CHROM. 4510

The separation of biochemical acids and bases on columns of Sephadex G-10

Many organic acids and bases, when ionised, are partially excluded from Sephadex dextran gels, whereas, when unionised, they diffuse freely into the gel and are frequently reversibly adsorbed by it[1]. Hence the retention times of organic acids and bases on columns of Sephadex G-10 depend on their acid dissociation constants and the pH of the eluent[2,3]. The shape of a plot of retention time against pH of a simple organic acid is shown in Fig. 1. Retention times for Sephadex gels are usually expressed[1] as a partition coefficient K_d. When the pH is less than A, the acid is unionised and, when greater than B, it is fully ionised. Point C gives the approximate pK_a of the acid. The plot for an organic base is similar but reversed. Consequently, plots of K_d of organic acids and bases against pH will indicate the pH at which the separation of a particular mixture of acids and bases is most efficient.

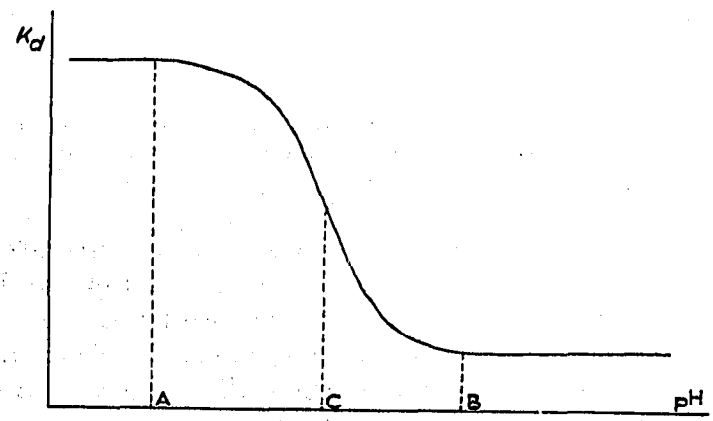

Fig. 1. The effect of pH on the K_d value of an organic acid.

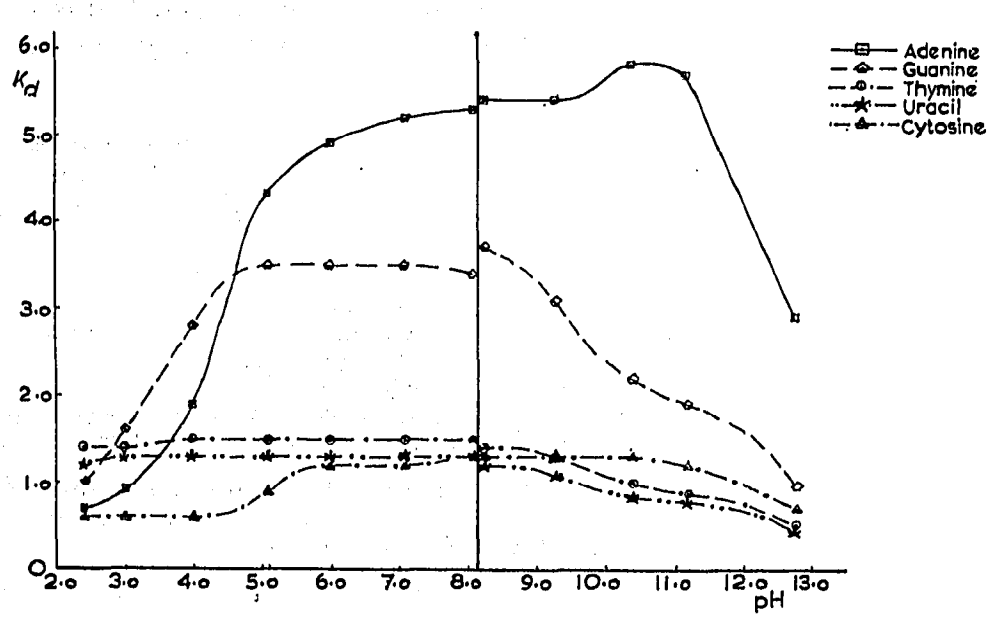

Fig. 2. K_d–pH plots for typical nucleotide bases.

TABLE I

A COMPARISON OF DERIVED DISSOCIATION CONSTANTS WITH LITERATURE VALUES

	pK_a estimated from Fig. 2	*Literature values of pK_a*
Adenine	(i) 4.3; (ii) 9.8	(i) 4.2; (ii) 9.8
Guanine	(i) —*; (ii) 9.4; (iii) —*	(i) 3.3; (ii) 9.2; (iii) 12.3
Cytosine	(i) 4.9; (ii) —*	(i) 4.7; (ii) 12.2
Uracil	(i) 9.5; (ii) —*	(i) 9.4; (ii) 12
Thymine	(i) 9.9; (ii) —*	(i) 9.8; (ii) >13

* These values cannot be estimated from Fig. 2.

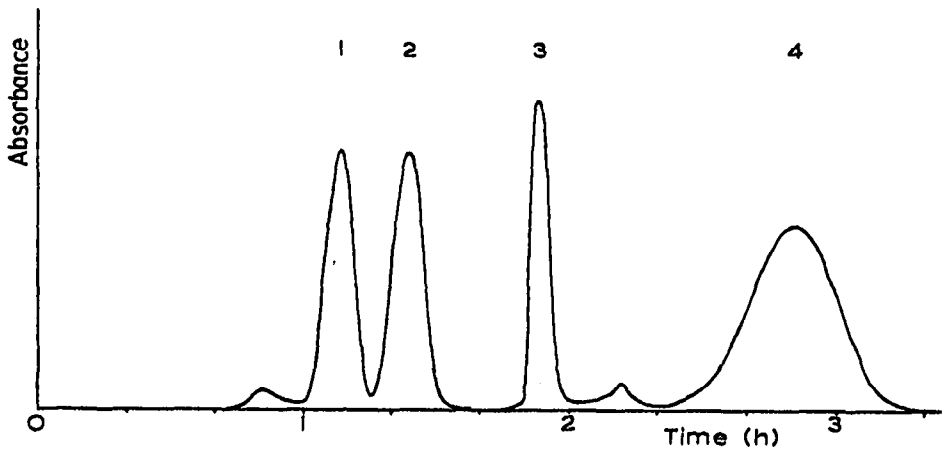

Fig. 3. Separation of RNA bases on an 87 × 1.5 cm column of Sephadex G-10. Peaks: 1 = uracil; 2 = cytosine; 3 = guanine; 4 = adenine.

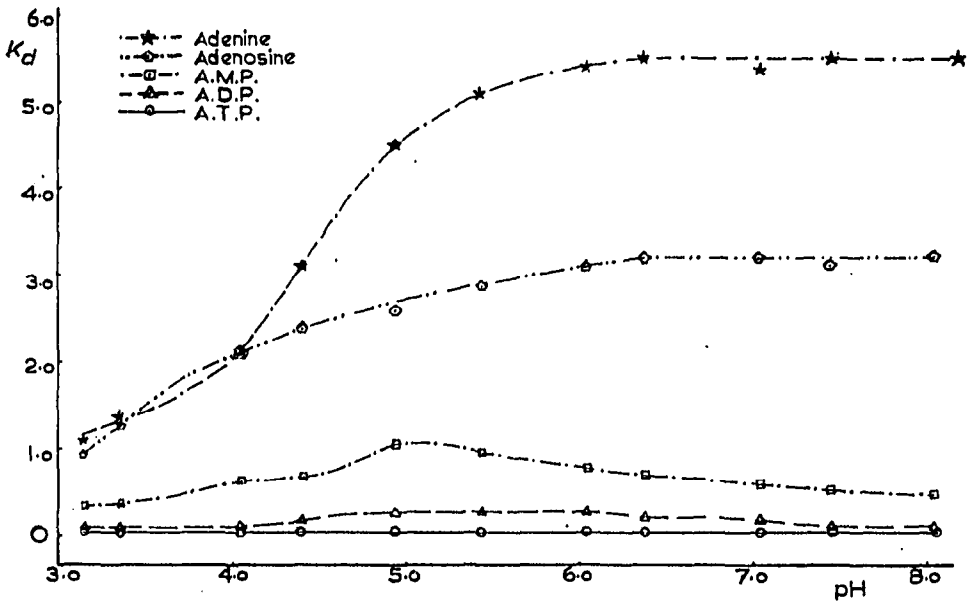

Fig. 4. K_d–pH plots for adenine, adenosine, AMP, ADP, and ATP.

This approach should have a wide application to the separation of low-molecular-weight biochemicals having acidic and basic groups. For example, K_d–pH plots of five typical nucleotide bases are shown in Fig. 2. Values of K_d were measured with a 87 × 1.5 cm column. At pH 8.2, the buffers were changed from McIlvain to Sörenson–Walbum. Literature values of pK_a (Table I) of guanine[4], cytosine[5], uracil[6] and thymine[7] compare well with those derived from Fig. 2.

Adenine is anomalous in that the K_d should decrease at pH 9.8 since a proton is lost from an –NH group in the iminazole ring[4].

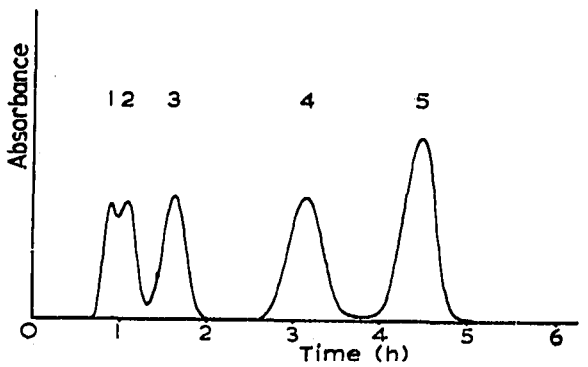

Fig. 5. Separation on a 115 × 1.5 cm column of Sephadex G-10. Peaks: 1 = ATP; 2 = ADP; 3 = AMP; 4 = adenosine; 5 = adenine.

Fig. 2 shows that the optimum pH for the separation of typical RNA bases is 10.2. This separation is shown in Fig. 3. Uracil and cytosine were eluted with a buffer of pH 10.2, then, to reduce the separation time, guanine and adenine were eluted with 0.1 *N* sodium hydroxide solution. The bases were recovered quantitatively from the column. Separation on Sephadex G-10 compares favourably with ion-exchange methods[8].

As a second example, K_d–pH plots of adenine, adenosine, AMP, ADP, and ATP are shown in Fig. 4. The separation of these compounds is shown in Fig. 5.

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1 B. GELOTTE, *J. Chromatog.*, 3 (1960) 330.
2 A. J. W. BROOK, *J. Chromatog.*, 39 (1969) 328.
3 A. J. W. BROOK AND S. HOUSLEY, *J. Chromatog.*, 42 (1969) 112.
4 A. ALBERT AND D. J. BROWN, *J. Chem. Soc.*, (1954) 2060.
5 S. LEWIN AND D. A. HUMPHREYS, *J. Chem. Soc.*, (*B*), (1966) 210.
6 A. ALBERT AND J. N. PHILLIPS, *J. Chem. Soc.*, (1956) 1294.
7 D. SHUGAR AND J. J. FOX, *Biochim. Biophys. Acta*, 9 (1952) 199.
8 S. P. KELEMEN AND E. T. DEGENS, *Nature*, 211 (1966) 857.

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