

# Oxygen Availability in Polyethylene Glycol Solutions and Its Implications in Plant-Water Relations<sup>1</sup>

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JOHN MEXAL,<sup>2</sup> JAMES T. FISHER, JANET OSTERYOUNG,<sup>3</sup> AND C. P. PATRICK REID  
*Department of Forest and Wood Sciences and Departments of Civil Engineering and Microbiology,  
Colorado State University, Fort Collins, Colorado 80523*

## ABSTRACT

The solubility of O<sub>2</sub> in polyethylene glycol 4000 and 6000 solutions of varying concentrations was determined iodimetrically (titrimetrically) and electrochemically using a rotating glassy carbon electrode and a PAR Model 174 Polarograph. The titrimetric determination resulted in the formation of an unexpected precipitate at 2% (w/v) polyethylene glycol corresponding to the approximate critical micelle concentration of the two polyethylene glycol homologs. Beyond 5% polyethylene glycol, O<sub>2</sub> concentration was inversely proportional to polyethylene glycol concentration, and was higher in polyethylene glycol 4000 solutions than in polyethylene glycol 6000. The electrochemical data are a direct measure of O<sub>2</sub> transport to the electrode surface, rather than O<sub>2</sub> activity or concentration. Results indicate that even at relatively high H<sub>2</sub>O potentials, the transport of O<sub>2</sub> to the root surface might be insufficient to meet the plant's respiratory requirements.

Osmotic agents are frequently used to subject plant tissues to known levels of water stress. Unfortunately, in addition to controlling the water potential of tissues, artificial media may result in undesirable plant responses. For example, sugars and salts may be absorbed and utilized by plants, resulting in nutritional or osmotic adjustments. Toxic effects may also result.

Lacking many of the undesirable properties of salts and sugars, polyethylene glycols have been used successfully with such plants as pepper (8), wheat (12), pine (19, 30), basidiomycetes (17), and yeasts (1). PEGs<sup>4</sup> are not easily broken down by living organisms and have been shown to be nontoxic in most cases. Furthermore, PEG molecules are not readily absorbed by intact plants (8, 11), and molecules with mol wt greater than 3000 are apparently not absorbed at all (28).

Widespread acceptance of PEG as an osmotic agent, however, has been impeded by reports linking PEG with toxic effects. Although high mol wt PEGs were reportedly toxic due to high concentrations of aluminum and magnesium ions required in the synthesis of PEG (10), toxic responses linked to lower mol wt homologs have been attributed to the PEG molecule *per se* (6, 13). For example, deionized PEG 1540 proved toxic to algal cultures (6).

The possibility also remains that plant injury may result from a substantial reduction of O<sub>2</sub> availability due to the addition of PEG. Since low levels of O<sub>2</sub> in culture media may induce plant growth responses similar to those caused by water stress (5) this possibility especially deserves consideration. The objective of the following research was therefore to determine the degree to which PEG concentration and mol wt govern the availability of O<sub>2</sub> in aqueous solutions. Since PEG 4000 and PEG 6000 are commonly used for plant research, the relation of O<sub>2</sub> availability to the concentration of PEG 4000 and 6000 required to bring a solution to a particular water potential was investigated.

## MATERIALS AND METHODS

The oxygen solubilities of PEG 4000 (J. T. Baker Chem. Co., average mol wt = 3000-3700) and PEG 6000 (J. T. Baker Chem. Co., average mol wt = 6000-6700) in air-saturated aqueous solutions were determined polarographically with a PAR Model 174 polarographic analyzer. Solutions were adjusted to about pH 11.8 with NaOH pellets and maintained at 25 C in a water bath. The wave pattern of O<sub>2</sub> was measured with a rotating (60 rps) glassy carbon electrode referenced against a saturated calomel electrode. The polarograph was adjusted to a range of 1.5 v, a rate of 20 mv/sec, an initial potential of -200 mv, and a current range of 0.1 milliamperes (full scale). Current for the reduction of O<sub>2</sub> to peroxide was measured at -550 mv.

Oxygen concentrations of various PEG 4000 and 6000 solutions were also determined iodimetrically using the modified micro-Winkler technique (27).

## RESULTS

Titrimetric determination of the oxygen concentration of air-saturated PEG 4000 and 6000 solutions by the micro-Winkler method revealed that any addition of PEG reduced the O<sub>2</sub> concentration of the solution when compared to water (Fig. 1). Solutions of PEG 4000 and 6000 with concentrations less than 15% (w/v) exhibited similar O<sub>2</sub> solubility properties, while solutions of PEG 6000 more concentrated than 15% contained less dissolved O<sub>2</sub> than similar concentrations of PEG 4000.

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<sup>2</sup> Present address: Southern Forestry Research Center, Weyerhaeuser Company, Hot Springs, Ark. 71901.

<sup>3</sup> Departments of Civil Engineering and Microbiology.

<sup>4</sup> Abbreviations: PEG: polyethylene glycol; CMC: critical micelle concentration; DMT: dimensionless mass transport; ODR: oxygen diffusion rate.

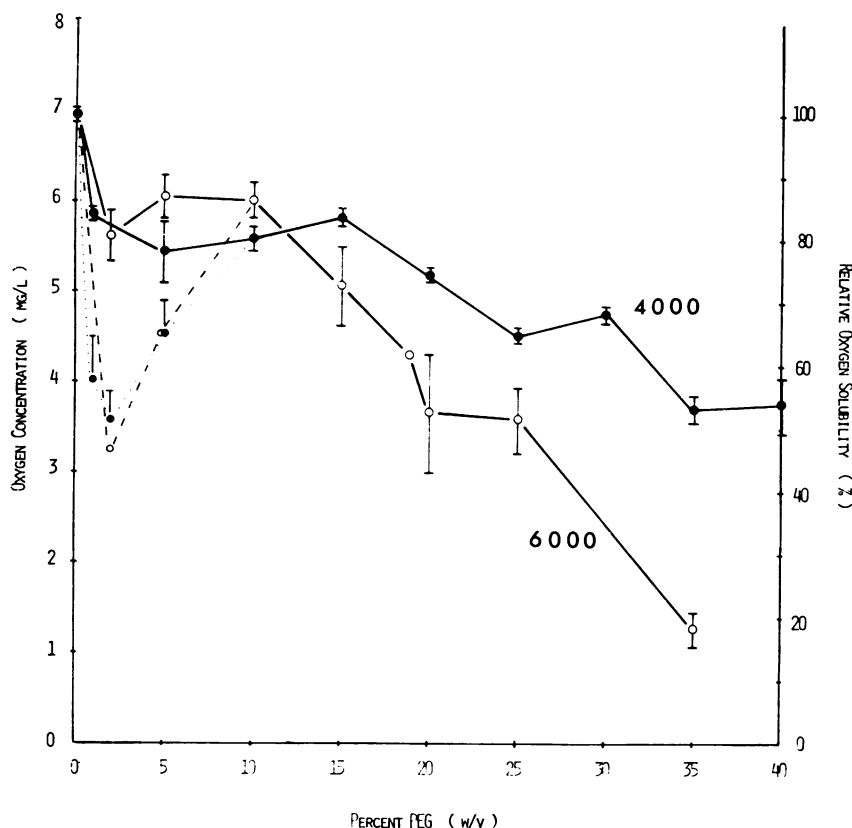


FIG. 1. Oxygen concentration of PEG solutions as determined by the micro-Winkler technique. PEG 4000 (●); PEG 6000 (○). Vertical bars represent SE of the mean.

The determination of  $O_2$  concentration in dilute PEG solutions (1–5% w/v) was hampered by the anomalous behavior of these solutions. At these PEG concentrations, a dark precipitate formed during the determination when concentrated  $H_2SO_4$  acid was added. This precipitate did not form at higher concentrations. If the precipitate was titrated with the thiosulfate solution slowly, the small volume added greatly increased the  $O_2$  content (Fig. 1, solid line) when compared to the determination without the precipitate (Fig. 1, dashed line). Except for this anomalous behavior at low PEG concentrations, the  $O_2$  concentration was inversely proportional to the PEG concentration. The formation of the precipitate at low PEG concentrations may be attributed to the critical micelle concentration of PEG solutions.

The term CMC represents the concentration at which micelles of polymers begin to aggregate in a solvent-solute system. If the properties such as surface tension, conductivity, and osmotic potential of a surfactant are plotted against the concentration of the polymer up to the CMC, at which point there will be a marked deviation in linearity. The CMC for nonionic surfactants is usually about  $10^{-4}$  moles/liter (24, 25). Since the maximum interference with the determination of  $O_2$  concentration of both PEGs as determined by the Winkler method occurs at about  $5 \times 10^{-4}$  moles/liter, this may be indicative of the CMC of PEG 4000 and 6000. Possibly these polymer micelles serve as nuclei which facilitate precipitation of hydrous manganese oxides during the micro-Winkler determination.

With ideal solutions, the voltammetric method is the most suitable for determining the concentration of an electroactive species such as  $O_2$ , because the current is proportional to the

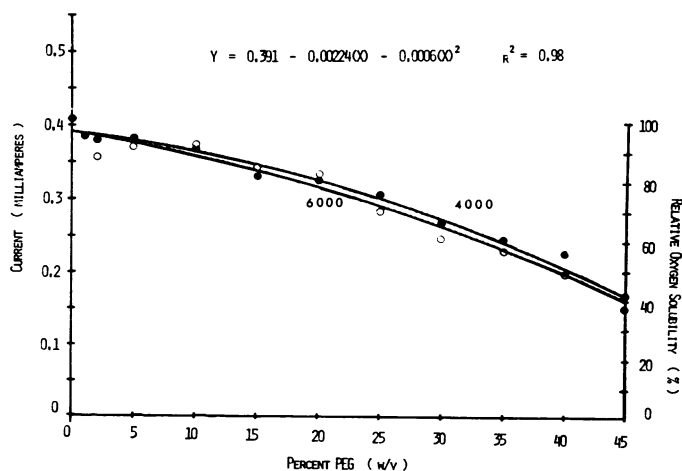


FIG. 2. Polarographic oxygen current as a function of PEG concentration. Symbols as in Fig. 1.

concentration of the electroactive species, viz.:



Data obtained for the steady state limiting current for reduction of  $O_2$  to  $H_2O_2$  for both PEG 4000 and PEG 6000 are presented in Figure 2. This current is predicted by the Levich equation:

$$i = \frac{nFAD^{3/2}C\omega^{1/2}}{1.6116\nu^{1/6}} \quad (2)$$

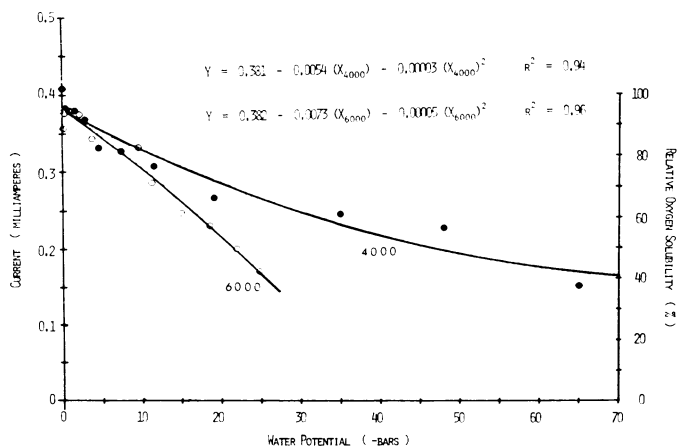


FIG. 3. Polarographic oxygen current as a function of the water potential of PEG solution. Symbols as in Fig. 1.

where  $i$  is current in microamperes,  $n$  the number of equivalents/mole (two),  $A$  the electrode area in  $\text{cm}^2$ ,  $D$  the diffusion coefficient in  $\text{cm}^2/\text{sec}$ ,  $C^b$  the concentration of  $\text{O}_2$  in millimoles/liter,  $\omega$  the electrode rotation rate in radians/sec, and  $\nu$  the kinematic viscosity in  $\text{cm}^2/\text{sec}$ .

As seen in Figure 2, the current outputs for PEG 4000 and PEG 6000 are almost identical within the range of concentrations examined. The polarographic data points also reveal a shoulder, although very slight, at 2% PEG.

Of more practical importance is the relationship of relative  $\text{O}_2$  solubility to the concentration of PEG 4000 or 6000 required to bring a solution to a particular water potential. The water potential of PEG 4000 solutions was determined by thermocouple psychrometry by Fisher and Reid (cited in 17). Water potentials ( $\Psi$ ) of PEG 6000 solutions were determined from the following regression equation fitted to the water potential values of PEG solutions expressed on a percentage (w/v) basis as reported by different authors (18, 20, 29, 31):

$$\Psi = -0.10 - 0.01 (\%) + 0.026 (\%)^2 - 0.0003 (\%)^3$$

$$r^2 = 0.92 \quad (3)$$

The relative  $\text{O}_2$  solubilities of PEG 4000 solutions are discernably higher than those of the PEG 6000 solutions at corresponding water potentials (Fig. 3). It is important to note that solutions of both PEGs showed relative  $\text{O}_2$  solubilities greater than 50% at corresponding water potentials greater than  $-20$  bars. Osmotic solutions with water potentials less than  $-20$  bars are rarely used in plant research. Since  $\text{O}_2$  is rarely limiting to plants if the relative solubility is above 50% (4), these data would indicate that aerated PEG solutions contain sufficient  $\text{O}_2$  to sustain optimum growth. However, the availability of  $\text{O}_2$  to plants is related to its activity coefficient and transport properties as well as solubility.

## DISCUSSION

Under the experimental conditions used for air saturation, one can reasonably assume that the fugacity of  $\text{O}_2$  in air is constant, and equal to the partial pressure of  $\text{O}_2$  in air. Since the solutions are brought to equilibrium with air, the activity of  $\text{O}_2$  must also be constant over changes in PEG concentration, and therefore, the pronounced changes in the observed  $\text{O}_2$  concentrations are a reflection of the corresponding changes in the activity coefficient of dissolved  $\text{O}_2$ . Equation 2, which can be solved for  $\text{O}_2$  concentration given current, viscosity, and diffusion coefficient describes only ideal solutions. Com-

parison of  $\text{O}_2$  concentration as determined by the Winkler method with results predicted by equation 2 indicate that the solutions are far from ideal, and that the resultant  $\text{O}_2$  concentrations of the PEG solutions (Fig. 2) are misleading.

In situations which depend upon the rate of mass transport in solution, such as  $\text{O}_2$  diffusion to an electrode surface or a root surface, the important parameter is not the absolute concentration or the activity, but rather the rate of mass transport itself (16), which depends on both concentration and such parameters as the diffusion coefficient of the diffusing species, the solution viscosity, and so on (21).

A voltammetric method is the most suitable for determining mass transport characteristics for an electroactive species such as  $\text{O}_2$ , because the current is a direct measure of the rate of the electrochemical reaction. If the experimental design is chosen such that the limiting rate process is mass transport to the electrode, then the current is a direct measure of the rate of the mass transfer process. According to Newman (22) the dimensionless mass transport parameter under conditions of forced laminar flow at a rotated disk electrode is given by:

$$\text{DMT} = \frac{i}{nFA C^b (\nu \omega)^{1/2}} \quad (4)$$

where the symbols are defined above. Although this equation contains the concentration of the electroactive species,  $C^b$ , in fact this involves an approximation in common use, and the proper expression uses not the concentration but rather the activity. In this case, the  $\text{O}_2$  activity is the same for all solutions and is equal to the  $\text{O}_2$  concentration in pure water saturated with air under our experimental conditions. Notice that the right hand side of the equation contains only known ( $n, F$ ) or directly measurable quantities. Values of this DMT function are given in Tables I and II along with data used in its calculation. Also included in Tables I and II are values of the diffusion coefficient for  $\text{O}_2$  in these solutions calculated from the DMT function. According to Newman (22), with Schmidt numbers greater than 100, the DMT is given by:

$$\text{DMT} = \frac{0.62048(\text{Sc})^{-2/3}}{1 + 0.2980(\text{Sc})^{-1/3} + 0.14514(\text{Sc})^{-2/3}} \quad (5)$$

Table I. Physical Properties of Polyethylene Glycol 4000 Solutions

% PEG	Molarity <sup>1</sup>	$\Psi$ <sup>2</sup>	Viscosity <sup>3</sup>	Relative Oxygen Solubility	DMT	Diffusion Coefficient
w/v		-bars	centistokes	$i/i_0 \times 100$		$(\text{cm}^2/\text{sec}) \times 10^{10}$
0	0	0	0.9	100.0	0.01832	4.91
1	0.00030	0.4	1.0	94.1	0.01660	4.74
2	0.00060	1.0	1.1	94.1	0.01566	4.76
5	0.00150	1.5	1.5	93.1	0.01341	5.11
10	0.00298	2.7	2.4	90.2	0.01027	5.43
15	0.00448	4.5	3.7	81.4	0.00746	5.14
20	0.00597	7.3	6.0	80.4	0.00579	5.66
25	0.00746	11.5	9.5	75.5	0.00432	5.74
30	0.00895	19.0	15.3	65.7	0.00296	5.20
35	0.01045	35.0	21.5	60.3	0.00229	4.96
40	0.01194	48.0	32.0	55.9	0.00174	4.87
45	0.01343	65.0	48.0	37.3	0.00095	2.93

<sup>1</sup> Based on an average mol wt of 3350.

<sup>2</sup> Calculated from Ref. 17.

<sup>3</sup> Extrapolated from "Carbowax Polyethylene Glycols," Union Carbide, 1972.

Table II. *Physical Properties of Polyethylene Glycol 6000 Solutions*

% PEG	Molarity <sup>1</sup>	$\Psi^2$	Viscosity <sup>3</sup>	Relative Oxygen Solubility	DMT	Diffusion Coefficient
$w/v$		-bars	centistokes	$i/i_0 \times 100$		$(cm^2/sec) \times 10^{10}$
0	0	0	0.9	100.0	0.01905	5.22
2	0.00032	0	1.2	87.3	0.01405	4.40
5	0.00079	0.3	1.9	92.2	0.01179	5.32
10	0.00156	1.9	3.8	91.7	0.00829	6.20
15	0.00237	4.1	7.6	84.3	0.00539	6.43
20	0.00315	6.7	14.4	81.4	0.00378	7.10
25	0.00394	9.9	26.0	70.1	0.00242	6.52
30	0.00474	13.5	44.0	60.8	0.00182	6.01
35	0.00552	17.7	75.0	56.4	0.00115	6.10
40	0.00630	22.3	125.0	49.0	0.00077	5.59
45	0.00709	27.5	200.0	41.7	0.00052	4.92

<sup>1</sup> Based on an average mol wt of 6350.

<sup>2</sup> Calculated from equation 3.

<sup>3</sup> Extrapolated from "Carbowax Polyethylene Glycols," Union Carbide, 1972.

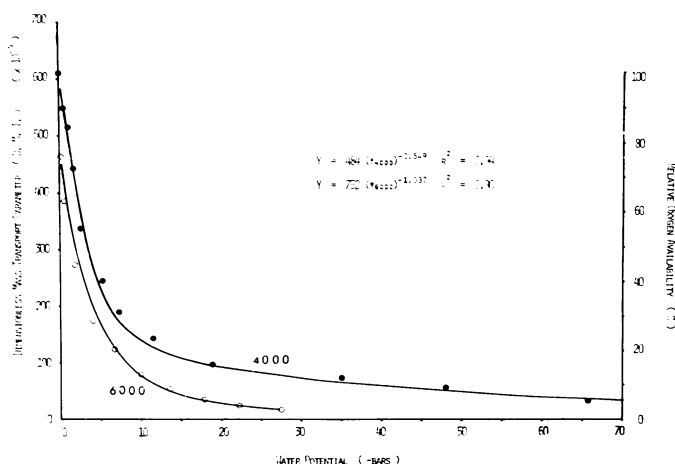


FIG. 4. Dimensionless mass transport parameter of oxygen as related to the water potential of PEG solution. Symbols as in Fig. 1.

where  $Sc$ , the dimensionless Schmidt number, is given by:

$$Sc = \nu/D \quad (6)$$

The diffusion coefficients are calculated using the DMT values from equation 4 and equations 5 and 6. The variation in the diffusion coefficient with solution composition is probably accounted for by combined experimental uncertainties in current and viscosity measurements.

Relative values of the DMT function, using pure water as the reference solvent, are presented in Figure 4. This curve may be interpreted as showing the relative availability of  $O_2$  in PEG-water solutions of varying water potentials. Note that  $O_2$  concentration values in Figure 1 have a range of about 3.2 mg/l between 0 and 40% for PEG 4000, and about 5.8 mg/l between 0 and 35% for PEG 6000. However, the availability, as measured by the mass transport parameter (Fig. 4), has a range of about 90 and 95%, respectively. The DMT values for PEG 6000 drop below 0.004 at about -6 bars, whereas the value for PEG 4000 is 0.004 at -12 bars (Fig. 4).

The DMT values present a more realistic estimate of the

" $O_2$  availability" than either the titrimetric  $O_2$  concentration or the individual current ( $i$ ) readings from the polarograph, since neither consider the effects of viscosity and diffusion coefficient.

The DMT equation is similar to the ODR equation used to quantify anoxic effects on plant growth (14, 16, 26), except that the DMT equation considers the effect of viscosity and convection. Values of ODR less than  $52 \times 10^{-8} g O_2/cm^2 \cdot min$  (corresponding to a 10%  $O_2$  content in air) resulted in severe limitations in root growth of three desert shrubs (15). Of course, desert shrubs are probably adapted to a high ODR which would result from coarser soils and lower soil-water content. Previous work has indicated that other plant species are restricted by ODRs of less than  $40 \times 10^{-8} g O_2/cm^2 \cdot min$  (26). The criticisms of relating ODR to growth have been discussed by others (7), but are primarily related to the heterogenous nature of a soil-water system including the air spaces in such a system. Such arguments do not apply to hydroponic systems.

Oxygen availability of PEG solutions as measured by the DMT parameter may possibly be limiting even at relatively high water potentials. Oxygen availability reaches 50% of that in water at a water potential of -3 and -2 bars for PEG 4000 and 6000, respectively. Oxygen availability may become severely limiting (relative  $O_2$  solubility = 20%) at water potentials of about -12 bars and -7 bars for PEG 4000 and 6000, respectively.

It is possible that some of the toxic principles alluded to by others could in fact have been associated with decreased  $O_2$  availability. The gelatinous bacterioglucosae which developed on pine roots placed in PEG solutions as reported by Lesham (13) may have been the result of decreased  $O_2$  availability. The substance could arise from facultative anaerobic bacteria in the rhizosphere. We have noticed very high respiration rates associated with slimy roots of pine grown in solutions of PEG. Pine roots in Hoagland's solution do not develop a gelatinous surface, and do not have high respiration rates (unpublished). Plants grown in soil do have bacteria intimately associated with the root surfaces (3, 9), and enhanced bacterial growth could possibly result from decreased  $O_2$  availability.

Gingrich and Russell (5) found that low  $O_2$  tensions in nutrient solution could reduce growth of corn roots over a range of soil moisture tensions. The inhibition was most pronounced at high water potentials (low stress). Growth was inhibited only slightly at an oxygen content of 10.5% (50% relative  $O_2$  solubility). However, a reduction in relative  $O_2$  solubility of 75% gave a response similar to lowering the soil-water potential to -5 bars. The  $O_2$  tension had to be above 10.5% (50% relative solubility) to allow maximum growth at high water potentials.

Others have found that low  $O_2$  tensions are inhibitory to root growth of a variety of plants (4). Oxygen is not only important in root growth but it also appears to be important in the formation of mycorrhizal roots (23). Read and Armstrong (23) found that on agar substrates, active growth of the fungal symbiont occurred only if sufficient  $O_2$  was evolved by the roots or a capillary surrogate. The same investigators have shown that conifer seedlings are capable of translocating  $O_2$  down to the roots if the medium is limiting in  $O_2$  (2).

The results reported here indicate that plants growing in nutrient solutions containing PEG could suffer from  $O_2$  deficiency. This problem might be averted by either limiting the PEG concentration or possibly by supplying considerable aeration to the system. Aeration by use of frits might allow more  $O_2$  to reach the root surface through direct contact of numerous small bubbles. Oxygen concentration in the bubbles would

be greater than the solution in equilibrium with air, and the diffusion pathway length through liquid would be at a minimum. Use of very fine air bubbles in aeration would also reduce root damage caused by too vigorous solution agitation.

Within the range of concentrations of PEG studied,  $O_2$  solubility was found to be inversely related to the mol wt and concentration of PEG. Therefore, the selection of a particular mol wt PEG for use in osmotic adjustment in plant studies should be based upon its  $O_2$  properties in solution as well as its absorption potential by plant roots. Regardless of the molecular size chosen for osmotic studies, PEG solutions with concentrations near the critical micelle concentration should probably not be used, not only because of the abrupt changes in osmotic potential of the solution (25), but also because the micelles could possibly interfere with other properties of the solution. Fortunately, the CMC corresponds to a water potential of  $-1$  bar or greater, and would seldom be required for use in physiological studies.

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