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Relative Chemical Shifts for Obtaining Accurate Chemical Shifts of Hydrogen Atoms in CH Groups

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(Dated: Received on September 4, 2013; Accepted on November 12, 2013)

Accurate chemical shifts of hydrogen atoms in CH groups are difficult to obtain. To solve this problem, relative chemical shifts are introduced. Internal and external standard methods were used to measure the chemical shifts in a whole-concentration of *N*-methylacetamide-water system. Determination of the chemical shifts of hydrogen atoms, especially those of CH groups, according to the two methods yielded significant differences. Relative chemical shifts were proven to be independent of the reference and may be applied to other systems.

Key words: Relative chemical shift, CH group, Internal and external method

I. INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is a highly powerful technique to investigate structures and interactions in mixtures [1−10]. However, spectral data over the entire composition range of binary mixtures, especially in aqueous mixtures, are limited. Obtaining accurate concentration- and temperature-dependent chemical shifts is difficult [11−13]. NMR measurements are usually obtained by internal and external standard methods. The chemical shifts of some aqueous solutions have been measured by the external reference method [14−17]. This method, especially the external double reference method, can obtain generally accurate chemical shifts. In this method, the reference is separated from the sample solution; thus, no interactions occur between the sample and the reference. However, the values of the chemical shifts must be corrected for magnetic susceptibility [18]. The method also requires special devices and experimental skills. The internal reference method is often adopted to qualitatively measure the concentration- and temperature-dependence of chemical shifts [19−22]. In this method, the reference is added into the sample solution, and the values of the chemical shifts do not need to be corrected. However, the reference can interact with the sample, which will probably affect the chemical shifts. Thus, neither the internal nor the external reference methods can determine accurate chemical shifts.

The chemical shifts of hydrogen atoms, which can form traditional strong hydrogen bonds, are greatly affected by temperature or concentration. These changes are not significantly affected by different measurement methods. For hydrogen atoms in CH groups, changes in chemical shifts with temperature or concentration are minimal. These changes are significantly affected by the used method. Accurate chemical shifts of alkyl protons are difficult to obtain. Thus, an approach to obtain accurate chemical shifts in CH groups is necessary.

In the present work, we use the internal and external reference methods to measure the chemical shifts of a whole-concentration *N*-methylacetamide (NMA)-water mixture. This mixture is extensively investigated as a model of peptide bonds at different temperatures [23−25].

II. EXPERIMENTS

Chemical shifts were measured using a Bruker DMX 500 spectrometer operating at 500 MHz at different temperatures and with an accuracy of ±0.1 K. Both internal and external methods were used in the NMR experiment. In the internal reference method, a 2 mm capillary tube, in which deuterated dimethyl sulfoxide (DMSO-d_6) was sealed, was placed at the center of a 5 mm sample tube filled with the chemical shift reference, sodium 2,2-dimethyl-2-silapentane-5-sulfonate, and the sample solution. In the external reference method, a 2 mm capillary tube, in which DMSO-d_6 and tetramethylsilane were sealed, was placed inside the 5 mm tube filled with the sample solution. The ^{1}H NMR spectra of the NMA-water mixture at temperatures of 308, 323, and 338 K were measured using the internal and external reference methods.

III. RESULTS AND DISCUSSION

A. Chemical shifts using two methods

Different types of hydrogen atoms are present in an NMA molecule. NMA molecules contain amide hydro-

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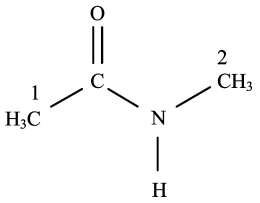

FIG. 1 Structure of NMA molecule.

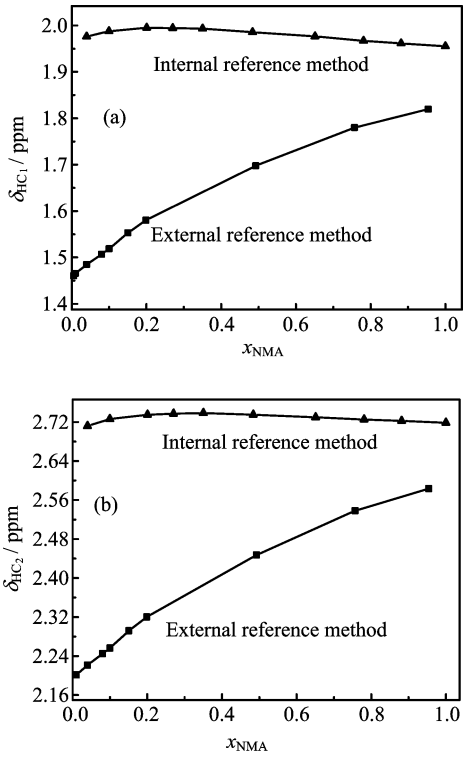

FIG. 2 Chemical shifts of different alkyl hydrogen atoms in the NMA-water mixtures against x_{NMA} at 308 K by two different methods. (a) Chemical shifts of HC_1 atom (δ_{HC_1}). (b) Chemical shifts of HC_2 atom (δ_{HC_2}).

gen atoms and CH groups. Figure 1 shows the structure of an NMA molecule, the definitions of the hydrogen atom symbols are as follows: HN is the amide hydrogen atom in NMA, HC_1 is hydrogen atoms in CH group connected with C=O, and HC_2 is hydrogen atoms in CH group connected with amide. Hydrogen atoms in the amide group can form traditional hydrogen bonds, and hydrogen atoms in the CH group can form weak C−H···O bonds [26−30]. Hydrogen bonding interactions are sensitive to temperature and concentration. The chemical shifts of good donors, such as amide protons, change greatly with the concentration, and the effect of the reference may be ignored. However, the change in chemical shifts of alkyl protons is minimal over the entire concentration measured. Strong hydrogen bonds lead to greater shifts than weak hydrogen bonds.

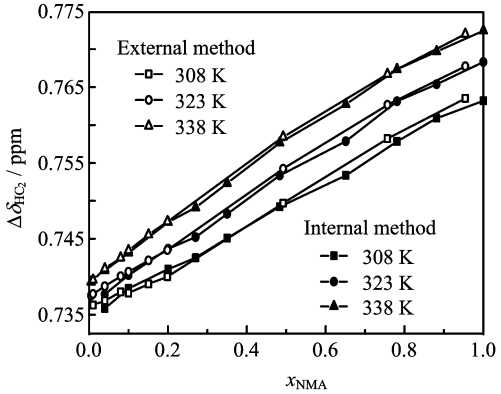

FIG. 3 Relative chemical shifts with temperature dependence of the HC_2 atom ($\Delta\delta_{HC_2}$) by the two methods.

Figure 2 shows the chemical shifts of alkyl protons obtained using the internal and external reference methods against concentration at a temperature of 308 K. The chemical shifts obtained using the external reference methods are not corrected for magnetic susceptibility. The chemical shifts of alkyl protons (δ_{HC_2} and δ_{HC_1}) show significant variations in values and tendencies. These results imply that the used method greatly affects the value of chemical shifts in CH groups. Finding an approach to solve this problem is thus necessary.

B. Relative chemical shifts in NMR

To avoid variations in chemical shifts obtained using different methods, the relative chemical shift is used. This shift is defined as follows:

$$\Delta\delta = \delta - \delta_{\mathrm{ref}} \tag{1}$$

where $\Delta\delta$ is the relative chemical shift, δ is the direct value of the chemical shifts obtained in the NMR experiment, and δ_{ref} denotes the chemical shifts of the reference standard. The chemical shifts of alkyl protons can be easily affected by changes in the reference. Compared with the chemical shifts of the HC_1 atom, the relative chemical shifts ($\Delta\delta_{HC_1}$) are independent of the reference because the HC_1, HC_2, and amide hydrogen atoms are in the same NMA molecule. Thus, δ_{HC_1} was used as a reference standard in this work.

Figure 3 presents the effects of concentration and temperature on the relative chemical shifts of alkyl protons ($\Delta\delta_{HC_2}$) determined using the external and internal reference methods. Values of $\Delta\delta_{HC_2}$ obtained using the two methods against concentration with temperature dependence are close. The relative chemical shifts of amide hydrogen atoms ($\Delta\delta_{HN}$) are shown in Fig.4. The deviations between the two methods of both $\Delta\delta_{HC_2}$ and $\Delta\delta_{HN}$ are less than 0.005 ppm, and the precision of the NMR apparatus is ±0.005 ppm. Thus, variations in the relative chemical shifts of the HC_2 atoms are independent of the reference.

DOI:10.1063/1674-0068/27/02/240-242

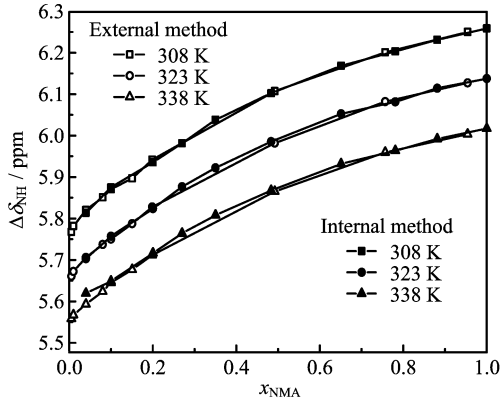

FIG. 4 Relative chemical shifts with temperature dependence of the amide hydrogen atom ($\Delta\delta_{NH}$) by the two methods.

IV. CONCLUSION

External and internal reference methods are used to obtain the chemical shifts of the hydrogen atoms in an NMA-water system. The chemical shifts obtained using the two methods exhibit very different values and tendencies. Relative chemical shifts are thus introduced to solve these variations, and the chemical shifts of HC_1 atoms are used as a reference standard. The deviations of the relative chemical shifts between the two methods are less than 0.005 ppm. Changes in the relative chemical shifts of the HC_2 atoms are independent of the reference. If the chemical shifts of one hydrogen atom are chosen as a reference standard, the relative chemical shifts of other hydrogen atoms in the same molecule will be independent of this reference. Relative chemical shifts solve the problem of variations in chemical shifts obtained using different measurement methods.

V. ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No.20903026), the Talents Introduction Foundation for Universities of Guangdong Province (No.2011), Scientific Research Foundation of the Natural Science Foundation of Guangdong Province, China (No.S2011010002483), and Science and Technology Planning Project of Guangzhou (No.2013J4100071).

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DOI:10.1063/1674-0068/27/02/240-242