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Rose M. McConnell
University of Arkansas at Monticello

Walter E. Godwin
University of Arkansas at Monticello

Brant Stanley
University of Arkansas at Monticello

M. Shane Green
University of Arkansas at Monticello

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Rose McConnell, Walter Godwin, Brant Stanley, and M. Shane Green
Division of Mathematics and Sciences
University of Arkansas at Monticello
Monticello, Arkansas 71656

Abstract

Structure based drug development is currently considered to be an important strategy for drug discovery. This strategy requires that critical knowledge of the three dimensional binding site on receptor molecules be known. NMR studies are frequently employed to ascertain important structural data on individual proteins as well as complexes of proteins with ligands. Deuterium labeled acids and bases are frequently used as buffers in deuterium oxide solutions for NMR studies on the structural conformations of bioactive molecules. Since pH is an important factor in any study of the confirmational and stereochemical aspects of biologically active molecules, deuterated buffers are an essential part of the NMR experiments. However, the ionization constant for deuterium oxide (1.95×10^{-15}) is significantly different from that of water. Therefore, a pH comparison of deuterium-labeled acids and bases in deuterium oxide with nondeuterated aqueous acids and bases was conducted. Titration curve comparisons for deuterated and non-deuterated hydrochloric acid, acetic acid, sodium formate, and TRIS (tris[hydroxymethyl]amino methane) are described. Also, the average pK_as of deuterated and non-deuterated acetic acid, formic acid, and TRIS are compared.

Introduction

Over the last 20 years the development of structural chemistry and biochemistry has provided more and more detailed information about the chemical nature of living organisms. Each new insight into the chemical make-up of organisms provides additional guidance for the construction of compounds that have a desirable pharmacological effect. Prior to some of the recent advances in structural chemistry, the discovery of new drugs was made largely through serendipity. In many cases systematic searches for new drugs were made by screening compounds through biological assays. Once a compound was found to have a desirable pharmacological effect, it became a lead compound. However, simply determining that a compound exerts a biological effect did not automatically lead to the development of a new drug. Transport of the compound to the site of action, specificity of the interaction of the compound with a target receptor in an environment where a multitude of other receptors are present, and the economics of synthesizing, investigating, and marketing the compound were all to be considered in developing a promising lead compound into a drug. This process is limited by budget, is not guaranteed to produce a favorable result, and may miss potential drugs if the assay chosen is not appropriate. Although random screening has led to the discovery of several antibiotics and other important drugs, it is clearly inefficient.

New knowledge of biological systems could suggest new approaches to drug discovery and development. This is especially true if specific macromolecules can be identified as the receptor or target for a possible drug. Critical knowledge about the three dimensional nature of the binding site of the receptor is of greatest value. X-ray and NMR methods are now frequently used to provide these three dimensional structures of individual proteins and complexes of proteins with ligands at a rate of several hundred per year (Perutz, 1992). In 1996, the protein data base at Brookhaven National Laboratories contained over 4000 structures of proteins and nucleic acids. There is considerable evidence that the tertiary structure of a protein observed in a X-ray crystal structure is similar to the average structure of the protein in solution as observed by NMR. However, the consensus at present seems to be that the X-ray crystal structures of nucleic acids are imperfect when compared to the average structures of these molecules as seen by NMR. Accurate three-dimensional structure information of macromolecule receptors is critical to structure-based drug development.

Techniques for interpreting NMR spectral data and the reliability of the structures proposed from these data have been extensively described (Braulin, 1995; Billeter, 1995; Markus et al., 1994). NMR methods of structure determination use non-crystalline samples, typically aqueous solutions. The solution conditions (pH, temperature, nature and concentration of buffers or added salts, and con-

centration of the macromolecule) can be varied. NMR methods are unique in that they can be used to detect the presence of two or more major conformations (Schaefer et al., 1995; Cheong and Lee, 1995) and to obtain quantitative information on dynamic processes, including the rates of confirmational interconversion, rates of exchange of labile protons (particularly amide NH protons), as well as the rates of backbone and side chain motions (Markus et al, 1994; Houk et al., 1994). Like many other spectroscopic methods, NMR can be used in titration experiments to demonstrate the stoichiometry of ligand-receptor interaction and to provide a value for the equilibrium constant that characterizes the formation and dissociation of the receptor-ligand complexes. Since these experiments can not only provide three dimensional structural information but can also indicate structural flexibility, the knowledge base for discovery of new pharmaceutical agents by structure based drug design can be considerably expanded by NMR.

Virtually all molecules in a biological system include protons in their structure, and the NMR signals from these hydrogens are readily detectable. Although the spectral lines for ^{13}C and ^{15}N signals from a biomolecule can be resolved better than proton NMR signals from the same molecule, ^{13}C and ^{15}N are present only in 1.1% and 0.37% natural abundance, respectively. The amounts of these isotopes can be enriched to essentially 100% by appropriate synthetic efforts. However, this can be costly and time consuming. Therefore, most NMR experiments focus on ^{1}H NMR in deuterium-labeled solvents. In order to differentiate the biomolecule of interest from the solvent, NMR solvents generally consist of deuterium oxide buffered by deuterium labeled weak acids and bases. However, the ionization constant for deuterium oxide ($K_{Deuterium\ oxide}$), reported to be 1.95×10^{-15} at 25°C (Merck Index, 1989), is significantly different from the ionization of water ($K_w = 1.0 \times 10^{-14}$ at 25°C). The ionization constant for deuterium oxide (D_2O) was calculated in a similar fashion as K_{Water} (the product of the D_3O^+ concentration and the ODconcentration). Little or no information is available on the K_a and K_b values for the deuterated form of commonly used buffers, such as acetic acid-d_4, sodium formate-d, TRIS-d_3, and phosphoric acid-d_3. Therefore, the question arises as to whether there is a variation in the pH of solutions prepared in deuterated and non-deuterated buffers. Even a small change in pH arising from the use of deuterated buffers in deuterium oxide could cause a significant effect on the three dimensional structure of the protein or other biomolecule under investigation. There have been reports in the literature of primary and secondary deuterium isotope effects on the ^{13}C NMR chemical shifts of aldehydes (Vjanic et al., 1995), carboxylic acids (Yonemitsu et al., 1995), and intramolecularly hydrogen-bonded olefins (Hasen et al., 1995), as well as the amide bonds of proteins (Markus et al., 1994) in deuterium oxide solutions. These deuterium isotope chemical shift effects may be related to a pH deuterium effect. Therefore, we have undertaken an investigation of the effects of deuterium on pH. The results of this investigation are reported below.

Materials and Methods

Materials Used

Hydrochloric Acid Concentrate
(Fisher SA49-100) standard
volumetric solution

Sodium Hydroxide Concentrate
(Fisher SS267-100) standard
volumetric solution

Acetic Acid Concentrate
(Anachemia 206-06) standard
volumetric solution

Formic Acid, Sodium Salt
(Sigma F-6502) anhydrous 99.9+%

TRIS (Tris[hydroxymethyl]amino methane
(SigmaT-1503) 99.9%

Deionized Water

Deuterium Chloride, 37 wt %
solution in Deuterium Oxide
(Aldrich 22,707-2), 99.5 atom % D

Sodium Deuteroxide, 40 wt %
solution in Deuterium Oxide
(Aldrich 37,207-2)

Acetic-d_3 acid-d, 99.9 atom % D
(Aldrich 23,7000)

Formic-d, Sodium Salt, 98 atom%D
(Aldrich 37,384-2) anhydrous

TRIS-d_3 (Tris[hydroxy-dmethyl]
amino - d_2 -methane
(Aldrich 32,994-0), 98 atom %D

Deuterium Oxide, 100.0 atom %D
(Aldrich 15, 189-0)

A standardized (Aldrich Zl l, 343-3) calomel combination pH electrode (ultra-thin, long stem) accompanied by a

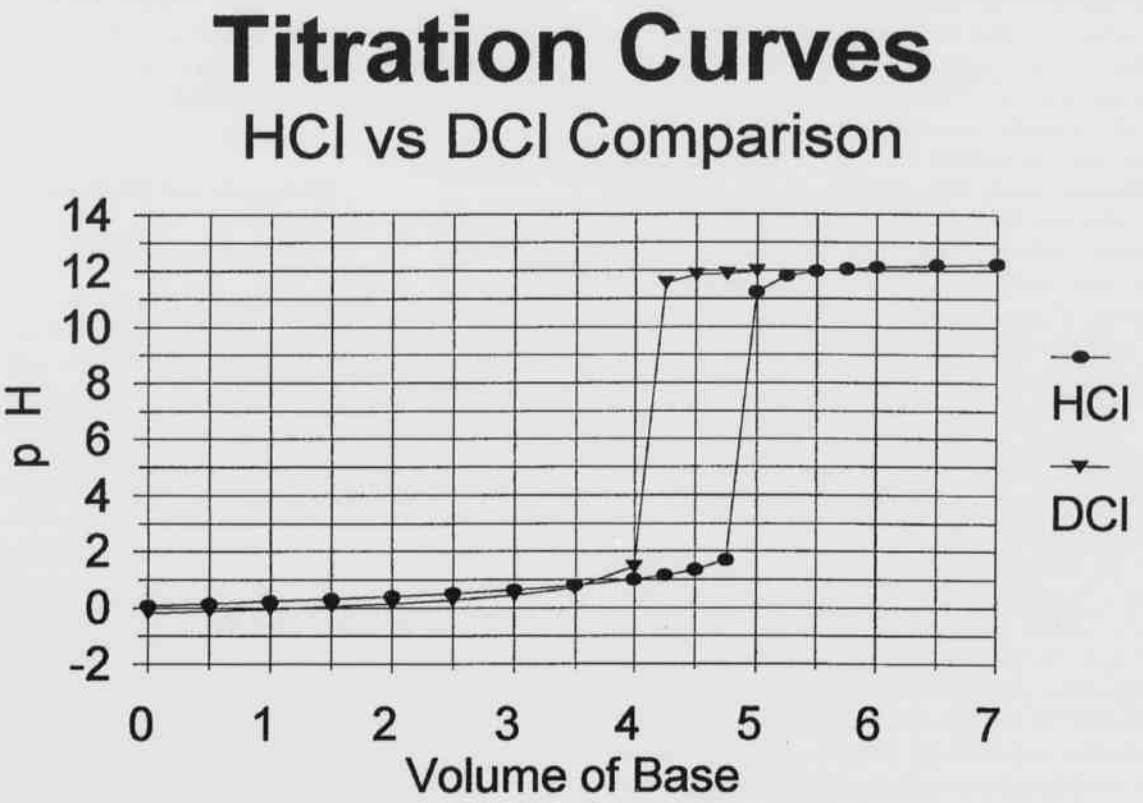

Fig. 1. Titration curve for HC1/DC1 comparison.

temperature compensator probe was used in all titrations. American Scientific Products pH reference buffer solutions of pH 4 and 7 were used in the standardization. All titrations were repeated a minimum of three times for consistency.

Titration Method 1.--This method was used for all compounds purchased as liquids or solutions (sodium hydroxide, sodium deuteroxide, hydrochloric acid, deuterium chloride, acetic acid, and acetic acid-d_4). 5.00 mL of a 1.00 M acid solution (HCl, DCl, acetic acid, or acetic acid-d_4) was transferred by volumetric pipette (Pyrex No. 7102, 5.00 mL) into a 30 mL conical shaped glass centrifuge tube. A small stir bar was added to the centrifuge tube, and the tube was placed in a circulating water bath (22° C). The long, narrow pH electrode was placed in the centrifuge tube and the temperature compensator probe was placed in the water bath. A 10.00 mL buret (increments = 0.05 mL) was filled with exactly 1.00 M base solution (sodium hydroxide in deionized water or sodium deuteroxide in D_2O). The pH was recorded with each 0.10 mL addition of the base solutions to the appropriate acid solutions. Hydrochloric acid and acetic acid solutions in deionized water were titrated with a 1.00 M sodium hydroxide solution in deionized water, whereas, deuterium chloride and acetic acid-d_4 solutions in D_2O were titrated with a 1.00 M sodium deuteroxid solution in D_2O.

Titration Method 2.--This method was used for all compounds purchased as solids (sodium formate, sodium formate-d, TRIS, and TRIS-d_3). Each base was dried for 1 hour in an oven (105° C), and then cooled in a desiccator for 15 minutes. Each base was then massed into conical shaped centrifuge tubes. Three samples, each appropriated to give 5 mL of a 1.0 M solution, of each base were prepared. The samples were then dissolved in about 5 mL of either deionized water or deuterium oxide. The ~1.0 M base solutions were placed in a water bath (22° C). A long thin pH electrode was placed in the centrifuge tube and the temperature compensator probe was placed in the water bath. A 10.00 mL buret (increments = 0.05 mL) was filled with exactly 1.00 M acid solution (either HCl in deionized water or DCl in deuterium oxide). The pH was recorded with every 0.10 mL addition of the acid to the centrifuge tube. Sodium formate and TRIS were titrated with 1.00 M hydrochloric acid in deionized water, whereas, sodium formate-d and TRISd_3 were titrated with 1.00 M deuterium chloride in deuterium oxide.

Results and Discussion

Figure 1 shows a plot of a representative titration curve for both 1.0 M aqueous hydrochloric acid with 1.0 M aque-

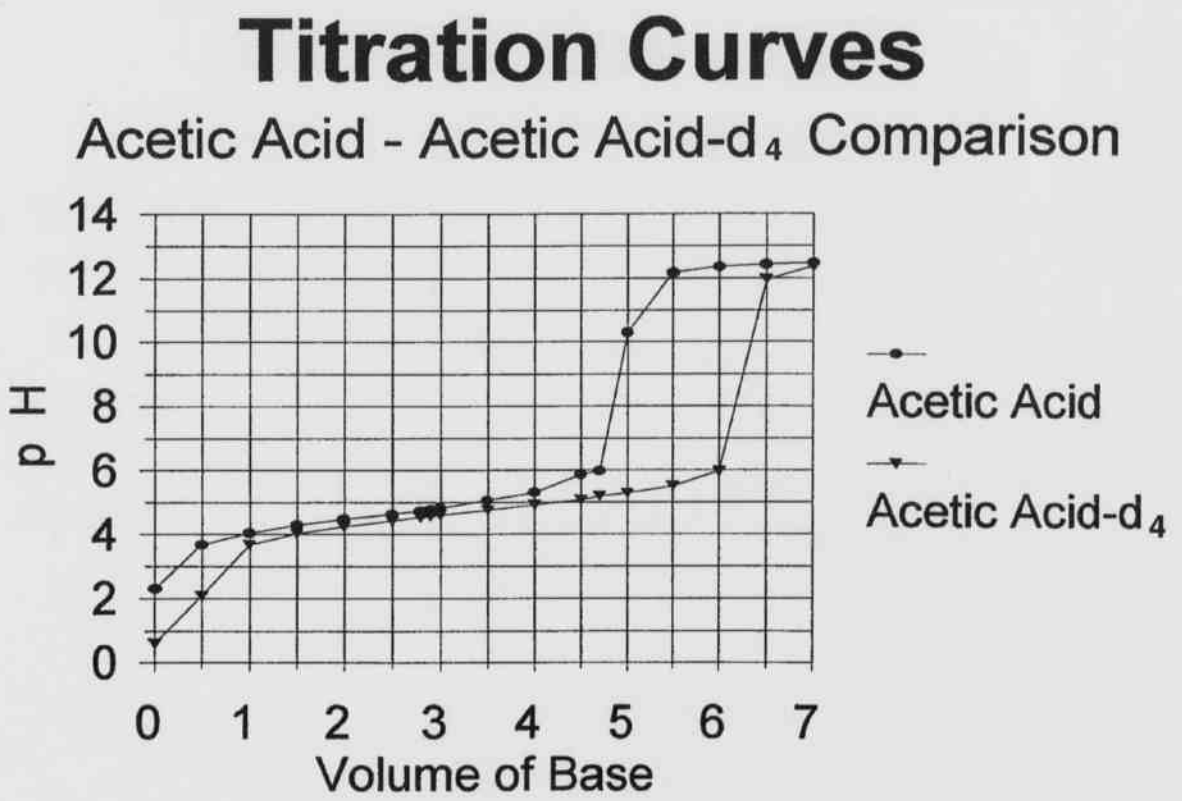

Fig. 2. Titration curve for acetic acid/acetic acid-d_4 comparison.

ous sodium hydroxide as well as for 1.0 M deuterium chloride in D_2O with 1.0 M sodium deuteroxide in D_2O. A significant difference in the equivalence points can be seen in the titration curves of the deuterated and non-deuterated strong acids. Figure 2 shows a similar plot of a representative titration curve for 1.0 M aqueous acetic acid titrated with 1.0 M aqueous sodium hydroxide as well as for the titration of 1.0 M acetic acid-d_4 in D_2O with 1.0 M sodium deuteroxide in D_2O. In both Fig. 1 (HCl/DCl comparisons) and Fig. 2 (acetic acid/acetic acid-d_4 comparison), there are major differences in the equivalence points for the deuterated vs. the non-deuterated acid. Representative titration curves for formic acid-d in D_2O and aqueous formic acid solutions titrated with deuterium chloride and hydrochloric acid solutions, respectively (Fig. 3), and for the titrations of deuterated and non-deuterated TRIS (tris[hydroxymethyl]-aminomethane) solutions with solutions of deuterium chloride and hydrochloric acid (Fig. 4) are shown below. Each of these representative titration curves (Figs. 3 and 4) of these weak bases shows differences in the equivalence points for the deuterated and nondeuterated bases.

Table 1 shows the pK_a's calculated from the titration data for each of the deuterated and non-deuterated acids and bases. The K_a's are determined from several titration curves utilizing the one half equivalence point on the titration curves. The average pK_a is shown on the right side of Table 1. In each case the average pK_a is somewhat higher for the deuterated acid or base in D_2O than the average pK_a for the non-deuterated aqueous form of the acid or base. The differences in the average pK_a's are especially significant for sodium formate-d/sodium formate and TRIS-d_3/TRIS. This increase in the average pK_a for the deuterated acids and bases may result from a difference in the ionization constants of deuterium oxide (1.95×10^{-15} at 25°C) and water (1.00×10^{-14} at 25°C). Deuterium oxide is significantly less ionized than normal distilled water; therefore, weak acids and bases may also be significantly less ionized when dissolved in deuterium oxide.

Conclusions

From the results of our study, thus far, it is apparent that the use of deuterated weak acids or bases as buffers in deuterium oxide solutions will have some effect on the pH of the solution. Therefore, it may be extremely important to measure the pH of all NMR samples containing large proteins or other biomolecules in deuterated buffered solvents

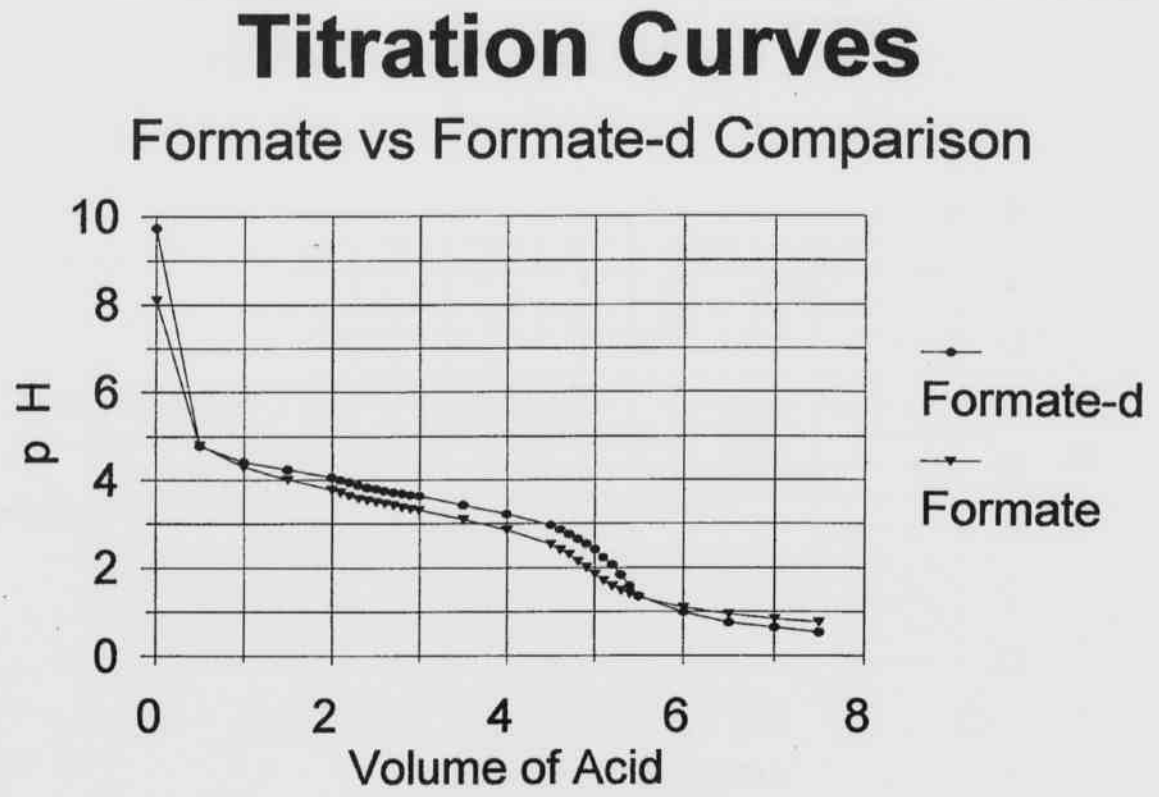

Fig. 3. Titratium curve for formate-d/formate comparison.

before collecting data on the three-dimensional structure.

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Literature Cited

Billeter, M. 1995. NMR for structural studies in drug discovery. Perspectives in Drug Discovery and Design, 3: 151-67.

Braulin, W.H. 1995. NMR studies of cation-binding environments on nucleic acids. Advances in Biophysical Chemistry, 5: 89-139.

Cheong, C., and **C. Lee** 1995. Synthesis of perdeuterated RNAs and its application to NMR studies. Bull. Korean Chem. Soc. 16: 383-6.

Hansen, P.E., S. Bolvig, F. Duus, M.V. Petrova, R. Kawecki, R. Krewski, and **L. Kozerski** 1995. Deuterium isotope effects on ^{13}C chemical shifts of intramolecularly hydrogen-bonded olefins. Magn. Reson. Chem. 33: 621-31.

Houk, K.N., Y. Li, M.A. McAllister, G. O'Doherty, L.A. Paquette, W. Siebrand, and **Z.K. Smedarchina** 1994. Mechanistic analysis of double hydrogen dyotropy in syn-sesquinorborene disulfones, a combined kinetic and theoretical evaluation of primary deuterium isotope effects. J. Am. Chem. Soc. 116:(10):895-913.

Markus, M.A., K.T. Dayie, P. Matsudaira, and **G. Wagner** 1994. Effects of deuteration on the amide proton relaxation rates in proteins. Heteronuclear NMR experiments on Villin 14T. J. Magn. Reson., Ser. B. 105: 192-5.

Merck Index 1989. S. Budvari (editor). 33: 246-30.

Perutz, M.F. 1992. Protein structure. New approaches to disease and therapy. Freeman Publisher, New York.

Schaefer, T., S. Kroeker, and **D.M. Mckinnon** 1995. ^{1}H Nuclear Magnetic Resonance and molecular orbital studies of 2-formyl styrene. Three significant non-planar conformations. Can. J. Chem. 73: 2208-16.

Vjanic, P., E. Gacs-Baitz, Z. Meic, T. Suste, and **V. Smrecki** 1995. Primary and secondary deuterium induced isotope effects for $^{13}CNMR$ parameters of benzaldehyde. Magn. Reson. Chem. 33: 426-30.

Yonemitsu, T., H. Tsuzuki, M. Mukumoto, S. Mataka, and **M. Tasiro** 1995. Deuterium isotope effects on the

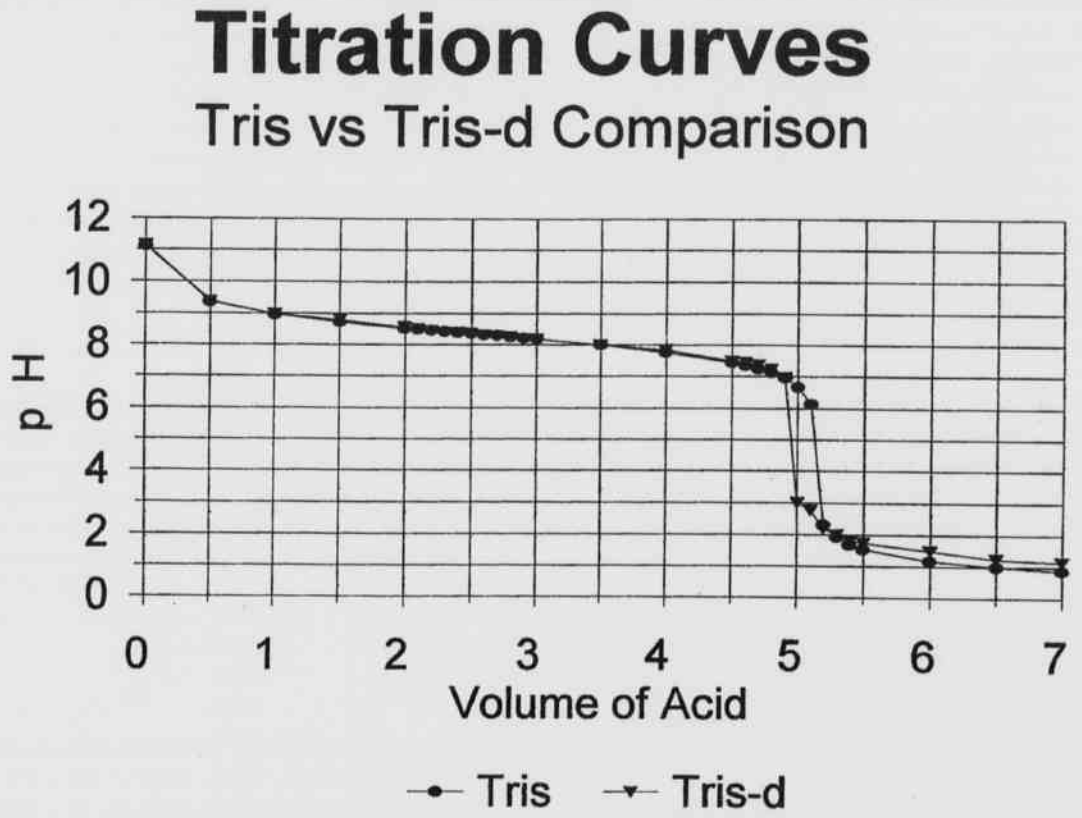

Fig. 4. Titration curve for TRIS/TRIS-d_3 comparison.

chemical shifts on NMR in deuterio benzoic acid, in synthesis and applications of isotopically labelled compounds. John Wiley & Sons Publishers, NY.

Table 1. pk_a data for deuterated and non-deuterated acids and bases.

run	compound	pK_a	average pK_a
I	acetic acid-d_4	4.66	
II	acetic acid-d_4	4.65	4.65
III	acetic acid-d_4	4.65	
I	acetic acid	4.64	
II	acetic acid	4.62	4.63
III	acetic acid	4.63	
I	sodium formate-d	3.63	
II	sodium formate-d	3.63	3.63
III	sodium formate-d	3.63	
I	sodium formate	3.54	
II	sodium formate	3.53	3.54
III	sodium formate	3.54	
I	TRIS-d_3	8.37	
II	TRIS-d_3	8.36	8.37
III	TRIS-d_3	8.37	
I	TRIS	8.28	
II	TRIS	8.33	8.30
III	TRIS	8.30	