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# Gibbs–Donnan Effects in Gel Chromatography

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The recent demonstration of osmotic effects in Sephadex (Edmond, Farquhar, Dunstone & Ogston, 1968) has provided convincing support for the model of gel filtration based on liquid–liquid partition of solute between the mobile phase and the gel medium, in which the velocity of solute is zero (Laurent & Killander, 1964). Such evidence for the function of the gel matrix as a semipermeable membrane implies that Gibbs–Donnan effects must also operate in gel chromatography of systems involving charged macromolecules. The present study of dextran sulphate on columns of Sephadex G-25 verifies the existence of the Donnan equilibrium in gel filtration, thereby substantiating the basic tenets of the liquid–liquid partition model.

Consider an aqueous solution of macroion P bearing $n$ negative charges, which are counterbalanced by $n$ cations A to preserve electroneutrality. After exhaustive dialysis of the solution against an aqueous solution of uni–univalent electrolyte AR, the Gibbs–Donnan equilibrium may be written (Svensson, 1946):

$$(m_A)_i = (m_A)_o[1 + nm_P/2(m_A)_o] \quad (1a)$$

$$(m_R)_i = (m_R)_o[1 - nm_P/2(m_R)_o] \quad (1b)$$

where $m_P$ is the molar concentration of macroion, $m_A$ and $m_R$ being the total molar concentrations of ions A and R respectively: the subscript i is used to distinguish concentrations within the dialysis sac from those in the diffusate (subscript o). It follows from eqn. (1b) that $(m_R)_i < (m_R)_o$, and thus the concentration of unbound AR within the dialysis sac is less than its concentration in the diffusate (eqn. 2):

$$(m_{AR})_i = (m_{AR})_o[1 - nm_P/2(m_{AR})_o] \quad (2)$$

We now wish to consider the situation resulting from frontal gel filtration of the dialysed solution of macroion on a column of Sephadex: for simplicity the grade of Sephadex is chosen such that the macromolecular species (P, $PA_n$) are excluded from the gel phase, whereas the supporting electrolyte ions A and R do penetrate the gel. It is assumed that A and R have the same elution volume ($V_{AR}$), which is, of necessity, greater than that of the macromolecular species ($V_o$). The use of water to elute a column equilibrated previously with the dialysed solution of macroion gives rise to the following boundary distribution in the trailing elution profile:

$$PA_n, A, R, H_2O\ (\alpha) \leftarrow A, R, H_2O\ (\beta) \leftarrow H_2O\ (\gamma)$$

This nomenclature originates from Longsworth (1959), the arrows denoting boundaries between the specified $\alpha$, $\beta$ and $\gamma$ regions. A moving-boundary equation may be written in terms of either the A or R constituent for the $\alpha\beta$ boundary, across which macroion (but not AR) disappears: for purposes of illustration we examine that pertaining to the R constituent (corresponding to unbound AR), although an entirely analogous result emerges from consideration of the A constituent in all of its forms. The appropriate equation, referring to the elution profile (denoted by the subscript e), is eqn. (20) of Nichol, Ogston & Winzor (1967), which may be written in the present terminology as:

$$(V_o - V_o/\mu_{AR}{}^{\alpha})(m_{AR}{}^{\alpha})_e = (V_o - V_o/\mu_{AR}{}^{\beta})(m_{AR}{}^{\beta})_e \quad (3)$$

where $\mu_{AR}{}^{\alpha}$ represents the ratio of the amount of free AR in the mobile phase to the total free AR in both mobile and stationary phases of the $\alpha$ region on the column: if subscripts m and s are used to denote concentrations in the mobile and stationary phases, respectively, $\mu_{AR}{}^{\alpha}$ is given by eqn. (4):

$$\mu_{AR}{}^{\alpha} = V_o(m_{AR}{}^{\alpha})_m/[V_o(m_{AR}{}^{\alpha})_m + (V_{AR} - V_o)(m_{AR}{}^{\alpha})_s] \quad (4)$$

An entirely analogous expression written for $\mu_{AR}{}^{\beta}$, the fraction of AR in the mobile phase of the $\beta$ region, simplifies to:

$$\mu_{AR}{}^{\beta} = V_o/V_{AR} \quad (5)$$

since the concentrations of AR in the mobile and stationary phases are identical in the absence of macroion. Combination of eqns. (3)–(5) yields:

$$(m_{AR}{}^{\beta})_e = (m_{AR}{}^{\alpha})_e(m_{AR}{}^{\alpha})_s/(m_{AR}{}^{\alpha})_m = (m_{AR}{}^{\alpha})_s \quad (6)$$

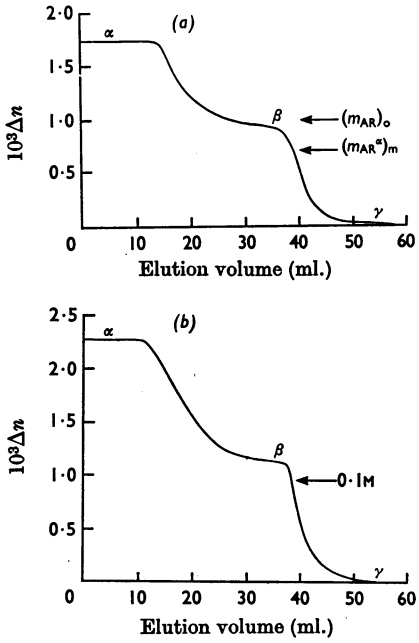


Fig. 1. Profiles resulting from the use of water to elute a 2·5cm.×8cm. column of Sephadex G-25 equilibrated previously with: (*a*) a 1% solution of dextran sulphate dialysed to equilibrium against 0·1M-NaCl; (*b*) a 1% solution of dextran sulphate dissolved directly in 0·1M-NaCl (see the text).

the latter simplification resulting from the demonstration (Nichol *et al.* 1967) that in frontal chromatography the composition of the inflowing solution is identical with that of the mobile phase of the α-plateau region on the column, and also with that in the corresponding region of the elution profile, i.e. that:

$$(m_{AR})_I = (m_{AR^\alpha})_m = (m_{AR^\alpha})_e \quad (7)$$

In the absence of Donnan effects on the column:

$$(m_{AR^\alpha})_m = (m_{AR^\alpha})_s$$

whereupon it follows that the concentration of AR separating as the pure solute phase (β) is identical with its concentration in the applied solution. This identity does not pertain when the Gibbs–Donnan equilibrium operates, since $(m_{AR^\alpha})_m$ and $(m_{AR^\alpha})_s$ are then related by the expression:

$$(m_{AR^\alpha})_m = (m_{AR^\alpha})_s[1 - nm_P/2(m_{AR^\alpha})_s] \quad (8)$$

From a comparison of eqn. (8) with eqn. (2) in the light of eqns. (6) and (7) it follows that when a Gibbs–Donnan equilibrium operates:

$$(m_{AR^\beta})_e = (m_{AR})_o \quad (9)$$

Thus the existence of a Donnan effect in frontal gel filtration of a dialysed solution of a macroion may be inferred from the trailing elution profile, where the concentration of AR in the β phase should correspond with that in the diffusate.

A 1% solution of dextran sulphate (17·5% S; prepared from Dextran 500 by Pharmacia, Uppsala, Sweden) in water was dialysed to equilibrium against 0·1M-NaCl, and the diffusate was used to equilibrate a 2·5cm.×8cm. column of Sephadex G-25, thermostatically controlled at 25°. The dialysed solution (45ml.) of dextran sulphate was applied to the column and then eluted with water at a flow rate of 1ml./min., the effluent being collected in 1ml. fractions. Each fraction was monitored by determining the refractive-index difference at 546nm., water being used in the reference compartment of a Brice–Phoenix differential refractometer. From these data (Fig. 1*a*) it is evident that $(m_{AR^\beta})_e$ approximates closely to the concentration of NaCl in the diffusate, but differs significantly from $(m_{AR^\alpha})_m$, which was calculated from eqn. (8) on the basis of two sulphate groups/glucose unit for dextran sulphate with 18% S content (Ricketts, 1952).

An experiment of different design was also conducted in which the initial dialysis step was omitted, the column being equilibrated with a 1% (w/w) solution of dextran sulphate in 0·1M-NaCl. The trailing elution profile resulting from elution with water is shown in Fig. 1(*b*), from which it is clear that the concentration of NaCl in the β phase is greater than 0·1M. Application of eqn. (8) to these data yielded an estimate of 1·9 for *n*, a value in excellent agreement with expectation.

Thus we conclude that these experiments implicate the operation of a Gibbs–Donnan equilibrium in gel filtration of charged macromolecules. In so doing they supplement the osmotic data of Edmond *et al.* (1968) in providing experimental evidence for the model of gel chromatography based on liquid–liquid partition, with the gel matrix acting as a semipermeable membrane between the mobile and stationary phases.

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