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Theoretical and experimental studies on freezing point depression and vapor pressure deficit as methods to measure osmotic pressure of aqueous polyethylene glycol and bovine serum albumin solutions

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Abstract

For survival in adverse environments where there is drought, high salt concentration or low temperature, some plants seem to be able to synthesize biochemical compounds, including proteins, in response to changes in water activity or osmotic pressure. Measurement of the water activity or osmotic pressure of simple aqueous solutions has been based on freezing point depression or vapor pressure deficit. Measurement of the osmotic pressure of plants under water stress has been mainly based on vapor pressure deficit. However, differences have been noted for osmotic pressure values of aqueous polyethylene glycol (PEG) solutions measured by freezing point depression and vapor pressure deficit. For this paper, the physicochemical basis of freezing point depression and vapor pressure deficit were first examined theoretically and then, the osmotic pressure of aqueous ethylene glycol and of PEG solutions were measured by both freezing point depression and vapor pressure deficit in comparison with other aqueous solutions such as NaCl, KCl, $CaCl_2$, glucose, sucrose, raffinose, and bovine serum albumin (BSA) solutions. The results showed that: (1) freezing point depression and vapor pressure deficit share theoretically the same physicochemical basis; (2) theoretically, they are proportional to the molal concentration of the aqueous solutions to be measured; (3) in practice, the osmotic pressure levels of aqueous NaCl, KCl, $CaCl_2$, glucose, sucrose, and raffinose solutions increase in proportion to their molal concentrations and there is little inconsistency between those measured by freezing point depression and vapor pressure deficit; (4) the osmotic pressure levels of aqueous ethylene glycol and PEG solutions measured by freezing point depression differed from the values measured by vapor pressure deficit; (5) the osmotic pressure of aqueous BSA solution measured by freezing point depression differed slightly from that measured by vapor pressure deficit.

Keywords: Drought stress; Freezing point depression; Polyethylene glycol; Salt stress; Vapor pressure deficit; Water stress

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1. Introduction

Plants cannot move away from adverse environments, and some have mechanisms to enable survival under such environments as high salt concentrations, water stress, drought and/or low temperature. Some plants can synthesize glycine betaine [1–7], glycerol [8,9], amino acids [10–13] and proteins called dehydrins [14–17], late embryogenesis-abundant proteins [18,19], proteins with enzymatic functions such as aldehyde dehydrogenase [20], small heat shock proteins [21] and some other proteins [13,22,23] in response to high external salt concentration while not inducing plasmolysis and/or loss of water from cells under drought or low temperature.

Recently, many genes of plants have been shown to have a strong effect on their responses to physical stresses which induce loss of turgor and/or heighten ion concentration in the surroundings of the cell membrane [2–4,6,7,16,17,24–31].

Plants seem to synthesize such biochemical substances in response to change in water activity or osmotic pressure of their cells (cf. [32]). Plant and animal cells are enclosed by a cell membrane which is semipermeable to many aqueous nonelectrolyte and electrolyte solutions and is less semipermeable to other aqueous solutions. In other words, the cell membrane passes water molecules with very large permeability, such small molecules as ethylene glycol and glycerol with small permeabilities [33], and such large molecules as mannitol and sucrose with actually no permeability [34,35].

Osmotic pressure, which is dependent on the permeability of the cell membrane for solute molecules or the reflection coefficient, is generated across the cell membrane due to the difference in the chemical potential of water inside and outside a cell. The osmotic pressure π originates essentially from the difference in the chemical potential of water across an ideal semipermeable membrane as expressed by the equation

$$\pi_{\text{ideal}} = -(RT/V\text{m}_1)\ln(x_1^{\text{I}}/x_1^{\text{II}}) \quad (1)$$

where R is the gas constant, T the absolute temperature, $V\text{m}_1$ the partial molar volume of water, x^{I} and x^{II} the mole fraction of water in Phase I (e.g. inside a cell) and that in Phase II (e.g. external solution). The subscript 1 indicates water which is the solvent and 'ideal' indicates an ideal semipermeable membrane. Usually, the osmotic pressure of an ideal aqueous solution is expressed in relation to that of pure water [36], namely,

$$\pi_{\text{ideal}} = -(RT/V\text{m}_1)\ln x_1 \quad (2)$$

For ordinary solutions in an ideal semipermeable membrane

$$\pi_{\text{ideal}} = -(RT/V\text{m}_1)\ln f_1 x_1 \quad (3)$$

where f_1 is the activity coefficient of water and f_1x_1 is the activity of water in terms of mole fraction.

For ordinary solutions in the cell membrane, the osmotic pressure π is expressed by the equation

$$\pi = \sigma\pi_{\text{ideal}} \quad (4)$$

where σ is called the reflection coefficient and has a numerical value of $1 \geqslant \sigma \geqslant 0$ [37–39]: $\sigma = 1$ for aqueous solutions whose solute molecules cannot pass through the cell membrane at all and $\sigma = 0$ for aqueous solutions whose solute molecules can freely pass through the cell membrane.

Many plant physiologists have adopted the physiological quantity of the water potential Ψ which is defined by the equation

$$\Psi = \mu_1/V\text{m}_1 \quad (5)$$

where is μ_1 the chemical potential of water expressed by the equation

$$\mu_1(T,p) = \mu_1^0(T,p) + RT\ln x_1 \quad (6)$$

$$= \mu_1^*(T) + pV\text{m}_1 + RT\ln x_1 \quad (7)$$

where $\mu^0(T, p)$ and $\mu_1^*(T)$ are the chemical potential of water as a function of temperature and pressure, and temperature alone, respectively, in the standard state and p indicates pressure exerted

on the water of the cell sap inside a plant cell and/or the external solution.

Thus,

$$\Psi = \mu_1^*(T)/V\mathrm{m}_1 + p + (RT/V\mathrm{m}_1)\ln x_1 \quad (8)$$

Therefore, if we define the water potential in relation to pure water as we do the osmotic pressure (cf. Eqs. (2) and (3)), we have the equation

$$\Psi = p - \pi \quad (9)$$

Since the osmotic pressure of the plant cells is usually higher than that of the external solution, water is about to enter the cell. However, plant cells are enclosed by a cell wall which has mechanically strong elasticity. Thus, a hydrostatic pressure which pushes the water back is generated across the cell membrane. This hydrostatic pressure is called turgor and is the term p in Eq. (9) itself. Turgor is equal to the difference in the osmotic pressure between the inside and outside the cell, that is, $\Psi = 0$ in Eq. (9) in an equilibrium state.

Thus, when the osmotic pressure of the external solution is raised, the turgor pressure p decreases towards 0 approximately maintaining the following linear relation until plasmolysis occurs

$$p = \pi_{in} - \pi_{ex} \quad (10)$$

where π_{in} indicates the osmotic pressure inside the cell in question and π_{ex} indicates the osmotic pressure of the external solution. The osmotic pressure of the cell π_{in} increases only a little with the decrease in the turgor pressure p because the cell volume decreases only a little during this process.

Therefore, the water activity inside a plant cell is reduced only a little until plasmolysis occurs. Beyond incipient plasmolysis, π_{in} increases with an increase in π_{ex} maintaining the osmotic relation

$$\pi_{in} = \pi_{ex} \quad (11)$$

Thus, the water activity inside the cell decreases with an increase in π_{ex} in the manner expressed by Eq. (11). However, this does not deny that the high external salt concentration [40–43] and the high external osmotic pressure [44,45], which are so low that they cannot induce plasmolysis, would affect the functions of the cell membrane.

Some plant cells such as *Valonia* [46,47], *Codium* [48], *Chara longifolia* [49,50] and *Lamprothamnium* [51,52], which grow in the sea or brackish water, can sense a change in turgor accompanying a change in the osmotic pressure of the external solution and regulate ion transport processes across the cell membrane.

However, many other plant cells seem to be insensitive to the change in turgor p. On the other hand, all of the plant cells plasmolyze in the external solution whose osmotic pressure is higher than that of the cells, which would be drastic events to plant cells. Therefore, it is very important to measure the osmotic pressure of cells and the external solution.

The osmotic pressure has often been determined by measuring the vapor pressure of plant materials [16,53]. This method shares the same physicochemical principle as the vapor pressure deficit method for measuring the osmotic pressure of simple aqueous solutions.

To modify the osmotic pressure of the external solution, polyethylene glycol (PEG) of various number average molecular weights has sometimes been used [23,54–64]. Thus, some researchers have measured the osmotic pressure of aqueous PEG solutions using freezing point depression, vapor pressure deficit method or a semipermeable membrane [54,58,65–71].

The values of the osmotic pressure of aqueous PEG solutions measured with the vapor pressure deficit method do not always agree with those measured with the freezing point depression method [68,69].

Recent trends in experiments on water stress seem to be focused on plant responses to dehydration due to drought and high salt concentrations in culture media. In these experiments, however, what must be measured are the activity of water or the osmotic pressure of the plants.

Plant cells contain or even can synthesize a group of water-soluble proteins called ‘dehydrins’ [14–17] and probably also other water-soluble proteins [13,18,19,21,23] which are polymers such

as PEG. The values of their osmotic pressure may differ depending on the method of measurement, i.e. freezing point depression or vapor pressure deficit.

For both freezing point depression and vapor pressure deficit measurements, the apparatus for determining osmotic pressure is calibrated using standard aqueous NaCl solutions of at least two different concentrations before measurements and the water activity of aqueous solutions, plant and animal cells is expressed in terms of Osmol kg^{-1}; 1.000 Osmol kg^{-1} is equivalent to 1.000 molal concentration of ideal aqueous nonelectrolyte solution with a freezing point depression of 1.862 °C by the freezing point depression method and a depression of dew point of 0.303 °C by the vapor pressure deficit method at 25 °C (cf. Theory).

To clarify any inconsistencies in the value of the osmotic pressure of aqueous PEG solutions expressed in terms of Osmol kg^{-1} between the two methods, the following three points should be made clear: (1) whether or not both the vapor pressure deficit method and the freezing point depression method share theoretically the same physicochemical basis and similar theoretical equations; (2) whether or not the standard aqueous NaCl solutions of the same concentration give the same value of osmotic pressure in both the vapor pressure deficit method and the freezing point depression method; (3) whether or not aqueous PEG solutions actually give different values of the osmotic pressure expressed in terms of Osmol kg^{-1} depending on the vapor pressure deficit method or the freezing point depression method.

To compare and verify the differences between osmotic pressure values measured by the freezing point depression and vapor pressure methods, both methods were also used to measure the osmotic pressure expressed in terms of Osmol kg^{-1} for aqueous ethylene glycol, KCl, $CaCl_2$, glucose, sucrose, raffinose, and bovine serum albumin (BSA) solutions, which are important in plant physiology.

Let us start constructing theories of the freezing point depression method and the vapor pressure deficit method and then compare them.

2. Theoretical

2.1. A theory of the freezing point depression method

A precise thermodynamical theory and formulation of freezing point depression has been offered by Kiyosawa [72]. However, the conclusive equation for expressing freezing point depression has complex terms which cannot be determined precisely by experiments because mathematical exactitude is preferred to thermodynamical simplicity. In the present paper, a simple approximate equation to express the freezing point depression was derived from Kiyosawa's procedure [72] through reasonable thermodynamical approximations. In the present paper, the procedure is applied to obtain thermodynamically more comprehensive expressions.

2.1.1. Procedure to measure freezing point depression

Freezing point depression of an aqueous solution is measured as follows (cf. Fig. 1). An aqueous solution of approximately 2.0 cm^3 is taken into a test tube for measurement. The test tube is cooled using approximately 3.3 M aqueous ethylene glycol solution in a cooling bath brought to approximately −6.5 °C beforehand. The aqueous solution is gradually cooled down to beyond 273.15 K, T^0 K, and supercooled to several degrees below 0 °C as shown in Fig. 1. At this point, mechanical shock is given to the solution to induce rapid ice formation which generates heat equivalent to that of ice fusion. This causes a rapid temperature rise in the aqueous solution, followed by a gradual approach to equilibrium temperature, T K, at which the chemical potential of liquid water $\mu^{0\ l}(T)$ of the aqueous solution becomes equal to that of ice, i.e. solid water, $\mu^{0\ s}(T)$ formed in the solution; where subscript 1 indicates the solvent, i.e., water and the superscript l and s indicate liquid state and solid state, respectively.

2.1.2. Theory

Freezing point depression can be regarded as one of the problems of the phase transition of water molecules going from the liquid state to a

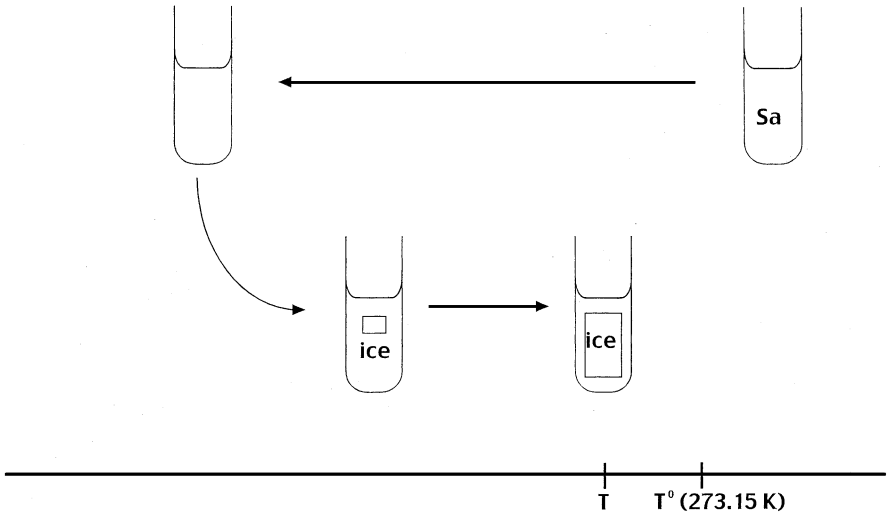

Fig. 1. Schematic illustration of processes of freezing point depression as a function of the temperature T of an aqueous solution. The aqueous solution Sa to be measured in a test tube is cooled to below the freezing point of pure water T^0, 273.15 K, to about minus several degrees centigrade in 3.3 M aqueous ethylene glycol solution. To this aqueous solution in a supercooled state, mechanical shock is given. This causes ice to form, generating the heat equivalent to that of fusion of ice, $\Delta_{fus}Hm_1(T)$, and raising the temperature of the aqueous solution rapidly then gradually as it approaches a constant temperature T which is lower than T^0 and determined primarily by its molal concentration. The difference between T^0 and T is the freezing point depression of the aqueous solution. For further explanation, see the text.

solid one in an aqueous solution. In this phenomenon, solute molecules in an aqueous solution cannot dissolve in the phase of solid water, i.e., ice, and only water molecules in an aqueous solution can be transformed to solid water by being transported from the liquid phase in an aqueous solution to the solid one during the phase transition.

When an equilibrium is attained between liquid water of an aqueous solution and ice in it at T K, it should be expressed by the equation

$$\mu_1^l(T) = \mu_1^s(T) \tag{12}$$

where

$$\mu_1^l(T) = [Hm_1^l(T) - TSm_1^l(T)] + RT\ln x_1^l \tag{13}$$

and

$$\mu_1^s(T) = [Hm_1^s(T) - TSm_1^s(T)] \tag{14}$$

because the solute of an aqueous solution cannot dissolve into ice in it and thus, ice is pure solid water

$$R\ln x_1^s = R\ln 1 = 0 \tag{15}$$

which corresponds to $R \ln x_1^l$ in Eq. (13).

Here, $Hm(T)$ indicates the molar enthalpy as a function of temperature; $Sm(T)$ indicates the molar entropy as a function of temperature; R is the gas constant; T indicates the absolute temperature.

The change in the freezing point of aqueous solutions of which solute molecules can dissolve in ice formed in them on freezing differs from the so-called freezing point depression in ordinary aqueous solutions where solute molecules cannot dissolve in ice formed in them on freezing [73–75]. In the present paper, the so-called freezing point depression in ordinary aqueous solutions is discussed.

Since under constant pressure, the change in the molar enthalpy $dHm(T)$ is equal to the quantity of the heat added dq, $dHm(T)$ and the change in the

molar entropy $dSm(T)$ can be expressed by the equations

$$dHm(T) = dq \tag{16}$$

$$= Cm(T)dT \tag{17}$$

and

$$dSm(T) = dq/T \tag{18}$$

$$= Cm(T)dT/T \tag{19}$$

$Hm(T)$ and $Sm(T)$ can be calculated, respectively, from the molar heat capacity of solid water (ice) $Cm_1^s(T)$, that of liquid water $Cm_1^l(T)$ and the heat of fusion of ice at T^0 K, $\Delta_{fus}Hm_1(T^0)$ from the equations

$$Hm_1^s(T) = \int_0^T Cm_1^s(T')dT' \tag{20}$$

$$Sm_1^s(T) = \int_0^T Cm_1^s(T')dT'/T' \tag{21}$$

$$Hm_1^l(T) = \int_0^{T^0} Cm_1^s(T')dT' + \Delta_{fus}Hm_1(T^0) + \int_{T^0}^T Cm_1^l(T')dT' \tag{22}$$

$$Sm_1^l(T) = \int_0^{T^0} Cm_1^s(T')dT'/T' + \Delta_{fus}Hm_1(T^0)/T^0 + \int_{T^0}^T Cm_1^l(T')dT'/T' + RT\ln x_1^l \tag{23}$$

From the conservation of energy, we have the equation [72]

$$\Delta_{fus}Hm_1(T) = \Delta_{fus}Hm_1(T^0) + \int_T^{T^0} Cm_1^s(T')dT' - \int_T^{T^0} Cm_1^l(T')dT' \tag{24}$$

In pure water, since the equilibrium between liquid water and ice is attained at T^0 K,

$$\mu_1^l(T^0) = \mu_1^s(T^0) \tag{25}$$

Namely,

$$Hm_1^l(T^0) - T^0Sm_1^l(T^0) = Hm_1^s(T^0) - T^0Sm_1^s(T^0) \tag{26}$$

Introducing Eqs. (13), (14), (20)–(23) into Eqs. (12) and (26), rearranging the equation thus obtained, and then further introducing the conservation of energy expressed by Eq. (24), we have the equation

$$\begin{aligned}\Delta_{fus}Hm_1(T^0)(1/T - 1/T^0) &= -\int_T^{T^0} Cm_1^l(T')dT'/T' + \int_T^{T^0} Cm_1^s(T')dT'/T' \\ &\quad -(1/T)\int_T^{T^0} Cm_1^s(T')dT' \\ &\quad +(1/T)\int_T^{T^0} Cm_1^l(T')dT' - R\ln x_1^l\end{aligned} \tag{27}$$

Eq. (27) is a precise expression of the freezing point depression [72]. However, since T is nearly equal to T^0, the entropy of liquid water between T and T^0, $\int_T^{T^0} Cm_1^l(T')dT'/T'$, is nearly equal to the enthalpy between T and T^0, $\int_T^{T^0} Cm_1^l(T')dT'$, which is divided by the freezing point T of an aqueous solution in question, namely,

$$\int_T^{T^0} Cm_1^l(T')dT'/T' \approx (1/T)\int_T^{T^0} Cm_1^l(T')dT \tag{28}$$

With the same logic, we have the equation

$$\int_T^{T^0} Cm_1^s(T')dT'/T' \approx (1/T)\int_T^{T^0} Cm_1^s(T')dT \tag{29}$$

Thus, we have the following simple equation as a good approximation, namely,

$$\Delta_{\mathrm{fus}}\mathrm{Hm}_1(T^0)(1/T-1/T^0)=-R\ln x_1 \tag{30}$$

For ordinary aqueous solutions

$$\Delta_{\mathrm{fus}}\mathrm{Hm}_1(T^0)(1/T-1/T^0)=-R\ln f_1 x_1 \tag{31}$$

Transforming Eq. (30), we have the equation

$$T^0-T=-\left\{TT^0/\Delta_{\mathrm{fus}}\mathrm{Hm}_1(T^0)\right\}R\ln x_1 \tag{32}$$

For dilute aqueous solutions in which $1\gg M_1 m$, where

$$x_1=1/(1+M_1 m) \tag{33}$$

M_1 is the molecular weight of solvent in terms of kg mol^{-1} and m is the molal concentration of the aqueous solution in question, Eq. (32) can be approximated as follows:

$$T^0-T=-\left\{R(T^0)^2/\Delta_{\mathrm{fus}}\mathrm{Hm}_1(T^0)\right\}M_1 m \tag{34}$$

Introducing numerical values of $R=8.314$ J K^{-1} mol^{-1}, $T^0=273.15$K, $\Delta_{\mathrm{fus}}\mathrm{Hm}_1(T^0)=$

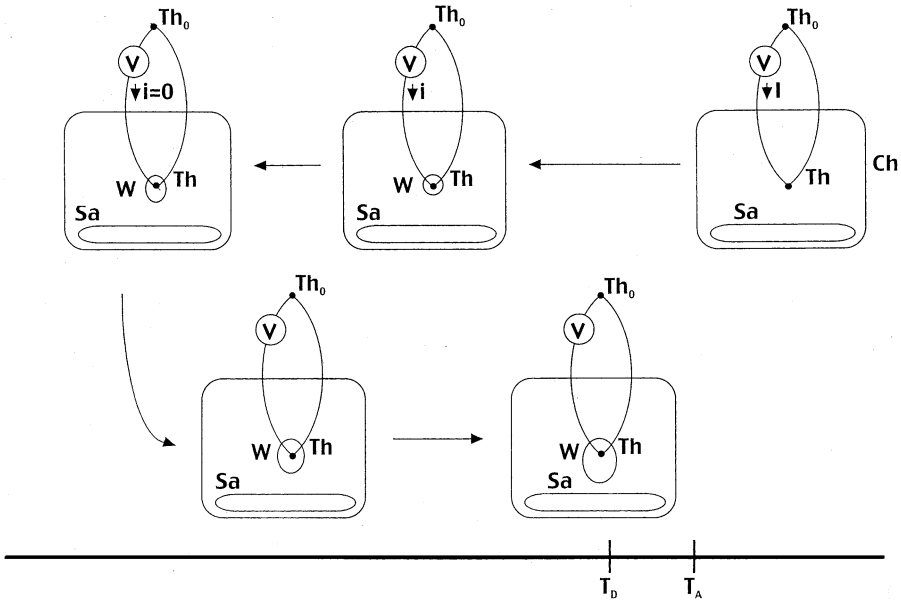

Fig. 2. Schematic illustration of processes of the vapor pressure deficit as a function of the temperature T of a small droplet of water condensing on the surface of a thermocouple. A small amount of the aqueous solution to be measured, Sa, is allowed to be absorbed onto a small paper disk in a small closed chamber Ch. The small chamber is equipped with a thermocouple Th which measures the temperature of a small droplet of water w condensing on its surface. $\mathrm{Th_o}$ is another thermocouple which is kept at a constant temperature. V represents a volt meter to measure the difference in the electromotive force between the two thermocouples Th and $\mathrm{Th_o}$; the difference in the electromotive force indicates the temperature of a small droplet of water w on the surface of thermocouple Th. When electrical current is allowed to flow through the thermocouples, the temperature surrounding thermocouple Th decreases due to the Peltier effect beyond the dew point of pure deionized water T_{A}, making vapor water condense onto the surface of the thermocouple. When the temperature surrounding the thermocouple is lowered far beyond the dew point of pure water T_{A} due to the Peltier effect, the electrical current stops flowing. As vapor water continues to condense onto the surface of the thermocouple generating the heat equivalent to that of vaporization of liquid water, the temperature of a droplet of liquid water on the surface of the thermocouple rises and approaches a constant temperature T_{D} which is determined primarily by the molal concentration of the aqueous solution Sa in question. The difference between T_{A} and T_{D} is the vapor pressure deficit. For further explanation, see the text.

6003.1 J mol^{-1} [76] and $M_1 = 0.018016$ kg mol^{-1}, respectively, we have the equation

$$T^0 - T = 1.8616m \quad (35)$$

Namely, the freezing point depression $(T^0 - T)$ is proportional to the molal concentration m of an aqueous nonelectrolyte solution and the numerical value of the proportional coefficient is the well-known 1.862 °C kg mol^{-1}.

The apparatus to measure the osmotic pressure of an aqueous solution based on the freezing point depression is designed so as to express $(T^0 - T)/1.862$ in terms of Osmol kg^{-1}, or the molal concentration of ideal aqueous nonelectrolyte solution. Thus, the freezing point T of the aqueous solution in question can be obtained.

Introducing the T value into Eq. (30) or Eq. (31), we can obtain the water activity of a usual aqueous solution in terms of $R \ln f_1 x_1$ or $f_1 x_1$. Thus, introducing the $R \ln f_1 x_1$ or $f_1 x_1$ obtained in Eq. (30) or Eq. (31) into Eq. (3), we can calculate the osmotic pressure of the aqueous solution in question in terms of Pa if we have the numerical value of the partial molar volume of water $V\mathrm{m}_1(T, p)$ as a function of the solute concentration.

2.2. A theory of the vapor pressure deficit method

2.2.1. Procedure for measuring vapor pressure deficit

An apparatus to measure vapor pressure deficit can be constructed with a small closed chamber equipped with a thermocouple (cf. Fig. 2). A small amount of the aqueous solution to be measured is placed in a chamber maintained at a constant temperature (usually 25 or 37 °C). When electric current is passed through a thermocouple connected to the chamber, the temperature of the vapor water surrounding the thermocouple gradually decreases due to the Peltier effect.

When the temperature decreases further beyond the dew point, there is condensation of vaporous water, which is in an equilibrium state with the chemical potential of water of the aqueous solution to be measured, onto the surface of the thermocouple in the chamber. This transformation of vaporous water to liquid water generates the heat equivalent to that of vaporization of liquid water at T' K, $\Delta_{\mathrm{vap}}\mathrm{Hm}_1(T')$. Thus, when electric current stops flowing through the thermocouple below the dew point, the temperature of liquid water condensing on the surface of the thermocouple rises rapidly due to successive condensation of vaporous water onto the surface of the thermocouple and approaches the equilibrium value T_{D} determined by the water activity of the aqueous solution being measured. Here, let us designate the dew point of pure deionized water at 25 or 37 °C as T_{A}. For usual aqueous solutions, $T_{\mathrm{A}} > T_{\mathrm{D}}$.

The notations of T_{A} and T_{D} are used according to the manufacturer's (Wescor, Inc., USA) instructions. However, it should be pointed out that the explanation of the principle of the apparatus is not based on a thermodynamically quantitative theory.

2.2.2. Theory

Condensation of vaporous water to liquid water at a temperature below the dew point can be considered as a kind of phase transition of water molecules in vaporous water to liquid water similar to the transformation of liquid water to ice in freezing point depression. Thus, the theory would seem to be, in principle, equal to that of the freezing point depression method. What we must consider are: (1) the equilibrium between the chemical potential of liquid water and that of vaporous water of the aqueous solution in question at T_{D}; (2) the equilibrium between the chemical potential of liquid water and vaporous water of pure water at T_{A}; and (3) how to formulate the heat of vaporization of water at T_{D}, $\Delta_{\mathrm{vap}}\mathrm{Hm}_1(T_{\mathrm{D}})$, as a function of the molar heat capacities of liquid water $\mathrm{Cm}_1^{\mathrm{l}}(T')$, that of vaporous water $\mathrm{Cm}_1^{\mathrm{vap}}(T')$ and the heat of vaporization of water at T_{A}, $\Delta_{\mathrm{vap}}\mathrm{Hm}_1(T_{\mathrm{A}})$. These factors are similar to those for the freezing point depression. The subscript 1 and vap indicate water and vaporization, respectively, and superscripts l and vap indicate liquid and vaporous states, respectively.

The equilibrium in the chemical potential of water between liquid water condensed onto the surface of the thermocouple in the small chamber and the vaporous water of the aqueous solution to be measured at T_{D} can be expressed by the equation

$$\mu_1^{\mathrm{l}}(T_{\mathrm{D}}) = \mu_1^{\mathrm{vap}}(T_{\mathrm{D}}) \quad (36)$$

where

$$\mu_1^{l}(T_D) = [\mathrm{Hm}_1^{l}(T_D) - T_D \mathrm{Sm}_1^{l}(T_D)] + RT\ln x_1^{l,th} \quad (37)$$

$$\mu_1^{vap}(T_D) = [\mathrm{Hm}_1^{vap}(T_D) - T_D \mathrm{Sm}_1^{vap}(T_D)] + RT\ln x_1^{l} \quad (38)$$

In the formulation of Eqs. (36)–(38), it has been assumed that (1) the solute in an aqueous solution to be measured does not dissolve in the water condensed onto the surface of the thermocouple and thus, the water is pure, that is, $R \ln x_1^{l,th} = R \ln 1 = 0$ in Eq. (37): where the superscript th indicates the thermocouple and that (2) the temperature of vaporous water surrounding the thermocouple is in an equilibrium state with that of the condensed water on the surface of the thermocouple.

$\mathrm{Hm}_1{}^{l}(T_D)$ and $\mathrm{Sm}_1{}^{l}(T_D)$ in Eq. (37) are equal to those in Eq. (13). $\mathrm{Hm}_1{}^{vap}(T)$ and $\mathrm{Sm}_1{}^{vap}(T)$ are calculated by the equations

$$\mathrm{Hm}_1^{vap}(T) = \mathrm{Hm}_1^{l}(T^0) + \int_{T^0}^{T} \mathrm{Cm}_1^{l}(T')\mathrm{d}T' + \Delta_{vap}\mathrm{Hm}_1(T) \quad (39)$$

and

$$\mathrm{Sm}_1^{vap}(T) = \mathrm{Sm}_1^{l}(T^0) + \int_{T^0}^{T} \mathrm{Cm}_1^{l}(T')\mathrm{d}T'/T' + \Delta_{vap}\mathrm{Hm}_1(T)/T \quad (40)$$

respectively, where $\Delta_{vap}\mathrm{Hm}_1(T')$ indicates the heat of vaporization of liquid water at T' K.

On the other hand, the equilibrium in the chemical potential between liquid water condensed onto the surface of the thermocouple and vaporous water of pure water at T_A can be expressed by the equation

$$\mu_1^{l}(T_A) = \mu_1^{vap}(T_A) \quad (41)$$

Namely,

$$\mathrm{Hm}_1^{l}(T_A) - T_A\mathrm{Sm}_1^{l}(T_A) = \mathrm{Hm}_1^{vap}(T_A) - T_A\mathrm{Sm}_1^{vap}(T_A) \quad (42)$$

From the conservation of energy, we have the equation (cf. Eq. (24))

$$\Delta_{vap}\mathrm{Hm}_1(T_D) = \Delta_{vap}\mathrm{Hm}_1(T_A) + \int_{T_D}^{T_A} \mathrm{Cm}_1^{l}(T')\mathrm{d}T' - \int_{T_D}^{T_A} \mathrm{Cm}_1^{vap}(T')\mathrm{d}T' \quad (43)$$

From Eqs. (36)–(40), (42) and (43), we have the equation

$$\begin{aligned}\Delta_{vap}\mathrm{Hm}_1(T_A)(1/T_D - 1/T_A) &= \int_{T_D}^{T_A} \mathrm{Cm}_1^{l}(T')\mathrm{d}T'/T' - \int_{T_D}^{T_A} \mathrm{Cm}_1^{vap}(T')\mathrm{d}T'/T' \\ &\quad - (1/T_D)\int_{T_D}^{T_A} \mathrm{Cm}_1^{l}(T')\mathrm{d}T' \\ &\quad + (1/T_D)\int_{T_D}^{T_A} \mathrm{Cm}_1^{vap}(T')\mathrm{d}T' - R\ln x_1 \end{aligned} \quad (44)$$

Since T_D is nearly equal to T_A in dilute aqueous solutions, the following approximations can be permissible (cf. Eqs. (28) and (29)), namely,

$$\int_{T_D}^{T_A} \mathrm{Cm}_1^{l}(T')\mathrm{d}T'/T' \approx (1/T_D)\int_{T_D}^{T_A} \mathrm{Cm}_1^{l}(T')\mathrm{d}T' \quad (45)$$

$$\int_{T_D}^{T_A} \mathrm{Cm}_1^{vap}(T')\mathrm{d}T'/T' \approx (1/T_D)\int_{T_D}^{T_A} \mathrm{Cm}_1^{vap}(T')\mathrm{d}T' \quad (46)$$

Thus, we have the simple equation

$$\Delta_{vap}\mathrm{Hm}_1(T_A)(1/T_D - 1/T_A) = -R\ln x_1 \quad (47)$$

as an approximation. This equation is similar to that for freezing point depression, Eq. (30).

For dilute aqueous solutions, from Eq. (47), we have the equation

$$T_A - T_D \approx \{RT_A^2/\Delta_{vap}\mathrm{Hm}_1(T_A)\}M_1 m \quad (48)$$

When $T_A = 298.15$ K ($=25$ °C), introducing numerical values of $\Delta_{vap}\mathrm{Hm}_1(298.15\ \mathrm{K}) = 43\,979$

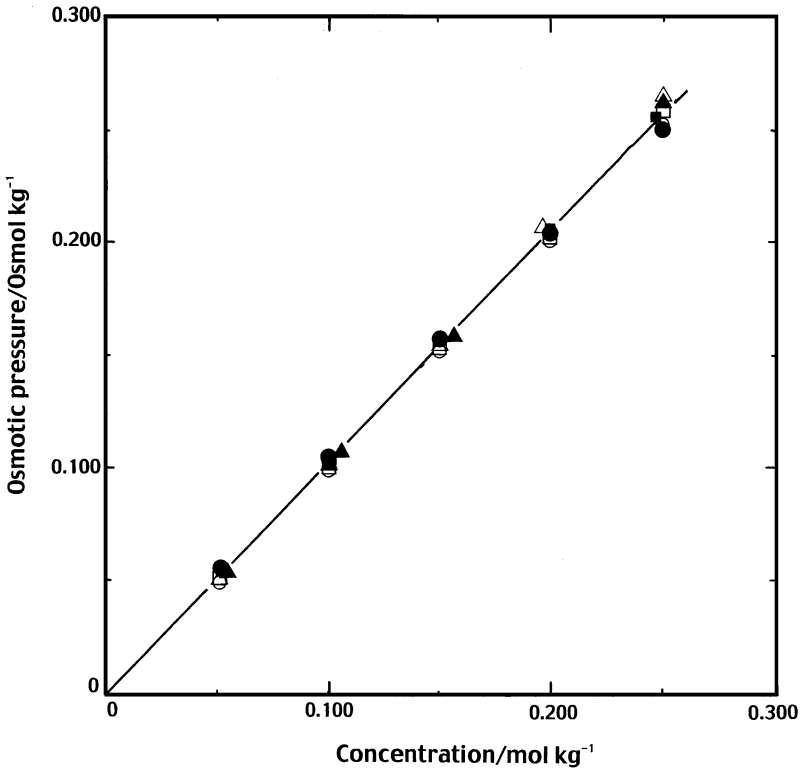

Fig. 3. Linear relationship between the freezing point depression and vapor pressure deficit of aqueous glucose, sucrose, and raffinose solutions expressed in terms of Osmol kg^{-1} and their molal concentration. Circles indicate aqueous glucose solutions; squares, aqueous sucrose solutions; triangles, aqueous raffinose solutions. Open symbols indicate the freezing point depression and closed symbols indicate the vapor pressure deficit. For further explanation, see the text.

J mol^{-1} [76] and $M_1 = 0.018016$ kg mol^{-1} into Eq. (48) we have the linear equation

$$T_A - T_D = 0.3027m \quad \text{(at 25 °C)} \tag{49}$$

Eq. (49) indicates that the vapor pressure deficit of an ideal aqueous solution is proportional to the molal concentration m. This is the same as the freezing point depression of an ideal aqueous solution.

An apparatus to measure the osmotic pressure of an aqueous solution based on the vapor pressure deficit is designed so as to express $(T_A - T_D)/0.303$ in terms of Osmol kg^{-1}; 1.000 Osmol kg^{-1} is equivalent to 1.000 mol kg^{-1} of an ideal aqueous nonelectrolyte solution whose vapor pressure deficit $(T_A - T_D)$ is 0.303 °C.

Thus, using Eqs. (47) and (49), we can calculate the water activity of usual solution in terms of $R \ln f_1 x_1$ or $f_1 x_1$. Introducing this numerical value into Eq. (3), we can calculate the osmotic pressure of an aqueous solution in question in terms of Pa if we have the numerical value of the partial molar volume of water $V\mathrm{m}_1(T, p)$ as a function of the solute concentration.

3. Materials and methods

Reagent grade glucose, sucrose, raffinose, NaCl, KCl, and $CaCl_2$ were purchased from Wako Pure Chemicals, Ltd, or Nacalai Tesque, Inc., PEG and ethylene glycol used were purchased from Nacalai Tesque, Inc. and Merck, respectively. BSA (fraction V; fatty acid free) was purchased from Bayer Corporation. The water used was 17–18 M cm^{-1}.

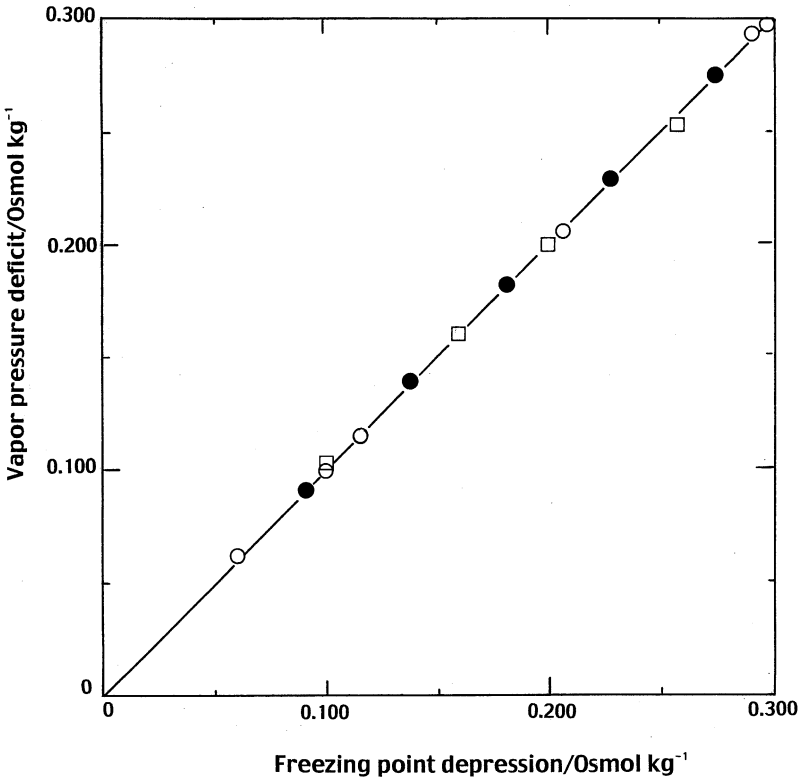

Fig. 4. Consistency between the freezing point depression and the vapor pressure deficit expressed in terms of Osmol kg^{-1} of aqueous NaCl, KCl, and $CaCl_2$ solutions. Open circles indicate aqueous NaCl solutions; closed circles, aqueous KCl solutions; open squares, aqueous $CaCl_2$ solutions. For further explanation, see the text.

The freezing point depression was measured with an osmometer (Type 3W, Advanced Instruments, Inc., USA) which had been calibrated with standard aqueous NaCl solutions [77,78]. This apparatus takes advantage of the supercooling method. The vapor pressure deficit was measured with a Wescor vapor osmometer (Type 5520, Wescor, Inc.) which had also been calibrated with standard aqueous NaCl solutions.

4. Results

Fig. 3 shows the freezing point depression and vapor pressure deficit in terms of Osmol kg^{-1} in aqueous glucose, sucrose and raffinose solutions as functions of their molal concentration. Clearly, plotting their freezing point depression and vapor pressure deficit in terms of Osmol kg^{-1} against the molal concentration m on the same graph paper, we have one linear curve over dilute concentrations as predicted by Eqs. (35) and (49).

Namely, Fig. 3 shows that the freezing point depression and vapor pressure deficit give the same values for aqueous glucose, sucrose, and raffinose solutions over the concentrations examined, irrespective of the difference in the temperature at which the osmotic pressure of their aqueous solutions was measured by freezing point depression or vapor pressure deficit; the freezing point depression measures the osmotic pressure of aqueous solutions just below 0 °C while vapor pressure deficit gives the value at just below 25 °C in the present experiments.

Fig. 4 is a plot of the vapor pressure deficit in terms of Osmol kg^{-1} against the freezing point depression in terms of Osmol kg^{-1} for aqueous

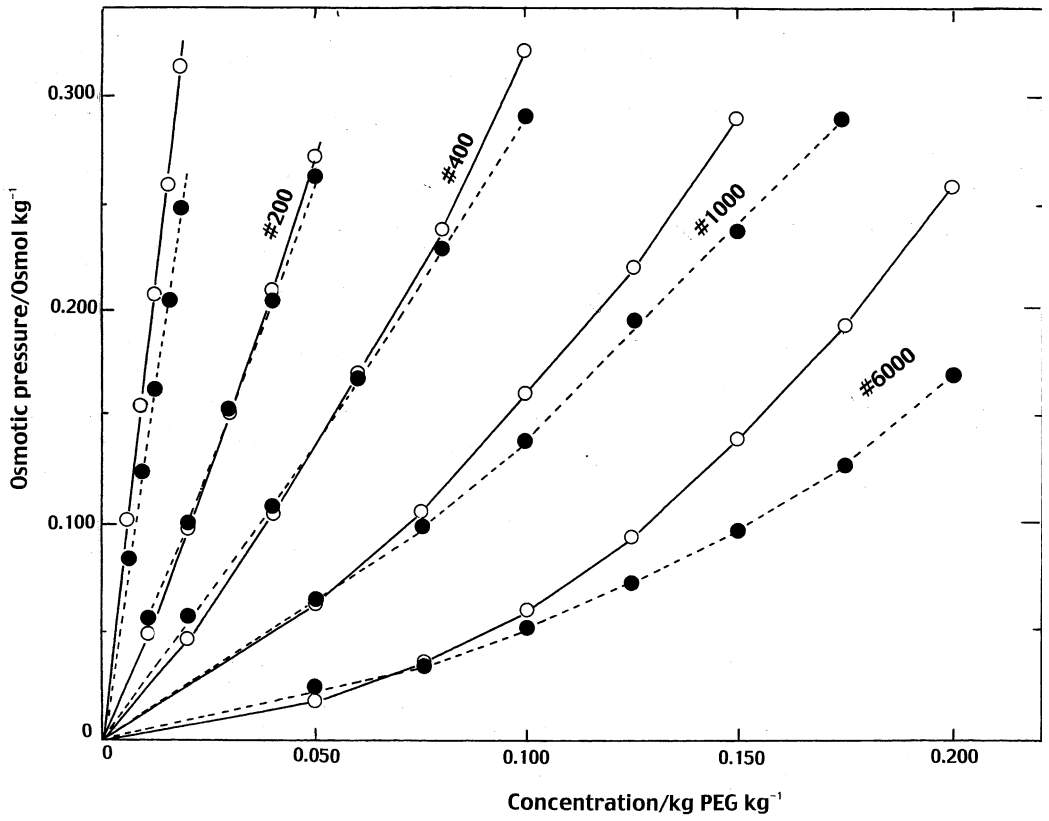

Fig. 5. Inconsistency of freezing point depression expressed in terms of Osmol kg^{-1} with the vapor pressure deficit of aqueous PEG solutions. Solid lines with open circles indicate the freezing point depression and dashed lines with closed circles indicate the vapor pressure deficit, respectively. #200, #400, #1000, and #6000 indicate the degree of polymerization of PEG used; #200 indicates the number-average molecular weight of 190–210 g mol^{-1}: #400, that of 380–420 g mol^{-1}: #1000, that of 950–1050 g mol^{-1}: #6000, that of 7300–9000 g mol^{-1}: no number, ethylene glycol. For further explanation, see the text.

NaCl, KCl, and $CaCl_2$ solutions. There is a good linear curve with a slope of 1 which passes through the origin. The figure shows a good consistency between the freezing point depression and vapor pressure deficit examined in terms of Osmol kg^{-1} over the concentrations examined for aqueous electrolyte solutions such as NaCl, KCl, and $CaCl_2$, irrespective of any difference in temperature at which the osmotic pressure of their aqueous solutions was measured based on freezing point depression or vapor pressure deficit.

However, the values of the osmotic pressure measured by freezing point depression were not consistent with those measured by the vapor pressure deficit in aqueous PEG solutions. Fig. 5 is a plot of the vapor pressure deficit and the freezing point depression in terms of Osmol kg^{-1} against the concentration in terms of kg PEG kg^{-1}. The freezing point depression in terms of Osmol kg^{-1} gave always larger numerical values than the vapor pressure deficit in terms of Osmol kg^{-1} for the same aqueous PEG solutions. The difference between them became larger with an increase in the concentration and the degree of polymerization. This deviation in the osmotic pressure of aqueous PEG solutions is qualitatively consistent with the results reported by Steuter et al. [68] and Schiller et al. [69].

The deviation in the value of the osmotic pressure between those measured by the freezing point depression and those measured by the vapor pressure deficit can be observed even in aqueous ethylene glycol solutions although they are good linear functions of the molal concentration (Fig. 6).

Fig. 7 shows plots of the freezing point depression and vapor pressure deficit of aqueous BSA solutions against the concentration in terms of kg BSA kg^{-1}. They are good linear functions of the concentration, but do not coincide with each other at low concentrations. Namely, numerical values of the osmotic pressure of aqueous BSA solutions measured by the freezing point depression were a little smaller than those measured by the vapor pressure deficit. This is contrary to the case of aqueous PEG solutions.

The BSA used was contaminated with 1.6 gNa^{+} (kg BSA)$^{-1}$ and 2.5 gCl^{-} (kg BSA)$^{-1}$ probably as NaCl and 2.6 gNa^{+} (kg BSA)$^{-1}$ probably as Na azide. Thus, the freezing point depression of aqueous BSA solutions examined seem to be explainable as due to the contaminating NaCl and/or Na azide, except for BSA. What remains to be explained is why the vapor pressure deficit in terms of Osmol kg^{-1} is larger than the freezing point depression in terms of Osmol kg^{-1} in aqueous BSA solutions.

5. Discussion

To express the water activity in terms of the osmotic pressure precisely in Pa units, numerical values are necessary for the partial molar volume of water $V\mathrm{m}_1(T, p)$ in Eq. (3) of the aqueous solutions under study. However, numerical values were not examined for the partial molar volumes of water of all the aqueous solutions. Thus, in the present study, the osmotic pressure has been expressed only in terms of Osmol kg^{-1} as the freezing point depression and the vapor pressure deficit.

In the present theories, what should be noticed is that Eqs. (30) and (35) and also Eqs. (47) and (49) are only conventional approximations for measuring the water activity or the osmotic pressure of aqueous solutions, respectively. Generally, the water activity or osmotic pressure of ordinary aqueous solutions can be expressed as functions of the temperature, species of the solute, and the concentration under constant pressure.

The results obtained in the present study can be summarized as follows. (1) Both the freezing point depression and vapor pressure deficit thermodynamically share the same basis and are theoretically proportional to the molal concentration of aqueous solutions over dilute concentrations. (2) The osmotic pressure in terms of Osmol kg^{-1} of aqueous glucose, sucrose, and raffinose solutions are proportional to their molal concentrations over dilute concentrations (cf. [79]) independent of the difference in methods, based on either the freezing point depression or the vapor pressure deficit. (3) Aqueous electrolyte solutions such as NaCl, KCl, and $CaCl_2$ give equal values of osmotic pressure for the same molal concentrations, as theoretically predicted, irrespective of the difference in method. (4) However, aqueous solutions of ethylene glycol, which is a monomer of PEG, give different values of osmotic pressure with different methods, although the osmotic pressure values obtained by both methods were proportional to the molal concentration. (5) Aqueous solutions of PEG gave different values of the osmotic pressure in terms of Osmol kg^{-1} dependent on the methods as reported by Steuter et al. [68] and Schiller et al. [69]. (6) Aqueous solutions of BSA gave slightly different values of osmotic pressure depending on the methods.

Since both freezing point depression and vapor pressure deficit are in principle based on the colligative properties of solutions, they have been expected to express the water activities or the osmotic pressures of aqueous solutions of glucose, sucrose, raffinose, ethylene glycol, PEG, NaCl, KCl, $CaCl_2$, and BSA with unique numerical values in terms of Osmol kg^{-1} irrespective of the methods used.

The apparatus to measure vapor pressure deficit can give different values for aqueous solutions of volatile solutes or solvents from those for nonvolatile ones, according to the manufacturer's (Wescor, Inc.) explanation. The boiling point of ethylene glycol is 197.9 °C, which suggests that it may be a volatile solvent, while PEGs and BSA

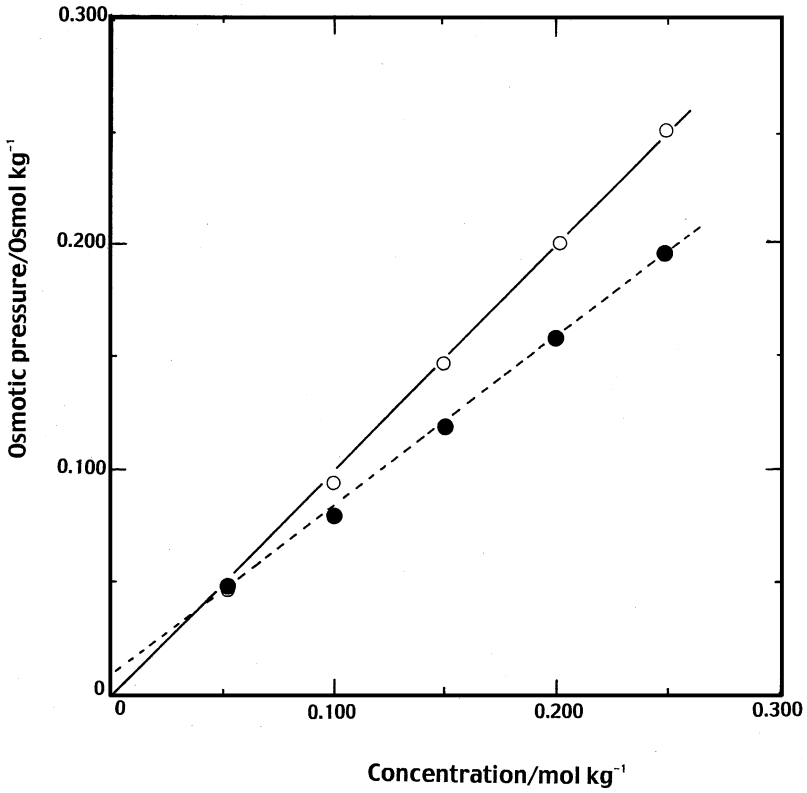

Fig. 6. Inconsistency of the freezing point depression expressed in terms of Osmol kg^{-1} with the vapor pressure deficit of aqueous ethylene glycol solution. A solid line with open circles indicates freezing point depression and a dashed line with closed circles indicates vapor pressure deficit, respectively. Linearity is maintained between the freezing point depression and the molal concentration of aqueous ethylene glycol and that between the vapor pressure deficit and the molal concentration. For further explanation, see the text.

do not seem to be volatile. The question remains of why the osmotic pressures of aqueous solutions of PEGs and BSA have different values depending on the method used to measure them.

Recent work in plant physiology has demonstrated that certain kinds of proteins called 'dehydrins' [14–17] and some other proteins [13,18, 19,21,23] play an important roles in plant survival in drought conditions. However, the present findings that aqueous solutions of a kind of protein, BSA, as well as PEGs, which are polymers, give different values of the osmotic pressure expressed in terms of Osmol kg^{-1} depending on the method used, i.e. either freezing point depression or vapor pressure deficit, can raise serious problems when measuring the water activity of plants and plant cells under water stress. These findings urge us to provide a clear physicochemical basis for the osmotic pressure of the cytoplasm as well as protein solutions, because the water activity of plants has been measured mainly by the vapor pressure deficit method [16,53] while that of aqueous solutions has been measured mainly by freezing point depression [72,77].

In the freezing point depression method, aqueous PEG solution is put into a glass tube. On the other hand, in the vapor pressure deficit method, aqueous PEG solution is absorbed by a thin sheet of filter paper in a small chamber of the apparatus. Thus, Hardegree and Emmerich [71] have tried to explain the difference in the osmotic pressure measured by freezing point depression and vapor

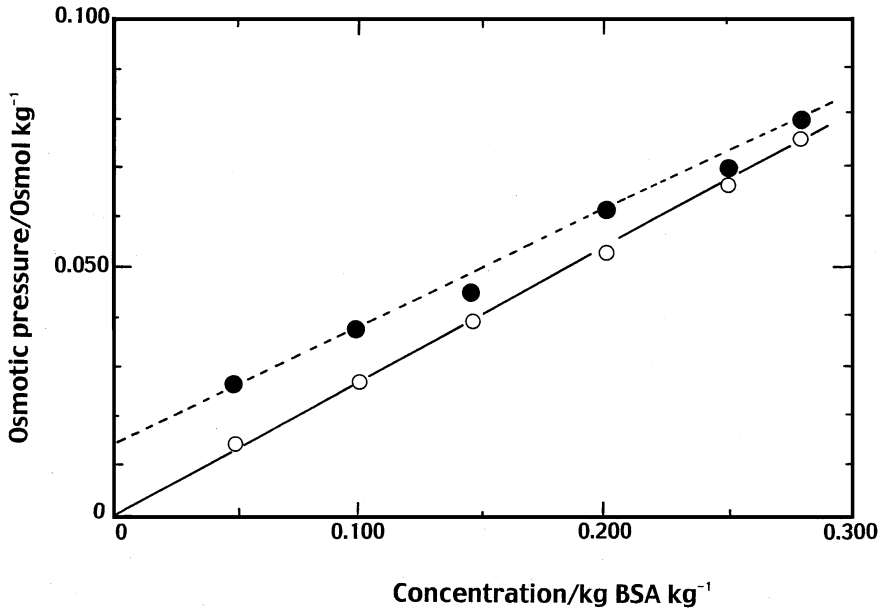

Fig. 7. Low inconsistency of freezing point depression in terms of Osmol kg^{-1} with the vapor pressure deficit of aqueous BSA solution. A solid line with open circles indicates the freezing point depression and a dashed line with closed circles indicates the vapor pressure deficit. The freezing point depression is a linear function of the concentration of aqueous BSA solution in terms of kg BSA kg^{-1} and the vapor pressure deficit is also a linear function of the concentration of aqueous BSA solution in terms of kg BSA kg^{-1}. For further explanation, see the text.

pressure deficit in terms of errors associated with filter paper exclusion of PEG which are polymers of high molecular weights. They have suggested that, since filter paper is made from plant cell fibers, it contains a volume fraction accessible to water but not high molecular weight PEG.

The chemical potential of water $\mu_1(T, p)$ of ordinary aqueous solutions is expressed by the equation (cf. Eq. (7))

$$\mu_1(T,p) = \mu_1^*(T) + pV\mathrm{m}_1 + RT\ln f_1 x_1 \quad (50)$$

$$= \mu_1^*(T) + pV\mathrm{m}_1 + RT\ln x_1 + RT\ln f_1 \quad (51)$$

Thus, Ψ in Eq. (9) can be expressed by the equation

$$\Psi = p - \pi + (RT/V\mathrm{m}_1)\ln f_1 \quad (52)$$

Many of plant physiologists express the water potential by attaching subscript w as Ψ_w by the equation

$$\Psi_w = \Psi_p + \Psi_s + \Psi_m \quad (53)$$

where Ψ_p is called the pressure potential and $\Psi_p = p$ in Eq. (52); Ψ_s is called the osmotic potential due to the solute and is usually expressed by the van't Hoff equation

$$\Psi_s = -\pi \quad (54)$$

$$= -RTc \quad (55)$$

where c is the molar concentration of cell sap; Ψ_m is called the matic potential and is thought to be due to colloids and surfaces in cells and cell walls (cf. [80]).

Comparing Eq. (53) with Eqs. (52) and (55) shows that Ψ_m would consist of $(RT/V\mathrm{m}_1)\ln f_1$

and deviation of the van't Hoff equation from Eq. (3).

Since cellulose fibers composing filter paper, which is used in the vapor pressure deficit method, can be thought to be a species of colloidal particle, it can be expected to affect the activity coefficient of water f_1 of aqueous PEG solutions and thus, the osmotic pressure as well as Ψ_m.

The concept of 'water potential' which is the chemical potential of water $\mu_1(T, p)$ itself divided by the partial molar volume of water V_{m_1} may not need to be employed. 'The chemical potential of water' is sufficient to describe water relations in plants and plant cells.

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