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Note

Determination of the solvent regain of Sephadex gels by column chromatography

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The solvent regain (Sr) of a gel or resin can be defined as

$$Sr = \frac{w_{sa}}{w_a} \qquad (1)$$

where w_{sa} is the weight of solvent absorbed by a weight w_a of dry gel. The determination of dry weight is often difficult because the conditions required for complete removal of the solvent may cause some decomposition of the gel[1]. This is a particularly difficult problem with the water-swollen and thermolabile polysaccharide gels such as Sephadex. There seems to be no completely satisfactory solution to this problem and the only answer may be to standardize the drying procedure and trust, in this way, that a standard solvent or water regain value is obtained.

The weight of solvent absorbed by the gel may be determined as the difference between the weight of fully solvated and dry gel. It is therefore necessary to obtain a sample of gel which is fully swollen but which does not contain any excess of solvent. Centrifugation has been used to remove the interstitial solvent[2]. However, if the particles are very irregular, complete removal of the external solution will be more difficult[1].

In the exclusion method, dry gel is mixed with a solution containing a known concentration of an excluded solute. The solvent regain can then be calculated from the concentration change due to mixing. This is true if the solute itself neither influences the solvent regain nor penetrates (is not absorbed by) the gel. Provided that very low solute concentrations are used, an influence on the solvent regain is very unlikely but the probability of the lack of penetration or absorption can only be inferred indirectly when a number of different solutes yield the same regain value.

The solute exclusion can be measured either by the above "static" mixing method or by "dynamic" column chromatography, provided that the conditions in the latter are quasi-equilibrial, for which there is much evidence, provided that flow-rates are sufficiently slow. Chromatography is easier to perform in that it has much less stringent demands on analytical accuracy and, further, repeated measurements can be made on the same gel without any intervening drying process. It is necessary to dry the gel only once, either before or after the chromatographic measurements.

THEORY OF CHROMATOGRAPHIC METHOD

In eqn. 1

$$w_{sa} = V_{sa}/V'_{sa} \qquad (2)$$

where V'_{sa} is the specific volume of the solvent within the gel particles.

In a chromatographic column

$$V^*_{sa} = V_s - V_0 \qquad (3)$$

where V_s and V_0 are the peak elution volumes of an isotopically labelled solvent sample and an excluded solute, respectively, and the asterisk in V^*_{sa} indicates that it is calculated from a column experiment. If it is assumed that static mixing and dynamic column experiments are equivalent, then

$$V^*_{sa} = V_{sa}$$

Substituting eqns. 2 and 4 into eqn. 1, we obtain

$$Sr = \frac{V^*_{sa}}{w_a \cdot V'_{sa}} \qquad (5)$$

In the case of water, V_s (eqn. 3) can be measured by the use of $H_2{}^{18}O$, while tritiated water (H^3HO) gives an overestimate of V_s in gels that contain exchangeable protons (*e.g.*, polyhydroxylic and polyacrylamide gels)[3]. Measurement of ^{18}O is a laborious procedure for most laboratories but other reference solutes can be used provided that the relationships between their elution volumes and that of $H_2{}^{18}O$ are known. Thus, tritium activity (V_{H^3HO}) loaded on to the column as H^3HO is retarded relative to $H_2{}^{18}O$ owing to protium–tritium exchange, *i.e.*

$$V_{H^3HO} = V_s + V_x$$

where s refers to $H_2{}^{18}O$ and x to the isotope exchange retardation. Defining V^*_{ta} analogously with V^*_{sa}, as the volume that the tritium activity would occupy in the gel if it were all in solution as H^3HO at the same concentration as outside, instead of partially "adsorbed", it then follows that

$$V^*_{ta} = V^*_{sa} + V_x \qquad (6)$$

and, rearranging after dividing both sides by V^*_{sa}

$$V^*_{sa} = \frac{V^*_{ta}}{\left(1 + \frac{V_x}{V^*_{sa}}\right)} = \frac{V^*_{ta}}{q} \qquad (7)$$

where q ($H_2{}^{18}O$) is the ratio between the distribution coefficients of tritium of H^3HO and $H_2{}^{18}O$ (ref. 3).

Combining eqns. 5 and 7:

$$Sr = \frac{V^*_{ta}}{q \cdot w_a \cdot V'} \qquad (8)$$

TABLE I

VARIATION OF φ ($H_2^{18}O$) WITH Wr

Sephadex gel	*Nominal Wr*	φ ($H_2^{18}O$)
G-10	1.0	1.091*
G-15	1.5	1.075**
G-25	2.5	1.060**

* Taken from ref. 3.
** Taken from ref. 4.

The value of φ does not vary greatly in relation to the water regain value (Wr), as Table I shows, and it is therefore probably unnecessary even to determine the $H_2^{18}O$ elution volume on each gel batch.

EXPERIMENTAL

Column method

Amounts of Sephadex G-15 (Batch No. 2014) and G-25 (Batch No. 5336) gels approximately sufficient to fill the columns (bed dimensions *ca.* 40 cm × 0.8 cm²) were weighed in tared vacuum filtration flasks and then dried to constant weight at 60°. The flasks were transferred from the drying oven into a desiccator and allowed to cool over silica gel before weighing. The gels were then swollen in an excess of water for at least 18 h, after which they were degassed. The slurry was then poured into the column, the flask being rinsed several times until only trace amounts of the gel, which were difficult to wash out, remained in the flask. The weight of this residual gel was then determined by re-weighing the flask after drying it at 105° for several hours.

The column was then allowed to settle by allowing about 250 ml of deionized water to flow through it. During subsequent column operation, the bed length did not shorten by more than 0.3 cm. Tritiated water and [^{14}C]glucose (Radiochemical Centre, Amersham, Great Britain) were eluted together with dextran 500, mol. wt. 450,000 (Pharmacia, Uppsala, Sweden) as the void volume indicator. The volume of the loading solution was 0.5 ml and the eluent was deionized water at a linear flow-rate of about 2 cm·h^{-1}. Dextran was determined by an anthrone method and ^{14}C and ^{3}H activities by liquid scintillation counting (Beckman CPM 200).

Equilibration method

For equilibration with [^{14}C]Dextran ("mol. wt." 60,000–90,000, New England Nuclear, Boston, Mass.), 0.2 g of Sephadex G-25, dried as above, was mixed with 2 ml of [^{14}C]Dextran solution (100,000 cpm) and the mixture shaken for 5 h at 25°. The suspension was filtered (Millipore) and five 0.1-ml samples of the filtrate were counted.

Centrifuge method

Water-swollen gel was centrifuged at 500 *g* for 10 min in a plastic tube closed at the bottom by 40 μm mesh nylon net. Then 2–3 g of the centrifuged gel were first weighed and afterwards dried to constant weight at 60°.

Table II shows the results of the different methods. Although the results are limited, we believe that they indicate that the column method is promising, but it

TABLE II

WATER REGAIN VALUES

Values are means ± standard deviation; numbers of measurements in parentheses.

Sephadex gel	*Method*		
	Centrifuge	*[^{14}C]Dextran*	*Column*
G-15	1.480(3) ± 1.5%	—	1.245(5) ± 3.8%
G-25	2.547(3) ± 3.9%	2.047(6) ± 6.1%	2.347(3) ± 0.23%

must be tested much more extensively before a final verdict can be given. All of the column values were lower than those obtained by centrifugation, which agreed well with the water regain values stated by the manufacturer.

The differences between the centrifuge and column methods were similar for the two gels, *viz.*, 0.21 $ml \cdot g^{-1}$ for Sephadex G-15 and 0.20 $ml \cdot g^{-1}$ for Sephadex G-25. If it is assumed that the average bead diameter was 100 μm and that the column method gives the true value, the excess values for the centrifuge method could be accounted for by a water layer about 4 μm thick surrounding the beads. The gel surface is, however, almost certainly not smooth and may be expected to hold some residual water[5].

It is possible that centrifugation at higher speeds would reduce the excess further[5], but deformation of these rather soft gels may occur. Some water might also be held by capillarity in fissures in the gel beads, although judging from their infrequent occurrence in scanning electron micrographs of dry Sephadex G-15 and G-25 beads this is unlikely to occur[6].

The values obtained from equilibration with [^{14}C]dextran were rather low. This was probably due to the entry of some radiation decomposition product of the dextran into the gel and should be repeated with inactive dextran. It still remains to be proved that dextran 500 is totally excluded, but this seems to be a reasonable assumption.

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