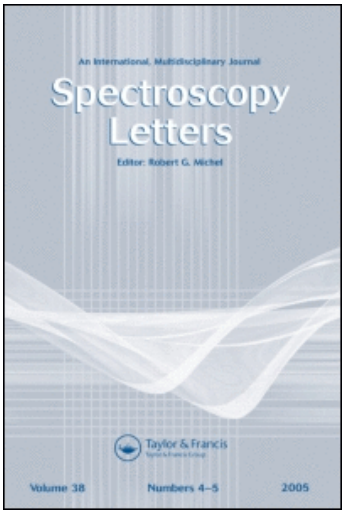

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Protonation Sequence Study of the Solution Structure of DTPA by ^{1}H NMR

Key words: DTPA, PMR, protonation sequence, aminocarboxylate

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ABSTRACT

The protonation sequence of DTPA is evaluated using a 300 MHz ^{1}H NMR instrument. A terminal nitrogen is protonated first ($pK_{a1,2}$=10.02, 8.45). Upon the first protonation a dynamic hydrogen bonded five membered ring is formed between the terminal nitrogen and the central nitrogen. The second equivalent of protons protonates the opposite terminal amine and causes the ring to open. The third protonation (pK_{a3}=4.65) takes place on the central acetate and forms a ring with the central amine. The fourth protonation is assigned to a terminal acetate. The formation of five membered rings involving hydrogen bonding gives a better quantitative and qualitative account for the unusual shifts observed in the ^{1}H NMR spectra than previous explanations.

INTRODUCTION

In Sudmeier and Reilley's classic paper[1] on the protonation sequence of polyamine and aminocarboxylate compounds in aqueous solution, they evaluated diethylenetriaminepentaacetic acid (DTPA) using a 60 MHz ^{1}H NMR instrument over the pH range 4-12. In their interpretation of the data, they concluded that the first protonation took place at the central nitrogen (41 %) with some distribution of protons at the terminal nitrogens (26 % each). Their model proceeds to describe the second equivalent of acid protonating a terminal nitrogen and forcing the proton located on the central nitrogen to migrate to the opposite terminal nitrogen via an electrostatic repulsion model. The third equivalent of acid populates the central nitrogen and central acetate.

Using the pK_a values stated by Sudmeier and Reilley[1], speciation curves for the various species of DTPA (L^{-5},HL^{-4},H_2L^{-3},H_3L^{-2},H_4L^{-1}) plotted as a function of pH are illustrated in Fig. 1. The difference in pK_a values (10.02, 8.45)[1] suggests two distinct protonation steps. At pH 10.02 the aminocarboxylate backbone (L^{-5}) is 50 % protonated (HL) but approximately 1 % of the form H_2L also exists at this pH. If the distribution of protons over the central amine and terminal amines is as even as Sudmeier and Reilley suggest (41%:52%, respectively)[1] one would expect the pK_a values to lie closer together.

Kula and Sawyer[3] are in general agreement with Sudmeier and Reilley[1] when they proposed that the first protonation ($pK_a \approx 10.5$)[3] occurred at the central nitrogen with the second protonation ($pK_a \approx 8.6$)[3] occurring at one of the end nitrogen atoms plus a migration of the proton on the central nitrogen to the other end nitrogen. They also agree that the third protonation ($pK_a \approx 4.3$)[3] takes place at the central nitrogen.

Choppin *et al.*[4] used ^{13}C NMR spectroscopy to study the protonation sequence of DTPA and TTHA. Their proposed mechanism matched that of Sudmeier and Reilley's early work. Sudmeier and Reilley's[1] results at n=1, where n represents the equivalents of acid, indicated that the central nitrogen atom was more strongly basic than the end

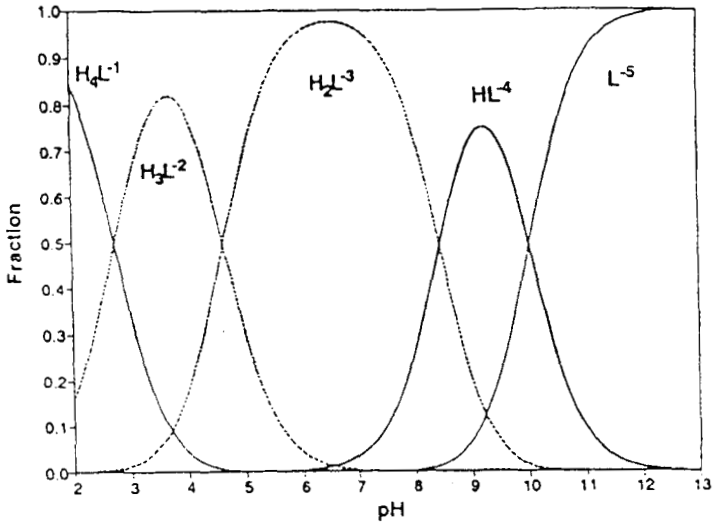

Figure 1. Speciation plot of DTPA as a function of pH.

nitrogen atoms. This view opposed that of Nakamoto *et al.*[5]. who found that the terminal nitrogen atoms were more strongly basic at n=1. In their studies, Nakamoto *et al.*[5] used a dispersive IR instrument to obtain their data. This present study agrees with the sequence proposed by Nakamoto *et al.*[5] for n=0 to n=1.

The paper by Sudmeier and Reilley[1] has been used as a key reference for polyaminocarboxylate protonation sequences for the past three decades. It is important to know the relative basicities of the molecules in order to understand thermodynamic trends, dissociation and exchange kinetics, and solution geometry of lanthanide-aminocarboxylate interactions[6]. Specifically the Gd(III)-DTPA complex has been heavily investigated in Magnetic Resonance Imaging studies[7], a medical diagnostic technique in which DTPA acts as a molecular cage utilized to contain toxic paramagnetic shift reagent. The relative toxicity of Gadolinium is sharply reduced because of the high stability constants encountered with this molecule (eg. Gd(III)-DTPA, $\log \beta = 22.1$)[7]. This

paper will examine the protonation sequence of DTPA and offer an alternative mechanism to that proposed by Sudmeier and Reilley[1].

EXPERIMENTAL

The ^1H NMR spectra were recorded using the 300 MHz ^1H NMR instrument located at the University of Florida (Gainesville). All spectra were recorded at 25 °C and at an ionic strength of 0.10 M in $NaClO_4$. The DTPA samples were dissolved in D_2O at an approximate concentration of 2 mM. Chemical shift values were measured relative to DSS (Sodium 3-trimethyl-silylpropane sulfonate) which served as the internal standard during the PMR analyses. DTPA obtained from the Aldrich Chemical Co. was used without further purification. All pH measurements were made with an Accumet 950 pH/ion meter at 25°C which was calibrated with pH 4.00 and 7.00 standard buffers.

RESULTS AND DISCUSSION

Figs. 2 (a) and (b) show DTPA's structure and the proposed five membered rings formed, respectively. The chemical shift data of DTPA in the pH range of approximately 2-12 is illustrated by Fig. 2 (c). Fig. 3 shows the ^1H NMR spectra at pH 11.58 and 9.86. From these data and by using the methylenic substituent shielding constants from Sudmeier and Reilley[1], an argument is presented to reevaluate the protonation sequence of DTPA.

a) **n=0** In Fig. 3 (a), the three peaks are easily assigned. The peak located at 3.167 ppm is assigned to the eight protons located on the acetates (designated a). The peak at 3.126 ppm is assigned to the central acetate (designated d) and the peak at 2.656 ppm is assigned to the ethylenic backbone (b, c). The peak integrations performed match the peak assignments. The basicities of each nitrogen atom are very similar at

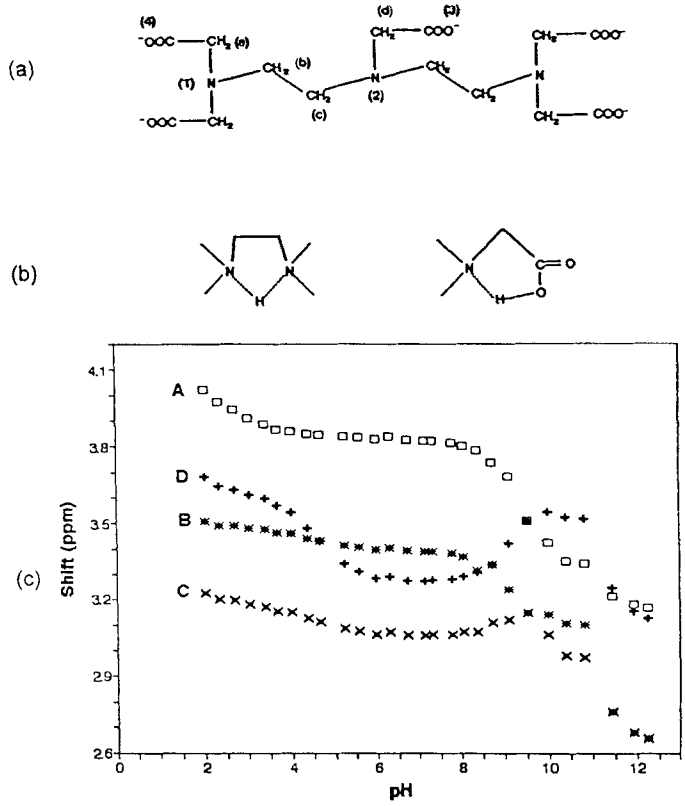

Figure 2. (a) Structure of DTPA with appropriately labeled protons and protonation sites.

(b) Five membered rings formed between the central and terminal amines and between the central amine and the central carboxylate group.

(c) Chemical shift of DTPA plotted as a function of pH.

n=0 due to the symmetry of the DTPA structure resulting in each nitrogen having a similar character.

b) **n=1** In this model it is proposed that the first equivalent of acid protonates a terminal nitrogen. This bound proton then forms a five membered ring with the central nitrogen. After the first protonation and the formation of the five membered ring (see Fig.

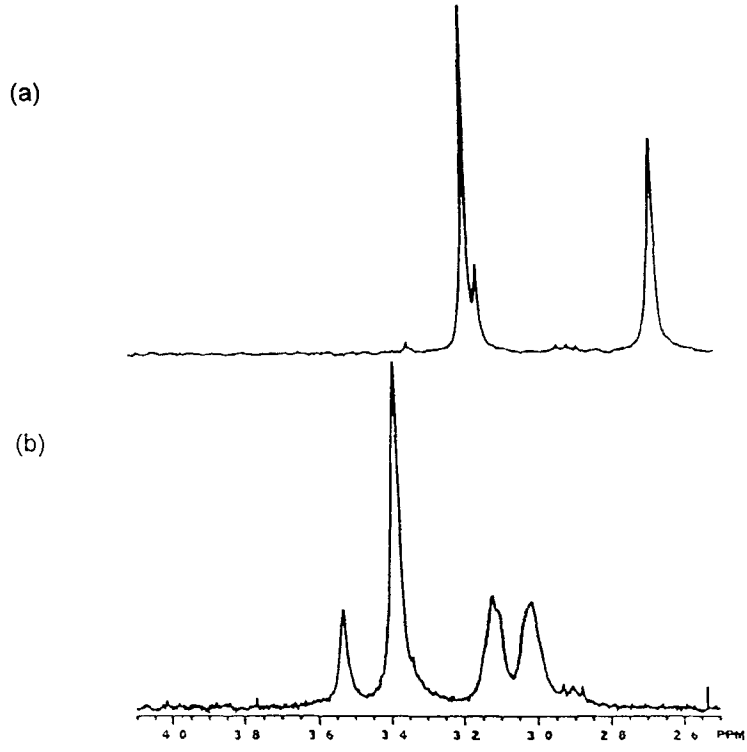

Figure 3. ^{1}H NMR spectral data of DTPA at (a) pH 11.58 and (b) pH 9.86. Spectra were recorded at 25°C with a 300 MHz ^{1}H NMR instrument.

2 b), the basicities of the central nitrogen and the terminal nitrogen differ but the central amine still attracts the bound proton. The proton member of the ring forms bonds with both the central nitrogen and the terminal nitrogen in a very dynamic process causing each nitrogen to have similar environments.

In order to accurately account for the magnitude of shifts observed in protons b and c, one must consider that a terminal nitrogen is populated, on average, fifty percent of the time but it interacts with the central nitrogen via a five membered hydrogen

bonded ring on a 1:1 ratio (see Fig 2). Considering that there are two terminal sites averaged in the PMR spectra, 25 % of the terminal sites are populated. The c proton shifts 0.45 ppm compared to the b proton shift of 0.32. The predicted shift of the c proton from Sudmeier and Reilley[1,2], considering the 25 % population, should be approximately 0.19 (0.75/4). The discrepancy (0.45 vs. 0.19 ppm) may be attributed to the effects of increased deshielding caused by the formation of the five membered ring. The shifts in the b proton (0.32) are accounted for by considering: (1) the secondary effects of protonating the terminal nitrogen (0.35/2), (2) the primary effects of weakly protonating the central nitrogen caused by ring formation, and (3) the effects of increased deshielding caused by the formation of the five membered ring.

c) **n=2** It is proposed that the second equivalent of acid protonates the second terminal nitrogen. This causes the five membered ring that formed upon the addition of the first equivalent to open and form a more rigid diethylenetriamine backbone with a maximum charge distribution and minimum energy. The breaking apart of the five membered ring induced by the second protonation is caused in part by electrostatic repulsion and a reduction in the electron density at the central amine causing the basicity of this nitrogen to be lowered.

The deshielding and shielding of proton d over the pH range 12-8 is attributed to the formation (pH 12-10) and breaking apart (pH 10-8) of the portion of the five membered ring associated with the central nitrogen. There is also a slight deshielding of proton c upon addition of the second equivalent that is caused by the breaking apart of this same portion of the ring. The shift measured for proton b (0.77) and c (0.40) over the pH range 12-6 can be attributed to the protonation of the terminal nitrogens. These values compare favorably to the difference in methylenic substituent shielding constants of 0.75 (the difference between NR_2 and NR_2H^+) and 0.35 (the difference between $-CH_2NR_2$ and $-CH_2NR_2H^+$) respectively as taken from Sudmeier and Reilley[1]. The small discrepancies

can be attributed to the effects that the different structures of the R group have on the basicity of nitrogen.

d) **n=3** It is proposed that the third equivalent of acid protonates the carboxylate on the central acetate and this forms a five membered ring with the central nitrogen. The predicted shift for the d proton should be on the order of 0.2 ppm but is 0.35 ppm. The relatively large shift incurred by the d proton indicates that the central COO^- is not only the population site at this pH but that the central acetate arm bonds to the central nitrogen to form a thermodynamically stable five membered ring. It is proposed that this ring formation deshields the d protons to almost twice (0.35 ppm vs. 0.2 ppm) the expected amount. The c and b protons shift only 0.25 and 0.12 ppm respectively, much lower than 0.75 and 0.35 ppm which would be expected if the central nitrogen was fully protonated. The ratio of shifts (c/b) in protons c and b indicates that the relatively weak protonation of the central nitrogen is what induces the deshielding of these ethylenic backbone protons but the magnitude indicates that it is not a complete protonation. The slight deshielding of protons b and c in the pH range 4-2 indicates that the stability of the ring increases as the $[H^+]$ increases.

e) **n=4** It is proposed that the fourth equivalent protonates a carboxylate on a terminal acetate. This is observed in the shifting of the a protons. It must be remembered that there are four terminal acetates and the shifts induced in the a protons are caused by the protonation of one site averaged over all four sites.

CONCLUSIONS

In addition to reevaluating the mechanism for the protonation sequence of DTPA, it is important to note that the central nitrogen is not a primary protonation site over the pH range 2-12 but rather it forms thermodynamically stable five membered rings with the terminal nitrogens in the pH range 9-11 and with the central carboxylate in the 2-5 pH

range via hydrogen bonding. This is an important point when studying the complexation of DTPA to various metals. It is well known that the sum of the pK_a's of a aminocarboxylate ligand is directly proportional to its stability constant in a 1:1 complex (eg. Gd(III)-DTPA)[8]. It follows that knowing the basicities of the individual protonation sites will give insight into the relative lifetime and strength of individual binding sites.

In Sudmeier and Reilley's studies[1,2] there is no mention of ring formation. In their work they study diethylenetriamine, the backbone of DTPA, and conclude that the terminal nitrogens are protonated first. This explanation corroborates other ^1H NMR studies and offers a more plausible quantitative and qualitative picture of the protonation sequence of DTPA.

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