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## Letter to the Editor

### The gel chromatographic behaviour of eluent ions on Sephadex G-10

Sir,

Ujimoto *et al.*<sup>1</sup> present some interesting data on the behaviour of eluent ions on Sephadex G-10. They observed that at high pH the partition coefficient of  $\text{Na}^+$  is considerably larger than of  $\text{Cl}^-$ , and explain this qualitatively as the result of a Donnan equilibrium due to the presence of some carboxylate groups in Sephadex gels.

However, by means of a quantitative analysis it can be shown that the main cause of the large value of the partition coefficient of  $\text{Na}^+$  at high pH is the preference of the gel for NaOH over NaCl. Further, the difference between the information that is obtained from the retention volume of a concentration disturbance in the eluent (monitored by refractometry or potentiometry) and from the retention volume of a radioactive component should be taken into account.

As regards the second point, from the retention volume of a radioactive component the  $K_d$  value of that component can be calculated, at its prevailing concentration in the eluent ( $K_d = M_g/M_e$ , where  $M$  denotes molarity,  $g$  the gel and  $e$  the eluent). On the other hand, from the retention volume of a concentration disturbance in the eluent the derivative of the sorption isotherm with respect to the concentration can be calculated, *i.e.*,  $K_d^* = dM_g/dM_e$ . This was illustrated clearly by Slaats *et al.*<sup>2</sup>. Obviously, the difference between  $K_d$  and  $K_d^*$  vanishes when the monitored component is present at trace concentration, *i.e.*, when it is absent from the eluent. The difference can be appreciable, however, when the retention of one of the eluent components is monitored, as in the present case.

As for the first point, according to Ortner and Pacher<sup>3</sup>, Sephadex G-10 contains about 4  $\mu\text{equiv}$ . COOH groups per g of dry gel or per 2 ml of swollen gel. According to De Ligny *et al.*<sup>4</sup>, swollen Sephadex beads occupy 57% of the bed volume. If the density of the gel matrix is equal to the density of sucrose, *i.e.*, 1.58 g/ml, 1 g of Sephadex gel occupies a volume of 0.63 ml. This leaves 0.51 ml for the water inside 1 g of Sephadex G-10 beads. The concentration of COOH groups in this interior water is thus 0.008  $M$ . In the low pH range investigated by Ujimoto *et al.*<sup>1</sup> (pH = 1.51–2.84) these COOH groups are virtually unionized; in the high pH range (pH = 12.05–12.50) they are fully ionized.

So, in the low pH range the only compounds that we must take into account are HCl and NaCl, both in the gel and in the eluent. The following equations hold for this case:

$$K_{d,\text{Na}^+} = \frac{M_{\text{Na}^+,g}}{M_{\text{Na}^+,e}} = \frac{M_{\text{NaCl},g}}{M_{\text{NaCl},e}} = K_{d,\text{NaCl}} \quad (1)$$

$$K_{d,Cl^-} = \frac{M_{Cl^-,g}}{M_{Cl^-,e}} = \frac{(M_{HCl} + M_{NaCl})_g}{(M_{HCl} + M_{NaCl})_e} \quad (2)$$

The distribution coefficients in these equations have been determined by Ujimoto *et al.* with radioactive isotopes. By means of refractometry and potentiometry they determined:

$$K_{d,HCl}^* = dM_{HCl,g}/dM_{HCl,e} \quad (3)$$

$$K_{d,NaCl}^* = dM_{NaCl,g}/dM_{NaCl,e} \quad (4)$$

The values of the distribution coefficients are given in the upper part of Table I.

Further, we can define:

$$K_{d,HCl} = M_{HCl,g}/M_{HCl,e} \quad (5)$$

This distribution coefficient has not been measured, but it can be calculated from other data, as will be shown below.

In the high pH range we must take into account NaOH and NaCl, both in the gel and in the eluent, and the COONa groups in the gel. It holds that:

$$K_{d,Cl^-} = \frac{M_{Cl^-,g}}{M_{Cl^-,e}} = \frac{M_{NaCl,g}}{M_{NaCl,e}} = K_{d,NaCl} \quad (6)$$

$$K_{d,Na^+} = \frac{M_{Na^+,g}}{M_{Na^+,e}} = \frac{(M_{NaOH} + M_{NaCl} + M_{COONa})_g}{(M_{NaOH} + M_{NaCl})_e} \quad (7)$$

$$K_{d,NaOH}^* = dM_{NaOH,g}/dM_{NaOH,e} \quad (8)$$

$$K_{d,NaCl}^* = dM_{NaCl,g}/dM_{NaCl,e} \quad (9)$$

TABLE I

$K_d$  AND  $K_d^*$  VALUES ON SEPHADEX G-10 IN 0.1 M NaCl AS ELUENT AT VARIOUS pH VALUES AT 20°C

Eluent pH	$K_d$		$K_d^*$		
	Na <sup>+</sup>	Cl <sup>-</sup>	NaCl	HCl	NaOH
1.51	0.64	0.69	0.66	0.83	
1.83	0.65	0.68	0.67	0.84	
2.37	0.66	0.67	0.67	0.95	
2.84	0.66	0.66	0.67	1.23	
12.05	0.87	0.54	0.66		2.07
12.50	0.96	0.51	0.62		1.58

The first two distribution coefficients have been measured with radioactive isotopes, the last two by means of refractometry and potentiometry. They are given in the lower part of Table I. Further we can define (not measured, but calculable):

$$K_{d,\text{NaOH}} = M_{\text{NaOH},g}/M_{\text{NaOH},e} \quad (10)$$

The calculations of the distribution coefficients in eqns. 5 and 10 proceed as follows.  $M_{\text{NaCl},e} = 0.1$ ;  $M_{\text{HCl},e}$  and  $M_{\text{NaOH},e}$  are calculated from

$$-\log M_{\text{HCl},e} = \text{pH} + \log y \quad (11)$$

$$-\log M_{\text{NaOH},e} = \text{p}K_w - \text{pH} + \log y \quad (12)$$

where  $y$  is taken to be equal to the activity coefficient of NaCl in a solution of the same ionic strength as the eluent<sup>5</sup> and  $K_w$  is the ionization constant of water<sup>6</sup>. The concentrations in the gel follow from eqns. 1, 2, 6 and 7. One of the results of the calculations is that at  $\text{pH} = 12.05$  the expression for  $K_{d,\text{Na}^+}$  (eqn. 7) reads as follows:

$$K_{d,\text{Na}^+} = \frac{0.034 + 0.054 + 0.008}{0.010 + 0.100} \quad (13)$$

This result shows that the large values of  $K_{d,\text{Na}^+}$  at high pH (large compared to the corresponding values for  $\text{Cl}^-$ , see the second and third columns of Table I) are not so much caused by the presence of 0.008  $M$  of COONa groups in the gel, *i.e.*, by the Donnan equilibrium, but by the affinity of the gel for NaOH, which gives rise to the first term in the numerator and the denominator of the right-hand side of eqn. 13. This affinity is also reflected in the large values of  $K_{d,\text{NaOH}}^*$  (see the last column of Table I). These values are even larger than 1, which demonstrates that NaOH (presumably as its hydroxyl ion) is adsorbed by Sephadex.

The calculated values of  $K_{d,\text{HCl}}$  and  $K_{d,\text{NaOH}}$  are given in Table II. These data, which are based on measurements of  $K_{d,\text{Na}^+}$  and  $K_{d,\text{Cl}^-}$  with radioactive isotopes, are clearly different from the data on  $K_d^*$  in Table I, which are measured by means of refractometry or potentiometry. This is not surprising, as they have different mean-

TABLE II

$K_d$  VALUES CALCULATED FROM THE DATA IN TABLE I WITH EQNS. 5 AND 10

Eluent pH	$K_d$	
	HCl	NaOH
1.51	0.81	
1.83	0.85	
2.37	0.76	
2.84	0.66	
12.05		3.5
12.50		2.3

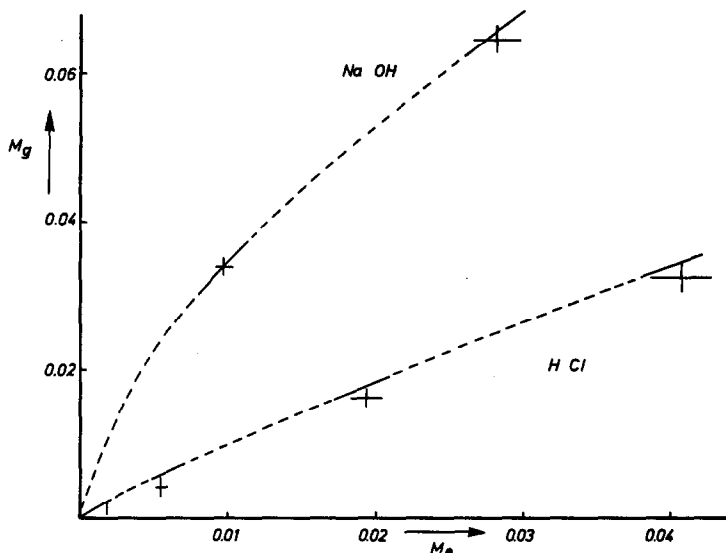


Fig. 1. Plots of  $M_{\text{HCl},g}$  and  $M_{\text{NaOH},g}$  against  $M_{\text{HCl},e}$  and  $M_{\text{NaOH},e}$  respectively.

ings (*cf.*, eqns. 3 vs. 5, and 8 vs. 10). The same conclusion applies to the values of  $K_{d,\text{Cl}^-} = K_{d,\text{NaCl}}$  and  $K_{d,\text{NaCl}}^*$  at high pH, in the third and fourth columns of Table I.

The values of  $K_{d,\text{HCl}}$  or  $K_{d,\text{NaOH}}$  in Table II can be compared graphically with the values of  $K_{d,\text{HCl}}^*$  or  $K_{d,\text{NaOH}}^*$  in Table I. This is done in Fig. 1 where  $M_{\text{HCl},g}$  is plotted as a function of  $M_{\text{HCl},e}$  and  $M_{\text{NaOH},g}$  is plotted as a function of  $M_{\text{NaOH},e}$ . The data on  $K_d$  yield the values of these functions (the sorption isotherms of HCl and NaOH in 0.1 M NaCl), while the data on  $K_d^*$  yield the slopes of the functions. The values and the slopes of the functions are obtained at the investigated pH values, *i.e.*, at a number of values of  $M_{\text{HCl},e}$  and  $M_{\text{NaOH},e}$ , respectively. The agreement is good.

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