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# High-Resolution $^{15}N$ NMR Study of Solid Homopolypeptides by the Cross-Polarization–Magic Angle Spinning Method: Conformation-Dependent $^{15}N$ Chemical Shifts Characteristic of the $\alpha$-Helix and $\beta$-Sheet Forms

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ABSTRACT: Natural abundance 27.4-MHz $^{15}N$ NMR spectra of various homopolypeptides having right-handed $\alpha$-helix ($\alpha_R$-helix) and $\beta$-sheet conformations in the solid state were measured by using the cross-polarization–magic angle spinning (CP-MAS) technique. It was found that the $^{15}N$ chemical shifts in the peptide backbone of these polypeptides exhibit a significant conformation-dependent change. The $\alpha_R$-helix conformation absorbs upfield of the $\beta$-sheet ($\alpha_R$-helix form $\delta$ 97.0–99.2; $\beta$-sheet form $\delta$ 99.5–107.0). Furthermore, the $^{15}N$ chemical shift was found to be displaced by as much as 1.2–10.0 ppm between the $\alpha_R$-helix and $\beta$-sheet forms, depending on the nature of the amino acid residue.

## Introduction

$^{15}N$ NMR spectroscopy offers many possibilities for study on the structure and dynamics of synthetic polypeptides and natural proteins.[1-6] Recently, a high-resolution $^{15}N$ NMR technique in the solid state has been increasingly applied to the investigation of polypeptides, proteins, and biopolymers.[7-14] However, with the exception of the group of Kricheldorf,[10] little attempt has been made to relate the $^{15}N$ chemical shift to conformational features such as the secondary structure determined by the peptide bonds of the backbone. The secondary structure plays a particularly important role in such a system and enables us to test if the $^{15}N$ chemical shifts can be examined as a possible source of information about the microstructures of solid polypeptides and proteins with these spectra. This is because, in polypeptides and proteins, most of the nitrogen sites are in the amide linkage of the backbone and also because the structure and dynamics of the backbone strongly reflect the conformation and the flexibility of these macromolecules. Kricheldorf and co-workers have reported extensively that $^{15}N$ NMR in solution can be used to determine sequence distributions of polypeptides.[15-19] It should be mentioned that comparisons between the solid and solution $^{15}N$ NMR spectra give us some information concerning the conformational difference between the solid and solution states.

In previous investigations,[18-35] it has been demonstrated that the $^{13}C$ NMR chemical shifts of a number of polypeptides and proteins in the solid state as determined by the CP-MAS method are significantly displaced depending on their particular conformations, such as $\alpha$-helix, $\beta$-sheet, $3_1$-helix, collagen-like triple helix, $\omega$-helix, and so on. In particular, the $^{13}C$ chemical shifts of an individual amino acid residue in a peptide or protein are mainly influenced by the local conformation, as defined by the torsional angles ($\phi$ and $\psi$) of the skeletal bonds, and not strongly influenced by the specific amino acid sequence.[18,19,24-26,30] This view was supported by our theoretical calculations of the contour map of the $^{13}C$ chemical shift utilizing the finite perturbation (FPT)-INDO theory[23,27] and the sum-over-states tight-binding MO theory.[28,29] These approaches permit one to use the conformation-dependent $^{13}C$ chem-

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ical shift as an intrinsic probe to elucidate the conformational features in the solid states in terms of the individual amino acid residues under consideration.

Since a nitrogen atom possesses lone-pair electrons, it is of interest to know how the presence of the lone-pair electrons influences the $^{15}N$ chemical shift, which is a measure of the electronic structure. In addition, nitrogen shieldings are probably most useful for the investigation of molecular interactions, since they are expected to be sensitive to various types of interactions.

In order to test systematically the power of $^{15}N$ CP-MAS NMR for the structural analysis of polypeptides and proteins in the solid state, in this paper, we have studied various kinds of model homopolypeptides such as poly(L-alanine), poly(L-leucine), poly($\beta$-benzyl L-aspartate), poly($\gamma$-benzyl L-glutamate), poly($\gamma$-methyl L-glutamate), poly(L-valine), and poly(L-isoleucine), which show characteristic differences in conformation.

## Experimental Section

**Materials.** Poly(L-leucine) (PLLeu-100), poly($\gamma$-benzyl L-glutamate) (PBLGlu-100), poly($\gamma$-methyl L-glutamate) (PMLGlu-100), poly(L-valine) (PLVal-100), and poly(L-isoleucine) (PLIle-100) were prepared by polymerizing the *N*-carboxyl anhydrides (NCAs) of corresponding amino acids in acetonitrile at 30 °C with n-butylamine as initiator (monomer and initiator ratio $A/I = 100$).

Poly($\beta$-benzyl L-aspartate) (PBLAsp-5, low molecular weight) was prepared from $\beta$-benzyl L-aspartate NCA in acetonitrile at 30 °C with *n*-butylamine as initiator ($A/I = 5$). Poly($\beta$-benzyl L-aspartate) (PBLAsp-100) was prepared from $\beta$-benzyl L-aspartate NCA in dichloroethane at 30 °C with triethylamine as initiator ($A/I = 100$). The sample of poly($\beta$-benzyl L-aspartate) (PBLAsp-100-III, $\beta$-sheet form) was obtained by heating at 220 °C for 1 h in vacuo by PBLAsp-100 film casting from the chloroform solution.

The Nps-[L-Glu(OMe)]$_4$-OH was prepared by fragment condensation of Nps-[L-Glu(OMe)]$_2$-ONSu with HCl·H-[L-Glu(OMe)]$_2$-OH in a tetrahydrofuran/aqueous $NaHCO_3$ system, by a method similar to that described previously.[36,37] The tetrapeptide was obtained as a purified solid after gel filtration (Sephadex G-10) in dimethyl sulfoxide.

The syntheses of poly(L-alanine) (PLAla-50), Z-(L-Ala)$_7$-NHBu, Z-(L-Leu)$_6$-OEt, and Nps-[L-Glu(OBzl)]$_6$-NHBu have been described previously.[21,25,38]

The conformational characterization of these homopolypeptides was made on the basis of conformation-dependent $^{13}C$ NMR chemical shifts determined from the $^{13}C$ CP-MAS method and also from the IR and far-IR spectra.

**$^{15}N$ and $^{13}C$ NMR Measurements.** The $^{15}N$ and $^{13}C$ CP-MAS NMR measurements were performed on a JEOL GX-270 spectrometer operating at 27.4 and 67.80 MHz, respectively, equipped with a CP-MAS accessory. A contact time of 2 ms was chosen and the repetition time was 5 s. A 90° pulse width was typically 5.7 $\mu$s for both $^{15}N$ and $^{1}H$ under CP conditions and 5.3 $\mu$s for both $^{13}C$ and $^{1}H$. Spectral width and data points were 20 kHz (for $^{15}N$) and 27 kHz (for $^{13}C$) and 8K points, respectively. Spectra were usually accumulated ca. 200–10 000 times to achieve a reasonable signal-to-noise ratios for natural abundance samples. The $^{15}N$ chemical shifts were calibrated indirectly through external glycine-$^{15}N$ ($\delta$ 11.59; line width = 17 Hz) relative to saturated $^{15}NH_4NO_3$ ($\delta$ 0) solution in $H_2O$. $^{13}C$ chemical shifts were calibrated indirectly through external adamantane [29.5 ppm relative to tetramethylsilane ($(CH_3)_4Si$)]. The experimental errors of the $^{15}N$ and $^{13}C$ chemical shift values are estimated to be within ±0.5 and ±0.2 ppm, respectively.

**$^{15}N$ Chemical Shift Calculation.** In this work, we calculated relative $^{15}N$ NMR chemical shifts (isotropic magnetic shielding constants) of a dipeptide fragment, *N*-acetyl-L-alanine methylamide (forming hydrogen bonds with two formamide molecules), employing the FPT-INDO method, as shown details in a previous paper.[23] The structural data, including the distance between nitrogen and oxygen atoms, 2.83 and 2.86 Å for the $\beta$-sheet and $\alpha_R$-helix forms, respectively, were taken from X-ray diffraction studies of poly(L-alanine) by Arnott et al.[39-41]

A Hitachi M200H computer at the Computer Center of the Tokyo Institute of Technology was used for the $^{15}N$ chemical shift calculation.

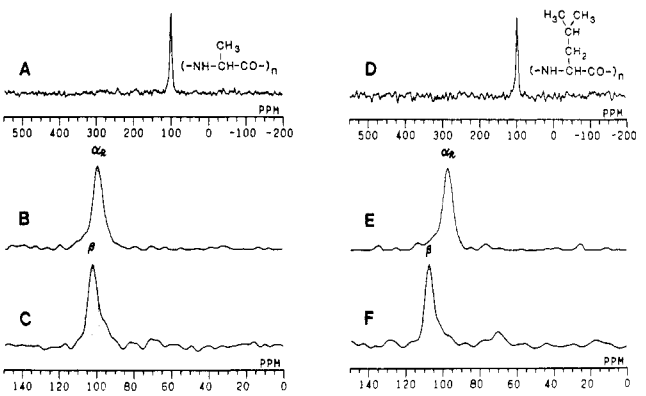


**Figure 1.** Natural abundance 27.4-MHz $^{15}N$ CP-MAS NMR spectra of poly(L-alanines) and poly(L-leucines) in the solid state. (A and B) PLAla-50 ($\alpha_R$-helix form, 2874 scans); (C) Z-(L-Ala)$_7$-NHBu ($\beta$-sheet form, 1927 scans); (D and E) PLLeu-100 ($\alpha_R$-helix, 850 scans); (F) Z-(L-Leu)$_6$-OEt ($\beta$-sheet, 1142 scans).

## Results and Discussion

**Conformation-Dependent $^{15}N$ Chemical Shifts.** Figure 1 shows natural abundance 27.4-MHz $^{15}N$ CP-MAS NMR spectra of poly(L-alanines) and poly(L-leucines) in the solid state. It is noteworthy that a sharp resonance due to main-chain peptide nitrogen-15 of both homopolypeptides appears around 100 ppm with good sensitivity by the CP-MAS technique despite of low natural abundance of $^{15}N$. This good sensitivity of the $^{15}N$ resonance is due to the large ratio of $|\gamma_{^1H}/\gamma_{^{15}N}|$ (where $\gamma$ is gyromagnetic ratio) and to the short proton $T_1$.[42] Thus, it now seems possible to examine the relation between the $^{15}N$ chemical shift and the conformation of natural abundance polypeptides in the solid state with these spectra. As shown in Figure 1, the resonance signal appears at 98.6 ppm for PLAla-50 ($\alpha_R$-helix form) and at 101.8 ppm for Z-(L-Ala)$_7$-NHBu ($\beta$-sheet form), respectively. The signal appears at 97.0 ppm for PLLeu-100 ($\alpha_R$-helix) and at 107.0 ppm for Z-(L-Leu)$_6$-OEt ($\beta$-sheet), respectively. Thus, the $^{15}N$ chemical shift of the $\alpha_R$-helix form appears at higher field than that of the $\beta$-sheet form in both homopolypeptides. Moreover, the $^{15}N$ chemical shift displacements between these two different conformations in poly(L-alanines) and poly(L-leucines) were 3.2 and 10.0 ppm, respectively, which are obviously significant. Accordingly, it is clear that this $^{15}N$ chemical shift displacement is mainly related to conformational changes.

To confirm this point, we have further studied the $^{15}N$ chemical shifts of other typical homopolypeptides having the $\alpha_R$-helix form and/or the $\beta$-sheet form in the solid state. Table I summarizes the $^{15}N$ chemical shifts for various homopolypeptides in the solid state obtained in this work by 27.4-MHz $^{15}N$ CP-MAS NMR, together with the results on their conformational analysis determined by the $^{13}C$ CP-MAS NMR, IR, and far-IR methods. As expected, the $^{15}N$ chemical shifts for the $\alpha_R$-helix form of all homopolypeptides studied here appear at higher field than those for the $\beta$-sheet form. Interestingly, the $^{15}N$ chemical shifts of the solid homopolypeptides having an $\alpha_R$-helix form were in range of 97.0–99.2 ppm, and those having the $\beta$-sheet form were in the range of 99.5–107.0 ppm. Although the $^{15}N$ chemical shift difference is rather small, we feel on safe grounds for homopolypeptides. The applicability to copolypeptides has still to be investigated. The present result suggests that there is significant con-

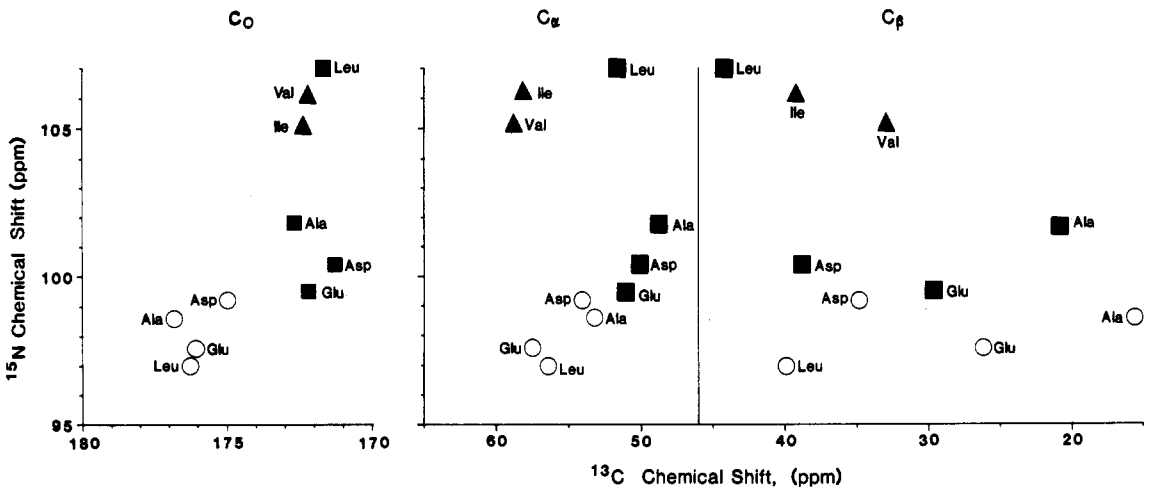


**Figure 2.** Relation between $^{15}$N chemical shift and $^{13}$C chemical shift (C=O, $C_\alpha$, and $C_\beta$) of various homopolypeptides in the solid state; open symbol (○) and closed symbols (■, ▲) denote the $\alpha_R$-helix form and the $\beta$-sheet form, respectively.

**Table I**
**Comparison of $^{15}$N Chemical Shifts of $\alpha_R$-Helix and $\beta$-Sheet Forms of Some Homopolypeptides in the Solid State[a] (ppm from $^{15}NH_4NO_3$, ±0.5 ppm)**

| sample[a] | conformation[b] | $^{15}$N δ | Δδ[c] |
|---|---|---|---|
| PLA1a-50 | $\alpha_R$-helix | 98.6 | −3.2 |
| Z-(L-Ala)$_7$-NHBu | $\beta$-sheet | 101.8 | |
| PLLeu-100 | $\alpha_R$-helix | 97.0 | −10.0 |
| Z-(L-Leu)$_6$-OEt | $\beta$-sheet | 107.0 | |
| PBLAsp-100 | $\alpha_R$-helix | 99.2 | −1.2 |
| PBLAsp-5 | $\beta$-sheet | 100.4 | |
| PBLAsp-100-III | $\beta$-sheet | 100.4 | |
| PBLGlu-100 | $\alpha_R$-helix | 97.6 | −1.9 |
| Nps-(L-Glu(OBzl))$_6$-NHBu | $\beta$-sheet | 99.5 | |
| PMLGlu-100 | $\alpha_R$-helix | 97.6 | −1.9 |
| Nps-(L-Glu(OMe))$_4$-OH | $\beta$-sheet | 99.5 | |
| PLVal-100 | $\beta$-sheet | 105.9 | |
| PLIle-100 | $\beta$-sheet | 106.1 | |

[a] Abbreviations: Ala, alanine; Leu, leucine; BLAsp, β-benzyl L-aspartate; BLGlu, γ-benzyl L-glutamate; MLGlu, γ-methyl L-glutamate; Val, valine; Ile, isoleucine; Z, benzyloxycarbonyl; Bu, butyl; Et, ethyl; Nps, (o-nitrophenyl)sulfenyl; Bzl, benzyl; Me, methyl. [b] Conformations of the samples were determined by the IR, far-IR, and $^{13}$C CP-MAS NMR spectroscopic methods. [c] Differences of the $^{15}$N chemical shifts of the $\alpha_R$-helix relative to those of the $\beta$-sheet form.

formational dependence of $^{15}$N chemical shifts. In addition, it is seen in Table I that the $^{15}$N chemical shift value of PBLAsp-5 (low molecular weight; $\beta$-sheet form) and that of PBLAsp-100-III (high molecular weight; $\beta$-sheet form) are identical, which indicates that the $^{15}$N chemical shift of solid polypeptide is not dependent on chain length. Accordingly, the $^{15}$N chemical shift could be principally used for observation of the main-chain conformations of solid homopolypeptides.

**Amino Acid Sequence Dependent $^{15}$N Chemical Shifts.** In order to clarify the unique $^{15}$N chemical shift displacement, we compared the $^{15}$N chemical shift data with the $^{13}$C chemical shift data. Figures 2 and 3 show the relation between the $^{15}$N chemical shifts and the $^{13}$C chemical shifts (C=O, $C_\alpha$, and $C_\beta$ peaks) and the relation between relative $^{15}$N chemical shift difference and relative $^{13}$C chemical shift differences of the $\alpha_R$-helix with reference to the $\beta$-sheet form, respectively.

As reported previously,[18-35] the $^{13}$C chemical shifts for the C=O, $C_\alpha$, and $C_\beta$ peaks are conformation dependent. That is to say, the main chain C=O and $C_\alpha$ peaks in the $\alpha_R$-helix form are generally displaced downfield with respect to those in the $\beta$-sheet form, while the side-chain $C_\beta$ peak of the $\alpha_R$-helix is displaced upfield with respect to

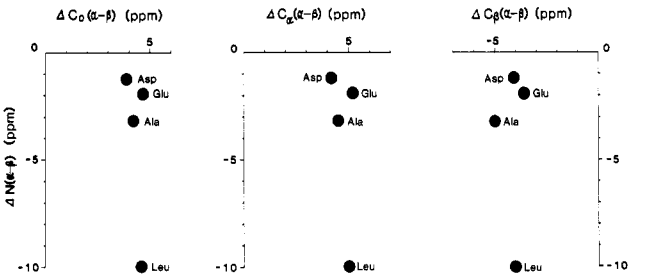


**Figure 3.** Relation between relative $^{15}$N chemical shift difference and relative $^{13}$C (C=O, $C_\alpha$, and $C_\beta$) chemical shift difference of the $\alpha_R$-helix form with reference to those of the $\beta$-sheet form for various homopolypeptides in the solid state.

that of the $\beta$-sheet form. The absolute $^{13}$C chemical shifts of the C=O and $C_\alpha$ carbons are not strongly affected by the structure of the individual amino acid residues, whereas the $C_\alpha$ peaks of the $\beta$-sheet form of poly(L-valine) and poly(L-isoleucine), in which the $C_\beta$ carbon is disubstituted, are displaced downfield by ca. 8–10 ppm in comparison with that of the other amino acid residues. On the other hand, the absolute $^{13}$C chemical shifts of the $C_\beta$ carbons are strongly affected by the chemical structure of the individual amino acid residues. Thus, absolute $^{13}$C chemical shifts give us much information about the conformation and the chemical structure of individual amino acid residues. However, $^{13}$C chemical shift differences for the C=O, $C_\alpha$, and $C_\beta$ peaks between the $\alpha_R$-helix and $\beta$-sheet forms were almost identical (ca. 4–5 ppm). Moreover, the variations of the $^{13}$C chemical shift difference are very small (1.0–1.5 ppm), and they are therefore almost independent of the structure of the individual amino acid residues at the present stage of the $^{13}$C CP-MAS NMR resolution.

On the other hand, it is notable, as is seen in Figures 2 and 3, that the $^{15}$N chemical shift of the $\alpha_R$-helix form appears at higher field by ca. 1.2–10.0 ppm than those of the $\beta$-sheet form of the same homopolypeptides and is obviously dependent on the structure of individual amino acid residues. Moreover, the variations of the $^{15}$N chemical shifts for various kinds of homopolypeptides are ca. 2.5 ppm in the $\alpha_R$-helix form and ca. 7.5 ppm in the $\beta$-sheet form. It is noteworthy that the $^{15}$N chemical shifts of the $\beta$-sheet form of the L-Leu, L-Val, and L-Ile residues, which possess hydrophobic side chains, appear downfield from that of the L-Ala residue; on the other hand, those of the L-Asp(OBzl), L-Glu(OBzl), and L-Glu(OMe) residues, which possess side-chain esters, appear upfield from that of the L-Ala residue. These results indicate that the $^{15}$N chemical

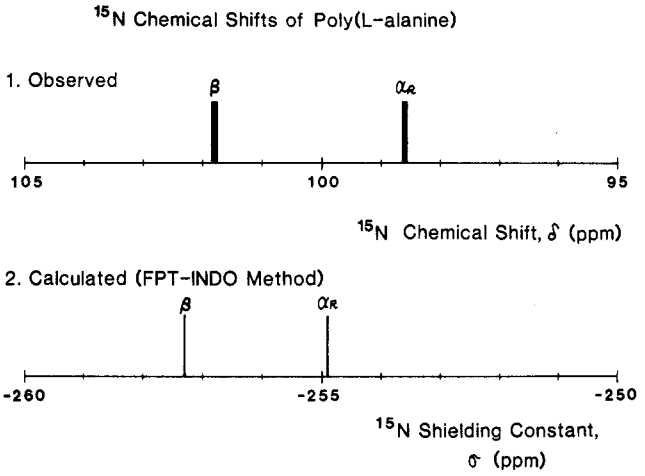


**Figure 4.** Observed $^{15}N$ chemical shift diagram of poly(L-alanine) in the solid state (A) and calculated $^{15}N$ shielding constant diagram of *N*-acetyl-L-alanine methylamide (taking hydrogen bonds with two formamide molecules), as a dipeptide model of poly(L-alanine), by means of the FPT-INDO method (B).

shift differences between the $\alpha_R$-helix and $\beta$-sheet forms are dependent on the side-chain structure of individual amino acid residues.

Förster et al.[10] have recently published a preliminary report on the observation of the secondary structure of solid polypeptides by $^{15}N$ CP-MAS NMR. They assumed that the $^{15}N$ chemical shift difference between the $\alpha_R$-helix and $\beta$-sheet forms is independent of the amino acid residues of homopolypeptides, from the results obtained for poly(L-leucine), poly(DL-leucine), and poly(L-phenylalanine). Our experimental results do not support their assumptions, as described above. Accordingly, $^{15}N$ chemical shifts may be very effective for the elucidation of the chemical structure, especially for the amino acid sequence analysis, as well as for the conformational analysis of polypeptides. However, to draw such a conclusion, it is necessary to get more informations on the $^{15}N$ chemical shifts for the secondary structures other than the $\alpha_R$-helix and $\beta$-sheet forms. Therefore, further $^{15}N$ NMR studies on conformation and amino acid sequence of copolypeptides in the solid state are needed, which will be published elsewhere.

**Theoretical Calculation of $^{15}N$ Shielding Constants for Poly(L-alanine).** In order to support the view that $^{15}N$ chemical shifts are conformation dependent, theoretical calculation of $^{15}N$ chemical shifts is required, on the basis of the electronic states derived by quantum chemical methods.

We have already calculated the theoretical $^{13}C$ chemical shifts (isotropic magnetic shielding constants) of the C=O, $C_\alpha$, and $C_\beta$ carbons of *N*-acetyl-L-alanine methylamide as a dipeptide model of poly(L-alanine) by means of the finite perturbation INDO (FPT-INDO) theory.[23] As a result, the calculated $^{13}C$ chemical shifts are found to exhibit conformation-dependent changes comparable with the experimental data. Furthermore, in theoretical calculations of the $^{13}C$ chemical shifts of the C=O and $C_\alpha$ carbons, it was also found that it is essential to take into account the intra- or intermolecular hydrogen bonds.

Figure 4 shows in diagrammatic form the observed $^{15}N$ chemical shift for poly(L-alanine) and the $^{15}N$ shielding constants (chemical shifts) for *N*-acetyl L-alanine methylamide calculated by the FPT-INDO method. Note that the calculated $^{15}N$ shielding constants are expressed as negative values, in contrast to the experimental $^{15}N$ chemical shifts, which refer to saturated $^{15}NH_4NO_3$ solutions in $H_2O$; smaller negative values correspond to the upfield shift, whereas larger negative values correspond to the downfield shift. As shown in Figure 4, the calculated $^{15}N$ chemical shifts for the $\alpha_R$-helix and $\beta$-sheet forms of poly(L-alanine) were -254.9 and -257.3 ppm, respectively; the chemical shift in the $\alpha_R$-helix form appears upfield by 2.4 ppm with respect to that of the $\beta$-sheet form. This calculated $^{15}N$ chemical shift displacement is qualitatively in good agreement with the observed results. Accordingly, it may be concluded that the $^{15}N$ chemical shifts of poly(L-alanine) are dependent on conformation and that the conformation can be interpreted mainly in terms of the changes of the electronic structure.

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**Registry No.** z-(L-Ala)$_7$-NHBu, 109721-21-3; z-(L-Leu)$_6$-OEt, 107868-02-0; Nps-(L-Glu(OBzl))$_6$-NHBu, 109721-22-4; Nps-(L-Glu(OMe))$_4$-OH, 109721-23-5; L-Leu NCA, 3190-70-3; L-Glu(OBzl) NCA, 3190-71-4; L-Glu(OMe) NCA, 1663-47-4; L-Ile NCA, 45895-90-7; L-Asp(OBzl) NCA, 13590-42-6; Nps-(L-Glu(OMe))$_2$-ONSu, 109721-24-6; H-(L-Glu(OMe))$_2$-OH·HCl, 109721-25-7; poly(L-leucine), 25248-98-0; poly(L-leucine) (SRU), 25322-63-8; poly(γ-benzyl L-glutamate), 25014-27-1; poly(γ-benzyl L-glutamate) (SRU), 25038-53-3; poly(γ-methyl L-glutamate), 25086-16-2; poly(γ-methyl L-glutamate) (SRU), 25036-43-5; poly(L-valine), 25609-85-2; poly(L-valine) (SRU), 25667-19-0; poly(L-isoleucine), 34464-35-2; poly(L-isoleucine) (SRU), 33220-75-6; poly(β-benzyl L-aspartate), 25248-99-1; poly(β-benzyl L-aspartate) (SRU), 25736-41-8; poly(L-alanine), 25191-17-7; poly(L-alanine) (SRU), 25213-34-7.

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# Comparison of Multicomponent Gas Chromatography Measurements of Vapor-Liquid Equilibrium with Static Measurements Using a Polymer/Two Solvent Ternary System

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ABSTRACT: Perturbation gas-liquid chromatography (GLC) was used to determine the equilibrium properties of the ternary cyclohexane/polybutadiene (PBD)/benzene system at finite concentrations for temperatures of 319.30, 333.15, and 348.15 K. Calculations using the multicomponent chromatography theory of Glover and Lau[1] showed an opposite temperature dependence for GLC-based solvent/solvent parameters compared with binary vapor-liquid equilibrium based solvent/solvent parameters. This confirms the trend previously reported by Ruff et al.[2] for the same system as in the present work. Static sorption data were also collected at 302.35, 319.30, and 333.15 K. Binary data for the benzene/PBD and cyclohexane/PBD systems were also obtained by both methods at the same temperatures. Excellent agreement was found between the two sets of binary data, reinforcing the utility of the GLC method to obtain polymer/solvent vapor-liquid equilibria. A comparison of calculated equilibria compositions using GLC-based parameters and the multicomponent chromatography of Glover and Lau[1] gave reasonable agreement with the ternary static sorption data at 302.35 and 319.30 K; however, due to the difficulties involved in these experiments, no definitive conclusion may be drawn from the data as to the adequacy of the multicomponent chromatography theory of Glover and Lau.[1]

## Introduction

Numerous measurements of binary systems have been made by both chromatographic and static methods. Although some studies have indicated that agreement between the two methods is not always achieved, it is generally accepted that, in principle, gas-liquid chromatography (GLC) can be used to obtain accurate binary vapor-liquid equilibrium (VLE) when proper precautions are taken with experimental technique. Recently, Glover and Lau[1] extended the theory of perturbation chromatography (also called elution-on-a-plateau or step-and-pulse chromatography) to multicomponent systems. This work examines the results of the theory to calculate ternary GLC-based parameters and discusses the experimental difficulties in obtaining static ternary data.

**Development of Chromatographic Techniques for Binary Systems.** The use of GLC for the measurement of equilibrium properties of polymer/solvent systems was established by Guillet and co-workers.[3-5] Early experimental work by Schreiber and co-workers[6-8] justified the use of the GLC technique for equilibrium measurements by showing good agreement between GLC and equilibrium sorption (static) data. This work also established guidelines for the useful application of the GLC method to polymer solution thermodynamics.

In contrast to the work of Schreiber et al.,[8] several authors have reported discrepancies between static and GLC results. Bonner[9] notes systematic differences between extrapolated finite concentration static and infinite dilution GLC measurements. Lichtenthaler, Newman, and Prausnitz[10a] followed by Lichtenthaler, Prausnitz, Su, Schreiber, and Patterson[10b] reported notable disagreement between static and GLC-based activity coefficients as well as between retention volumes. Chang and Bonner,[11a] in examining the effects of polymer degradation on GLC results, found only fair agreement with other workers. In another article, Chang and Bonner[11b] note good agreement of GLC measurements with static measurements except at low benzene concentrations. Sharma and Lakhanpal[12] obtained correct qualitative predictions based on Flory's equation of state theory, but did not obtain quantitative agreement between theory and experiment.

Ashworth, Laub, et al.[13] attribute differences between static and GLC activity coefficients and interaction pa-

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