# Chem 80 Report

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## Introduction

The helical structure of nucleic acids were deduced from x'ray diffraction studies by Watson and Crick.[1] The hydrogen bonding scheme between purine and primidine provided the explanation of the specificity of pairing and stability of the helical structure. It has been shown that in aqueous solution the contribution of hydrogen bonded pairs to the association energy of polynucleotide chains is small and may not even be positive.[2] The view has shifted to hydrophobic forces and/or stacking of bases as the forces in maintaining the stability of the helical structure.

Hydrogen bonding interactions of free nucleosides have been studied and observed by measuring the chemical shift of protons involved in bonding by nuclear magnetic resonance.[3] This however can be done only in nonaqueous solvents, since in acqueous solvents the rapid exchange of hydrogen bonding protons with water is too fast to be observed on NMR. The "stacking" of bases and nuclecsides in aqueous solution was first observed by Chan, Schweizer, and T'so.[4] The evidences suggested a vertical stacking model for the interaction.

It is well-known that nuclear magnetic shielding is a sensitive probe of inter and intra molecular forces. The ver-

tical stacking interaction can be distinguished from hydrogen bonding in principle. The vertical stacking was observed with 6-methylpurine.[5] For aromatic systems with the presence of pi elections, ring current can be induced by external magnetic field perpendicular to the plane of the molecule. This leads to high field shift on NMR spectra, upon dilution. This was confirmed in nucleosides and bases. The effect is not observed for primidine bases because of the lack of ring-current magnetic anisotropy.

Following the same line dinucleotides were studied with NMR.[6] The proton magnetic resonance spectra of TpT is compared to that of thymidine. Base protons of TpT are indistinguishable from base protons of thymidine. The H6 proton is broader in TpT compared to thymidine. This suggests that the two thymine bases in TpT are not equivalent. The unsymmetric attachment of the phosphate ester linkage affects the magnetic environment of the bases differently. When purine was added, the resonance shifts upfield. This suggests a stacking interaction; the purine molecule is inserted between the bases of the dinucleotide forming a sandwich-like complex. With the addition of purine, the $CH_3$ and $H_6$ lines are split. The nonequivalence increases with purine/dinucleotide ratio. This suggests that the two sets of thymine protons are no longer equivalent as a result of insertion of purine into the dinucleotide. The purine resonance increases with purine/dinucleotide ratio.

This effect is studied with CMP( cytidine-mono-5'-phosphate) and CpC(cytidinyl-3'-5'-cytidine) by NMR.

## Experimental

Materials. CpC, CMP, and purine were obtained from California Corporation for Biochemical Research. Purine(grade A) was used without further purification. The $NH_4^+$ salt of CpC and CMP were prepared by passing the CpC acid and $Na^+$ salt of CMP through a Dowex 50 cation exchange. The solution was then lyophilized and then dried over $P_2O_5$.

Instrumentation. All NMR spectra were recorded on Varian A60A spectrometer. Probe temperature at $26^\circ \pm 2^\circ C$. Varian 1024 Computer Averaged Transients were used to improve signal/noise on some spectras. Chemical shift was measured using $CHCl_3$-TMS as an external reference. $CHCl_3$ resonance at 436.0±0.5 cps. The bulk susceptiblity have not been corrected for. All pH measurements were made with Leeds and Northrup #124138 pH meter with microelectrode assembly. Accuracy 0.02 pH units. Whenever $D_2O$ was used, pD was coverted to pH by adding constant-0.40 pH units.[7]

## Results and Discussions

### CMP pH effect

To get acquainted with the CpC system, CMP·$NH_4$ salt was with NMR. The assignment of peaks for cytidine had been done by Galtin and Davis in DMSO.[8] pH was adjusted with HCl and $NaHCO_3$ whenever necessary. $H_6$ proton, $H_5$ proton, and $H_1'$ ribose proton resonance can be observed. The $H_5$ proton resonance are sharp enough to be observed at pH 2.0 and lower. pK of cytidine is 4.6. The amine group determines the pK. At low pH when essentially all the amine groups are protonated, the exchange with water is small, thus the $H_5$ are sharp enough to be observed.

pK can be determined graphically by plotting chemical shifts versus pH.[9] The pH dependence of chemical shift for an acid -base equilibrium is described by

$$\delta = \delta_B f_B + \delta_{BH^+} f_{BH^+}$$

$\delta_B$ -unprotonated chemical shift

$\delta_{BH^+}$ -protonated chemical shift

$f_{BH^+}$ -mole fraction of protonated form

$f_B$ -mole fraction of unprotonated form

and $f_{BH}$ is $$f_{BH} = \frac{[BH^+]}{[B]+[BH^+]}$$

The equilibrium expression is

$$K = \frac{[BH^+]}{[B][H^+]} = Ka$$

We then get $$\delta = \delta_B + (\delta_{BH^+} - \delta_B)\frac{K[H^+]}{1+K[H^+]}$$

pK was found to be 4.10 0.5.(Appendix 1)

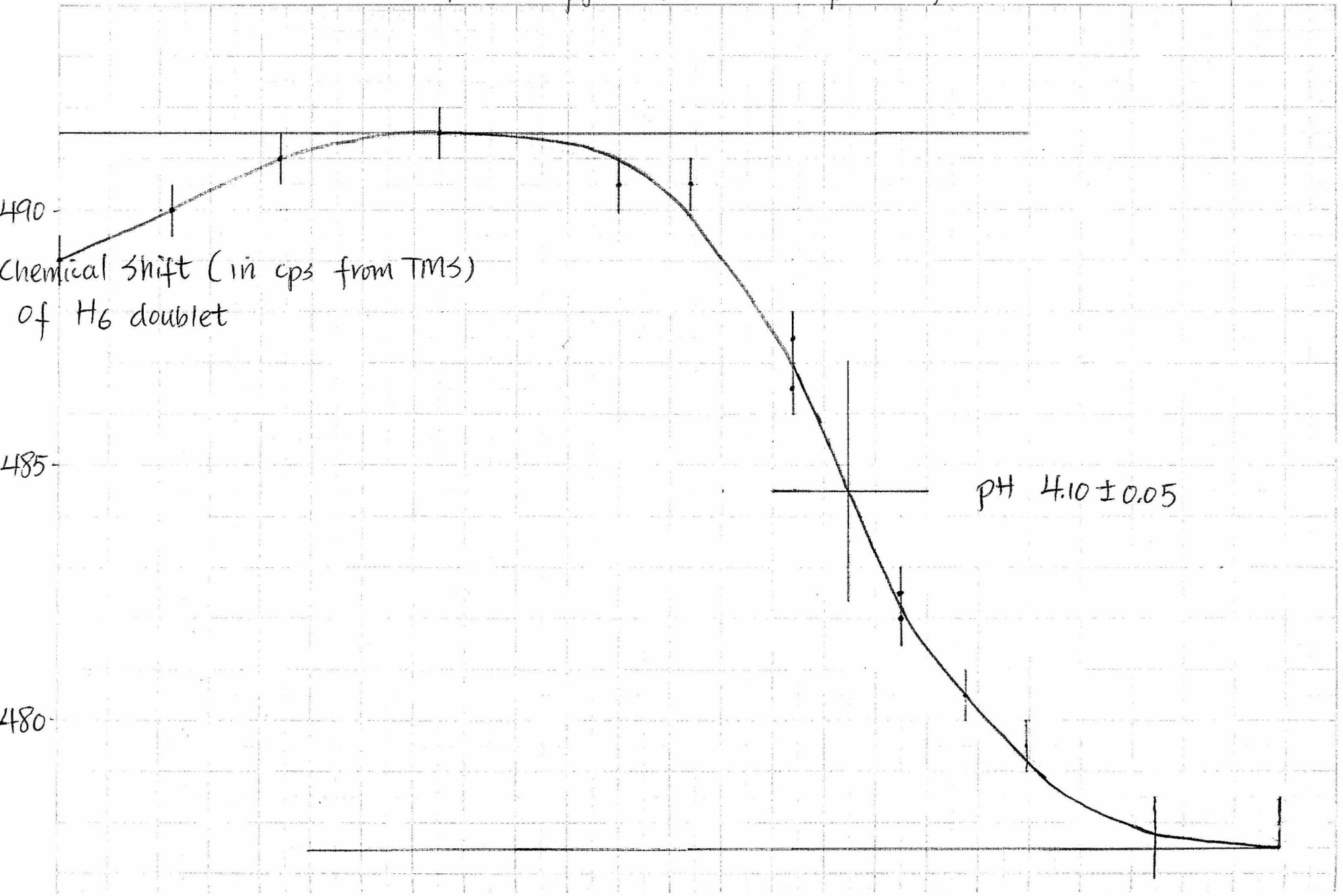
CpC·$NH_4$

The $H_6$, $H_5$ and $H_1'$ proton resonances appeared very similiar to CMP. $H_5$ could be resolved at pH 3.5 and lower. pK was determined graphically as CMP. pK was 4.15±0.5. (Appendix 2) This result is identical to that of CMP.

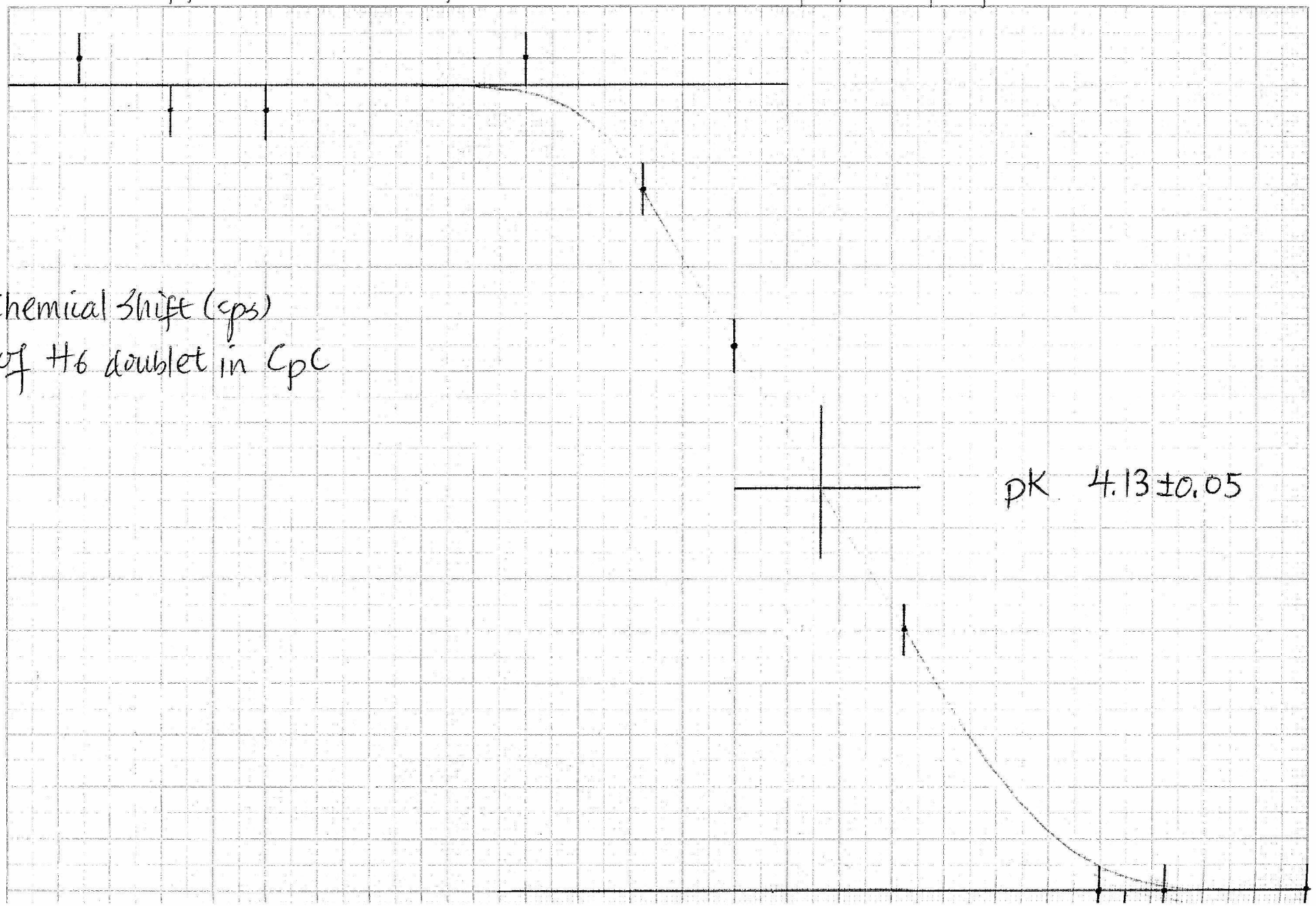
CpC and Purine

The CpC solution used in pH study was neutralized to pH 7.0 and purine was added. The high field shift due to ring current effect was observed. This suggests the purine-CpC stacking interaction. However, no non-equivalence of the $H_6$ proton was observed. In TpT experiments, the addition of purine induced non-equivalence of the $H_6$, $H_1'$ and $CH_3$ protons.[10] This effect suggested an inserted sandwich model. The non-equivalence increased with purine/dinucleotide. This purine induced splitting had been observed by Mr. Bangerter in CpC at neutral pH.[11] The non-equivalence increased with purine/dinucleotide as TpT. However this non-equivalence disappeared when the solution was acidified. This suggests that CpC was cleaved by acid. This destroyed the sandwich -like stack and therefore the purine induced non-equivalent because of the unsymmetric 3'-5' ester linkage. These results supports the theory advanced for purine-dinucleotide stacking interactions.

CMP Appendix 1. Graphical pK determination of CMP



# Appendix 2. Graphical determination of pK of CpC



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