Effect of Polyethylene Glycol Exclusion on the Water Potential of Solution-Saturated Filter Paper

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ABSTRACT

Solutions of high molecular weight polyethylene glycol are often used to control water potential in seed germination studies. There is an implicit assumption that the seed support materials do not alter the water potential of the osmotic solution. Filter paper, however, contains a hydrophylic volume fraction that is inaccessible to high molecular weight polymers. Water absorbed by filter paper fibers was found to concentrate polyethylene glycol and lower water potential in solution-filter paper mixtures. The magnitude of this concentration effect is a function of the original concentration of polyethylene glycol and the ratio of solution volume to filter paper weight.

PEG solutions are often used to control ψ^1 in seed germination studies (11). Seed have been germinated on PEG solution-saturated filter paper (7), absorbent cellulose cloth (9), and ground polystyrene (8) or in soil equilibrated with PEG solution across a cellulose membrane (2). In all of these studies, there is an implicit assumption that seed support materials do not alter ψ of the osmotic solution. Filter paper, cellulose cloth, and cellulose membrane, however, are made from plant cell fibers that contain a volume fraction accessible to water but not high molecular weight PEG (1, 10). These materials may, therefore, absorb and remove water from a saturating solution, concentrate high mol wt PEG, and lower ψ of the system. A high solution-to-fiber ratio (2) or a hydrophobic substrate (8) would reduce or eliminate the problem of PEG concentration by organic materials. PEG solution ψ estimates, however, would remain in error if the original measurements were made with solution-saturated filter paper in a thermocouple psychrometer (5, 6).

We hypothesize that filter paper absorption of water lowers the ψ of PEG solutions and that previously derived equations for estimating PEG solution ψ (5, 6) are in error because measurements were made with solution-saturated filter paper discs. The objectives of this study are to quantify ψ measurement errors associated with filter paper exclusion of PEG and to determine whether these errors warrant reexamination of previous studies in which PEG was used for ψ control.

MATERIALS AND METHODS

PEG Solution Density as a Function of Concentration

PEG (8000, Union Carbide)^2 solution density at 25°C was determined on three replicates at 23 different [PEG] over the range of 0.01 to 0.86 gPEG/gH$_2$O. PEG of known weight was placed in volumetric flasks, and distilled water was added to bring the solutions to volume. Weight of water was recorded for [PEG] determination and solution density calculated at each [PEG] by dividing solution weight by solution volume. A polynomial regression equation was derived relating solution density (D, g/mL) to [PEG]:

$$D = -0.0634[PEG]^2 + 0.1483[PEG] + 0.9977 \quad (1)$$

with an r^2 of 0.9989. Subsequent estimates of PEG solution volume were calculated by dividing solution weight by density.

Effect of Filter Paper on PEG Concentration in Free Solution

The magnitude of PEG exclusion from filter paper fibers was first determined gravimetrically by measuring the concentration change of solutions equilibrated with air-dry filter paper. Four solutions with a [PEG] of 0.151, 0.253, 0.360, and 0.428 gPEG/gH$_2$O were mixed. PEG solution aliquots of approximately 10 mL were weighed and added to known weights of filter paper (Whatman No. 1) inside plastic vials with snap top lids. PEG solution weight was converted to solution volume from the concentration-density relationship derived earlier. Fifty-one solution-filter paper combinations with a Vs/Wfa ratio of between 2.5 and 30 were mixed for each [PEG] with an additional six vials containing PEG solution but no filter paper. The vials were periodically shaken for 48 h and a sample of the PEG solution drawn off, immediately weighed and dried at 65°C to constant weight. Postequilibration [PEG] was calculated and plotted against Vs/Wfa.

Water Content of Filter Paper as a Function of PEG Concentration

A method similar to that of Tarkow et al. (10) was used to estimate the amount of water that filter paper will remove from an aqueous PEG solution. Wfa was measured for 12 filter paper circles and Wfo obtained by drying for 24 h at 65°C. The water content of air-dry filter paper can then be determined as a fraction of Wfo:

$$Wfw = (Wfa - Wfo)/Wfo \qquad (2)$$

PEG was added to 500 mL of water in 24 plastic containers to yield approximate [PEG] values of from 0.08 to 0.72 gPEG/gH$_2$O. Six filter paper circles with a Wfa of approximately 0.3 g each were weighed (Wfa) and placed in each container, and the container was sealed and periodically shaken. After 48 h, [PEG] was determined for the bulk solution by oven drying three samples from each container to constant weight at 65°C. The solution-saturated filter paper was removed, weighed (Wfs), and dried at 65°C to constant weight (Wfd). Wfs must have been the sum of four components: Wfo, Wp, Wsw, and Wnsw. Wfd would include only the first two components. Thus, we can write:

$$Wnsw = Wfs - Wfo - Wp - Wsw \qquad (3)$$
$$Wp = Wfd - Wfo \qquad (4)$$

where Wfo was obtained from Wfa using Equation 2, and Wsw was obtained by assuming Wp/Wsw to be equal to [PEG] of the bulk solution. Percent Wnsw was plotted against [PEG], and a regression equation relating %Wnsw to [PEG] was derived.

PEG Solution Water Potential Without Filter Paper

PEG and distilled water were mixed to make 30 solutions varying in [PEG] from 0.08 to 0.72 gPEG/gH$_2$O. ψ of each solution was measured without filter paper, in random order, three times each with both a C-52 (Wescor Inc, Logan, UT) and SC-10 (Decagon Devices, Pullman, WA) thermocouple psychrometer. Psychrometer microvolt output was measured with a CR-7 Measurement and Control System (Campbell Scientific Inc, Logan, UT) for the C-52 and a Series 85 Digital Thermocouple Psychrometer Meter (J.R.D. Merrill Specialty Equipment, Logan, UT) for the SC-10. ψ measurements with the C-52 are usually made on single solution-saturated filter paper discs in the smallest sample chamber. Free solution, however, forms a bead that extends above the sides of the smallest C-52 chamber so measurements were made on 50 μL samples in the medium size chamber. SC-10 measurements were made on 1 mL samples. Both psychrometers were calibrated without filter paper using NaCl solutions of known ψ (3) interspersed every fourth measurement. The SC-10 psychrometer was enclosed in a 0.5 m^3, wood and Plexiglas box to minimize air flow over the unit during sample equilibration. The C-52 chamber was enclosed in a form-fitting Styrofoam box. All measurements were made in a controlled temperature room at 25°C. PEG solution ψ was compared to that predicted by Equation 1 of Michel (5):

$$\psi = 0.129[PEG]^2 T - 14.0[PEG]^2 - 0.40[PEG] \qquad (5)$$

for solutions of the same [PEG] and temperature.

Filter Paper-Solution Water Potential Interactions

PEG was added to distilled water to make four solutions with a [PEG] of 0.150, 0.274, 0.357, and 0.424 gPEG/gH$_2$O.

PEG Solution Water Potential as a Function of Vs/Wfa

Filter paper discs of an aggregate Wfa of between 0.03 and 0.12 g were weighed and placed into a tared SC-10 thermocouple psychrometer sample cup. PEG solution was added to the filter paper to generate a range of mixtures with a Vs/Wfa of between 0.02 and 30, and ψ of the mixtures measured. This procedure was repeated with NaCl solutions of the same approximate ψ as the PEG solutions. ψ of free solution without filter paper was also measured. ψ of the free solutions and the solution-filter paper mixtures were compared to those predicted by Equation 5 for PEG solutions and by Lang (3) for NaCl.

PEG Solution Water Potential as a Function of Filter Paper Saturation Technique

PEG solution ψ is commonly measured in a C-52 type thermocouple psychrometer using solution-saturated filter paper discs (5, 6). Five disc saturation treatments were tested to determine the effect of saturation method on Vs/Wfa and ψ as measured with the C-52 psychrometer. The treatments were:

1. Two filter paper discs, immersed in PEG solution, immediately removed, and touched to the side of the container to draw off excess solution.
2. Two filter paper discs to which approximately 20 μL of solution were added.
3. Two filter paper discs, immersed in PEG solution, immediately removed and not touched to the side of the container.
4. Two filter paper discs to which approximately 40 μL of solution were added.
5. Two filter paper discs, immersed in PEG solution, stirred for several seconds, and not touched to the side of the container.

An estimate of Vs/Wfa for the saturated discs was determined 10 times for each saturation treatment and for all four solutions by weighing filter paper discs before and after saturation. Solution weight was converted to volume from the solution density-concentration relationship derived earlier. ψ for each dipping treatment and a control containing no filter paper was determined on 10 additional samples at each of the four levels of [PEG]. C-52 measurements are usually made on single solution-saturated discs in the smallest sample chamber. Two discs were used here to minimize the volume of air space over the sample because a larger than normal chamber had to be used for the control treatment. The hypothesis was tested that dipping treatment had no affect on Vs/Wfa or ψ by Analysis of Variance. Tukey's Studentized range test was

used to distinguish differences between dipping treatments for each [PEG].

RESULTS AND DISCUSSION

PEG solutions of known initial concentration were concentrated by the addition of air-dry filter paper and the magnitude of this effect was a function of the Vs/Wfa ratio (Fig. 1).
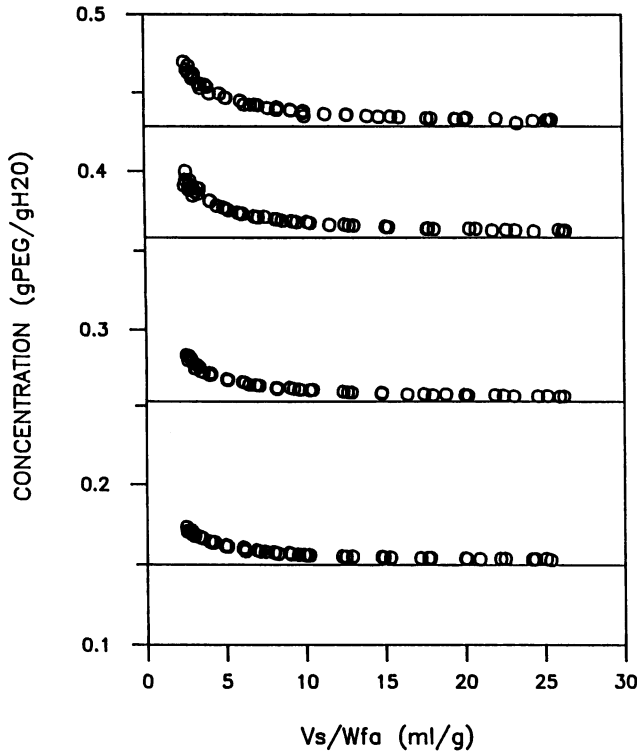

Figure 1. Effect of Vs/Wfa on the concentration of PEG solutions to which filter paper was added. Horizontal lines reflect the concentration of the four PEG solutions in the absence of filter paper.
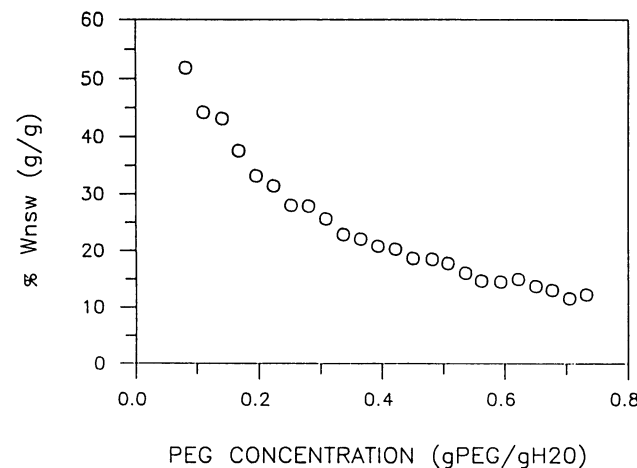

Figure 2. Relationship between %Wnsw and PEG solution concentration. The regression equation: %Wnsw = 102.22[PEG]^2 − 135.61 [PEG] + 58.297 with an r^2 of 0.9793 was derived to fit the data points. It should be noted that %Wnsw was calculated based upon oven- and not air-dry filter paper weight.

These data support the observation that materials derived from plant cells contain a volume fraction inaccessible to high mol wt polymers (1, 10). The fraction of water removed from solution by filter paper fibers, however, was a function of [PEG] (Fig. 2). There are two hypotheses that might explain the dependency of %Wnsw on [PEG]. The cell wall component of filter paper fibers may remove a constant amount of water but also act as a membrane to exclude PEG from the fiber lumina. The hydrophylic lumina would then absorb and hold pure water at a negative pressure equal to the osmotic potential of the external solution and fill and drain as a function of external [PEG]. Water tension induced by the nonpenetrating osmoticum may also cause the cell walls to shrink and thus hold less water at higher [PEG].

There is a seeming discrepancy between Figures 1 and 2 in that the PEG concentration effect of filter paper is greater (Fig. 1) but %Wnsw is smaller (Fig. 2) in the more concentrated PEG solution. The same volume of a more concentrated PEG solution, however, contains proportionally less water. Water removal by filter paper fibers, therefore, has a proportionally greater effect in the solutions of higher initial [PEG].

The concentration of PEG solutions by filter paper can also be inferred from ψ measurements made with a thermocouple psychrometer (Figs. 3 and 4; Table I). PEG solution ψ was less negative than predicted by Equation 5 when filter paper was not used to hold the sample (Fig. 3). When filter paper was used, ψ was dependent upon the Vs/Wfa ratio (Fig. 4; Table I). Below a Vs/Wfa ratio of 12 the effect of filter paper on PEG solution ψ became pronounced (Fig. 4). Low mol wt salts are not excluded from filter paper cell walls; therefore, salt solution ψ was not affected by the presence of filter paper down to a Vs/Wfa ratio of about 4 (Fig. 4). ψ deviations for salt solutions below a Vs/Wfa ratio of 4 can be attributed to negative pressures that develop in unsaturated capillary matrices at low water content. The matrix effect is unrelated to
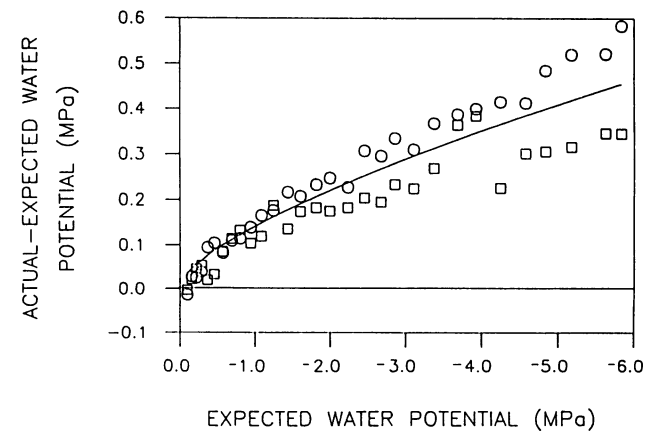
Figure 3. Relationship between actual ψ (measured without filter paper) minus expected ψ (Eq. 5) and expected ψ (Eq. 5) for measurements made with an SC-10 (O) and C-52 (□) thermocouple psychrometer. Solid line represents the difference between Equation 6 and Equation 5 for ψ measurements made with a vapor pressure osmometer, without filter paper, and a thermocouple psychrometer, with filter paper, respectively (5).
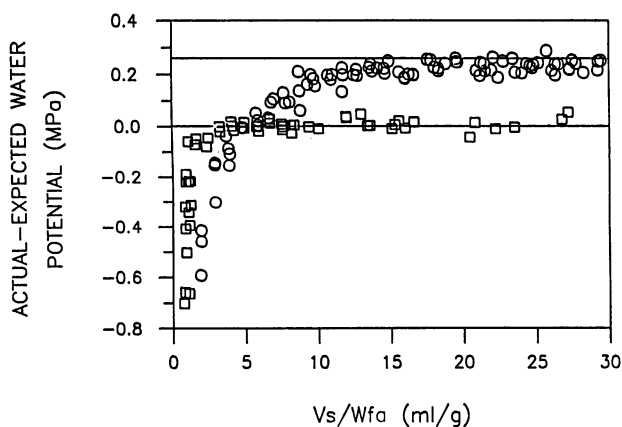
Figure 4. Relationship between actual ψ (measured) minus expected ψ (Eq. 5; 3) for a 0.424 gPEG/gH$_2$O solution of PEG (O) and a 0.4 M solution of NaCl (□) as a function of Vs/Wfa. Upper horizontal line represents a PEG solution to which no filter paper was added.

filter paper exclusion of PEG and would also occur if pure water were used. Negative pressures may develop at a Vs/Wfa ratio greater than 4 with PEG solutions, however, because they contain less water for a given volume.

The most common method for estimating PEG solution ψ is to measure solution-saturated filter paper discs in a small chamber thermocouple psychrometer (5, 6). Of the disc saturation treatments tested here with the C-52 psychrometer, all had a Vs/Wfa ratio of less than 12 and two had a ratio less than 4 (Table I). These data support the hypothesis that Equation 5 is in error because the measurements upon which it is based were made with saturated filter paper discs that concentrated the PEG solution.

Michel (5) found a discrepancy between Equation 5 and ψ measurements made in a vapor pressure osmometer without filter paper (6). Michel and Kaufmann (6), however, noted that the discrepancy between psychrometer and osmometer measurements disappeared when filter paper was introduced to the osmometer chamber. The equation Michel derived for predicting ψ from vapor pressure osmometer measurements (5, Eq. 4) was:

$$\psi = 0.130[PEG]^2 T - 13.7[PEG]^2 \qquad (6)$$

The evidence presented in this paper suggests that Equation 6 is probably correct and that Equation 5 is in error. We subtracted Equation 5 from Equation 6 and plotted the difference in Figure 3 for ψ measurements made at 25°C. The discrepancy between equations forms a reasonable match for our data (Fig. 3). Since our measurements were made at only one temperature, we suggest that Equation 6 be used in place of Equation 5 for predicting PEG solution ψ as a function of [PEG] and temperature.

A difference was found between measurements made with the two types of psychrometer used in this experiment (Fig. 3). Both psychrometers measure the microvolt output associated with evaporative cooling of a thermocouple junction but the method for obtaining water on the thermocouple, and the sample chamber size are different. Calibration with standard salt solutions, however, should have compensated for differences in instrument design. We hypothesize that there

Table I. *Vs/Wfa and ψ of Two Saturated Filter Paper Discs as a Function of Disc Saturation Treatment*

Treatment 1, dipped and touched to side of container; treatment 2, 20 μL added with pipette; treatment 3, dipped and not touched to side of container; treatment 4, 40 μL added with pipette; treatment 5, dipped and stirred for several seconds and not touched to side of container; control, 50 μL in chamber with no filter paper.

PEG Solution Concentration	Saturation Treatment	Vs/Wfa		ψ	
		Mean[a]	SE	Mean[a]	SE
gPEG/gH$_2$O		mL/g		MPa	
0.424	1	2.2a	0.18	−2.50a	0.072
	2	3.6b	0.17	−2.37b	0.093
	3	5.1c	1.31	−2.24c	0.144
	4	7.1d	0.34	−2.15cd	0.044
	5	8.5e	1.51	−2.05de	0.054
	Control			−2.01e	0.060
0.357	1	2.4a	0.25	−1.82a	0.104
	2	3.5b	0.24	−1.61b	0.050
	3	5.1c	0.93	−1.55bc	0.076
	4	7.2d	0.22	−1.41de	0.072
	5	8.0d	0.90	−1.44cd	0.084
	Control			−1.32e	0.096
0.274	1	3.1a	0.57	−1.10a	0.140
	2	3.5a	0.16	−0.96a	0.144
	3	6.4b	1.22	−0.81b	0.057
	4	7.1b	0.23	−0.71bc	0.050
	5	6.4b	1.30	−0.80b	0.105
	Control			−0.63c	0.080
0.150	1	2.9a	0.27	−0.34a	0.120
	2	3.4a	0.19	−0.34a	0.128
	3	4.6b	0.81	−0.27ab	0.047
	4	5.9c	0.63	−0.21bc	0.054
	5	7.3d	0.38	−0.22bc	0.054
	Control			−0.14c	0.049

[a] Values within a column for each concentration followed by the same letter were not significantly different (P ≤ 0.05).

is an interaction between psychrometer function and mol wt of the solute. Water is condensed on the C-52 thermocouple junction by Peltier cooling just prior to measurement but in the SC-10, water is obtained on the thermocouple by dipping the junction in a reservoir of water. The C-52 dehydrates the atmosphere in the sample chamber before measurement, whereas the SC-10 slightly hydrates it. We hypothesize that water evaporates from the sample to compensate for atmospheric drying by the C-52, and a concentration gradient of PEG is temporarily formed at the solution surface. Michel (4) hypothesized that concentration gradients in high molecular weight PEG solutions would tend to persist if the sample is not stirred, whereas lower mol wt solutes can be expected to adjust quickly to concentration gradients (4). If surface concentration of PEG occurred during a C-52 measurement, the psychrometer would estimate a more negative ψ than existed in the bulk solution. Surface dilution of PEG during the SC-10 measurement would cause this psychrometer to estimate a less negative ψ, but the magnitude of the discrepancy would be less because the SC-10 sample chamber has a larger air space relative to sample surface area. The discrepancy between

measured and expected ψ for the vapor pressure osmometer data of Michel (5) support this hypothesis since these data represent measurements taken at equilibrium when water was neither evaporating or condensing on the thermocouple junction. In the ψ range normally associated with plant growth, the difference between instruments is small compared to the discrepancy between measurements taken with and without filter paper (Fig. 3).

CONCLUSIONS

There have been numerous studies in which ψ in the germination environment has been controlled with solutions of PEG. Evidence presented here indicates that these studies may have contained errors in ψ measurements. The need for reinterpretation of previous results depends upon the experimental design used, the magnitude of the error, and the conclusions that were drawn. For germination systems that used a Vs/Wfa ratio greater than 12, a nonabsorbing structural matrix such as polystyrene (8), or a large volume of PEG solution equilibrated across a cellulose membrane (2), the concentration effect of the germination substrate would be minimal. If the initial ψ estimate was made with a solution-saturated filter paper disc, however, any error in the original ψ estimate would persist (Fig. 3). Figure 3 indicates that this error may be as large as 0.2 to 0.3 MPa in the range of ψ normally associated with germination and plant growth studies. Ironically, ψ measurement errors derived from psychrometer measurements are offset by PEG exclusion from filter paper used as the germination substrate. Psychrometer measurement errors would be nullified if Vs/Wfa of the germination substrate was exactly equal to that of the solution-saturated discs used to calibrate the psychrometer. It is not possible to determine if this has ever been the case for previous studies because Wfa and, therefore, Vs/Wfa are not usually recorded. For studies that use the same germination substrate for all treatments, errors in ψ measurements would have no effect on relative ranking of water stress between treatments. PEG exclusion errors would most seriously affect the interpretation of results where comparisons were made between a PEG solution-saturated substrate and purported isopotential treatments using a different substrate (2, 8, 9), or low mol wt salts (7-9) that can penetrate filter paper fibers.

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