

# POTENTIOMETRIC AND SPECTROPHOTOMETRIC DETERMINATION OF THE PROTONATION CONSTANT OF HEXAMETHYLENETETRAMINE

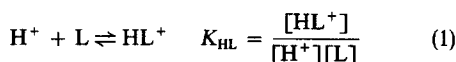
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Hexamethylenetetramine (urotropine) has found extensive use as a buffer, and is especially suitable for this purpose, because it forms relatively weak complexes with metals. In medicine it has long been used in the treatment of urinary tract infections. More recently it has been found to have an inhibiting effect on the growth of tumours.<sup>1</sup>

Very few equilibrium data can be found in the literature for this reagent.<sup>2</sup> Below, both a potentiometric and a spectrophotometric method are utilized for the determination of the stability constant of the reaction



where L denotes the ligand hexamethylenetetramine, and the constant is determined as a concentration constant

## EXPERIMENTAL

### Reagents

Hexamethylenetetramine and other chemicals were Merck *pa* grade. Potassium chloride was used to keep the ionic strength at 0.1 and 0.5. The titrant was potassium hydroxide solution. All measurements were made at 25°C in an inert atmosphere.

### Apparatus

The potentials were recorded with an Orion 801 pH/mV meter (precision  $\pm 0.1$  mV) using Beckman glass and calomel electrodes. The hydrogen-ion concentrations were calculated from the measured potentials by a method slightly modified from the one given by Ingman *et al.*<sup>3</sup> The spectrophotometric measurements were performed with a Coleman model 46 UV-VIS Spectrophotometer equipped with a quartz flow-cell. The volumes were measured with Metrohm piston and micro burettes.

For mathematical calculations a programmable Monroe 1860-44 calculator was used.

### Procedures

**Potentiometric determination** A potentiometric method not previously used for determination of stability constants of acids was employed. It is due to Ivaska and Wanninen,<sup>4</sup> and uses the equation for titration of weak acids derived by Ingman and Still.<sup>5</sup> The data obtained from a potentiometric titration are processed with a desk-calculator. The program calculates both the equivalence volume and the stability constant of the acid by an iterative procedure. The stability constant is a mean of four values calculated from four points, two on each side of the half-titration point. The equivalence volume is determined by the method of Ingman and Still,<sup>5</sup> using titration points near the equivalence point.

The titration is started by performing an  $E_0$ -titration<sup>3</sup> and then adding solid hexamethylenetetramine to the solution and hydrochloric acid in excess in order to protonate the reagent. The titration is then performed in the ordinary manner, the electrodes remaining in the solution throughout.<sup>3</sup> The titration curve,  $-\log[\text{H}^+]$  vs  $V$ , the volume of added base, shown in Fig. 1a, is the one obtained when protonated hexamethylenetetramine and excess of hydrochloric acid are titrated with potassium hydroxide at  $\mu = 0.5$ . The excess of hydrochloric acid, *i.e.*, the starting point for the hexamethylenetetramine titration, can be determined by the Gran method,<sup>6</sup> using points before the first potential jump (Fig. 1a). The data given in Table 1 represent the titration of hexamethylenetetramine, and the proton stability constant calculated from them is also given. The value of the constant at  $\mu = 0.1$  is determined in a similar way and is given in Table 3.

**Spectrophotometric determination** Spectrophotometric methods are widely used for the determination of protonation constants.<sup>7</sup> However, in this work a less common

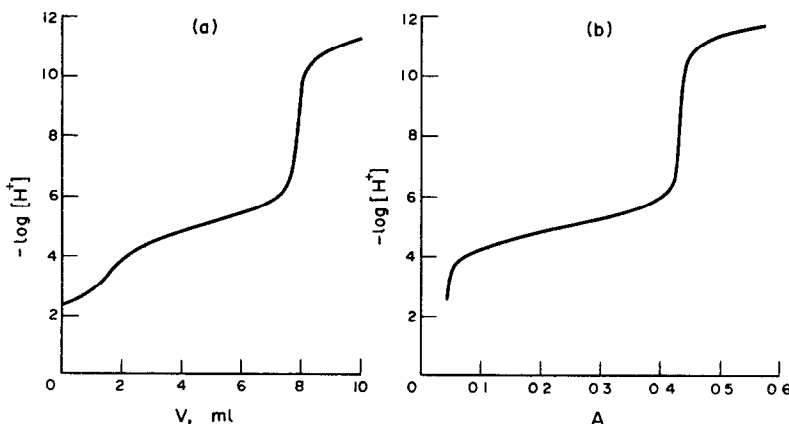


Fig. 1 (a)  $-\log [\text{H}^+]$  as a function of the volume of added potassium hydroxide for a titration of  $1.26 \times 10^{-2} \text{ M}$  hexamethylenetetramine containing an excess of hydrochloric acid  $V_0 = 48.33 \text{ ml}$ ,  $\mu = 0.5$  (KCl),  $C_{\text{OH}} = 0.09816 \text{ M}$  (b)  $-\log [\text{H}^+]$  as a function of absorbance for  $5 \times 10^{-3} \text{ M}$  hexamethylenetetramine, which is neutralized by addition of potassium hydroxide  $V_0 = 100.0 \text{ ml}$ ,  $\mu = 0.5$  (KCl),  $\lambda = 215 \text{ nm}$

Table 1 Titration of the proton complex of hexamethylenetetramine for determination of its stability constant

$V, \text{ml}$	$-\log[\text{H}^+]$	$V, \text{ml}$	$-\log[\text{H}^+]$
1.05	4.389	4.05	5.340
1.55	4.590	4.30	5.421
2.05	4.755	4.55	5.511
2.55	4.905	4.80	5.610
2.80	4.977	5.05	5.727
3.05	5.050	5.30	5.864
3.30	5.121	5.55	6.049
3.55	5.190	5.80	6.328
3.80	5.263	6.05	7.075

Found  $\log K_{\text{HL}} = 5.04_8$   
 $C_L = 1.239 \times 10^{-2} M$

Initial volume  $V_0 = 48.33 \text{ ml}$ , KOH concentration  $C_{\text{OH}} = 0.09816 M$ , total initial concentration of hexamethylenetetramine  $C_L = 0.0126 M$ ,  $T = 25^\circ$  and  $\mu = 0.5 \text{ (KCl)}$

titration procedure is employed. The absorbance and hydrogen-ion concentration of a solution that is gradually neutralized by addition of potassium hydroxide are simultaneously measured. The hydrogen-ion concentration is measured as described above. The advantages of this procedure are that no buffers are needed and the electrodes need not be removed from the solution during the experiment. An absolute necessity for precise spectrophotometric measurements is a fixed cell, *e.g.*, of flow-through type.

A suitable wavelength for the measurement of the absorption by hexamethylenetetramine was 215 nm. An example of an absorbance curve plotted as a function of  $-\log[\text{H}^+]$  is given in Fig. 1b. The shape of the absorbance curve implies that one proton is liberated at  $-\log[\text{H}^+]$  about 5. At  $-\log[\text{H}^+]$  above *ca.* 10 there is another rise in the curve, owing to absorption by the hydroxide ion.

When only the complex  $\text{HL}^+$  is formed, the following equations are valid:

$$A = a_{\text{HL}}[\text{HL}^+] + a_L[\text{L}] \quad (2)$$

$$C_L = [\text{HL}^+] + [\text{L}] \quad (3)$$

$A$  is the measured absorbance and  $a_{\text{HL}}$  and  $a_L$  denote absorptivities, which can be determined from the pH regions where respective species dominate. Thus equations (2) and (3) contain only two unknowns and can easily be solved. Because a titration is used, the change in volume must be taken into account in calculating  $C_L$ , equation (3).

Typical results are given in Table 2. The optimal region for the determination of the ratio  $[\text{HL}^+]/[\text{L}]$  and thus also the protonation constant is at or near the point of

Table 2 Spectrophotometric determination of the protonation constant of hexamethylenetetramine

$V, \text{ml}$	$-\log[\text{H}^+]$	$A$	$\log K_{\text{HL}}$
0.73	4.515	0.1305	5.056
0.85	4.734	0.1700	5.054
0.97	4.941	0.2135	5.052
1.10	5.137	0.2575	5.051
1.28	5.428	0.3175	5.051
1.42	5.724	0.3630	5.059

$\log K_{\text{HL}} = 5.05_2$

$V_0 = 100.0 \text{ ml}$ ,  $C_{\text{OH}} = 0.5 M$ ,  $C_L = 5 \times 10^{-3} M$ ,  $\mu = 0.5 \text{ (KCl)}$ ,  $T = 25^\circ$  and  $\lambda = 215 \text{ nm}$

Table 3 Summary of the stability constants of the proton complex of hexamethylenetetramine

$\log K_{\text{HL}}$	Temp., $^\circ\text{C}$	Ionic strength	Reference
4.92	—	—	Kolthoff <sup>9</sup>
6.30	25	0.5*	Pummerer and Hofmann <sup>9</sup>
4.9	—	—	Evstratova <i>et al.</i> <sup>11</sup>
4.85	—	—	Reilley and Schmid <sup>12</sup>
4.88 <sub>6</sub>	25	0.1 (KCl)	This work
5.04 <sub>8</sub>	25	0.5 (KCl)	(potentiometric method)
4.88 <sub>7</sub>	25	0.1 (KCl)	This work
5.05 <sub>2</sub>	25	0.5 (KCl)	(spectrophotometric method)

\* 0.5M hexamethylenetetramine

half-titration, because small errors in the absorbance measurements then have little effect on the value of  $[\text{HL}^+]/[\text{L}]$ . By taking four points around the point of half-titration the mean value  $\log K_{\text{HL}} = 5.05_2$  was obtained for the example given in Table 2.

## DISCUSSION

In Table 3 the protonation constants determined in this work are summarized and compared with published values. The values obtained by the potentiometric and spectrophotometric methods are in very good agreement. (It is perhaps motivated to give  $\log K_{\text{HL}}$  to three decimals, especially when the potentials are measured with 0.1 mV and absorbances with 0.001 units accuracy.) The difference between a potentiometric and a spectrophotometric method for determination of stability constants can be seen in Fig. 1. In potentiometry the amount of added titrant and in spectrophotometry the measured absorbance are used to calculate the mole ratio of  $[\text{HL}^+]$  to  $[\text{L}]$ . At alkaline pH the two curves are similar, because hydroxide ions absorb at the wavelength used, but in the acid region, the additional HCl affects the shape of the potentiometric curve.

The values of the protonation constant of hexamethylenetetramine obtained in this paper are in satisfactory agreement with the values reported by Kolthoff,<sup>9</sup> Evstratova *et al.*<sup>11</sup> and Reilley and Schmid<sup>12</sup> although the exact temperature and medium for their experiments are not given. The value given in "Stability Constants"<sup>2</sup> could not be found by the present authors in the original paper of Pawelka.<sup>10</sup>

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**Summary**—The protonation constant of hexamethylenetetramine (urotropine) was determined by a potentiometric and a spectrophotometric method. The calculations gave  $\log K_{\text{HL}}$  (concentration constants) 4.89 at  $\mu = 0.1$  and 5.05 at  $\mu = 0.5$ . The temperature was  $25^\circ$  and potassium chloride was used to adjust the ionic strength.