIP	30	standard	1.2	1.9	2.9	ns	ns	ns	6.2	4.2
HFIP	25	standard	1.2	1.9	2.9	ns	ns	ns	6.1	4.1
HFIP	12	standard	1.1	2.0	3.0	ns	5.9	ns	5.4	4.1
HFIP	6	standard	1.1	1.9	2.9	ns	6.0	ns	5.0	4.1

[a] $\delta_{^{1}H}$ relative to $\delta_{^{1}H}$ of external tetramethylsilane (Me$_4$Si) (=0 ppm); ^{1}H shifts accurate to ±0.2 ppm. [b] No signal.

ide/acid ratios go from ~1/11 in 6% (w/v) solution to ~1/2 in 30% (w/v) solution. Even as the amide/acid ratio approaches 1/1, only small changes in the ^{15}N shift data are expected since HFIP cannot form a complex like the amide/TFA system. The decrease in the HFIP concentration does cause a small increase in shielding of the ^{15}N nuclei. Studies of higher concentrations could not be done due to insolubility of higher concentrations of the nylon in HFIP.

At the lower nylon concentrations (<25% (w/v) in TFA), the degree of polymerization of nylon 66 (high amine content vs. standard) had little effect on the ^{15}N or ^{13}C chemical shifts. The ^{15}N signal of 35% standard nylon 66 solution is further upfield, however, than the ^{15}N signal of 45% high amine solution (45% standard solution gave no ^{15}N signal). This result is not accounted for by considering the neutralization of TFA by the basic amine end groups (amine/amide ratio is 1/16 for high amine sample and 1/66 for standard sample). The resulting amide/TFA ratios are calculated to be ~1/3 in the 45% high amine solution and ~1/4 in the 35% standard solution. The larger upfield shift of the ^{15}N signal in the latter would imply that formation of the amide/TFA complex is enhanced in the higher molecular weight sample. Further experiments to define the influence of degree of polymerization on complex formation are indicated.

The ^{1}H chemical shift data showed the least sensitivity to changes in concentration and degree of polymerization. The largest changes in the ^{1}H data were for the proton active in the protonation and/or hydrogen bonding. In TFA, $\Delta\delta_{^1\mathrm{H}}$ of the acidic proton over the concentration range (6–45%) was 2.6 ppm and in HFIP it was 1.2 ppm. The ^{1}H NMR spectrum for the 45% standard in TFA, however, showed extreme broadening, in support of fast proton exchange over the NH region (the 35% standard showed less broadening in the same region). In the 45% high amine sample in TFA solution, the NH signal was still present but was dominated by a peak at 7.1 ppm, which may be attributed to hydrogen bonding at the carbonyl oxygen. Thus, looking at the proton spectra, we can quickly determine if the cross-polarization techniques can be used in doing ^{15}N NMR spectroscopy.

Conclusions

The ^{15}N chemical shifts for nylon 66 in trifluoroacetic acid and hexafluoro-2-propanol solutions are more sensitive to changes in polymer concentration than corresponding ^{13}C data. The ^{15}N data exhibit deshielding with decreasing polymer concentration and with increasing solvent acidity. The site of hydrogen bonding and/or protonation in excess acid is the oxygen of the amide carbonyl as shown by the deshielding in the ^{15}N and ^{13}C data of the amide group. In the presence of excess acid, the degree of polymerization of nylon 66 has little effect on ^{15}N chemical shifts, while at higher nylon concentrations, there is an apparent change in the nature of the hydrogen bonding and/or protonation. As the amide/acid ratio approaches 1/1, there is an increase in the rate of proton exchange at the NH proton. This results from a possible complex formation between amide and TFA and/or a conformational change.

Utilization of the adiabatic *J* cross-polarization (AJCP) technique has provided major signal enhancements and time savings, has eliminated the need to exactly match the radio-frequency amplitudes of the two nuclei during polarization transfer, and, thus, has increased the practicality of ^{15}N NMR. The advantages of using ^{15}N NMR in conjunction with ^{1}H and ^{13}C NMR data in the elucidation of solute–solvent interactions are great, especially when the ^{15}N data are more responsive than the corresponding ^{1}H and ^{13}C data as they proved to be for the polyamide systems studied.

References and Notes

(1) Holmes, B. S.; Chingas, G. C.; Moniz, W. B.; Ferguson, R. C. *Macromolecules* **1981**, *14*, 1785.
(2) Schilling, G. S.; Kricheldorf, H. R. *Makromol. Chem.* **1975**, *176*, 3341.
(3) McClelland, R. A.; Reynolds, W. F. *J. Chem. Soc., Chem. Commun.* **1974**, 824.
(4) Kricheldorf, H. R.; Hull, W. E. *J. Polym. Sci., Polym. Chem. Ed.* **1978**, *16*, 583.
(5) Bertrand, R. D.; Moniz, W. B.; Garroway, A. N.; Chingas, G. C. *J. Am. Chem. Soc.* **1978**, *100*, 5227. *J. Magn. Reson.* **1978**, *32*, 465.
(6) Chingas, G. C.; Garroway, A. N.; Moniz, W. B.; Bertrand, R. D. *J. Am. Chem. Soc.* **1980**, *102*, 2526.
(7) Stewart, W. E.; Mandelkern, L.; Glick, R. E. *Biochemistry* **1967**, *6*, 150.

Small-Angle Neutron Scattering on Bulk Polystyrene with Mismatched M_w[†]

Celia Tangari,[‡] John S. King,* and George C. Summerfield

Department of Nuclear Engineering, The University of Michigan,
Ann Arbor, Michigan 48109.

Received August 5, 1981

ABSTRACT: Small-angle neutron scattering data have been obtained from a number of bulk polystyrene samples containing high concentrations of deuterated molecules. An equation was earlier derived for the SANS scattering cross section for bulk samples in which the deuterated and protonated molecules have modest differences in physical properties. The limits of validity of the equation are examined here by deliberate mismatch of molecular weights for PSD and PSH. The equation is found to fit the data for a mismatch ratio of molecular weights (M_w(PSD)/M_w(PSH)) between 0.32 and 1.94 but does not apply for ratios greater than 2.0.

Introduction

It has been demonstrated[1–4] that high-concentration marked (deuterated) samples can be used to measure the single-chain structure factor, with consequent high experimental intensities. The technique requires that the two polymer species be identical except for their neutron scattering lengths. It is difficult, even under ideal experimental conditions, to produce samples in which the two species are identical. They may differ in melting temperatures, in Θ temperatures, and particularly in degree of polymerization (molecular weight).

[†] Work supported in part by National Science Foundation Grant No. DMR-79-26254.

[‡] Sponsored by Comissao Nacional de Energia Nuclear/Brazil.

0024-9297/82/2215-0132$01.25/0