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References for NMR Chemical Shift Measurements in Cyclodextrin Solutions

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Sodium methanesulfonate and sodium methyl sulfate are good internal references for chemical shift determination in aqueous solutions containing cyclodextrin and anionic guests. The chemical shift, referred to external standard and corrected by adding a term proportional to the cyclodextrin concentration, is in excellent agreement with that obtained using internal standard.

Chemical shifts are usually measured by using an external or internal standard.[1] The chemical shifts of cyclodextrin (CD) protons referred to external standard change with increasing CD concentration.[2,3] This change was ascribed to the self-association of CD in aqueous solutions and was corrected for determination of binding constants.[2] Furthermore, the chemical shift δ of tetramethylammonium (TMA) chloride referred to external sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) decreases linearly with increasing CD concentration.[4] Because DSS is included into the cavity of CD, TMA and methanol (MeOH) were recommended as internal standards for such systems.[4] There is no general agreement about the best standard among researchers of CD,[5] and this uncertainty may also be an important issue for other researchers.[1,5,6]

In this work we investigate better internal standards in CD solutions and demonstrate that our method for correcting chemical shifts referred to external standard is valid.[3]

Experimental

Materials. Commercial samples of TMA chloride (Nacalai Tesque Co.), MeOH (Wako Pure Chemicals Co.), sodium methanesulfonate (MS, Aldrich), sodium methyl sulfate (MeS, Aldrich), 99.9 atom% D deuterium oxide (Aldrich), sodium 3-(trimethylsilyl)2,2,3,3-tetradeuteropropionate (TSPd_4, Aldrich), DSS (Nacalai Tesque Co.), α-CD (Nacalai Tesque Co.), β-CD (Nacalai Tesque Co.), and γ-CD (Nacalai Tesque Co.) were used as received. Sodium benzenesulfonate (BS, Tokyo Kasei Organic Chemicals Co.) was recrystallized from a 50%–50% mixture of water and ethanol.

NMR Measurements. For the internal standards of TMA, MS, MeS, and MeOH, all 500 MHz ^{1}H NMR experiments were carried out in deuterium oxide at 298.2±0.5 K. The concentrations of these standards were kept constant at 0.1 mmol dm^{-3}. The NMR spectra were obtained with a JEOL Lambda 500 spectrometer. In reference to internal TSPd_4, the chemical shifts of internal secondary standards of MeOH, MeS, and MS were determined to be 3.343, 3.742, and 2.815 ppm, respectively. For the external reference of DSS, all 300 MHz ^{1}H NMR spectra were recorded with a Varian XL-300 NMR spectrometer at 294.2±0.5 K. All obtained spectra were deconvoluted with Nuts NMR data-processing software (Acorn NMR Inc.).

Results and Discussion

A convenient reference signal is one that is sharp and well separated from other signals in the NMR spectrum. The use of an internal reference signal has the advantage that no bulk-susceptibility corrections are necessary. These procedures are satisfactory only when specific solvent and solution effects are unimportant.[1]

The chemical shifts of three aromatic protons of BS, referred to internal standards of TMA, MeOH, HDO, MS, and MeS, were determined at the BS concentrations of 1, 5, 10, 15, and 20 mmol dm^{-3}. The peaks of MeS and MS gave sharp singlets at δ = 3.742 and 2.815 ppm, respectively. Regardless of the kinds of protons, the chemical shift referred to TMA increased almost linearly to increasing BS concentration with a slope of 0.42 ppm dm^3 mol^{-1}, whereas that referred to the others remained unchanged. Because BS is an anion, it can form a 1 : 1 ion-pair with TMA, a cation. On the other hand, because MeS and MS are anions, they will not form such ion-pairs with BS.

If MS and MeS form complexes with CDs, α-CD will have a larger binding constant than β-CD and γ-CD. Thus, we recorded the NMR spectra for 50 and 100 mmol dm^{-3} α-CD solutions containing MS or MeS as the internal reference. The chemical shifts of the CD protons and the half-widths of the peaks of MS and MeS did not change with the addition of α-CD within experimental errors. These results indicate that MS and MeS are not incorporated into the α-CD cavity. Thus, we recommend MS, MeS, and MeOH as internal standards for NMR studies of CD inclusion of anionic guests.

Alston et al. suggested that the dependence of the chemical shift, referred to external standard, on the α-CD concentration is due to the self-association of α-CD.[2] If α-CD forms a dimer, the observed chemical shift δ of a CD proton can be written as

$$\delta=\{[(1+8K_\mathrm{d}C_\mathrm{D})^{1/2}-1]\delta_1+[1+4K_\mathrm{d}C_\mathrm{D}-(1+8K_\mathrm{d}C_\mathrm{D})^{1/2}]\delta_2\}/4K_\mathrm{d}C_\mathrm{D} \tag{1}$$

Here K_d is the dimerization constant, C_D is the α-CD concentration, and δ_1 and δ_2 stand for the chemical shifts of monomer and dimer. According to Eq. 1, the observed chemical shift should change non-linearly with increasing CD concentration.

Referring to external DSS, we determined the chemical

Table 1. Slopes (ppm dm^3 mol^{-1}) per Glucose Unit of Linear Regression Lines in Plots of Chemical Shift Variations against CD Concentrations

Saccharide	CDav	TMA[a]	MeOH[a]	HDO[a]
α-CD	0.070	0.070	0.067	0.081
β-CD	0.073	0.067	0.076	0.095
γ-CD	0.075	0.075	0.072	0.085
MeG	-	0.068	0.059	0.065
G1-α-CD	-	0.070	0.058	0.078
G1-β-CD	-	0.070	0.067	0.085
G2-β-CD	-	0.068	0.066	0.083

a) Referred to external DSS at 298.2 K and taken from Ref. 4.

shifts of six CD protons as a function of the CD concentration (data not shown). At variance with Eq. 1, the chemical shift of each proton decreased linearly with increasing CD concentration:

$$\delta = \delta_0 - aC_D. \tag{2}$$

Here δ_0 stands for the chemical shift in the absence of CD. The slope a divided by the number of glucose units is shown in Table 1. This slope is small, though not negligible, and is almost independent of the kinds of the CD protons (data not shown). In Table 1, the average of the slopes over the 6 protons of CD is shown for α-CD, β-CD, and γ-CD. This slope increases with increasing CD size. For methyl α-D-glucopyranoside (MeG), α-CD, β-CD, γ-CD, 6-*O*-α-D-glucosyl-α-cyclodextrin (G1-α-CD), 6-*O*-α-D-glucosyl-β-cyclodextrin (G1-β-CD), and 6-*O*-α-D-maltosyl-β-cyclodextrin (G2-β-CD), the chemical shifts of TMA, MeOH, and HDO using external DSS were determined as a function of the saccharide concentration.[4] As Table 1 shows, these slopes per glucose unit for TMA are very close to ours for α-, β-, and γ-CDs. The slopes per glucose unit for MeOH and HDO are similar to those for TMA and ours, but slightly depend on the kinds of saccharides. The former result suggests that the slope plotted against the weight concentration (g dm^{-3}) is almost independent of the kinds of saccharides.

On the basis of these results, we corrected the observed chemical shifts of all protons in solutions of α-CD and γ-CD by adding a term of aC_D and determined the binding constants of short chain lecithin and CDs.[3] This procedure will be applied to other chemical shift data referred to an external standard. The chemical shift of 2.19 mmol dm^{-3} acetonitrile in α-CD solutions was determined by using external DSS and internal TMA.[4] Three sets of chemical shift changes for this system are shown in Fig. 1. The squares show the data determined by using internal TMA standard and gave a reasonable 1 : 1 binding constant of 4.6 dm^3 mol^{-1} and $\Delta\delta_{complex} = 0.19$ ppm. The circles were obtained by using external DSS standard and were not amenable to such analysis.[4] We corrected these chemical shift data by using the slope of $a = 0.421$ ppm dm^3 mol^{-1}, determined for α-CD by Matsui and Tokunaga.[4] These corrected chemical shifts shown by the triangles gave a 1 : 1 binding constant of 4.4 dm^3 mol^{-1} and a chemical shift variation of $\Delta\delta_{complex}$ = 0.196 ppm. Thus, our method for correcting the chemical shift referred to external standard is very good.

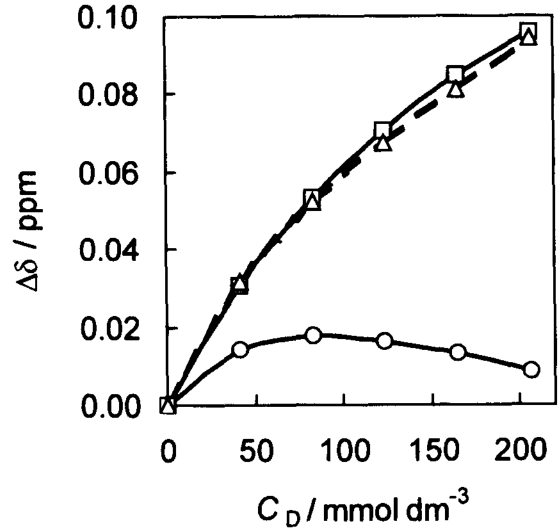

Fig. 1. Chemical shift variations of the acetonitrile proton with the addition of α-CD for three chemical shift standards: external DSS (○), internal TMA (□), and corrected external DSS (△). The dashed line was calculated using a binding constant of $K_a = 4.4$ dm^3 mol^{-1} and a chemical shift variation of $\Delta\delta_{complex} = 0.196$ ppm. The chemical shifts for external DSS and internal TMA were read from Ref. 4 by the use of a digitizer with a computer.

In conclusion, MeS and MS are good internal references in CD solutions, and the chemical shift referred to external standard must be corrected by the use of Eq. 2. This correction is not due to any self-association of CDs, but is probably due to the changes in the magnetic susceptibilities of water due to hydrogen bonding with the glucose units.[4,5] It is not certain whether compounds other than saccharides cause similar chemical shift variations.

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