

# An accurate numerical model for calculating the equilibration rate of a hanging-drop experiment

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A numerical model of the equilibration of a hanging-drop experiment has been developed and tested. To obtain accurate calculations with a given precipitant, the vapor pressure of water over water/precipitant solutions must be known for various concentrations of the precipitant. The calculations of the model are in excellent agreement with all available experimental data on hanging-drop equilibration when the necessary vapor pressures are known (ammonium sulfate and sodium chloride). By varying each of the relevant rate constants in the model, the rate-limiting step in the equilibration of a hanging drop is determined. This analysis clearly shows that the rate-limiting step is the diffusion of water vapor from the drop to the reservoir, which agrees with experimental findings. Since the diffusion of water vapor is the rate-limiting step, there is virtually no precipitant concentration gradient in the drop during equilibration. As a result, there is no gravity-induced convection owing to the equilibration. Thus, whereas gravity might have an effect during crystal growth, gravity does not affect the equilibration rate of a hanging-drop experiment to a significant extent, and the diffusion of water vapor will remain the rate-limiting step in the absence of gravity. Finally, the effects of several of the parameters, such as initial drop volume, drop-to-reservoir distance and temperature, are considered quantitatively. The equilibration rate was found to vary nearly linearly with drop volume. The equilibration rate decreases roughly by a factor of three as the temperature decreases from 293 to 276 K. This decrease in the equilibration rate is greater than would be expected when just considering the change in the diffusion coefficient of water vapor in air. This large dependence can, however, be attributed to the change in water-vapor pressure. Most surprisingly, a linear dependence on drop-to-reservoir distance is found, a result that agrees very well with experiment.

## 1. Introduction

Crystallization is often the rate-limiting step in protein structure determination *via* X-ray crystallography. Accordingly, more emphasis is being placed on understanding protein crystal growth (McPherson *et al.*, 1995) and on standardizing protein crystallization techniques (Ducruix & Giegé, 1992). There are several commonly used protein crystallization techniques: the most important ones being the batch, hanging-drop, sitting-drop, dialysis and capillary methods. Of these techniques, the hanging drop is one of the most frequently used.

Recently, there have been efforts to control nucleation through means such as varying the temperature (Rosenberger

& Meehan, 1988) and varying the precipitant concentration (Blow *et al.*, 1994) as functions of time. The technique of varying the temperature is beginning to show great promise (see for example DeMattei & Feigelson, 1992; Lorber & Geigé, 1992; Schall *et al.*, 1996). The goal is to lower the degree of supersaturation just before many nuclei cross the energy barrier and become stable micro-crystals, thereby preventing all but one or a few nuclei from becoming stable. As a result, enough free protein remains so that the stable nuclei can subsequently grow to diffraction-size crystals (Feher & Kam, 1985).

During a hanging-drop experiment, the concentration of precipitant in the drop can be controlled by varying the concentration of the precipitant in the reservoir. Several groups are using this technique with some success (see *e.g.* Gernert *et al.*, 1988; Przybylska, 1989; Wilson *et al.*, 1991; Sygusch *et al.*, 1996). Unfortunately, it is currently difficult to estimate *a priori* the effects on the drop of varying the precipitant concentration in the reservoir, and it is even more difficult to vary the precipitant concentration in the reservoir to obtain a desired equilibration rate. As a result, important information about nucleation as a function of precipitant concentration is being lost.

The equilibration of a hanging-drop experiment is driven by the difference between the chemical potential of the water in the drop and the chemical potential of water in the reservoir (in this case, the chemical potential of water is directly related to the vapor pressure of water; see, for example, Guggenheim & Stokes, 1969). The chemical potential of water will be the lowest where the concentration of precipitant is the highest. Thus, water will move from regions of low precipitant concentration (typically the drop) to regions of high precipitant concentration (typically the reservoir). In order to travel from the drop to the reservoir, the water first evaporates from the surface of the drop then travels *via* diffusion through the air space to the surface of the reservoir and finally condenses into the reservoir. As the water evaporates from the drop, the concentration of precipitant increases near the drop surface. Thus, as we shall see below, a small precipitant concentration gradient forms in the drop which then must be equilibrated *via* diffusion. Also, as the water condenses on the surface of the reservoir, a precipitant concentration gradient will form in the reservoir. As the reservoir is typically much larger than the drop, this concentration gradient is quite small and is not likely to have any influence on the equilibration rate.

Recently, the equilibration of a hanging drop has been studied both experimentally (Mikol *et al.*, 1990; DeTitta & Luft, 1995; Luft & DeTitta, 1995; Luft *et al.*, 1996) and theoretically (Fowles *et al.*, 1988; Sibille *et al.*, 1991). In Fowles *et al.* (1988), a model of the equilibration of a hanging drop is developed. They argue quite convincingly that in the absence of gravity the rate-limiting step should be the diffusion of the precipitant in the drop, but owing to gravity-induced convection precipitant concentration gradients equilibrate much more rapidly on earth and the rate-limiting step is the diffusion of the water vapor. Because of the complexity of the problem several approximations were necessary to obtain an

analytical solution. For example, it was necessary to assume that the cell was spherical in shape so that spherical coordinates could be used. Furthermore, the diffusion of precipitant in the drop could not be modeled explicitly: the authors had to assume the precipitant concentration was spatially constant. This assumption unfortunately made it impossible to conclusively determine the role of gravity in the equilibration process.

In Mikol *et al.* (1990), the first experimental study of the equilibration of a hanging drop was described. In particular, they investigated the importance of temperature, initial drop volume and type of precipitant. To approach the question of the rate-limiting step experimentally, DeTitta & Luft (1995) measured the equilibration rate of sitting drops at different residual air pressures in the vapor space. They found that the equilibration rate increased with decreasing air pressure. Since in theory the diffusion coefficient of water vapor in air is inversely proportional to the air pressure, this offered strong evidence that the diffusion of water vapor is the rate-limiting step. Finally, Luft *et al.* (1996) showed that the equilibration rate of a hanging-drop experiment decreased with increasing drop-to-reservoir distance. This showed conclusively that the rate-limiting step is indeed the diffusion of water vapor.

In using experimental methods to measure the equilibration rate, time-consuming experiments must be run for every possible set of starting conditions. It is, therefore, impractical to experimentally explore the hundreds if not thousands of vapor-diffusion protocols currently in use. Since theoretical methods do not suffer from such drawbacks, they could be a valuable tool.

The program *Drop* described in this manuscript is an attempt to unify these bodies of work. Using a numerical solution of standard diffusion-type equations, *Drop* attempts to calculate the rate of equilibration of a hanging drop with a varying reservoir concentration. Its calculations are in excellent agreement with available data.

## 2. Methods

### 2.1. The rate constants

There are three rate constants considered in the model:  $D_p$ ,  $D_v$  and  $E$ . The constant  $D_p$  is the diffusion coefficient of the precipitant in water and is typically around  $10^{-5} \text{ cm}^2 \text{ s}^{-1}$  (Robinson & Stokes, 1959). The constant  $D_v$  is the diffusion coefficient of water vapor in air and is typically around  $0.25 \text{ cm}^2 \text{ s}^{-1}$  (Handbook of Chemistry and Physics, 1996). The constant  $E$  is the evaporation/condensation coefficient for water. Loosely,  $E$  is the probability that a water molecule striking a water/air interface will change state. It is a dimensionless constant which has been estimated to range between 0.05 (Jones, 1992) and 1.0 (Jamieson, 1964). While this seems like a rather large amount of uncertainty, it turns out that in this range  $E$  has little effect on the equilibration rates. For *Drop* the value of 1.0 is used, as this seems to have the strongest experimental backing.

## 2.2. The mathematical model

The differential equations governing the diffusion of the precipitant in the drop and the water vapor in the air are

$$\frac{\partial u}{\partial t} - \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} \right) = 0 \quad (1)$$

and

$$\frac{\partial v}{\partial t} - \left( \frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial z^2} \right) = 0, \quad (2)$$

respectively (see, for example, Crank, 1975 and Cussler, 1984), where  $u$  is the concentration of the precipitant in the drop and  $v$  is the concentration of the vapor in the air space. Both  $u$  and  $v$  are functions of the spatial variables and of time. The equation governing the rate of evaporation,  $E_R$ , from the drop is (Jones, 1992)

$$E_R = [E/(2\pi RTM_w)^{1/2}](P_{\text{eq}} - P_{\text{act}}), \quad (3)$$

where  $E$  is the aforementioned evaporation/condensation coefficient,  $M_w$  the molecular weight of water,  $R$  the universal gas constant,  $T$  the absolute temperature,  $P_{\text{eq}}$  the equilibrium vapor pressure of the solution at the surface of the drop and  $P_{\text{act}}$  the actual vapor pressure at the surface of the drop. Equation (3) must then be integrated over the surface of the drop to calculate the rate of change of the volume of the drop. For the model,  $P_{\text{act}}$  is approximated *via* the ideal gas law, *i.e.*

$$P_{\text{act}} = (\Delta N/\Delta V)RT = \nu RT, \quad (4)$$

where  $\Delta V$  is a small volume element,  $\Delta N$  is the number of water molecules in  $\Delta V$  and  $\nu$  is again the concentration of water vapor in the cell [introduced in (2)]. The function  $P_{\text{eq}}$  depends upon the precipitant concentration  $u$  at the surface of the drop. The program *Drop* incorporates experimentally measured vapor-pressure data directly. Vapor-pressure data can take the form of chemical potentials, activities, osmotic coefficients or vapor pressures of water over solutions at various concentrations of the precipitant. For the results presented in this report, the tables of osmotic coefficients found in Robinson & Stokes (1959) were used. From the osmotic coefficient  $\varphi$  at a given concentration of precipitant  $u$ , the vapor pressure  $P(u)$  of the solution is calculated from

$$P(u) = P(0) \exp[\varphi(u)\eta r], \quad (5)$$

where  $\eta$  is the number of ions dissolved per precipitant molecule (for example, three for ammonium sulfate and two for sodium chloride) and  $r$  is the ratio of moles of precipitant to moles of water (Guggenheim & Stokes, 1969). These osmotic coefficients were typically measured at 298 K. An assumption for the results presented in this report is that the osmotic coefficient does not change with temperature. For the range of temperatures of interest this seems to be an adequate assumption. For more complicated precipitants such as polyethylene glycol this assumption will, however, need to be tested.

Notice that in the above discussion of the rate constants, the precipitant is assumed to be non-volatile. If it were volatile

(for example ethanol) then a fourth rate constant, the diffusion coefficient for the precipitant in air, would be necessary. Volatile precipitants can, in theory, be covered adequately by this model. The model used by *Drop*, however, does not currently consider volatile precipitants. Also, the diffusion of additional species, such as additives, buffers or proteins, in the drop could be handled by the model in a straightforward manner but currently are not.

Beyond the equations that govern the diffusion and the rate of evaporation, boundary conditions are necessary to obtain a solution. All the boundary conditions except that at the air-reservoir interface are derived from the following considerations.

(i) No precipitant flows out of the drop or reservoir, *i.e.* the precipitant is non-volatile.

(ii) No water/vapor flows out of the cell.

(iii) Any water that flows out of (or into) the drop must flow into (or out of) the vapor space (similarly for the reservoir).

These boundary conditions are derived from the conservation of mass of each species. The volume of the reservoir and the concentration of precipitant in the reservoir are assumed constant. The boundary condition at the surface of the reservoir simply becomes that the chemical potential of water is continuous at this interface. While in theory the assumption that the volume and precipitant concentration of the reservoir do not change violates the conservation of mass, in practice it has no effect because the volume of the reservoir is 100–1000 times larger than the volume of the drop.

There are two simplifying assumptions in the model. Firstly, the drop is assumed to be in the shape of a sphere and maintains a constant contact angle with the top of the cell throughout the simulation. Secondly, the problem is assumed radially symmetric. Thus, by using cylindrical coordinates, the three-dimensional problem is converted into a two-dimensional one. Cylindrical coordinates result in no loss in accuracy if a cylindrical cell is used.

## 2.3. The numerical methods

To numerically integrate the diffusion equation for the precipitant in the drop, a uniformly spaced rectangular grid is used. For the results presented in this report a  $20 \times 20$  grid proved to be sufficient. The size of the grid was determined by varying the grid until the calculated equilibration curves no longer changed. The program *Drop* explicitly integrates this diffusion equation (see, for example, Burden & Faires, 1985).

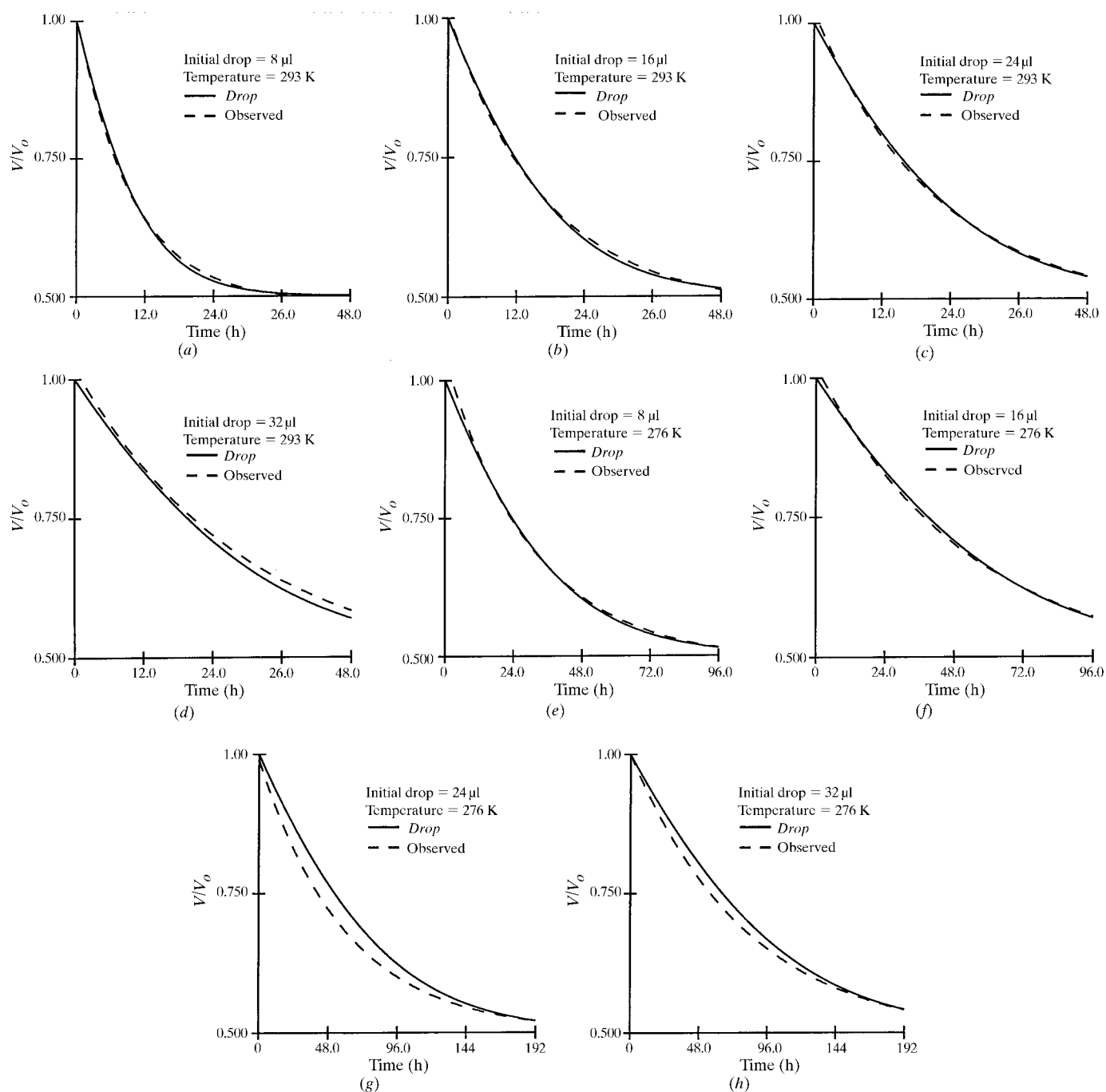
To numerically integrate the diffusion equation for the water vapor in the cell, a non-uniformly spaced locally rectangular grid is used. Smaller grid spacing near the drop and the reservoir proved to be more efficient than a uniformly spaced grid. For the results presented in this report eight grid points in the  $r$  direction and 20 in the  $z$  direction (drop-to-reservoir direction) proved to be sufficient. The size of this grid spacing was determined as that of the drop grid. The program *Drop* implicitly integrates this diffusion equation (see, for example, Burden & Faires, 1985).

To calculate the equilibration of four curves (8, 16, 24 and 32  $\mu\text{l}$  initial drop volumes at 293 K) for a time period of 96 h, *Drop* took a total of 1 h of CPU time on a Dec Alpha 4100/400. By increasing the grid spacing, this time could easily be cut by a factor of 10 without much loss in accuracy. The program *Drop* was written in C++ and was compiled using the GNU v2.4 C/C++ compiler. It should compile on any ANSI

C++ compiler and is freely available from the corresponding author.

### 3. Results

First *Drop*'s calculated equilibration curves were compared to experimentally determined equilibration curves. In Mikol *et*



**Figure 1**

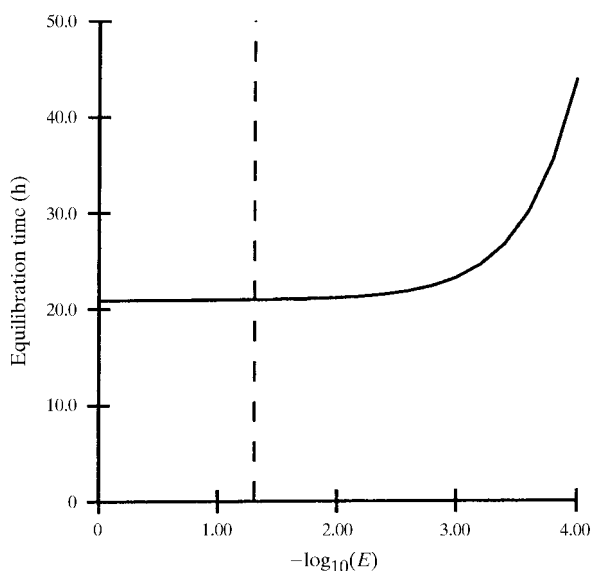
Calculated equilibration curves *versus* observed equilibration curves. All calculations were performed with a 1 cm cell height and a reservoir of 1.6 M ammonium sulfate. The solid line is *Drop*'s calculated equilibration curve and the dashed line is the observed equilibration curve from Mikol *et al.* (1990). (a) A temperature of 293 K and an initial drop of 4  $\mu\text{l}$  reservoir solution and 4  $\mu\text{l}$  water. (b) A temperature of 293 K and an initial drop of 8  $\mu\text{l}$  reservoir solution and 8  $\mu\text{l}$  water. (c) A temperature of 293 K and an initial drop of 12  $\mu\text{l}$  reservoir solution and 12  $\mu\text{l}$  water. (d) A temperature of 293 K and an initial drop of 16  $\mu\text{l}$  reservoir solution and 16  $\mu\text{l}$  water. (e) A temperature of 276 K and an initial drop of 4  $\mu\text{l}$  reservoir solution and 4  $\mu\text{l}$  water. (f) A temperature of 276 K and an initial drop of 8  $\mu\text{l}$  reservoir solution and 8  $\mu\text{l}$  water. (g) A temperature of 276 K and an initial drop of 12  $\mu\text{l}$  reservoir solution and 12  $\mu\text{l}$  water. (h) A temperature of 276 K and an initial drop of 16  $\mu\text{l}$  reservoir solution and 16  $\mu\text{l}$  water.

*al.* (1990) the equilibration rate for a hanging drop under many conditions was measured experimentally. A function of the form

$$V(t)/V(o) = A + B \exp(-t/\tau) \quad (6)$$

was then fit by these authors to their data and  $A$ ,  $B$  and  $\tau$  were reported for various precipitants, temperatures and initial drop volumes. Here,  $V(t)$  is the volume of the drop at time  $t$ . As a result, their work provides an excellent test of the validity of the *Drop* model. Of the three precipitants (MPD, PEG6000 and ammonium sulfate), ammonium sulfate is the only one for which vapor-pressure data (Robinson & Stokes, 1959) could be found. Accordingly, experiments E01–E04 and E09–E12 (Mikol *et al.*, 1990) were chosen as the test cases. Examples of *Drop*'s calculated equilibration curves *versus* the experimentally determined curves are shown in Fig. 1. The agreement is very good. The most important factor for obtaining accurate equilibration curves appears to be obtaining accurate vapor-pressure data.

Next, the rate-limiting constant in the model was determined. There are three rate constants (see §2.1), the diffusion coefficient of the precipitant in water  $D_p$ , the diffusion coefficient of water vapor in air  $D_v$  and the evaporation coefficient  $E$ . For these tests the base conditions of experiment E01 of Mikol *et al.* (1990) were used, *i.e.* a reservoir of 1.6 M ammonium sulfate, an initial drop of 4  $\mu$ l water plus 4  $\mu$ l reservoir solution, a temperature of 293 K and a cell height of 1 cm. Also, for these tests the equilibration time is defined as the time at which the drop is a fixed percentage equilibrated by volume (unless otherwise stated, 90% equilibration was used).

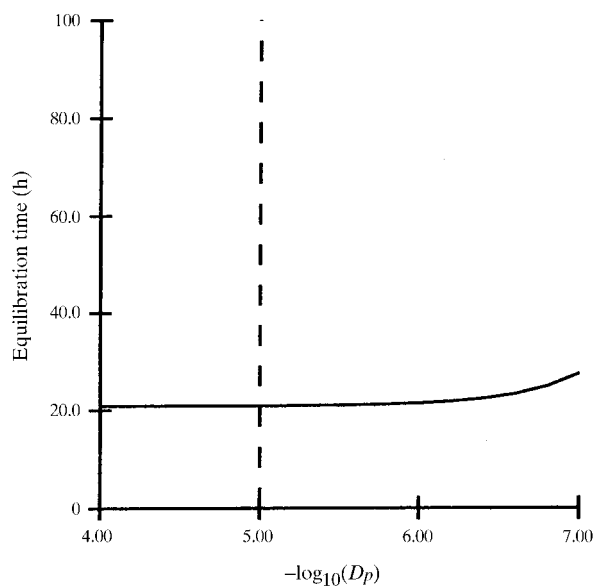


**Figure 2**  
The effects of the evaporation coefficient,  $E$ , on the equilibration. The calculations were performed with the baseline conditions of Fig. 1(a) and with  $-\log_{10} E$  varying from 0 to 4 in steps of 0.2. The estimated physical range for  $E$  is from 0.05 (Jones, 1992) to 1 (Jamieson, 1992). The first value is represented by the vertical dashed line. The equilibration time is the time at which the drop is 90% equilibrated by volume. The value of  $E = 1$  was used in all other calculations.

The evaporation coefficient  $E$  does not affect the predicted equilibration rate until it is less than  $10^{-3}$  (see Fig. 2), which is far outside the physical range (as mentioned before,  $E$  is believed to lie between 0.05 and 1). This offers an explanation as to why  $E$  has been difficult to measure (see Jones, 1992, for a discussion). The diffusion coefficient for the precipitant in water does not affect the calculated equilibration times (see Fig. 3) until  $D_p < 10^{-6} \text{ cm}^2 \text{ s}^{-1}$  (for ammonium sulfate  $D_p$  is around  $10^{-5} \text{ cm}^2 \text{ s}^{-1}$ ). Finally, the diffusion coefficient for water vapor in air,  $D_v$ , greatly affects the rate of equilibration (see Fig. 4). The equilibration time is roughly inversely proportional to  $D_v$ , which shows that the diffusion of water vapor through the air space is rate limiting.

The fact that the diffusion of water vapor through the air space is rate limiting is somewhat surprising. The standard scaling arguments (Fowles *et al.*, 1988) suggest that a concentration gradient of precipitant in the drop should equilibrate approximately 1000 times slower than a concentration gradient of water vapor in the air space. In this case, the scaling arguments are somewhat misleading because the concentration of water vapor in air over pure water at 298 K is only  $3.8 \times 10^{-7} \text{ mol cm}^{-3}$  (Handbook of Chemistry and Physics, 1996). Thus, for a 2  $\text{cm}^3$  cell at 298 K there is at most the equivalent of 0.015  $\mu$ l of water in the vapor state. As a result, a small amount of water evaporated from the drop leads to a large change in the concentration of vapor in the air space of the cell. Thus, the concentration of the precipitant in the drop does not get as far from equilibrium as does the concentration of water vapor in the cell.

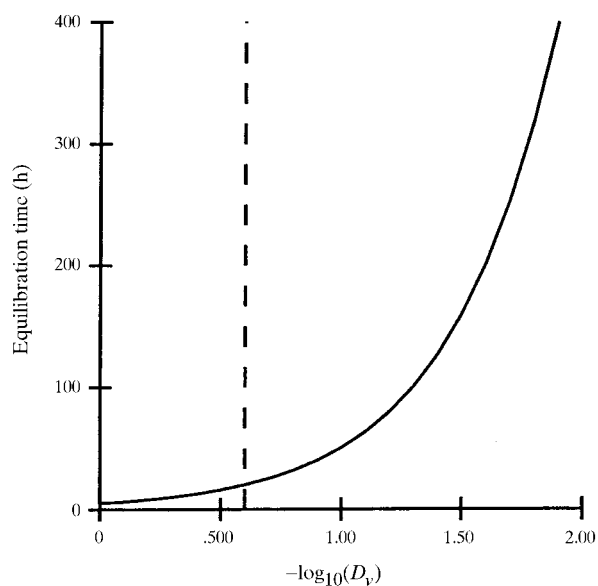
The fact that the diffusion of water vapor through air space is rate limiting has a significant implication. Since the diffusion of the water vapor is rate limiting, there is little variation in the



**Figure 3**  
The effects of the precipitant-diffusion coefficient  $D_p$  on the equilibration. The calculations were performed with the baseline conditions of Fig. 1(a) and with  $-\log_{10} D_p$  varying from 4 to 7 in steps of 0.15. The vertical dashed line represents a typical value for the precipitant-diffusion coefficient for a precipitant such as ammonium sulfate. The equilibration time is the time at which the drop is 90% equilibrated by volume.

precipitant concentration (spatially) throughout the drop (the model typically yields less than a 1% spatial variation in precipitant concentration in the drop). Since there is essentially no precipitant concentration gradient in the drop, there is no convection in the drop owing to gravity. As a result, gravity does not affect the equilibration rate of a hanging-drop experiment to a significant extent. As a side note, this result does not imply that gravity has no effect on crystal growth during a hanging-drop experiment. During crystal growth there might indeed be a large enough protein concentration gradient to cause gravity-induced convection.

Finally, the effects of some of the parameters in a hanging-drop experiment, in particular the contact angle, the initial drop volume, the temperature and the drop-to-reservoir distance, are considered quantitatively. The contact angle has little effect on the equilibration time. Varying the contact angle from 90 to 50° (the surface area increases by a factor of 1.17) decreases the predicted equilibration time (*i.e.* increases the equilibration rate) by about 7%. This is in good agreement with experimental results (Mikol *et al.*, 1990; see in particular experiments E09 *versus* E53 and E10 *versus* E54). The equilibration time depends almost linearly on the initial drop volume (Fig. 5). The slight degree of sub-linearity evident in Fig. 5 is likely to arise because the drop surface area increases with drop volume. Temperature also has a big effect on the equilibration time (see Fig. 6). The equilibration time changes by about a factor of three in going from 293 to 276 K. The diffusion coefficient, however, decreases by only 10% over this range. The primary reason for this large dependence on temperature is the change in the vapor pressure of water: the vapor pressure of pure water at 293 K is roughly three times that at 276 K. The temperature dependence of the calculations



**Figure 4**

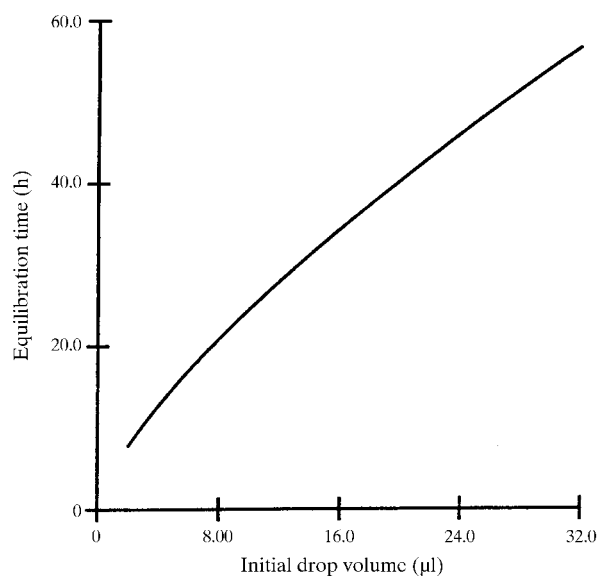
The effects of the vapor-diffusion coefficient  $D_v$  on the equilibration. The calculations were performed with the baseline conditions of Fig. 1(a) and with  $-\log_{10} D_v$  varying from 0 to 2 in steps of 0.1. The vertical dashed line represents the diffusion coefficient of water vapor in air at 293 K. The equilibration time is the time at which the drop is 90% equilibrated by volume.

is in excellent agreement with experimental data (see Figs. 1a *versus* 1e, 1b *versus* 1f, 4c *versus* 1g and 1d *versus* 1h).

Surprisingly, the equilibration time increases roughly linearly with the drop-to-reservoir distance (see Fig. 7). Typically, one expects the equilibration time for a diffusion process to vary quadratically (the units for a diffusion constant are length squared per time) with the length over which the species is diffusing. In a situation as complicated as a hanging drop, however, the simple scaling arguments that lead to the quadratic dependence are not valid. This *Drop* result is in excellent agreement with the experimental results of Luft *et al.* (1996) (see Fig. 7).

#### 4. Conclusions

In principle, the model proposed here is similar to that proposed in Fowles *et al.* (1988) and Sibile *et al.* (1991) with one key philosophic difference: *Drop* seeks a numerical solution rather than an analytical solution. The advantages of a numerical approach over an analytical approach include: fewer geometric assumptions are necessary, experimental data such as vapor pressure data can be incorporated directly rather than indirectly through a least-squares fit, the diffusion in the drop can be modeled explicitly and a numerical model is much easier to extend to more complicated situations such as volatile precipitants, multiple species in the drop *etc.* The potential disadvantages of any numerical scheme are excessive computational expense and numerical error. With modern computers, both the numerical error and computational expense required for *Drop* proved to be easily kept within reasonable limits. This is of significance since an accurate calculation of the equilibration rates can lend greater insight into the precipitant and protein concentrations when a protein



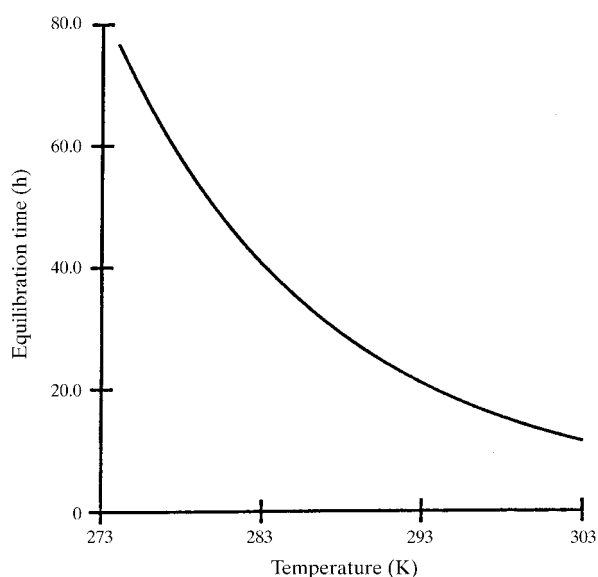
**Figure 5**

The effects of the initial drop volume on the equilibration. The calculations were performed with the baseline conditions of Fig. 1(a) and with the initial drop volume varying from 2 to 32  $\mu\text{l}$  in steps of 1  $\mu\text{l}$ . The equilibration time is the time at which the drop is 90% equilibrated by volume.

is nucleating and crystallizing. This information could in turn be useful for fine-tuning crystallization conditions.

Though all the comparisons with experimental results were performed with either NaCl or ammonium sulfate, some of the trends are applicable for other non-volatile precipitants such as polyethylene glycol (PEG) and 2-methyl-2,4-pentanediol (MPD). The three most important parameters in determining the equilibration time are initial drop volume, the temperature and the drop-to-reservoir distance. The conclusions that the equilibration time is roughly linearly dependent on the initial drop volume (see Fig. 5) and drop-to-reservoir distance (see Fig. 7) are likely to be independent of the precipitant. The conclusion that the equilibration rate changes by a factor of three when the temperature decreases from 293 to 276 K is likely to be less valid for the precipitants such as PEG which might adopt more compact conformations at lower temperatures. In most cases, this effect is likely to be very small and the factor of three difference in equilibration rate between 293 and 276 K is reasonably accurate.

In theory, in order to consider different precipitants two types of data are necessary: diffusion coefficients for the precipitant in water and water-vapor pressures at various precipitant concentrations. The diffusion coefficients are not likely to have a large practical effect. According to the Stokes–Einstein relation (Cussler, 1984) the diffusion coefficient of a solute should be inversely proportional to its radius as long as its radius is more than five times that of the solvent. Thus, even for the largest precipitants the diffusion coefficient is not likely to decrease by more than a factor of 10, which leaves it in the range of having no effect (see Fig. 3). The water-vapor pressures with the given precipitant, however, are crucial in obtaining reliable results. Unfortunately, we were unable to find the necessary vapor pressures for PEG or MPD as

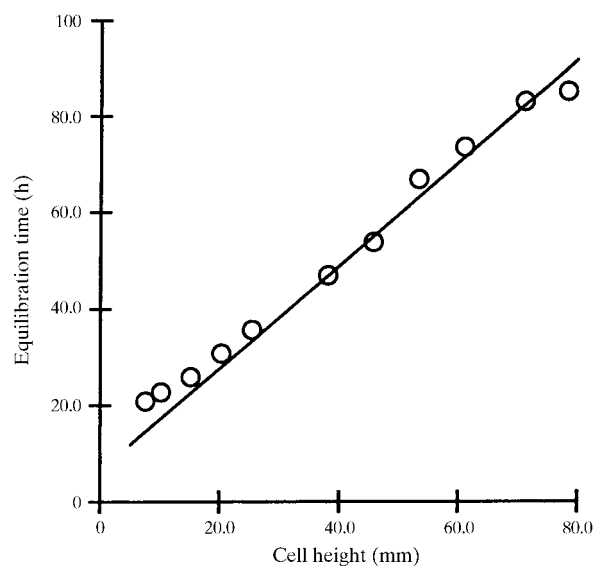


**Figure 6** The effects of temperature on the equilibration. The calculations were performed with the baseline conditions of Fig. 1(a) and with the temperature varying from 274 to 303 K in steps of 1 K. The equilibration time is the time at which the drop is 90% equilibrated by volume.

precipitating agents and it appears that they must be measured.

Though the numerical model of *Drop* is in excellent agreement with all available data on hanging-drop equilibration rates, there are also several potential improvements for *Drop*. As mentioned previously, *Drop* does not allow for volatile precipitants. The theory for volatile precipitants is largely the same as that presented in §2. To handle a volatile precipitant an additional diffusion equation similar to (1) and (2) to model the diffusion of precipitant in the vapor state and an equation similar to (3) to describe the evaporation of precipitant would be required. The primary difficulty in including volatile precipitants is that accurate physical parameters such as vapor-diffusion coefficients and chemical potentials are required. As an interesting side note, with a volatile precipitant the requirement that equilibrium is reached when the chemical potentials of all species are constant throughout the cell no longer uniquely determines the final drop volume. The relative rates at which the precipitant and water evaporate and diffuse not only determine the rate of equilibration but also determine the final drop volume.

A potential improvement to *Drop* would be to allow for a layer of oil on top of the reservoir. Again, the theory for such a model would be very similar to that presented in §2. To include the layer of oil in the model one new rate equation would be necessary: an equation similar to (2) and (3) describing the diffusion of water in oil. As with the case of the volatile precipitant, the difficulty in including the layer of oil would be to measure the chemical potential and diffusion coefficient of water in the oil being used.



**Figure 7** The effects of the cell height on the equilibration. The calculations were performed with the cell height varying from 5 to 80 mm in steps of 2.5 mm. The equilibration time is the time at which the drop is 50% equilibrated by volume. The circles are the observed equilibration times of Luft *et al.* (1996). The baseline conditions of their experiments and these calculations are initial drops of 24  $\mu$ l of 1 M NaCl, a reservoir solution of 2 M NaCl and a temperature of approximately 296 K.

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