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CHROMATOGRAPHIC DETERMINATION OF THE INTERNAL SOLVENT COMPOSITION, SOLVENT REGAIN AND DISTRIBUTION COEFFICIENTS IN GELS IN MIXED SOLVENT SYSTEMS

I. THEORY

N. V. B. MARSDEN

Institute of Physiology and Medical Biophysics, Uppsala University Biomedical Centre, Box 572, S-751 23 Uppsala (Sweden)

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SUMMARY

The theory of two chromatographic methods for determining the internal solvent composition of a gel in a mixed solvent system is described. In the first method, small isotopically labelled samples of the component solvents are eluted through a column and their positions in the effluent are measured. If water is present it must be labelled with an oxygen and not with an exchangeable hydrogen isotope. It is then possible to calculate the internal solvent composition, solvent regain and solvent distribution coefficients and also the distribution coefficient of any other solute present. In the second method, which was designed for use with radioactively labelled water, tritiated water (or another protic solvent tritiated at an exchangeable site) is used together with other solvents labelled isotopically in stable positions. Although protium–tritium exchange complicates this method, the internal solvent composition can be determined if independent estimates of the solvent regain and the magnitude of the hydrogen isotope exchange with the gel matrix are available. If the latter is unknown and is neglected, only a small error is introduced at all except the lowest solvent regain values. The magnitude of the hydrogen isotope exchange can be determined with water labelled by both oxygen and hydrogen isotopes provided that the degree of accessibility of the exchange sites is known.

INTRODUCTION

With water as the solvent, more tightly cross-linked neutral, or nearly neutral, hydrophilic gels such as those of dextran (Sephadex), polyacrylamide (Biogel) or cellulose exhibit considerable selectivity for low-molecular-weight non-electrolytes (for example, see refs. 1–4) and also for ions[5–13] (both anions and cations). Not unexpectedly, replacement of water with another solvent may result in drastic changes in the distributions of some solutes while having little effect on others. Thus, the mark-

ed affinity for the weakly polar aliphatic alcohols exhibited by the most tightly cross-linked Sephadex gels when water is the sole solvent disappears if the latter is replaced with formamide[14]. Dimethyl sulphoxide (DMSO) has a similar effect on some aromatic compounds[15]. With more polar solutes, however, such as oligosaccharides, replacement of water with formamide or DMSO has clear although less dramatic effects[16].

The effects of solvent changes on the selectivity of ion-exchange resins are well documented[17]. Ionic behaviour in Sephadex has been studied in water–methanol mixtures. Alkali[18,19] and alkaline earth cation[20] selectivities were considerably enhanced. Like other hydrophilic gels, aqueous Sephadex also has some selectivity towards anions, the "aqueous" order of affinity for halides[10] being $Cl^- < Br^- < I^-$. This order can be reversed and the selectivity increased in mixtures of water with methanol, ethanol or acetonitrile[21].

The effects of changing the external solvent composition are complex. The internal solvent composition is changed and also the solvent regain if the gel–solvent interactions are also changed. For example, in water–dioxane mixtures[22], the solvent regain of the unsubstituted dextran gel Sephadex G-25 is roughly proportional to the fraction of water in the solvent mixture. The solvent regain of the less polar hydroxypropylene-substituted derivative Sephadex LH-20 is, on the other hand, almost independent of the external composition if the water content exceeds about 40%.

As pointed out by Bush *et al.*[22] the effects of a changed solvent composition may be expected to be complex. The changed geometry of the gel matrix due to altered solvent regain may not only affect the degree, if any, of steric exclusion of a solute, but confinement of a solute, with restricted freedom of movement in a small space, may also affect the gel–solute interactions[23]. A difference between the internal and external solvent compositions will affect solute partitioning *per se* in addition to any effects that the gel–solvent interactions may have on solute molecules near to the gel matrix surface. (The gel matrix is the non-solvent moiety of the swollen gel bead, *i.e.*, the dextran chains and cross-links in the case of Sephadex). This surface can be expected to become increasingly important as the solvent regain declines to very low values.

These problems may be further complicated by the peculiar ability of water to achieve a considerable degree of ordering[24]. A polyhydroxylic surface may perturb the normal (bulk) order, thus creating a vicinal region of water with an altered structure[25,26]. The dextran–water interaction may be particularly strong, as the hydroxyl groups of glucose in the C1 conformation can be fitted into a water lattice with all hydrogen bonds unbent[27,28].

The ordering effect of such a surface may extend the vicinal layer to a distance corresponding to a few water molecules[29], *i.e.*, up to about 20 Å. Thus, in a tightly cross-linked gel, a further reduction in solvent regain, such as that which results from admixture of water with a few per cent of methanol or ethanol, may increase considerably the proportion of vicinal water molecules and in this way alter the affinity for the solute.

The lack of an adequate structural model for water that can account satisfactorily for its behaviour remains a major obstacle to our understanding of aqueous gel systems. This is not to suggest that all difficulties tend to disappear with other solvents; relationships in water–alcohol mixtures are far from simple[30]. Paradoxically,

solute behaviour, however, may prove easier to interpret in mixed solvent systems, which undoubtedly also have great potential practical usefulness.

Two variables that are of interest in this respect are the internal solvent composition and the solvent regain. Knowledge of the former enables the actual distribution coefficient of a component to be compared with that expected on the basis of the different solubilities in two bulk phases of similar composition.

The solvent regain value is a measure of two properties of the gel matrix chains: their dissolving tendency in the solvent and their average distance apart assuming a random network structure.

The two methods described below are based on the use of isotopically labelled solvents in order to determine their elution positions. The first method, which requires solvents labelled with isotopes that do not exchange, is applicable to any gel and solvent system provided that the gel swells in at least one of the solvents. The second method was designed specifically for aqueous mixtures because of the lack of a usable radioactive oxygen isotope and involves the use of tritiated water. This is complicated by tritium exchange, which occurs if exchange sites are present on the gel matrix or on the other solvent(s) present. This problem can be overcome provided that additional data about the gel structure are available. If neither the gel nor the other solvent(s) possess exchange sites, the second method does not differ from the first.

THEORY OF METHODS

The imbibed (internal) solvent composition in a binary system (first method)

The ratio of the chromatographic distribution coefficients of the two mutually miscible solvents can be used to determine the internal solvent composition under certain conditions that are discussed below. The so-called dynamic distribution coefficient[31], K_d^i, of a component, solvent or solute, can be defined as follows:

$$K_d^i = \frac{m^i - m^0}{m^s - m^0} \tag{1}$$

where m^i and m^0 are the weights of the effluent after which the peak concentrations of components i and 0, respectively, appear. The latter is a totally excluded solute or void volume indicator. In a single solvent system, m^s, the elution position of a solvent sample loaded on to the column together with components i and 0, can be determined by employing an isotopically labelled solvent; the difference $m^s - m^0$ represents the weight of solvent imbibed by the gel in the column. With a solvent mixture, there is no unique solvent indicator and the distribution coefficient cannot be determined directly. Ratios of distribution coefficients can, however, be estimated. Thus, with the two solvents, α and β, of a binary system, the ratio of their distribution coefficients can be obtained by eluting a small volume of the solvent mixture containing isotopically labelled samples of both solvents and a void volume indicator.

For the ratio, using eqn. 1, we then obtain

$$\frac{K_d^\alpha}{K_d^\beta} = \frac{m^\alpha - m^0}{m^\beta - m^0} \tag{2}$$

where m^{α} and m^{β} are the weights of effluent after which the peak concentrations (activities) of the isotopically labelled solvents are eluted.

The validity of the chromatographic method depends on the correctness of two assumptions. The first is of quasi-equilibrial conditions in the column, an assumption that appears to be valid, provided that the flow-rates are sufficiently low[32]. The second assumption, which seems reasonable, is that the specific activity of an isotopically labelled component is the same in the solvent spaces outside and inside the gel beads. The special case of exchangeable hydrogen isotopes will be discussed below. The distribution coefficient (K_{eq}), as measured in an equilibration experiment, can be defined as

$$K_{eq}^{i} = \frac{c_g^i}{c_0^i} \tag{3}$$

where c_g^i and c_0^i are the concentrations in the solvent imbibed by the gel and the solvent outside the gel bead, respectively. The concentrations of the solvents can be expressed conveniently as weight fractions (f_j^i) and in a binary system the distribution coefficients are thus

$$K_{eq}^{\alpha} = \frac{f_g^{\alpha}}{f_0^{\alpha}} \tag{4a}$$

$$K_{eq}^{\beta} = \frac{f_g^{\beta}}{f_0^{\beta}} \tag{4b}$$

The ratio of the coefficients ($K_{eq}^{\beta/\alpha}$) is then

$$K_{eq}^{\beta/\alpha} = \frac{K_{eq}^{\beta}}{K_{eq}^{\alpha}} = \frac{f_g^{\beta}}{f_g^{\alpha}} \cdot \frac{f_0^{\alpha}}{f_0^{\beta}} \tag{5}$$

In a binary system

$$f_g^{\alpha} + f_g^{\beta} = 1 \tag{6}$$

and, using this relation to eliminate f_g^{β} in eqn. 5 and solving for f_g^{α}, we obtain

$$f_g^{\alpha} = 1 \Big/ \left(1 + \frac{f_0^{\beta}}{f_0^{\alpha}} \cdot K_{eq}^{\beta/\alpha}\right) \tag{7}$$

Assuming that "static" equilibration and "dynamic" column experiments yield identical results, *i.e.*

$$K_d^i = K_{eq}^i \tag{8}$$

eqn. 2 can be substituted into eqn. 7 to yield

$$f_g^\alpha = 1 \Big/ \left(1 + \frac{f_0^\beta}{f_0^\alpha} \cdot \frac{m^\beta - m^0}{m^\alpha - m^0}\right) \tag{9}$$

and f_g^β and the two K_d values can then be calculated (eqn. 4). It should be noted that other column distribution coefficients such as K_{av} (ref. 33) can be used instead of K_d, as their ratios are equal to the K_d ratios. As noted above, the use of a hydrogen isotope for labelling a solvent is precluded unless it is at a virtually non-exchangeable site, such as a carbon-hydrogen[34], or unless the other solvent(s) and gel have no exchange sites. Unless the latter conditions are satisfied, water must be labelled with a stable oxygen isotope[35].

More complex solvent mixtures

More complex internal solvent compositions can be determined from the weight fraction ratios of pairs of the component solvents. Thus, in a ternary system with solvents α, β and γ (eqns. 2 and 5):

$$\frac{f_g^\beta}{f_g^\alpha} = \frac{f_0^\beta}{f_0^\alpha} \cdot \frac{m^\beta - m^0}{m^\alpha - m^0} \tag{10a}$$

and

$$\frac{f_g^\gamma}{f_g^\alpha} = \frac{f_0^\gamma}{f_0^\alpha} \cdot \frac{m^\gamma - m^0}{m^\alpha - m^0} \tag{10b}$$

As

$$f_g^\alpha + f_g^\beta + f_g^\gamma = 1 \tag{11}$$

then

$$f_g^\alpha = 1 \Big/ \left(1 + \frac{f_g^\beta}{f_g^\alpha} + \frac{f_g^\gamma}{f_g^\alpha}\right) \tag{12a}$$

$$f_g^\beta = f_g^\alpha \left(\frac{1}{f_g^\alpha} - \frac{f_g^\gamma}{f_g^\alpha} - 1\right) \tag{12b}$$

and

$$f_g^\gamma = 1 - f_g^\alpha - f_g^\beta \tag{12c}$$

More complex solvent mixtures can be treated in an analogous manner.

Determination of the solvent regain (Sr)

The *Sr* value is defined as the ratio between the weights of solvent (m^{sg}) and gel matrix (m^g) in a fully solvated bead, *i.e.*

$$Sr = \frac{m^{sg}}{m^g} \tag{13}$$

It has been proposed that solvent regain values in single-solvent systems can be determined chromatographically[36] and the same principles can be applied here.

Since

$$m^{sg} = m^s - m^0 \tag{14}$$

(see eqn. 1), if the weight of the gel matrix in the column is known and using eqns. 1 and 13, the *Sr* value can be calculated from the behaviour of any one solvent in a mixture, *viz.*,

$$Sr = \frac{m^i - m^0}{m^g \cdot K_d^i} \tag{15}$$

since the value of K_d^i can be obtained from f_v^i (eqn. 9).

An alternative expression, incorporating data from more, or all, of the solvents (r) in a mixture, is

$$Sr = \frac{\sum_{i=1}^{r} m^i - rm^0}{m^g \cdot \sum_{i=1}^{r} K_d^i} \tag{16}$$

It is perhaps also of interest to note that if, in a mixture, the weight fractions of all of the component solvents outside the gel are equal, then, from eqns. 4 and 6

$$\sum^{r} K_d^i = \sum^{r} \left(f_v^i \Big/ \frac{1}{r} \right) = r \tag{17}$$

Distribution coefficient of a solute

As there is formally no difference between a solute and a solvent, the distribution coefficient of a solute j can be obtained by rearrangement of eqn. 2:

$$K_d^j = \frac{(m^j - m^0) \cdot K_d^i}{m^i - m^0} \tag{18}$$

where i refers to any other component, solute or solvent.

In analogy with eqn. 16, data from all of the solvents in a mixture can be used to calculate a solute distribution coefficient:

$$K_d^j = \frac{(m^j - m^0) \sum^{r} K_d^i}{\sum^{r} m^i - rm^0} \tag{19}$$

The use of tritium-labelled water in determining the internal solvent composition in a binary solvent mixture (second method)

Although the tritium in tritiated water is exchangeable and therefore does not represent the behaviour only of water in a chromatographic column, it can be used instead of oxygen isotope-labelled water in determining the internal solvent composition.

Hydrogen atoms bonded to other atoms such as oxygen, nitrogen or sulphur are exchangeable[37,38]. This exchange depends on the relative affinities of the hydrogen isotopes for the exchange sites (isotope exchange effect) and also on the accessibility of the site to the exchanging isotope[39]. It is likely, in pure aqueous systems, that all, or nearly all, of the exchange sites (hydroxyl groups) on Sephadex gels are accessible, except perhaps in the most tightly cross-linked gels. For the present purpose, the degree of accessibility is irrelevant; all that matters is that it should not be changed significantly by change of solvent. Too large a decrease in the solvent regain induced by change to a solvent with a much lower dielectric constant may allow a sufficiently close mutual approach of gel hydroxyl groups to form intramolecular hydrogen bonds, which may prevent exchange. Constancy of the number of accessible sites is assumed here and, as will be evident below, this is unlikely to lead to a significant error, except with very low solvent regain values.

The principle of the method is as follows. In a column containing a gel bed with water as the only solvent, tritium introduced as labelled water is retarded relative to water labelled with an oxygen isotope, *e.g.* $H_2{}^{18}O$ (ref. 35). The retardation is a function of the relationship between the number of accessible sites on the gel matrix and the number of sites (two per water molecule) on the water imbibed by the gel.

Mixture of water and another protonic solvent. In such a mixture, exchangeable tritium (^3H, hereafter designated as T) will be distributed among exchange sites on the two solvents (H_2O, ROH) and gel matrix as described in the following reactions:

(I) $HTO + \text{Gel-OH} \rightleftharpoons \text{Gel-OT} + H_2O$ $(K_{\mathrm{I}}^{\mathrm{ex}})$

(II) $HTO + ROH \rightleftharpoons ROT + H_2O$ $(K_{\mathrm{II}}^{\mathrm{ex}})$

(III) $ROT + \text{Gel-OH} \rightleftharpoons \text{Gel-OT} + ROH$ $(K_{\mathrm{III}}^{\mathrm{ex}})$

Because of the relatively large differences between the vibrational zero-point energies of the hydrogen isotopes[37,38], the equilibrium exchange constants (K_r^{ex}) are likely to deviate from unity[39,40]. Thus, a value of 1.20 ± 0.06 was found for the ratio between the affinities of the hydroxyl groups of dextran and water for tritium[41].

$K_{\mathrm{II}}^{\mathrm{ex}}$ will affect the tritium distribution between the internal and external solvents only if their compositions are different. Preliminary measurements indicate that up to a methanol mole fraction of at least about 0.5 in water–methanol systems, the internal and external compositions do not differ by more than about 20%. $K_{\mathrm{I}}^{\mathrm{ex}}$ and $K_{\mathrm{III}}^{\mathrm{ex}}$ are then the main determinants of the occupancy of exchange sites on the gel matrix and contribute in proportion to the solvents' own molal exchange site ratios.

When the gel is suspended in a mixture of water (w) and another protic solvent such as methanol (p), the distribution coefficient of tritium (K_d^{T}) introduced as either HTO or CH_3OT can be expressed as

$$K_d^{\mathrm{T}} = \varphi \cdot \frac{n_g^{\mathrm{w}} + n_g^{\mathrm{p}}}{n_0^{\mathrm{w}} + n_0^{\mathrm{p}}} \qquad (20)$$

where the ns represent the concentrations of tritium exchange sites on the water and alcohol molecules in unit weights (1 kg) of internal (g) and external (0) solvent. K_{II}^{ex} is assumed to be unity and φ is a factor to correct for the occupancy of exchange sites on the gel matrix by tritium. One kilogram of internal solvent is associated with $1/Sr$ kg of gel matrix and φ is defined as

$$\varphi = \frac{K_b^{ex} \cdot n_g^{ex}/Sr + n_g^w + n_g^p}{n_g^w + n_g^p} \tag{21}$$

where n_g^{ex} is the molal concentration of exchange sites on the gel matrix. K_b^{ex} is a weighted average exchange constant defined as

$$K_b^{ex} = X_g^w \cdot K_I^{ex} + X_g^p \cdot K_{III}^{ex} \tag{22}$$

where X_g^w and X_g^p are the mole fractions of exchange sites on water and alcohol, respectively, in the internal solvent. As a water molecule has two exchange sites, the mole fraction of the exchange sites possessed by the water molecules is greater than the mole fraction of water itself.

Combining eqns. 20 and 21 and rearranging, we obtain

$$K_d^T = \frac{K_b^{ex} \cdot n_g^{ex} + (n_g^w + n_g^p) \cdot Sr}{(n_0^w + n_0^p) \cdot Sr} \tag{23}$$

In a binary system with only one degree of freedom, both solvent concentrations can be expressed in terms of the weight fraction of one solvent. Thus, eliminating the term for water and bearing in mind that it is bifunctional, the required relationship for water in both the internal and external solvents is

$$n_j^w = \frac{2\,M^h}{M^w} \cdot (1 - f_j^p) \tag{24a}$$

while for the monofunctional alcohol

$$n_j^p = \frac{M^h}{M^p} \cdot f_j^p \tag{24b}$$

where M^h is the atomic weight of hydrogen and M^w and M^p are the molecular weights of water and alcohol, respectively. Let $M^h \cdot (1/M^p - 2/M^w) = P$ and $2\,M^h/M^w = Q$, with $P = -0.08042$ and $Q = 0.119$.

Using the above terms when eqns. 24a and 24b are summed and substituted into eqn. 23, we obtain

$$K_d^T = \frac{K_b^{ex} \cdot n_g^{ex} + (Pf_g^p + Q) \cdot Sr}{(Pf_0^p + Q) \cdot Sr} \tag{25}$$

Because, as noted previously, the K_d^T value cannot be determined directly in a mixed

solvent system, the ratio between the tritium and alcohol distribution coefficients must be used instead. Assuming again the equivalence of column and equilibration experiments and using eqn. 4:

$$\frac{K_d^T}{K_d^p} = K_d^{T/p} = \frac{f_0^p}{f_g^p} \cdot \frac{K_b^{ex} \cdot n_g^{ex} + (Pf_g^p + Q) \cdot Sr}{(Pf_0^p + Q) \cdot Sr} \tag{26}$$

and thus

$$f_g^p = \frac{f_0^p \cdot (K_b^{ex} \cdot n_g^{ex} + QSr)}{Sr \cdot (Pf_0^p \cdot K_d^{T/p} + QK_d^{T/p} - Pf_0^p)} \tag{27}$$

Eqn. 27 can be solved if both $K_b^{ex} \cdot n_g^{ex}$ and Sr are known.

There are various independent non-chromatographic methods for determining solvent regain[42,43]. There then remains the problem of $K_b^{ex} \cdot n_g^{ex}$.

In a pure aqueous system when only reaction I (p. 7) occurs, the term $K_I^{ex} \cdot n_g^{ex}$ can be determined either chromatographically by comparing the elution weights of HTO and $H_2{}^{18}O$ (ref. 35), or by equilibrating a known weight of the gel matrix with tritiated water and determining the reduction in activity in the external water due to exchange.

With a pure aqueous system where $f_g^p = f_0^p = 0$, eqn. 25 becomes

$$K_d^T = \frac{K_I^{ex} \cdot n_g^{ex}}{Q \cdot Wr} + 1 \tag{28}$$

or

$$K_I^{ex} \cdot n_I^{ex} = 0.1119\ Wr \cdot (K_d^T - 1) \tag{29}$$

where Wr is the water regain value and the right-hand side of eqn. 28 is the factor φ in eqns. 20 and 21. In a pure aqueous system, the tritium K_d^T value (HTO) is thus equal to that of water (unity) plus the ratio between the tritium activity located on the exchange sites in the gel matrix and that on the imbibed water molecules in the water-swollen gel bead.

If tritium is used instead of an oxygen isotope for labelling water in a binary mixture, there are two sources of systematic error, related inversely to the solvent regain, in the calculated value of the weight fraction of the non-aqueous component in the external solvent.

Data on the isotope exchange effect indicate that the exchange constants probably lie between about 1.2 and 1.0 (refs. 39 and 41). The error resulting from this uncertainty varies inversely with the solvent regain, *i.e.*, in the term $K_b^{ex} \cdot n_g^{ex} + 0.1119\ Sr$ (eqn. 27). The assumption of a too high K^{ex} value will give an overestimate of f_g^p but this is small in Sephadex G-25 even at very low Sr values. This is shown in Table I

TABLE I

SEPHADEX G-25 IN METHANOL–WATER SYSTEMS AT 25°

Sr	$K_d^{T/p}$	f_0^p	f_g^p	K_d^p	K_d^T	f_g^{p*}	K_d^{p*}	K_d^{T*}	$E(K^{ex})\%$	$E(f_g^p)\%$
2.45	—	—	—	—	1.06	—	—	1.00	0.09	—
2.20	1.12	0.10	0.09_6	0.96	1.08	0.09_0	0.90	1.01	1.04	6.25
1.95	1.24	0.25	0.22_5	0.90	1.11	0.20_9	0.84	1.04	1.23	7.11
1.25	1.44	0.50	0.43_7	0.87	1.25	0.39_0	0.78	1.12	1.75	10.8
1.01	1.61	0.60	0.51_1	0.85	1.37	0.44_5	0.74	1.20	2.13	12.9
0.78	1.96	0.70	0.56_5	0.80	1.57	0.47_3	0.68	1.33	2.67	16.3
0.60	2.49	0.78	0.58_4	0.75	1.87	0.46_8	0.60	1.50	3.60	19.9
0.47	3.55	0.83	0.54_0	0.65	2.30	0.40_9	0.49	1.75	3.66	24.3

* The terms marked with asterisks represent values calculated assuming $n_g^{ex} = 0$. $E(f_g^p)\% = (f_g^p - f_g^{p*})\ 100/f_g^p$, and $E(K^{ex})\%$ is the percentage difference in f_g^p between $K^{ex} = 1.2$ and $K_d^{ex} = 1.0$ assuming a constant value of n_g^{ex} (0.01371).

which also gives the values of some other variables in different methanol concentrations.

The second and greater uncertainty arises from a lack of knowledge about the accessibility of the exchange sites. Assuming that all exchange sites in the dry gel matrix are accessible when the gel is solvated, the term n_g^{ex} represents a property of the gel that is dependent on the degree of cross-linking, as a hydroxyl-hydrogen atom is lost at each junction point between the anhydroglucose residue and the cross-linking unit. As each residue has three hydroxyl groups and a molecular weight of 162.14, the maximal value of n_g^{ex} in non-cross-linked dextran is 3.024/162.14 = 0.01865 mole/kg. In Sephadex G-25, solvated in water, $K_d^T = 1.06$ (ref. 2) and assuming $K_1^{ex} = 1.20$ (ref. 41) and $Wr = 2.45$ (Table I), n_g^{ex} is equal to 0.01371 mole/kg ($K_1^{ex} \cdot n_g^{ex} = 0.01645$ mole/kg).

While it is probable that the tendency to reduced accessibility is much less in dextran than in cellulose gels, very low Sr values must increase the likelihood of interchain hydroxyl coupling resulting in decreased accessibility for exchange with solvent protons[44]. Although complete loss of accessibility ($n_g^{ex} = 0$) which would cause large errors in f_g^p (Table I) is highly unlikely, even a moderate reduction would cause an appreciable error at low solvent regains. This problem is a limitation of the second method and does not arise with the use of $H_2{}^{18}O$, which is to be preferred especially with lower solvent regain values. $H_2{}^{18}O$ in conjunction with HTO can be used to determine $K_b^{ex} \cdot n_g^{ex}$ (eqn. 26) and this seems to be of promise for studies on accessibility although with some remaining uncertainty regarding the value of K_b^{ex} a precise value could not be assigned to n_g^{ex}.

Mixture of a protic and an aprotic solvent. In a mixture such as, for example, water (or alcohol) and the dipolar aprotic DMSO, exchangeable tritium can also be used to determine the internal solvent composition. In a water–DMSO mixture, DMSO does not participate in tritium exchange and thus, in analogy with eqn. 23

$$K_d^T = \frac{K_1^{ex} \cdot n_g^{ex} + n_g^w \cdot Sr}{n_0^w \cdot Sr} \tag{30}$$

whence, dividing by the equilibrium constant (K_d^a) for DMSO (eqn. 26), the weight fraction of DMSO in the gel (f_g^a) can be obtained, *viz.*:

$$f_g^a = \frac{f_0^a \cdot (K_l^{ex} \cdot n_g^{ex} + QSr)}{QSr \cdot (K_d^{T/a} - f_0^a K_d^{T/a} + f_0^a)} \tag{31}$$

For other tritiated protic solvents, the factor Q can be generalized to Q', *i.e.*,

$$Q' = \frac{n \cdot M^h}{M^{ps}} \tag{32}$$

where n is the number of tritium exchange sites per molecule and M^{ps} the molecular weight of the protic solvent.

The above second method could be extended to more complex mixtures.

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