Acta Cryst. (1995). D**51**, 772–779

Non-ideality of Aqueous Solutions of Polyethylene Glycol: Consequences for Its Use as a Macromolecular Crystallizing Agent in Vapor-Diffusion Experiments*

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(*Received* 11 *August* 1994; *accepted* 9 *January* 1995)

Abstract

Microisopiestic measurements of the concentrations of polyethylene glycol (PEG 8000) paired with the salts sodium chloride, ammonium sulfate and magnesium sulfate heptahydrate have been made in a sitting-drop arrangement with PEG in the droplet and salt in the reservoir. Resulting graphs of the concentrations of PEG and salt that are equivalent with respect to the vapor pressure of water are non-linear, do not intersect their origins, and demonstrate that relatively low (m*M*) salt concentrations are equivalent to relatively high PEG concentrations. The consequences of each of these observations for macromolecular crystallization by the vapor-diffusion technique when PEG is employed as the crystallizing agent are discussed.

Introduction

Pared to its essentials, the supported-drop method of macromolecular crystallization involves the equilibration of the droplet and a reservoir. The droplet contains a macromolecule and a crystallizing agent while the reservoir contains a dehydrating agent. Equilibration is effected as water, in the form of vapor, leaves the droplet, traverses the vapor chamber, and enters the reservoir (Fowlis, DeLucas, Twigg, Howard, Meehan & Baird, 1988). The driving force for the process is the inequality of the chemical potential of the volatile species, water, in droplet, vapor chamber, and reservoir. In the process of dehydration the concentrations of macromolecule and crystallizing agent in the droplet increase. In the favorable case, conditions evolve in the droplet that lead to nucleation and crystal growth. The practical and theoretical considerations of crystallization *via* vapor diffusion are discussed in detail by McPherson (1982) and by Ducruix & Giegé (1992).

In a frequently utilized protocol for crystallization experiments a stock solution containing the macromolecule is prepared. Typically the stock is buffered and often it contains other ingredients, such as detergents, co-factors, metal ions, and inhibitors. Separately a reservoir solution is prepared containing a dehydrating agent. The reservoir solution may or may not be buffered. The droplet is constituted at the time of the crystallization setup by combining aliquots of the stock and reservoir solutions. By design the crystallizing agent in the droplet is also the dehydrating agent in the reservoir. Although the same chemical species is used for both, it is worthwhile to make a clear distinction between the crystallizing agent in the droplet and dehydrating agent in the reservoir. They play very different roles in the crystallization process. Indeed, since the droplet and the reservoir are in contact only through the vapor there is no requirement that the crystallizing and dehydrating agents be the same chemical species. McPherson (1992) recently described a rapid-screening procedure in which nine distinct crystallizing agents are allowed to equilibrate with a common reservoir containing a single dehydrating agent. In the case of the screen it was principally a question of speed and ease of setup that dictated the choice to distinguish the two agents chemically, but in the case of a crystallization protocol we recently described (Luft, Arakali, Kirisits, Kalenik, Wawrzak, Cody, Pangborn & DeTitta, 1994) the choice was not one of convenience but of necessity. In the *Z*/3 crystallization protocol diffusion cells are employed as reservoirs; by varying their depths it is possible to tailor the kinetics of equilibration of the droplet and the reservoir solutions. The reservoirs are prepared by overlayering a solid sample (or highly concentrated solution) of the dehydrating agent with a solution containing the dehydrating agent at a comparatively low concentration. As the solid dissolves and diffuses to the surface of the reservoir the vapor pressure of water over the reservoir slowly decreases from its initial value. The droplet responds by further dehydration. Measurement of the surface concentration of dehydrating agent as a function of time (Arakali, Easley, Luft & DeTitta, 1994) suggests that salts such as sodium chloride, ammonium sulfate and magnesium sulfate heptahydrate dissolve, diffuse and equilibrate in times measured in days or weeks, even for the deepest reservoirs. A similar strategy involving the dissolution and diffusion of

* Presented at the American Crystallographic Association Meeting, Atlanta, GA, 1994.

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ISSN 0907-4449

polyethylene glycols (PEG) in the reservoirs might be impractical because of their much longer equilibration times. For example, the diffusion coefficients of PEG 1600 and PEG 50 000 are 2×10^{-10} and $3 \times 10^{-11}\,m^2s^{-1}$, respectively (Couper & Stepto, 1969; Brown, Stilbs & Johnsen, 1983); these are one and two orders of magnitude smaller than the diffusion coefficient for sodium chloride in aqueous solution, $1.5 \times 10^{-9}\,m^2s^{-1}$. Thus, crystallizations in which PEG is utilized in the $Z/3$ reservoirs as the dehydrating agent might take months, possibly years, to fully equilibrate. An obvious strategy when PEG is the crystallizing agent of choice is to utilize a more rapidly diffusing dehydrating agent, such as a salt, in the reservoirs. To proceed we need to know what concentrations of salt are appropriate to effect a dehydration of the droplet to a desired final PEG concentration.

In the $Z/3$ experiment there are a number of equilibration processes occurring simultaneously: in the droplet, across the vapor chamber and within the reservoir. In order to decouple the kinetic and thermodynamic aspects of the equilibration processes we report here concentrations of PEG and three salts that are equivalent with respect to the vapor pressure of water *at equilibrium* in a sitting-drop arrangement in which the droplet contains PEG and the reservoir contains salt. In the sequel we will make use of the term 'equivalent concentration' which is specifically meant to imply equality with respect to the vapor pressure of water in the experimental design to be described. It does not imply any other kind of equality; in particular it does not imply equality with respect to protein solubility. The results of our experiments with PEG are of interest to anyone employing it as a crystallizing agent, be it in a $Z/3$ plate or in a traditional vapor-diffusion setup, such as in a Linbro plate with homogeneous reservoirs.

Experiments and results

Solutions were prepared with PEG 8000 from Fluka and sodium chloride, ammonium sulfate and magnesium sulfate heptahydrate from Sigma. Water was distilled and deionized (Barnstead NANOpure II, > 17 MΩ cm) but not degassed. Solutions were prepared directly, and never by dilution of more concentrated stock solutions. 24 PEG 8000 solutions ranging evenly over PEG concentrations of 1 to 48%(*w*/*v*) were prepared and their refractive indices recorded on a Bausch and Lomb Abbé 3L refractometer at 297 K. A plot of refractive index *versus* PEG concentration is linear, Fig. 1, and serves as a basis for the measurement of equilibrium PEG concentration.

The classical isopiestic method (Bousfield, 1918; Robinson & Sinclair, 1934; Scatchard, Hamer & Wood, 1938) is an elegant technique to measure concentrations of solutes that are equivalent with respect to the vapor pressure of a common volatile solvent. Solutions are allowed to equilibrate in a sealed chamber that is carefully thermostatted and continously agitated. Equilibration takes place as volatile solvent distills from solutions of higher to solutions of lower vapor pressure. Concentrations are measured gravimetrically, as a difference in mass can only be due to the addition or removal of solvent. Though conceptually simple the classical isopiestic technique requires instrumentation not often found in the crystal-growth laboratory, and is cumbersome when many different conditions of equilibration need to be examined. We have designed a simple variant of the procedure that is easy to implement in the crystal-growth laboratory, requires no special equipment other than a refractometer, employs an arrangement of the equilibrating solutions particularly germane to crystal growth, and is capable of measuring equivalent concentrations with good precision.

The equilibration experiments are carried out in Linbro plates fitted with microbridges, Fig. 2. Salt solutions of known concentration were introduced into the wells, microbridges were inserted, and PEG solutions were introduced into the microbridge depressions. The plates were sealed with clear plastic label tape from Manco (Crystal Clear Package Tape) using a HANGMAN jig (Luft & DeTitta, 1992), and were stored in a constant temperature incubator (293 ±0.1 K) to equilibrate. Reservoir volumes were 1.0–1.5 ml and droplet volumes were 10–30 µl.

Preliminary experiments gave an approximate idea of the shapes of the graphs of $[PEG]^D_{eq}$ *versus* $[salt]^R_{eq}$, where $[PEG]^D_{eq}$ is the equilibrium concentration of PEG in the droplet and $[salt]^R_{eq}$ is the equilibrium concentration of salt in the reservoir. This permitted the design of more refined experiments where, for a given salt at a particular

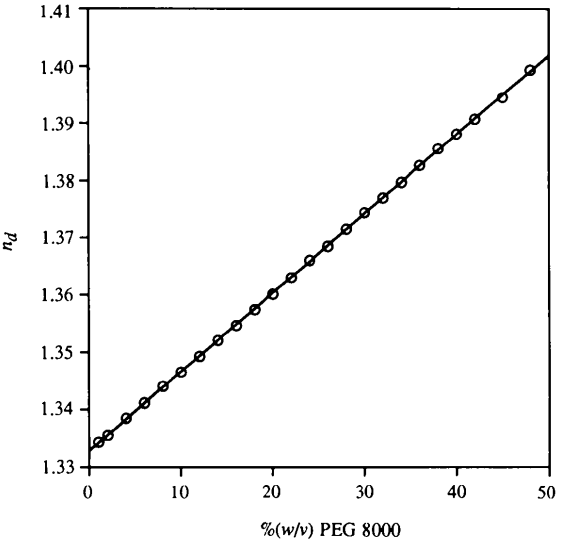

Fig. 1. The refractive index, n_d, of PEG 8000 at 297 K as a function of concentration, measured as a %(*w*/*v*). The concentration of PEG can be calculated from these data as $[PEG]\%(w/v) = (n_d - b)/a$, with $a = 0.0013889$ and $b = 1.3325$.

concentration in the reservoir, a series of PEG droplets, some initially higher and some initially lower in concentration than the expected equilibrium concentration, were allowed to equilibrate. Those with initial concentrations of PEG higher than $[PEG]^D_{eq}$ equilibrated by hydrating the droplet and dehydrating the reservoir; conversely those with initial concentrations of PEG lower than $[PEG]^D_{eq}$ equilibrated by dehydrating the droplet, as in a typical crystallization experiment, and hydrating the reservoir. Asymptotic approach to equilibrium both from above and below $[PEG]^D_{eq}$ is one test of the completeness of equilibration.

In addition, each initial condition of PEG and salt was set up in replicate. For the lower concentrations of salts and PEG as many as a dozen identical equilibrations were carried out. Typically half of the experiments were terminated and read after a month-long equilibration, while the other half were allowed to equilibrate an additional month before termination and reading. The maintenance of an equivalent concentration over an extended interval is a second test of the completeness of equilibration. An example of the two tests for equilibration is shown in Fig. 3. Note that this set of experiments, entailing the equilibration of many droplets/reservoirs, determines one point on the graph of equivalent concentrations.

The plates were recorded in a standard fashion. A droplet was made accessible by running a razor blade around the inner lip of a reservoir, cutting the tape and folding it back. The droplet was immediately retrieved from the microbridge with a micropipette and transferred to the refractometer prism where its refractive index was recorded. The prism was washed, dried and readied for the recording of the reservoir solution. From the time the seal was broken it took approximately 10 s to retrieve and record the refractive index of the droplet; it took another 35 s to prepare the prism and record the refractive index of the reservoir. The graph of refractive index *versus* PEG concentration, Fig. 1, was used to convert a droplet reading into a PEG concentration. The reservoir readings were checked against starting values to insure that the Manco tape had adequately sealed the wells. It is of some interest to note that in the thousands of wells employed in these experiments we have recorded only three catastrophic failures of the tape seals, each of which was explained by a crease in the tape at the lip of a well. This may be compared to the more frequent failures of coverslips to seal the wells of Linbro plates, at least in our hands. After visual inspection of the graphs of PEG concentration in the droplet *versus* time and starting concentration, such as shown in Fig. 2, average values were computed to determine $[PEG]^D_{eq}$. Since the lower salt concentrations could not be determined with the requisite accuracy by refractometry, and because the removal or addition of a few microliters of water from or to the reservoir was unlikely to change the reservoir concentration perceptibly, the starting concentrations of the salts were taken as $[salt]^R_{eq}$.

In general, the estimated precision in a value of $[PEG]^D_{eq}$ is better than $\pm 1\%(w/v)$. This estimate was based both on internal consistency from droplet to droplet in any single experiment and on consistency of the average value of $[PEG]^D_{eq}$ as found in a small number of replicate experiments set up independently by the investigators. What variation that does appear is unrelated to the accuracy or precision of the refracto-

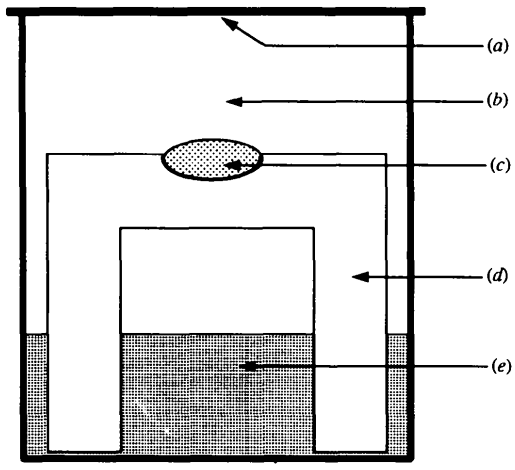

Fig. 2. Experimental arrangement for the equilibration experiments. Shown is one well of a 24-well Linbro plate with (*a*) the seal made with clear plastic label tape, (*b*) the vapor chamber, (*c*) the droplet containing PEG, (*d*) a clear plastic microbridge, and (*e*) a salt solution of known concentration.

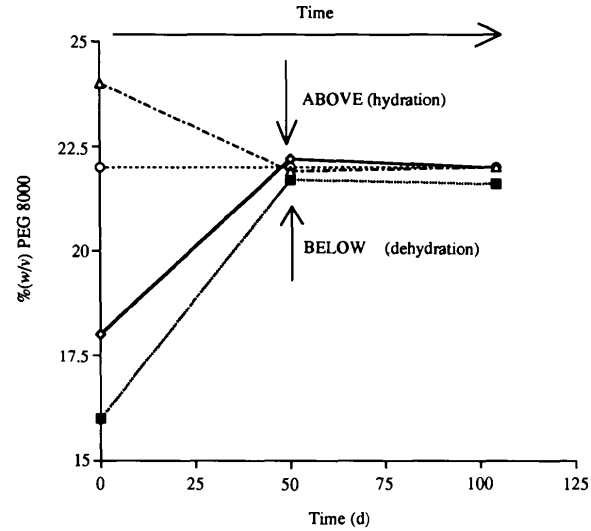

Fig. 3. The determination of a single point on the graph of $[PEG]^D_{eq}$ *versus* [ammonium sulfate]$^R_{eq}$. The reservoir solution is 125 m*M* ammonium sulfate. Initial concentrations in the droplets were 16, 18, 22, and 24%(*w/v*) PEG. The first readings were after 50 d, the second after 104 d of equilibration. The latter readings were averaged to give an equivalent concentration point of [21.9%(*w/v*) PEG, 125 m*M* ammonium sulfate].

metric measurements *per se*. That is, repeated measurements of the refractive indices of the stock solutions used to create the standard curve of Fig. 1 were consistent to better than ±0.2%(*w*/*v*) PEG. Therefore, the variation observed was because of actual variations in the equilibrated droplets. Those variations were greatest for the lowest values of $[PEG]^D_{eq}$ and were much smaller for larger $[PEG]^D_{eq}$ values.

Graphs of $[PEG]^D_{eq}$ *versus* $[salt]^R_{eq}$ for the polyethylene glycol PEG 8000 and the salts sodium chloride, ammonium sulfate, and magnesium sulfate heptahydrate are shown in Figs. 4, 5, and 6, respectively. Data employed to construct the graphs are given in Tables 1, 2 and 3, respectively. Salt concentrations are expressed in molarity units; PEG concentrations are expressed as %(*w*/*v*). As some confusion exists in the literature surrounding concentration units employed for PEG experiments, we note that %(*w*/*v*) is a unit related to molarity. For example a 15%(*w*/*v*) PEG solution is made by dissolving 15.0 g PEG to a total volume of 100.0 ml solution.

For sodium chloride and magnesium sulfate heptahydrate, reservoir concentrations as low as 5 m*M* had been allowed to equilibrate with PEG droplets of various concentrations, as just described. These experiments led to the unexpected observation that extrapolation to 0 m*M* salts, *i.e.* pure water, in the reservoir did not predict 0%(*w*/*v*) PEG, *i.e.* pure water, in the droplet at equilibrium. A special set of equilibrations was then carried out with freshly prepared PEG 8000 solutions, from 6 to 10%(*w*/*v*), against pure water. The same stock of water used in the reservoirs was used to prepare the PEG solutions. Five Linbro plates (24 wells) with pure water in the reservoirs and each of the five PEG concentrations were permitted to equilibrate for 35 d. In each plate four different drop sizes (12, 18, 24, and 30 µl) were set up in sextuplicate to examine the possibility that drop-volume effects would be observable. The droplets were harvested, their refractive indices recorded, and their PEG concentrations determined as previously described. All of the initially 6 and 7%(*w*/*v*) PEG droplets had equilibrated against pure water with dehydration; all of the 8, 9 and 10%(*w*/*v*) PEG droplets had equilibrated with hydration. No obvious drop-volume effects were observed. The range 7–8%(*w*/*v*) PEG clearly included the equivalence point for PEG in a droplet over a pure water reservoir. The average over the

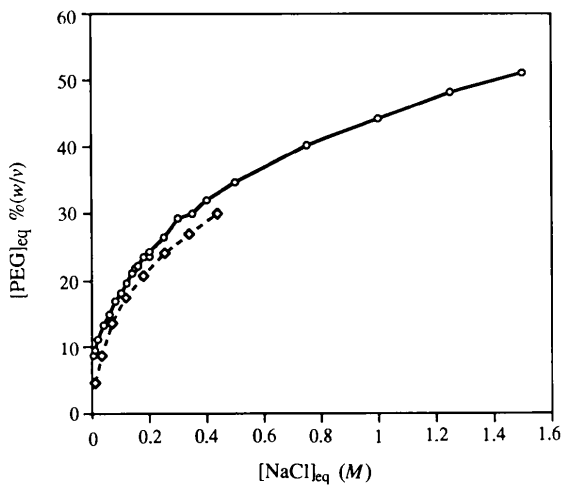

Fig. 4. Concentrations of PEG 8000 and sodium chloride that are equivalent with respect to the vapor pressure of water. Open circles are for our data, in which the PEG solution takes the form of a sitting droplet and the sodium chloride takes the form of a reservoir. Open diamonds are calculated from the equation (1) of Michel (1983) as described in the text and are representative of equilibrations in which both PEG and sodium chloride solutions approximate the bulk.

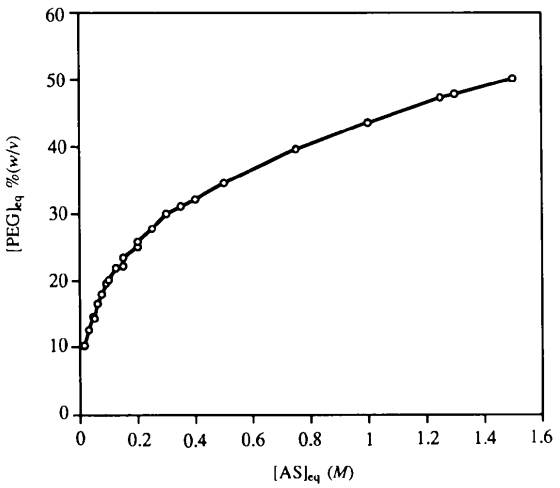

Fig. 5. Concentrations of PEG 8000 and ammonium sulfate (AS) that are equivalent with respect to the vapor pressure of water.

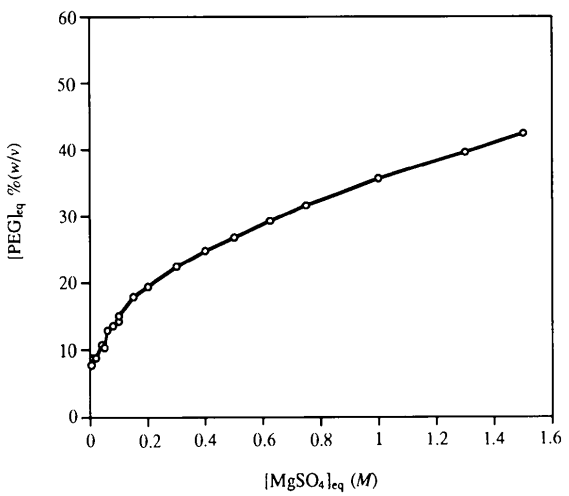

Fig. 6. Concentrations of PEG 8000 and magnesium sulfate heptahydrate that are equivalent with respect to the vapor pressure of water.

Table 1. *Concentrations of sodium chloride (reservoir) and PEG 8000 (droplet) that are equivalent with respect to the vapor pressure of water*

Concentration units are molarities for NaCl, %(*w*/*v*) for PEG.

$[NaCl]^R_{eq}$	$[PEG]^D_{eq}$	$[NaCl]^R_{eq}$	$[PEG]^D_{eq}$
0.005	8.7	0.200	23.6
0.010	9.5	0.200	24.3
0.020	11.2	0.250	26.5
0.040	13.3	0.250	26.5
0.060	14.9	0.300	29.3
0.080	16.9	0.350	30.0
0.100	18.2	0.400	32.0
0.120	19.7	0.500	34.7
0.140	21.2	0.750	40.2
0.150	21.9	1.000	44.2
0.160	22.2	1.250	48.2
0.180	23.5	1.500	51.1

Table 2. *Concentrations of ammonium sulfate (AS, reservoir) and PEG 8000 (droplet) that are equivalent with respect to the vapor pressure of water*

Concentration units are molarities for ammonium sulfate, %(*w*/*v*) for PEG.

$[AS]^R_{eq}$	$[PEG]^D_{eq}$	$[AS]^R_{eq}$	$[PEG]^D_{eq}$
0.015	10.2	0.250	27.8
0.030	12.5	0.300	30.0
0.045	14.6	0.350	31.1
0.050	14.3	0.400	32.1
0.060	16.6	0.400	32.1
0.075	18.0	0.500	34.6
0.090	19.6	0.750	39.7
0.100	20.1	0.750	39.6
0.125	21.9	1.000	43.7
0.150	22.2	1.000	43.6
0.150	23.4	1.250	47.4
0.200	25.0	1.300	47.9
0.200	25.8	1.500	50.1
0.200	25.0		

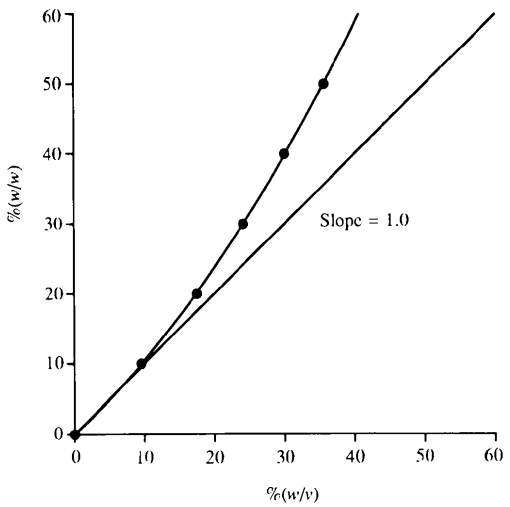

Fig. 7. Comparison of the two prototypical concentration units employed in the literature of PEG solutions. Solutions of accurately measured %(*w*/*w*) concentrations were prepared and their refractive indices measured. Comparison with the calibration chart in Fig. 1 yielded equivalent %(*w*/*v*) concentrations which are plotted one against the other.

Table 3. *Concentrations of magnesium sulfate heptahydrate (reservoir) and PEG 8000 (droplet) that are equivalent with respect to the vapor pressure of water*

Concentration units are molarities for the $MgSO_4$, %(*w*/*v*) for the PEG.

$[MgSO_4]^R_{eq}$	$[PEG]^D_{eq}$	$[MgSO_4]^R_{eq}$	$[PEG]^D_{eq}$
0.005	7.8	0.200	19.4
0.010	8.8	0.300	22.4
0.020	8.8	0.400	24.8
0.040	10.7	0.500	26.8
0.050	10.3	0.625	29.3
0.060	12.8	0.750	31.7
0.080	13.5	1.000	35.6
0.100	14.2	1.300	39.6
0.100	15.0	1.500	42.4
0.150	17.9		

Table 4. *Equivalent %(w/v) and %(w/w) concentrations of PEG 8000*

%(*w*/*v*)	%(*w*/*w*)
9.5	10.0
17.5	20.0
24.1	30.0
30.0	40.0
35.6	50.0

48 droplets comprising the two plates with original concentrations of 7 and 8%(*w*/*v*) PEG is 7.60%(*w*/*v*) PEG; the standard deviation is 0.25%(*w*/*v*). The individual plates had average values of 7.46 (25)%(*w*/*v*) (originally 7%) and 7.71 (20)%(*w*/*v*) (originally 8%). It appears that, at these very low concentrations of PEG, one month is not a sufficient amount of time to reach equilibrium.

In order to facilitate the interpretation of osmotic pressure data in the literature (*vide infra*) experiments relating the two prototypical concentration units, %(*w*/*w*) and %(*w*/*v*), were conducted. Solutions of PEG 8000 of known %(*w*/*w*) concentration were prepared by weighing out quantities of solid PEG, weighing out 100.0 g of distilled, deionized water, and dissolving the former in the latter. Samples of the solutions were analyzed by refractometry and the equivalent %(*w*/*v*) concentrations were determined by reference to the calibration chart, Fig. 1. A graph of %(*w*/*w*) PEG *versus* %(*w*/*v*) PEG is shown in Fig. 7. Data employed to construct the graph are in Table 4. The various concentration units employed in the literature are related in simple ways to one or the other of the prototypical units.

Discussion

Inspection of the graphs of equivalent concentrations of PEG and the salts, Figs. 4–6, leads to three observations: (i) the graphs are non-linear over their entire range; (ii) the curves do not intersect the origins of their graphs; and (iii) quite low (m*M*) concentrations of salts in the reservoirs are equivalent to relatively high concentrations

of PEG in the droplets. Each of these observations has practical consequences.

The non-linearity of the equivalent concentration graphs is a consequence of the non-ideality of aqueous PEG solutions and can be traced to factors of entropic origin (Flory, 1953). In their study of the kinetics of water equilibration in hanging drops Mikol, Rodeau & Giegé (1992) argue that the rate of equilibration is proportional to the difference in the partial pressure of water over the droplet and over the reservoir. They further argue that pressure difference is proportional to the concentration of dehydrating agent in the reservoir, assuming a traditional hanging drop arrangement where the dehydrating and crystallizing agents are the same chemical species. Thus, holding the temperature, drop size, drop shape, drop volume, the distance from the drop to the reservoir, and the dilution factor (initial ratio of crystallizing to dehydrating agent concentrations) constant, doubling the concentration of the dehydrating agent will halve the time necessary for the droplet to equilibrate with the reservoir. Such a development describes the equilibration process well for situations where salts are the crystallizing/dehydrating agents. However, the situation with PEG as the crystallizing agent is quite different. Our results imply that, in a traditional vapor-diffusion crystallization experiment, doubling the PEG concentration in the reservoir will more than double the rate of equilibration of droplet with reservoir.

The failure of the curves of equivalent concentration to intersect the origins of their graphs is not artifactual. Equilibration of droplets with various PEG concentrations over reservoirs of pure water indicates that a droplet 7.6%(*w*/*v*) in polyethylene glycol is in equilibrium with such a reservoir. Clearly this observation reflects the particulars of the experimental protocol in which a small droplet, supported by a plastic microbridge, equilibrates with a relatively large reservoir. Whereas the reservoir is sufficiently large to approximate the bulk properties of solution, the droplet is sufficiently small that surface tension effects become significant. The surface tension tends to increase the vapor pressure of water over the droplet (Daniels & Alberty, 1961) *vis-à-vis* bulk water, requiring the addition of PEG to a concentration of about 8%(*w*/*v*) to re-establish equilibrium with bulk water. Consequently, it is important to appreciate that in crystallizations involving PEG alone as the crystallizing/dehydrating agent it will be difficult to establish equilibrium conditions with PEG concentrations less than $\sim$ 8%(*w*/*v*) in the droplet.

The low concentrations of salt that are equivalent to relatively high concentrations of PEG in the droplet are of particular interest. Cudney (1994) surveyed the NIST/CARB/NASA Biological Macromolecule Crystallization Database, Version 2.0 (Gilliland & Bickham, 1990) and found that the majority of successful crytallizations reported with PEG 6000 employed polyethylene glycol concentrations in the range 6–15%(*w*/*v*). Our estimates suggest that 60 m*M* sodium chloride, 45 m*M* ammonium sulfate, and 100 m*M* magnesium sulfate heptahydrate are equivalent concentrations in the reservoir to 15%(*w*/*v*) PEG 8000 in the droplet. We note that 45–100 m*M* is a concentration range frequently employed for buffers and other additives. Inasmuch as vapor pressure lowering is a colligative property it depends, to a first approximation, on the additive effects of all the solutes present. It is frequently the case however that certain solutes, most notably the macromolecule itself, but also expensive detergents, inhibitors available in short supply, and occasionally buffers, are present in the droplet but not the reservoir. Under those circumstances it is possible, when using PEG as the crystallizing and dehydrating agent, to unwittingly constitute a vapor–diffusion experiment in which the droplet dehydrates the reservoir instead of the other way around. This is another way of stating that PEG in the concentration range most useful for crystallization has a very small effect on the vapor pressure of water. Thus, the emphasis on a restricted interpretation of the term 'equivalent concentration'. At PEG concentrations that are effectively inducing nucleation and crystal growth (Atha & Ingham, 1981), salts, in concentrations equivalent with respect to the vapor pressure of water, are at concentrations generally associated with the salting-*in* phenomenon. Thus, the attractive proposal (Schreuder, Groendijk, van der Laan & Wierenga, 1988; Wierenga, Zeelen & Noble, 1992) that equivalent concentrations, in the restricted sense of this development, might provide a rational strategy for the transfer of protein crystals from one mother liquor to another, for example from ammonium sulfate to PEG, may be untenable.

The osmotic properties of polyethylene glycols have long been of interest in the soil sciences and plant physiology community. Applegate (1960) showed that the freezing point depression of water caused by polyethylene glycol is non-linear in the concentration of PEG; likewise Lagerwerff, Ogata & Eagle (1961) demonstrated a comparable non-linearity in the osmotic pressure of aqueous PEG solutions. Subsequent studies by Zur (1966), Williams & Shaykewich (1969) and Vink (1971) confirmed the non-ideality of PEG solutions. Michel & Kaufmann (1973); Rogers & Tam (1977); Schrier, Bullock & Schrier (1980); Steuter, Mozafar & Goodin (1981) and McClendon (1981) studied further the quantitative relationship between the osmotic pressure (or potential) and PEG concentration as a function of temperature. Michel (1983) critically analyzed these and other unpublished data and fitted the body of it with a single equation that relates the osmotic potential ψ to the concentration of PEG and the temperature. The equation is quadratic in the PEG concentration when expressed in gPEG/gH_2O, a unit related to molality and %(*w*/*w*). Using the standard thermodynamic relationship between the osmotic pressure (the negative of the osmotic

potential) and the activity of water, Lewis & Randall (1961),

$$\ln a_{H_2O} = -\pi \bar{V}_{H_2O} R^{-1} T^{-1}$$

where a_{H_2O} is the activity of water, π is the osmotic pressure, $\bar{V}_{H_2O}$ is the partial molal volume of water, R is the gas constant, and T is the absolute temperature, values of the water activity can be calculated as a function of PEG concentration. Thereafter, concentrations of sodium chloride that have the same value of water activity can be interpolated from the tables of Robinson & Stokes (1965). After conversion to the appropriate concentration units the quadratic form fitted by Michel (1983) yields an independent graph of equivalent concentrations of PEG and NaCl, shown as a dotted line in Fig. 4. There are important, systematic differences between our results and those of Michel (1983). In particular, for a given concentration of sodium chloride we predict an equivalent concentration of PEG that is anywhere from 2 to 8%(*w*/*v*) higher than the values of Michel. The greatest discrepancies are at the lowest NaCl concentrations. The quadratic form of Michel predicts that the curve of equivalent concentrations will intersect the origin whereas our equilibrations over pure water yield ~ 8%(*w*/*v*) PEG concentrations in the droplet. The two approaches should be regarded as complementary rather than contradictory. Our data refer specifically to PEG in a sitting droplet arrangement were surface energy effects are important; those of Michel refer to bulk PEG solutions where surface energy effects are minimized. In particular we recommend the results of Michel in reference to a change of one reservoir solution for another, for example NaCl for PEG, and we recommend our results in reference to the water vapor properties for the droplet, as employed in crystallization experiments. In practice one should establish a desired final concentration value of $[PEG]^D$ for the experiment at hand, then consult either Figs. 4–6 or Tables 1–3 to determine a value of $[salt]^R$ that will yield the desired $[PEG]^D$, and prepare the salt solution for the reservoir accordingly. Michel & Radcliffe (1995) recently described a computer program for the calculation of osmotic potentials of aqueous solutions of PEG, sodium chloride, potassium chloride, mannitol, and sucrose.

Money (1989) revisited the osmotic properties of PEG solutions and his results are quite similar to those of Michel (1983). While we have confined most of our experimental studies to one molecular weight range of PEG, Money examined osmotic properties of PEG's ranging in molecular weight from 200 to 10000 Da. In the range most commonly utilized for protein crystallizations (PEG 1500–PEG 10000) Money found reasonably similar osmotic effects for a given PEG concentration when the latter is expressed in the units of %(*w*/*v*) employed in our studies. Below the molecular weight of ~ 1500 Da the osmotic effect for a given concentration is more pronounced as the molecular weight decreases. While the osmotic effects of PEG's of various molecular weights above 1500 Da are not too dissimilar, the solubilities of proteins, as shown by Atha & Ingham (1981), can be quite sensitive to the molecular weight of the PEG used as a precipitating agent.

The original aim of these studies was to identify appropriate amounts of salts to use as diffusants in $Z/3$ plates when PEG is the crystallizing agent of choice. The short answer is surprisingly little. Millimolar concentrations of salts cover the range of interesting PEG concentrations, meaning that diffusion experiments in the $Z/3$ plate will be quite slow, even in the shallowest reservoirs and with the fastest diffusing salts. But the interest in these results should extend well beyond the original aim of the work. They underscore the very modest effects that PEG in useful concentrations has on water vapor pressure, suggesting a number of practical consequences for its use in the macromolecular crystal growth problem.

We wish to thank our colleagues Robert H. Blessing and Walter A. Pangborn, and Professor Burlyn E. Michel of the Department of Botany, University of Georgia, for many enlightening discussions, our colleague Vivian Cody for her moral support of us all and financial support of JRL, Mary Jo Kirisits for setting up the water-equilibration experiments, and Farrah Greene and Jason Bray for setting up some of the magnesium sulfate equilibration experiments. This work was supported by NIH Grants GM46733, RR03103, DK41009, CA31922, The Buffalo Foundation, and the Harker Fund. Microbridges were supplied by Hampton Research.

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