

# Evaluation of the Water Potentials of Solutions of Polyethylene Glycol 8000 Both in the Absence and Presence of Other Solutes

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## ABSTRACT

Published and additional data for polyethylene glycol 8000 (PEG), formerly PEG 6000, solution water potentials ( $\Psi$ ) are compared. Actual bars  $\Psi$  over the concentration range of 0 to 0.8 gram PEG per gram H<sub>2</sub>O and temperature ( $T$ ) range of 5 to 40°C are best predicted (probably within  $\pm 5\%$ ) by this equation:  $\Psi = 1.29[\text{PEG}]^2 T - 140[\text{PEG}]^2 - 4.0[\text{PEG}]$ . Although transformable through division by  $[\text{PEG}]$  to virial equation form, results indicate that the coefficients are not virial. Mannitol (MAN) interacts with PEG to produce  $\Psi$  significantly lower than additive. Vapor pressure osmometer (VPO) data for MAN-PEG synergism compared favorably with those from thermocouple hygrometry; and VPO data showing the interactions between PEG and four salts are presented. The synergism of MAN-PEG and of NaCl-PEG are related linearly to the concentration of solute added with PEG.

Requests continue to be received for a table of PEG 6000 concentrations required for specific  $\Psi^1$  values to -20 bars from 15 to 35°C (4). Results obtained by others (2, 7) and in my laboratory (5) differ sufficiently with the former and each other to create concern. This paper will compare published values with previously unpublished data collected from several sources and additional measurements that extend both the concentration and temperature ranges. PEG represents only a name change (from PEG 6000) made by Union Carbide. The actual mol wt range, about which some confusion seems to exist, remains the same.

Although the importance of synergism between PEG and other solutes has been indicated (4), the infinite variety of solute, concentration, and temperature combinations possible discourage thorough investigation of this phenomenon. The magnitude of synergism found between dextran and PEG and between MAN and the combination of PEG and dextran (5) stimulated a comprehensive study of MAN-PEG synergism, which is reported here and compared with earlier results from a VPO.

Relationships found can be described best by equations. These and estimates of their reliabilities will be presented.

## MATERIALS AND METHODS

**Published Values.** Where only graphical presentations were available (2, 7-9), plotted points were measured carefully using a finely divided rule and a hand lens. Atmospheres were converted

<sup>1</sup> Abbreviations:  $\Psi$ , water potential; PEG, polyethylene glycol 8000; MAN, mannitol; VPO, vapor pressure osmometer;  $T$ , Celsius temperature; GLM, general linear models; SAS, statistical analysis system (SAS Institute, Inc.);  $r^2$ , correlation coefficient; CL, confidence limits;  $[\text{PEG}]$ , g PEG/g H<sub>2</sub>O;  $[\text{MAN}]$ , molal concentration of MAN.

to bars through division by 0.987. Concentrations were converted to g PEG/g H<sub>2</sub>O based on displacement of 0.84 g H<sub>2</sub>O/g PEG and a water density of 0.997 g/cm<sup>3</sup> at 25°C.

**Personal Communications.** In the mid to late 1960s, samples of PEG obtained as Carbowax 6000 from Union Carbide were sent to five laboratories for measurement of  $\Psi$ . Only the following two sets of data obtained appear to be useful. John S. Boyer returned three values obtained with standard Richards and Ogata thermocouple psychrometry (personal communication). H. D. Barrs, CSIRO, Australia, returned duplicate values for nine concentrations obtained with thermocouple psychrometry, type unspecified (personal communication).

**Author's Laboratory.** The VPO measurements were made in 1971 with a Hewlett-Packard model 302B calibrated with both NaCl and MAN solutions (4). Thermocouple hygrometer measurements were made in 1975 with two Wescor C-51 sample chambers in a growth cabinet having modulated temperature control that maintained the temperature evenly at 25°C. During 1981 up to six Wescor C-52 sample chambers were used for three sets of measurements at various temperatures. Other equipment for the latter and thermocouple hygrometer techniques are described elsewhere (5).

**Modeling.** Michel and Kaufmann (4) had found that  $\Psi$  of PEG solutions, varying quadratically with concentration and linearly with temperature, could be modeled by an equation in this form:  $\Psi = a[\text{PEG}]^2 T + b[\text{PEG}]^2 + c[\text{PEG}]T + d[\text{PEG}]$ , where the coefficients,  $a$ ,  $b$ ,  $c$ , and  $d$ , are constants. This model was used as a starting point for analyses reported here.

If one prefers to use Kelvin temperature rather than Celsius, because  $T = K - 273$ , the form of the equation and the values of  $a$  and  $c$  remain unchanged and only the values of  $b$  and  $d$  become different. Both  $b$  and  $d$  are still constants.

For any single temperature, the above equation reduces to  $\Psi = a[\text{PEG}]^2 + b[\text{PEG}]$ , with  $a$  and  $b$  having different numerical values from above. The latter equation was transformed through division by  $[\text{PEG}]$  to become  $\Psi/[\text{PEG}] = a[\text{PEG}] + b$  and treated as a virial equation (2, 6), in which  $a = RK A_2$  and  $b = RK/M$ , where  $R$  is the gas constant,  $K$  is Kelvin temperature,  $A_2$  is the second virial coefficient, and  $M$  is mol wt of solute. Division by concentration transforms the measurement values and modifies statistical analysis (5). That either approach is better statistically was not apparent (5) so both forms were used for each data set.

All analyses were by the GLM procedure of SAS. Parameters with coefficients not significantly different from zero were eliminated and the new model analyzed.

## RESULTS AND DISCUSSION

**PEG Alone.** Most available measurements of  $\Psi$  for PEG solutions have been made at or below 0.4 g PEG/g H<sub>2</sub>O and at 25°C. The points in Figure 1 represent all compatible, published, or personally communicated data for this concentration range and temperature (or 23°C corrected to 25°C). The curve was drawn

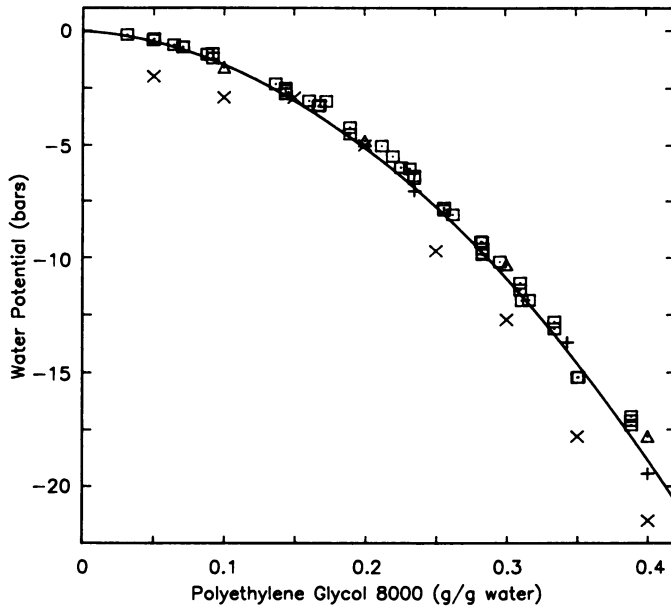


FIG. 1.  $\Psi$  of PEG solutions at 25°C as a function of concentration. Points from J. S. Boyer, 1965 (personal communication); Zur, 1966 (9); H. D. Barrs, 1969 (personal communication); Williams and Shaykewich, 1969 (8) ( $\square$ ); Michel and Kaufmann, 1973 (4) ( $\Delta$ ); Steuter *et al.*, 1981 (7) ( $\times$ ); and McClendon, 1981 (2) (+). Solid line is from Eq. 1, this paper, Michel *et al.*, 1983 (5). All measurements were by thermocouple hygrometry except those of McClendon (2).

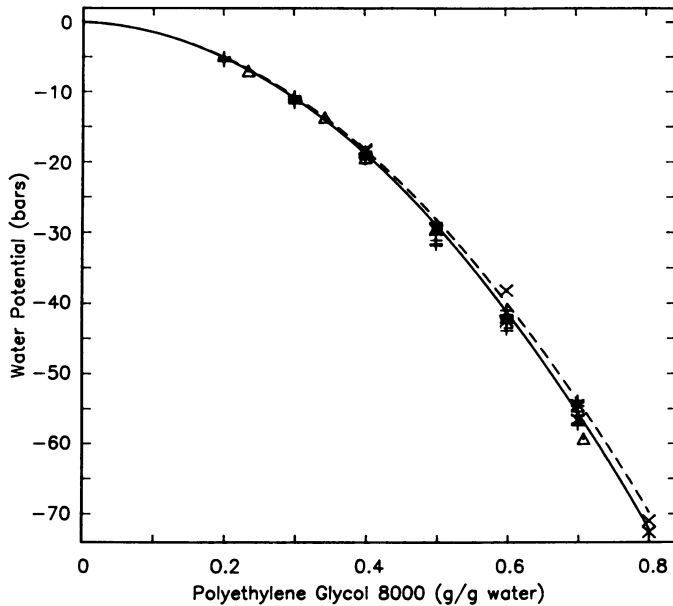


FIG. 2.  $\Psi$  of PEG solutions at 25°C as a function of concentration. Points from Michel, 1975 (unpublished) ( $\times$ —10 pts); McClendon, 1981 (2) ( $\Delta$ —6 pts) and Michel (+—60 pts). Solid line is from Eq. 1, this paper, Michel *et al.*, 1983 (5). Dashed line is from Eq. 2, this paper. All measurements were by thermocouple hygrometry except those of McClendon (2).

from Eq. 1 (5).

$$\Psi = 1.29[\text{PEG}]^2T - 140[\text{PEG}]^2 - 4.0[\text{PEG}] \quad (1)$$

Most points lie on or slightly above this curve. Determination from all but two [PEG] from Steuter *et al.* (7) fall well below the others.

Data for 25°C and the concentration range to 0.8 g PEG/g H<sub>2</sub>O were obtained from McClendon (2) and my measurements of 1975

and 1981 (Fig. 2). The lower curve (—) also represents Eq. 1 and fits these data even better than it does those of Figure 1.

Additional measurements from my laboratory covered the temperature range of 10 to 40.7°C and concentration range of 0.2 to 0.6 g PEG/g H<sub>2</sub>O. The complete data set also contained measurements for PEG + MAN (Table I). Eq. 2, for PEG in the absence of MAN, was derived from the means of those independent variable coefficients whose parameters did not contain [MAN] (Table I). The other terms are eliminated when [MAN] is zero.

$$\Psi = 1.22[\text{PEG}]^2T - 134[\text{PEG}]^2 - 4.4[\text{PEG}] \quad (2)$$

Eq. 2 is not significantly different from Eq. 1 (see Table III; Ref. 5), with differences in predicted  $\Psi$  less than 3% over most of the ranges of 5 to 40°C and 0.1 to 0.8 g PEG/g H<sub>2</sub>O. Biggest differences occur at the lowest temperature and highest concentrations, with 3.7% being the maximum difference. The upper curve (---) of Figure 2 represents Eq. 2. Eq. 1 seems to describe better the data for 25°C (Fig. 2). These two equations reflect quite similar responses to temperature. Considering all items, Eq. 1 probably is preferable to Eq. 2.

Michel and Kaufmann (4) reported an equation that fit data over the ranges of 15 to 35°C and 0.1 to 0.4 g PEG/g H<sub>2</sub>O. Eq. 3 is that equation, modified for expression of [PEG] as g PEG/g H<sub>2</sub>O.

$$\Psi = 0.84[\text{PEG}]^2T - 118[\text{PEG}]^2 + 0.267[\text{PEG}]T - 11.8[\text{PEG}] \quad (3)$$

Eq. 3 indicates a greater response to temperature but a reduced response to concentration in comparison with Eq. 1. The latter is partially illustrated in Figure 1. Over the temperature and concentration ranges used for the derivation of Eq. 3, the maximum difference between water potentials predicted by Eq. 1 and 3 is 10% except at the lowest temperatures and concentrations (−1.6 *versus* −1.8 bars at 15°C and 0.1 g PEG/g H<sub>2</sub>O). Extending the temperature range to from 5 to 40°C increases the percentage difference only at low concentrations of PEG. The probability of erroneous determination of temperature effects should be inversely related to the range of temperatures encompassed; thus, Eq. 1 would be favored over Eq. 3.

The data obtained using a VPO (4) make a valuable contribution toward resolving the effects of temperature. Results of analysis of that data (Table II) yielded Eq. 4.

$$\Psi = 1.30[\text{PEG}]^2T - 137[\text{PEG}]^2 \quad (4)$$

Eq. 4 is not significantly different from Eq. 1 without its last term. This means that temperature has identical effects on  $\Psi_{\text{PEG}}$  as measured by both the VPO and thermocouple hygrometry. On the other hand, Eqs. 3 and 4 predict  $\Psi_{\text{PEG}}$  measured by the two methods to be equal only at particular temperatures and [PEG]—ranging from 44.8°C at 0.1 g/g H<sub>2</sub>O to 50.6°C at 0.4 g/g H<sub>2</sub>O. Such cross-over points (see Fig. 1; Ref. 4) do not seem logical. Eqs. 1 and 4 indicate  $\Psi_{\text{PEG}}$  measured by the VPO will be 4[PEG] less negative than measured by thermocouple psychrometry at all temperatures. Observations were reported (4) which indicate that the VPO is in error, that error now appearing to range from about 0.4 bar at 0.1 g PEG/g H<sub>2</sub>O to about 3.2 bars at 0.8 g PEG/g H<sub>2</sub>O. I am unable to indicate how PEG is acting, but the error induced in the VPO appears to be directly proportional to [PEG]

Eq. 4 supports Eq. 1 as the equation of choice. The uncertainties involved preclude assigning a definite degree of reliability to predictions made from it; however, errors seem unlikely to exceed 5%.

To make possible simple calculation of [PEG] required for particular  $\Psi$  and temperatures, Eq. 5 was derived by the quadratic solution of Eq. 1.

$$[\text{PEG}] = (4 - (5.16\Psi T - 560\Psi + 16)^{0.5}) / (2.58T - 280) \quad (5)$$

Table I. Comparison between Models for PEG ± MAN Solutions from Data Obtained by Thermocouple Hygrometry and Analyzed by the GLM Procedure of SAS

[PEG](−MAN) = 0.2, 0.3, 0.4, 0.5, and 0.6 g/g H<sub>2</sub>O. [PEG](+MAN) = 0.1, 0.3, and 0.5 g/g H<sub>2</sub>O. [MAN] = 0.1, 0.3, 0.5, and 0.7 molal. Temperatures = 10, 20.4, 29.8, and 40.7°C. n = 175.

Dependent Variable	Independent Variables		<i>r</i> <sup>2</sup>	Max Ψ ± 95% CL Min Ψ ± 95% CL
	Parameter	Coefficient ± SE		
(Ψ + 0.078[MAN]T + 22.75[MAN])	[PEG] <sup>2</sup> [MAN]T	−1.0 ± 0.3	0.9993	bars −4.33 ± 0.09 −58.24 ± 0.44
	[PEG] <sup>2</sup> [MAN]	58 ± 10		
	[PEG][MAN]T	0.66 ± 0.13		
	[PEG][MAN]	−66 ± 4		
	[PEG] <sup>2</sup> T	1.22 ± 0.03		
	[PEG] <sup>2</sup>	−132 ± 2		
(Ψ + 0.078[MAN]T + 22.75[MAN]) [PEG]	[PEG]	−5.4 ± 0.7	0.9827	−4.19 ± 0.08 −58.31 ± 0.74
	[PEG][MAN]T	−1.1 ± 0.3		
	[PEG][MAN]	65 ± 10		
	[MAN]T	0.72 ± 0.09		
	[MAN]	−69 ± 3		
	[PEG]T	1.21 ± 0.06		
[PEG]	−136 ± 2	−3.5 ± 0.6		
Intercept	−3.5 ± 0.6			

Table II. Comparison between Models for PEG ± MAN Solutions from Data Obtained by Vapor Pressure Osmometry and Analyzed by the GLM Procedure of SAS

[PEG](−MAN) = 0.1, 0.2, 0.3, and 0.4 g/g H<sub>2</sub>O. [MAN] = 0.244, 0.488, and 0.731 molal. Temperatures (−MAN) = 25, 35, 45, 55, and 65°C. Temperatures (+MAN) = 25, 45, and 65°C. n = 56.

Dependent Variable	Independent Variables		<i>r</i> <sup>2</sup>	Max Ψ ± 95% CL Min Ψ ± 95% CL
	Parameter	Coefficient ± SE		
(Ψ + 0.0713[MAN]T + 22.96[MAN])	[PEG] <sup>2</sup> [MAN]	35 ± 4	0.9995	bars −0.52 ± 0.02 −46.66 ± 0.32
	[PEG][MAN]T	0.37 ± 0.03		
	[PEG][MAN]	−64 ± 2		
	[PEG] <sup>2</sup> T	1.32 ± 0.04		
	[PEG] <sup>2</sup>	−137 ± 2		
(Ψ + 0.0713[MAN]T + 22.96[MAN]) [PEG]	[PEG]	50 ± 4	0.9988	−0.53 ± 0.03 −46.41 ± 0.52
	[MAN]T	0.42 ± 0.04		
	[MAN]	−70 ± 2		
	[PEG]T	1.27 ± 0.06		
	[PEG]	−136 ± 3		

Consider whether or not this equation,  $\Psi/[PEG] = a[PEG] + b$  (see "Materials and Methods") which appears accurately to describe relationships for any single temperature, can be interpreted as a valid virial equation. Several lines of evidence indicate that  $b \neq RK/M$ . If  $b$  were constant, as is indicated by Eqs. 1 and 2, mol wt would vary directly with Kelvin temperature. That is absurd. The relationships between [PEG] and Ψ have been reported to be nearly identical for PEG 6000 and PEG 20,000 (8) as well as different (7). These relationships also have been found not to be significantly different between PEG 4000 and PEG 6000 (3, 7). If different mol wt of PEG have the same  $b$ , then  $b \neq RK/M$ . Similar phenomena have been reported for dextrans, where 'negative solvent pressures' were close to the same over the mol wt range of 20,000 to 150,000 (1). The conclusion was that long and short chains have essentially the same effect on Ψ at equal g dextran/g H<sub>2</sub>O.

If  $b$  is unrelated to mol wt then  $a/RK$  can hardly be a second virial coefficient. Values for this term can be calculated for various temperatures from Eqs. 1 to 4 and equations, which are quite similar, can be derived to show that the term is slightly curvilinear

rather than linearly (2) related to temperature; however, the meaning and utility of these equations are questionable.

**PEG plus Other Solutes.** Equations representing data from both thermocouple hygrometry and vapor pressure osmometry and showing the effects on Ψ of PEG, MAN, and their interaction were obtained by averaging coefficients reported in Tables I and II. These equations are presented in Tables III and IV along with representative values obtained as solutions of the equations. The parameters without [PEG] define the effect of pure MAN on Ψ; those without [MAN] define the effect of pure PEG on Ψ and also were given as Eqs. 2 and 4; and the parameters with both [PEG] and [MAN] define the effect of interaction between PEG and MAN on Ψ. Because the interaction is always greater than additive, it is synergistic. The temperatures and concentrations chosen for preparation of Tables III and IV represent overlap of conditions used during measurement by the two methods, except above 0.4 g PEG/g H<sub>2</sub>O and below 0.244 molal MAN for the VPO (Table IV).

As they should, both methods yield the same Ψ for MAN; and, as previously indicated, the VPO measures higher Ψ for PEG than

Table III. Influence of PEG, MAN, and Their Interaction on  $\Psi$  Measured by Thermocouple Hygrometry  
 $\Psi$  were obtained as:  $\Psi = -1.0[\text{PEG}]^2[\text{MAN}]T + 61[\text{PEG}]^2[\text{MAN}] + 0.69[\text{PEG}][\text{MAN}]T - 68[\text{PEG}][\text{MAN}] + 1.22[\text{PEG}]^2T - 134[\text{PEG}]^2 - 4.4[\text{PEG}] - 0.078[\text{MAN}]T - 22.75[\text{MAN}]$ . Sums and differences were computed before rounding.

<i>T</i>	MAN Concn.	Concn. of PEG (g/g H <sub>2</sub> O)						
		0.0 (Total $\Psi$ )	0.1		0.3		0.5	
			Total $\Psi$	Synergism	Total $\Psi$	Synergism	Total $\Psi$	Synergism
$^{\circ}\text{C}$	<i>molal</i>		<i>bars</i>					
25	0.0	0.0	-1.5	0.0	-10.6	0.0	-28.1	0.0
	0.1	-2.5	-4.4	0.5	-14.3	1.2	-32.2	1.6
	0.3	-7.4	-10.3	1.4	-21.6	3.6	-40.4	4.9
	0.5	-12.4	-16.2	2.4	-29.0	6.0	-48.6	8.2
	0.7	-17.3	-22.1	3.3	-36.3	8.4	-56.8	11.5
40	0.0	0.0	-1.3	0.0	-9.0	0.0	-23.5	0.0
	0.1	-2.6	-4.3	0.4	-12.6	1.0	-27.6	1.5
	0.3	-7.8	-10.2	1.1	-19.8	3.1	-35.7	4.5
	0.5	-12.9	-16.1	1.9	-27.0	5.1	-43.9	7.5
	0.7	-18.1	-22.1	2.7	-34.3	7.2	-52.1	10.5

Table IV. Influence of PEG, MAN, and Their Interaction on  $\Psi$  Measured by Vapor Pressure Osmometry  
 $\Psi$  were obtained as:  $\Psi = 43[\text{PEG}]^2[\text{MAN}] + 0.40[\text{PEG}][\text{MAN}]T - 67[\text{PEG}][\text{MAN}] + 1.30[\text{PEG}]^2T - 137[\text{PEG}]^2 - 0.0713[\text{MAN}]T - 22.96[\text{MAN}]$ . Sums and differences were computed before rounding.

<i>T</i>	MAN Concn.	Concn. of PEG (g/g H <sub>2</sub> O)						
		0.0 (Total $\Psi$ )	0.1		0.3		0.5	
			Total $\Psi$	Synergism	Total $\Psi$	Synergism	Total $\Psi$	Synergism
$^{\circ}\text{C}$	<i>molal</i>		<i>bars</i>					
25	0.0	0.0	-1.0	0.0	-9.4	0.0	-26.1	0.0
	0.1	-2.5	-4.0	0.5	-13.2	1.3	-30.4	1.8
	0.3	-7.4	-10.0	1.6	-20.8	4.0	-38.9	5.3
	0.5	-12.4	-16.1	2.6	-28.4	6.6	-47.4	8.9
	0.7	-17.3	-22.1	3.7	-36.0	9.3	-55.9	12.4
40	0.0	0.0	-0.8	0.0	-7.6	0.0	-21.3	0.0
	0.1	-2.6	-3.9	0.5	-11.4	1.1	-25.3	1.5
	0.3	-7.7	-10.0	1.4	-18.8	3.4	-33.4	4.4
	0.5	-12.9	-16.1	2.3	-26.3	5.7	-41.5	7.4
	0.7	-18.1	-22.2	3.3	-33.7	8.0	-49.6	10.3

Table V. Effects of Certain Salts and MAN on the  $\Psi$  of PEG Solutions as Measured by Vapor Pressure Osmometry at 45 $^{\circ}\text{C}$

Additive	Concn.	Concn. of PEG (g/g H <sub>2</sub> O)				
		0.0 (Meas- ured $\Psi$ )	0.2		0.3	
		Meas- ured $\Psi$	Syner- gism	Meas- ured $\Psi$	Syner- gism	
	<i>molal</i>	<i>bars</i>				
None		0.0	-3.1	0.0	-6.7	0.0
K <sub>2</sub> SO <sub>4</sub>	0.355	-20.0	-32.0	8.9	-39.3	12.6
KF	0.410	-20.1	-29.0	5.8	-35.0	8.2
NaCl	0.410	-19.9	-27.4	4.4	-32.8	6.2
KI	0.410	-20.1	-21.4	-1.8	-24.0	-2.8
MAN	0.731	-19.5	-27.6	5.0	-33.7	7.5

thermocouple hygrometers by the predicable amount of 4[PEG].

In general, the total  $\Psi$  measured by the two methods approach each other as [MAN] rises; therefore, apparent synergism is increasingly overestimated by the VPO as [MAN] becomes larger.

Nevertheless, the amounts of synergism indicated by the two methods do not differ greatly from each other and consideration of other VPO measurements of synergism seems justified.

Solutions of several salts and MAN having essentially the same  $\Psi$ , interacted quite differently with PEG; and synergism ranged from high to none ( $\Psi$  even less than additive). The data of Table V were obtained with a VPO at 45 $^{\circ}\text{C}$ . With synergism tending to be greater at lower temperatures (Tables III and IV) and differences in synergism indicated by the two methods not large, these data strengthen the argument that synergism between PEG and other solutes is sufficient that it must not be ignored. Each solute or combination thereof will require separate measurements; however, synergism may be related linearly to salt concentration as it is to [MAN]. This was true for the two NaCl concentrations tested (Table V), where the ratios of NaCl synergism (0.70 and 0.69) were not significantly different from the ratio of NaCl concentrations (0.67). The existence of additional predictable trends might permit accurate equations to be derived from relatively few measurements.

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