

# Lyophilization

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Elementary lyophilization involves thinking about processes that occur in an environment foreign to common human experience. The science of lyophilization is taught at the college level in two courses, over three to four semesters. The courses are physical chemistry and mechanics, and they are often split over departments, chemistry, physics and engineering. Obviously, the information from those courses is not tailored to freeze drying and encompass greater depth than required for freeze drying alone. The purpose of this document is to extract and introduce the relevant sections from those disciplines and to apply it only to lyophilization. Lyophilization is composed of three events. The first two are freezing and sublimation – physical phase changes. The third event, desorption, comes from surface science, a relatively new field that is still rapidly developing.

## ***Introduction***

Why freeze dry? Usually, the reason is to preserve food or drugs. Freeze drying lowers moisture content and consequently decreases hydrolysis reactions that ultimately degrade food and pharmaceutical products despite sterile conditions. Other reasons to freeze dry drugs are to permit product reconstitution at a higher concentration than it was at the time of freezing, or to permit storage of the product at ambient temperatures. The later can be particularly useful for hospital products in areas that do not have ready access to freezers, especially ultra-cold freezers. Although pharmaceutical regulatory authorities will not assign multi-decade expiration dating to any products, it is well known that the US smallpox vaccine (Dryvax) manufactured by Wyeth as a lyophilized live virus was found to be still active and still potent 30 years beyond its original date of manufacture. Freeze drying works.

Technically, lyophilization is the vacuum sublimation of ice crystals that are formed when an aqueous solution is frozen. Left behind are the solid non-volatile solutes which may have been excipients and pharmaceutical ingredients of the liquid product. Within the box that is called a lyophilizer there is a large amount of stainless steel in the way of shelves and tubing. In use, there is also a reasonable amount of glass as vials. The remainder of the box volume is largely empty. How empty is it, and how does that exert control over the rate of lyophilization?

Kinetic theory is that branch of physics for which the laws of mechanics (Newton's Laws) are used to calculate the properties of bulk matter. It is instructive to apply those laws at the molecular level to the gasses (usually nitrogen and water) that compose the atmosphere during lyophilization. The first derivation of kinetic theory was provided by Daniel Bernoulli in 1738. Through consideration of nothing more than the elastic collision of rigid spheres, he successfully derived Boyle's Law (*At constant temperature, gas volume varies inversely as the pressure*). Since lyophilization is all about temperature and pressure, and since pressure relationships can be obtained from idealized models of rigid spheres, then lyophilization, to the extent that it involves gas molecules, may also be understood through kinetic theory.

## ***The Steps of Lyophilization***

Products to be lyophilized are first frozen. Such freezing may or not involve thermal conditioning to promote the growth of larger ice crystals. Whether or not such treatments are detrimental to sensitive pharmaceutical ingredients, they do indeed accelerate sublimation. In the second step of lyophilization (primary drying), the pressure is reduced to between 7 and 55 Pa, and heat energy is put into the chamber. Of course, the pressure need not be within the range above, but most often it is. The applied heat may come

from warmed fluid flowing through the shelves or walls of the chamber, or from microwave energy, or indeed from any conceivable source. Sublimation, as it applies to ice, is the phenomenon whereby a molecule of water seemingly spontaneously arises from the ice and becomes a molecule of gas. As the bulk of the molecules perform such feat, the ice disappears. Non-crystalline, amorphous, or glass portions of the frozen mixture do not sublime, but rather remain adsorbed to the solids at the end of sublimation. By definition, sublimation is the phase transition from ice to gas and ice is defined as crystalline. The third step of lyophilization (secondary drying) is the desorption of water molecules from the surface of the remaining solids. This third step is not a continuum of the primary drying step. It is a phenomenon separate and distinct from sublimation which is applied only to solid surfaces on which gas molecules have adsorbed.

The mechanism of sublimation and desorption are different in that sublimation and its opposite, condensation, is accompanied by a phase change and the departing (arriving) molecule either takes away or contributes a proportionately large amount of energy. Conversely, sublimation and desorption are similar in that both processes can be modeled as a single molecule leaving a surface.

## **Freezing**

At zero degrees C and 1 atmosphere pressure, water crystallizes into ice. Hexagonal ice (1h), the ice we make in a freezer or that falls as snow, has a measured density of  $0.917 \text{ gm/cm}^3$ . Each water molecule is involved in four hydrogen bonds donating 2 electrons and accepting 2 electrons. Near the freezing point, the crystalline structure is not tight and there is often room for a water molecule within the crystal.

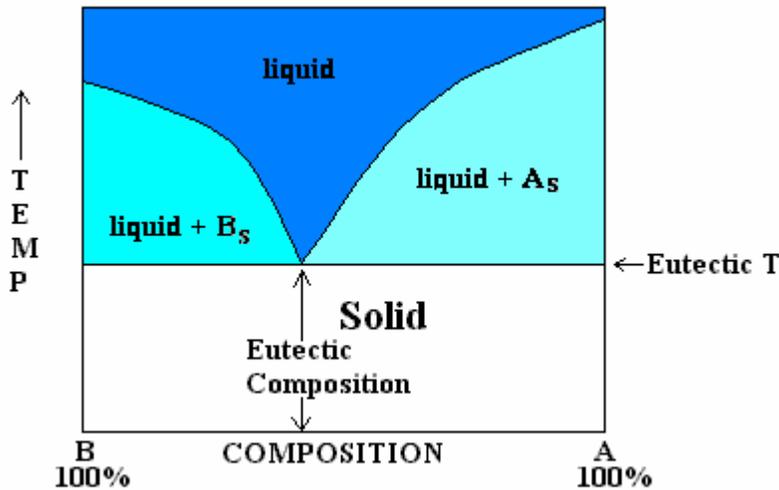
Consequently, there is plenty of “free” water to grow larger crystals if the temperature is maintained near the melting point. Similarly, there is extra water adsorbed onto the excipients and some of that can be crystallized under suitable conditions. Although water crystals can be exceedingly large, they are more often small and randomly arranged, with substantial amounts of non-crystallized water in the vicinity of the crystals. The amount of crystallization expressed as a percentage of the total water is called *the degree of crystallinity*. It is measured with a differential thermal analyzer (DTA). DTA involves heating a material at a controlled rate to a pre-determined temperature and observing emissions of heat (exothermic) or absorptions of heat (endothermic) from the material in comparison with a standard such as pure water. Although there is some debate about the product consequences of large crystals, it seems clear that larger ice crystals result in faster sublimation times. Presumably the difference in rate is due to having larger pores which do not inhibit the exit of gas molecules. There may be times when mass transfer (the exit of gas molecules) is rate limiting to sublimation. However, it is far more common for heat input to be limiting the sublimation rate.

In between the crystals of ice are all other ingredients and they are considerably more concentrated than when in solution. If the degree of crystallinity is about 80% (a typical expectation), then the remaining excipients, which are not crystallized and thus in liquid water solution, have experienced a 5 fold increase in concentration. Is an active protein soluble at a concentration 5 times higher than it was at the time of fill? If not, then one might expect it to precipitate – perhaps catastrophically. The same is true for any other excipient. If NaCl was an ingredient (a truly bad idea) and it experienced a similar 5 fold increase in concentration, what might that do to a protein? In addition to the salting out concern from changing concentration there is also a possibility of pH change because hydrogen ion may be one of the ingredients that gets concentrated.

## **Eutectic Point**

Eutectic points are uncommon in the mixtures associated with pharmaceutical lyophilized products. There was time, not long past, when the “eutectic” was something that one needed to know in order to perform a successful lyophilization. A “eutectic point” refers to a specific concentration of two solutes, A and B, such that upon solidification by the lowering of temperature, the lowest possible temperature is achieved prior to the solidification. For the purpose of lyophilization and understanding the diagram, let A be water and B be

a non volatile active (e.g. Dimethoxy- $\alpha$ -methylhydrocinnamic acid). When two crystallizable solutes, A and B, are cooled, one of the solutes will crystallize disproportionately to the other, thereby changing the solution concentration until a eutectic mixture is achieved. The eutectic temperature is that temperature where no liquid remains. The eutectic composition is that ratio of compound A and compound B that exists at the instant of liquid crystallization.

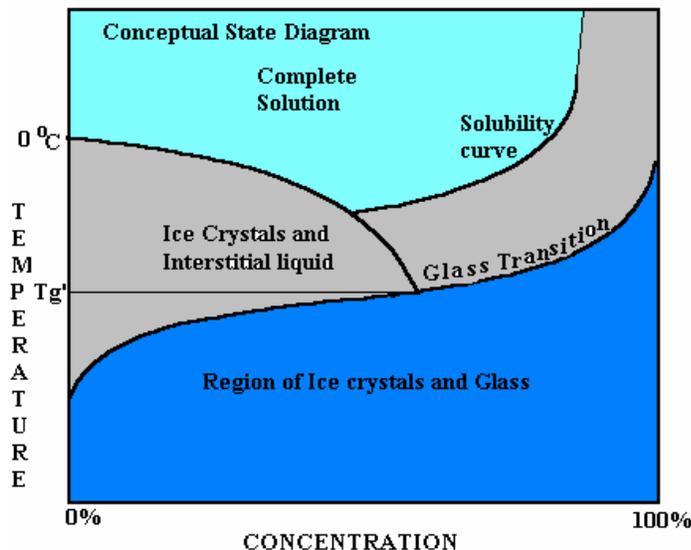


Most lyophilized pharmaceutical formulas do not have a true eutectic point, and instead have non-crystalline, amorphous regions that go through a rubbery glass transition phase as they gradually solidify. Freezing of liquid water into water ice happens prior to solidification of non-water molecules in association with liquid water. To minimize hydrolytic damage during primary drying as well as to avoid meltback, shrinkage, or collapse, it is necessary to bring the product ice temperature low enough to stop gross molecular motion of the

non-crystalline portion of the mixture. As the product ice temperature becomes warm enough for microscopic movement to be observed (as for example in a freeze drying microscope), then the product is in danger of melting at the sublimation interface. At the interface water ice will have sublimed away, and an interstitial liquid region remaining will dissolve the newly dried cake, causing it to physically reduce in bulk.

### *Collapse*

Shrinkage, collapses, and meltback all refer to the same physical phenomenon at different degrees of completion. Amorphous solids (solids that are not crystalline) that are mixtures of water and other molecules maintain their solid state as a function of temperature and exhibit melting at a temperature called  $T_g'$ , the glass transition temperature. A hypothetical eutectic point exists, but it is a non-event in that no crystallization or melt occurs. As temperature is decreased, ice crystals continue to grow until the point that a maximum degree of crystallization has occurred,  $T_g'$ , at which time the system goes into the ice plus glass phase.



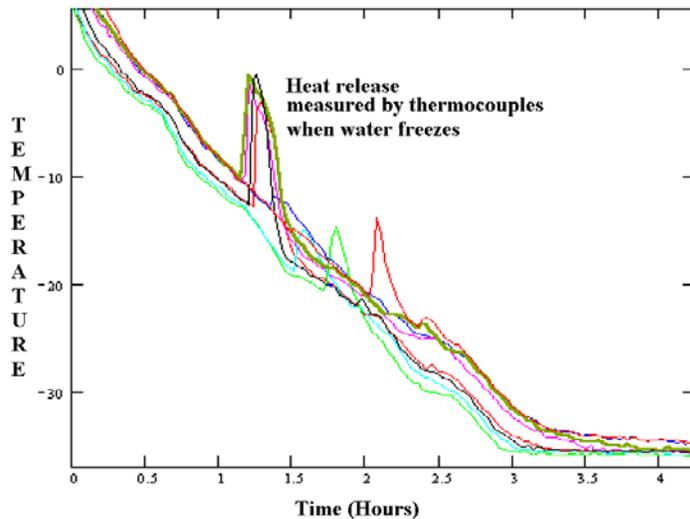
When water is limiting, the glass transition temperature is a moving target and can occur anywhere along a continuum. Thus, for example, during secondary drying at 6% moisture, if the temperature is raised too high it is possible (likely) that one will exceed the glass transition and "liquefy" the product, resulting in some degree of collapse. One can think of a glass transition as a rubbery state of high viscosity that lies between liquid and solid. Collapse is the consequence of gravity acting on that state.

### ***Thermocouple registration of the freezing event***

Crystallization occurs around distinct sites called nucleation sites. These may be microscopic particulates or scratches in the glass or most certainly a thermocouple in a vial. It has been shown that seeding water with nucleation sites can catalyze crystal formation. Of course, pharmaceutical preparations are by design low in particulate and thus low in nucleation sites. As the temperature of water falls below zero degrees, it is said to be supercooled. Nucleation then becomes a random event from vial to vial happening at different times and different temperatures.

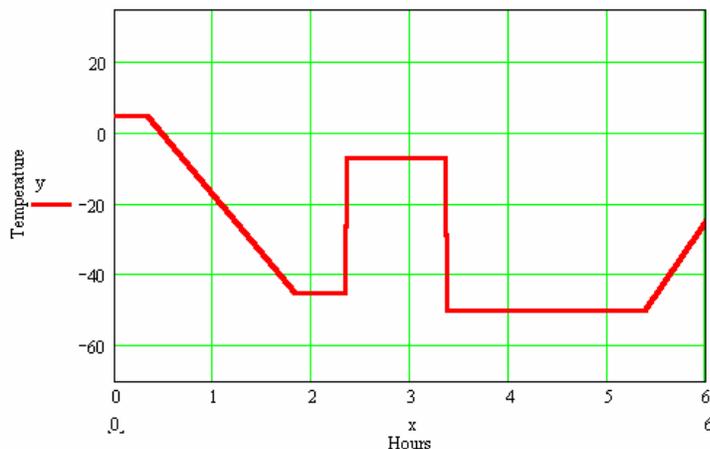
At the temperature and pressure of a phase transition, two phases (liquid water and solid ice) are in equilibrium. The addition of heat to ice at its melting temperature causes melting (the energy is used for molecular rearrangement) rather than raising the temperature of the ice. Conversely, when water freezes there is heat that must be released over the same time period that crystallization occurs. With a

thermocouple in the liquid, that heat is registered as a rise in temperature of the thermocouple.



### ***Annealing***

One can promote the growth of larger crystals and consequently accelerate the rate of primary drying by holding the ice temperature below the freezing point of water and above  $T_g'$  for the product. This was shown by Jim Searles, [J.A. Searles, J.F. Carpenter, T. W. Randolph, "Annealing to optimize the primary



*drying rate, reduce freezing-induced drying rate heterogeneity, and determine  $T(g)'$  in pharmaceutical lyophilization", *Journal of Pharmaceutical Sciences*, 90, 872-877, (2001)]. The apparent mechanism, as previously stated, is to increase the pore size of the frozen matrix. There are potentially two reasons not to anneal products. First, one may do harm to the ingredients as a consequence of chemical reactions that can take place in the liquid interstitial region during annealing. Second, secondary drying or desorption, may be less efficient due to less surface area and the desired final moisture content could be more difficult to*

achieve. With those disclaimers, it is worth considering an annealing step if the product bulk volume is large compared to the vial size.

## Primary Drying

Prior to the thermodynamic discussion of sublimation, it is appropriate to consider the chamber environment that the gaseous water molecule will encounter. At some moment in time a molecule will find it energetically favorable to arise from an ice crystal and fly about. If it isn't resorbed onto another crystal in the vicinity, then it must progress through a tortuous path of pores created by previously sublimed ice and the semi-dry cake structure. Finally, the steam molecule breaks free into the upper vial interior and then must locate the vial exit. Upon getting into the chamber and without navigational aids, it must randomly bounce around until it leaves the chamber and perchance encounters the condenser. At the condenser, temperature is such that the molecule should find it favorable to re-solidify as ice.

Elementary kinetic theory is based on the assumption that molecules do not attract or repel each other and therefore travel in straight lines between collisions. Collisions are perfectly elastic and are of two types – collisions with walls and collisions between molecules. How far the molecule goes before it bumps into a wall or another molecule is called the “mean free path”. The molecules do not all go the same speed. They travel at about 500 m/s and have a range of speeds for which we use the term “velocity distribution”. From the equations for “mean free path” and “velocity distribution” we can develop other equations that help to explain parts of the thermocouple behavior during a lyophilization cycle, as well as permitting the development of rate equations that predict the overall time needed to sublime ice.

Water and nitrogen molecules are of molecular weight 18 and 14 respectively. While neither is exactly spherical, they can nonetheless be treated as rigid spheres for modeling. They will collide and they will bounce, and the resultant vector positions are no more or less random than if they were in fact rigid spheres. Bodies that collide, exchange energy and direction according to two laws of mechanics. The first is conservation of momentum, where momentum is mass x velocity. The second is conservation of energy,  $K_E = \frac{1}{2} \times \text{mass} \times (\text{velocity})^2$ . When two rigid spheres undergo “head on” elastic collision, they exactly exchange momentum.

$$\mathbf{m}_1 \cdot \mathbf{v}_1 = \mathbf{m}_2 \cdot \mathbf{v}_2$$

When two spheres collide, and their momentum vector is not “head on”, then their momentum in the direction of their line of centers is exactly exchanged. Meanwhile, their respective momentum vector that is perpendicular to their line of centers is unaffected by the collision and continues to reside with the original sphere.

The mean free path (MFP) of a water molecule is defined as an average distance traveled by the molecule before it collides with another molecule.

$$\text{MeanFreePath} := \frac{R \cdot T}{\sqrt{2} \cdot \pi \cdot \text{WaterDia}^2 \cdot N_a \cdot P}$$

R = 8.31451 J/mol\*K

WaterDia =  $3.85 \times 10^{-10}$  m

T = Absolute temperature

P = Pressure in Pascals

Mean Free Path = meters

$N_a = 6.023 \times 10^{23} \text{ mol}^{-1}$

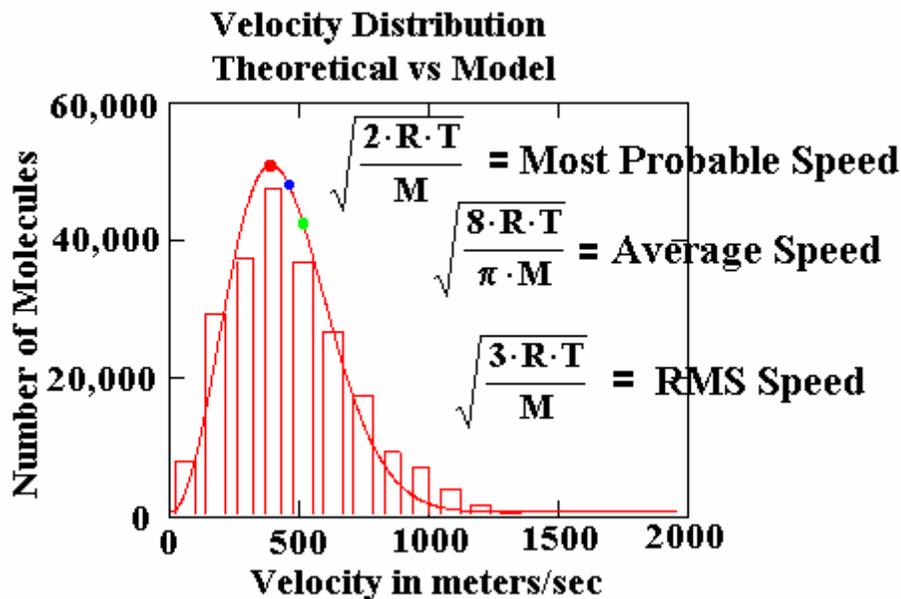
In a typical commercial lyophilizer, a water molecule must travel about 3 meters before adhering to a condenser surface, or being pulled into a vacuum exit. At a typical chamber pressure of 13 Pa, and 0°C the MFP for the molecule will be 440 μm.

Temp	Pressure	Volume (nm <sup>3</sup> )	Intermolecular Distance	Mean Free Path Length
0°C	Atmos.	37.0	3.3	56.5 nm
-20°C	200 mtorr	131,078	50.8	0.199 mm
-40°C	200 mtorr	120,723	49.4	0.183 mm
-40°C	100 mtorr	241,445	62.3	0.367 mm
-40°C	10 mtorr	2,414,452	134.0	3.666 mm
-40°C	1 mtorr	21,144,524	289.0	36.666 mm

Table 1: For various temperatures and pressures that might be attained in a lyophilizer, this table gives the cubic volume available to each molecule, the linear distance between molecules, and the mean free path length.

$$f(v) := 4 \cdot \pi \cdot \left( \frac{M}{2 \cdot \pi \cdot R \cdot T} \right)^{\frac{3}{2}} \cdot v^2 \cdot e^{\left( \frac{-M \cdot v^2}{2 \cdot R \cdot T} \right)}$$

In 1866, James Clerk Maxwell of Edinburgh, Scotland formulated the modern kinetic theory, and derived an equation for the distribution of velocities,  $f(v)$ , for different gas molecules. A modern derivation is given in the Appendices.



M = .018 kg/mol  
R = 8.31451 J/mol\*K  
T = -40°C (expressed in Kelvin)  
v = velocity

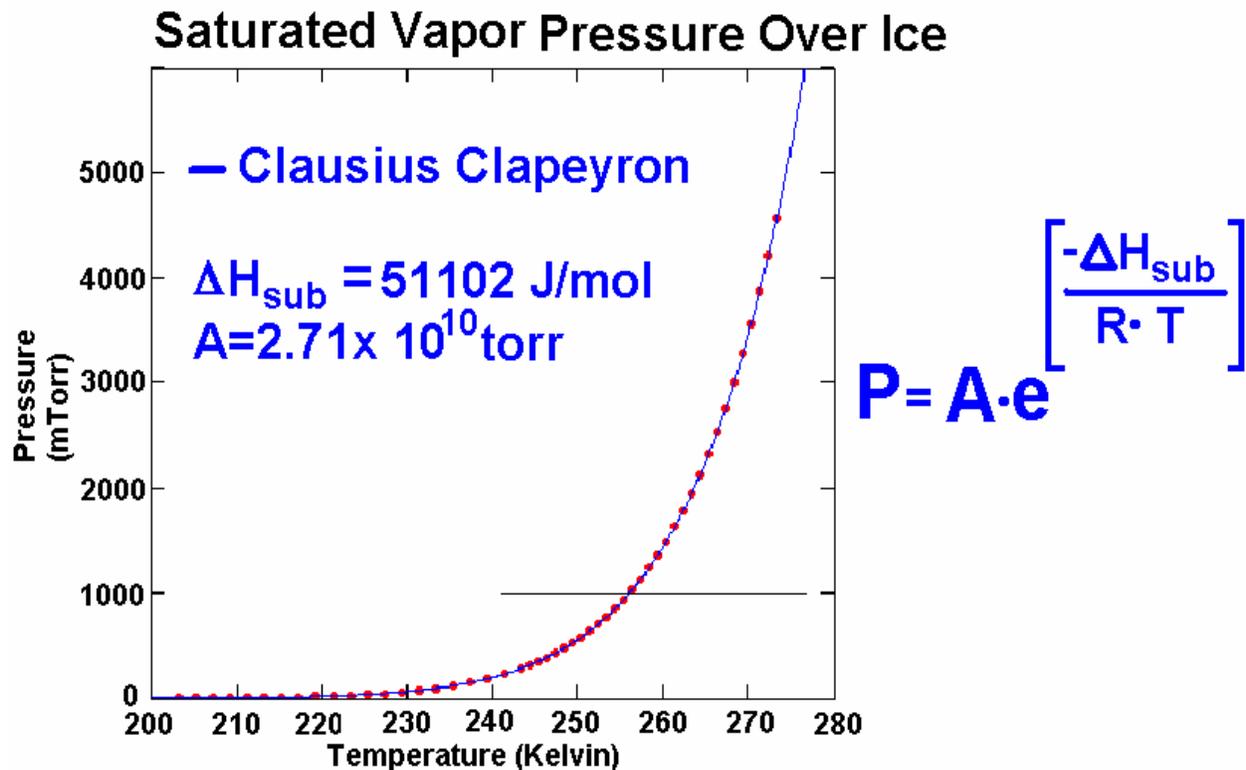
### ***Derived from Kinetic Theory***

The following five principles have been derived from simple kinetic theory.

- Dalton's Law of Partial Pressure  $P_T = P_1 + P_2 + P_3$  etc. The total pressure of a gas is the sum of the partial pressure of all the different gas species.
- Energy distribution among molecules.
- Number of Collisions per second. One can calculate the number of collisions that will occur in a three (or two) dimensional box and even though every molecule is started at a random place and a random speed and a random direction, the calculation is still within a fraction of 1% of the actual number of collisions counted by a computer simulation.
- Knudsen numbers and the effusion of gas from a small orifice. This concept may play an important role in secondary drying related to the theory of how a gas molecule escapes from the fibers.
- Viscosity of a gas, diffusion, and thermal conductivity

## Saturated Vapor Pressure Over Ice

There is an energy equilibrium between the ice and gas phase of water in the lyophilizer and it is expressed by the Clausius Clapeyron equation. A derivation from Gibbs Free Energy is given in the Appendices. The Clausius Clapeyron relation permits a sensible selection of pressure to achieve a product ice temperature that will be below the collapse temperature. The constant, A, can be derived in any desired pressure units. To do so, see the appendix.



From Dalton's Law of Partial Pressure, the total pressure of a gas is the sum of the partial pressure of all the different gas species. Data found in the Handbook of Chemistry and Physics, is specific to the situation where the only gas over the ice is water vapor. That is, only water vapor accounts for the pressure shown in Figure 2. Pressure is nothing more than a way of expressing gas concentration as a function of temperature.

$$P \cdot V = n \cdot R \cdot T \quad \text{Ideal Gas Law}$$

$$P = \frac{n}{V} \cdot R \cdot T$$

$$\text{but Conc} = \frac{n}{V} \quad \text{moles/Liter}$$

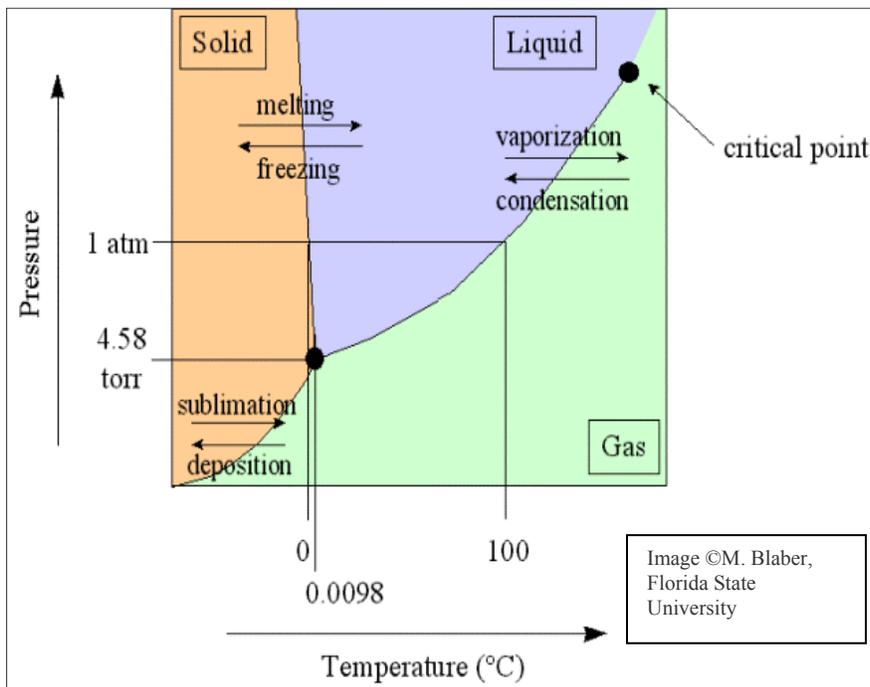
$$P = \text{Conc} \cdot R \cdot T \quad \text{by substitution}$$

$$\text{Conc}(P, T) := \frac{P}{R \cdot T} \quad \text{and algebra}$$

Were there to be substantial N<sub>2</sub> or air from a bleed source used to control pressure, then the ice temperature would not be the one shown, but would rather correspond to the partial pressure of water vapor. Consequently, in the situation where there is excess N<sub>2</sub> to make the total pressure, the ice temperature will correspond more to the partial pressure due to water than to the total pressure. While this concept is very simple, it is often misunderstood by operators. It is not the pressure in the lyophilizer that determines the ice temperature, but rather the partial pressure of water vapor in the lyophilizer that determines the ice temperature. These two, total pressure and partial pressure of water vapor, are probably not the same when the lyophilizer is being operated in a manner whereby pressure is controlled. Of course, the partial pressure of water can never exceed the total pressure! Further, during primary drying, so much water is coming off so fast that most of the chamber pressure is due to water vapor. Indeed, there are instances when the vacuum pumps cannot keep up and the chamber pressure during primary drying exceeds the vacuum set point.

From a phase diagram of water, it is clear that sublimation will not occur when the partial pressure of water vapor is above 4.58 torr (610.6 Pa). However, Dalton's Law clearly shows that the total pressure could be atmospheric (760torr) and sublimation could still occur if the partial pressure of water were less than 4.58 torr. Hence snow will sublime under atmospheric conditions. Further, if the outdoor temperature were -5°C, the Clausius pressure would be 4.466. With a relative humidity of 20%, the partial pressure of water would be only 0.8932 torr and sublimation would proceed nicely – which of course it does.

$$(RH = 100 * VaporPres / ClausiusPressure)$$



During primary drying in a lyophilizer, water vapor is likely to be the dominant partial vapor pressure right above the ice. Consequently, controlling water vapor pressure is going to control ice temperature. Do not confuse temperature of the ice with temperature of the shelf. They are less related to one another than chamber pressure and ice temperature. Temperature of the ice is what the product thermocouples read and temperature of the ice is what is important. The temperature of the shelf offers no direct information about the temperature of the product.

## Kinetic Theory of Gasses

In order to predict the vagaries of sublimation, it is essential to possess an understanding of kinetic theory. After all, the goal of lyophilization is to convert a solid (water ice) into a gas, thereby permitting it to be removed from the product and perhaps be recaptured at the condenser. Kinetic theory begins with a simple system of point molecular masses (molecules in 3d) and progresses logically to everyday rules such as  $PV=nRT$ . For a derivation of an expression for pressure and the ideal gas law, see Appendix I.

For those who skim over appendices, there is still a need to know something about pressure. First, pressure is expressed in more units, and more unfamiliar units than most anything else that we encounter. Second, zero pressure is defined as a complete vacuum. Third, pressure is a way of expressing concentration for a gas. That is, when we know the pressure and we know the gas identity, then we know exactly how much of it we have, or at least we know its concentration.

### Units

Common units of pressure among US engineers and thus on some lyophilization equipment are psi, pounds per square inch. The confusion starts here. PSI should be either psia or psig. PSIA is absolute pressure, where zero means no gas molecules. In that terminology, 1 atmosphere (1 atm) is 14.696 psia. PSIG is gauge pressure. In gauge pressure 1 atm is zero on the dial. If PSI is used, it must be interpreted in context. Some vacuum pumps use inches of mercury with a gauge pressure of zero. Thus vacuum is expressed in negative inches of mercury. In that case, absolute zero would be  $-29.921$  inches of mercury because one atmosphere is equivalent to  $+29.921$  inches of mercury.

Most modern lyophilizers record pressure in either mbars, microns, mtorrs, or Pascals. An atmosphere is defined as the pressure exerted by a 760mm column of mercury, with a density of  $13.5951 \text{ gm/cm}^3$  and subject to gravity of  $9.80665 \text{ m/s}^2$ . A bar and a Pascal (Pa) differ by exactly 5 orders of magnitude as a matter of convenience. One atm is 1.01325 bars. For the purpose of thinking about it, 1 bar is 1 atmosphere. One atmosphere is 101325 Pa. Meteorologists have historically given pressure in bars, but atmospheric pressure doesn't change enough to be significant, thus they have used millibars. A common atmospheric pressure would be 1013 mbars. The torr is also a common unit and is defined as a millimeter of mercury in the aforementioned column. A millitorr and a micron are synonymous.

mtorr	Pa	mbar	atm	in. of Hg	psia
10	1.333	0.013	1.32E-05	-29.921	1.93E-04
50	6.666	0.067	6.58E-05	-29.919	9.67E-04
100	13.332	0.133	1.32E-04	-29.917	1.93E-03
150	19.998	0.2	1.97E-04	-29.915	2.90E-03
200	26.664	0.267	2.63E-04	-29.913	3.87E-03

torr	kPa	mbarr	atm	in. of Hg	psia
25.857	3.447	34.474	0.034	-28.903	0.500
51.715	6.895	68.948	0.068	-27.885	1.000
413.720	55.158	551.581	0.544	-13.633	8.000
646.437	86.184	861.845	0.851	-4.471	12.500
760.000	101.325	1013.250	1.000	0.000	14.696

Reading across, the table shows equivalent measures of pressure in different units. The top section depicts common pressures that might be used during primary drying and the bottom section shows pressures that might be used during stoppering. In almost all instances, different transducers would be employed in the two

situations and they are likely to register in different units. Typically, conversion is not required. If it is, there are numerous tables and websites available to do it for you.

Pressure is an indirect way of giving concentration.

$$P \cdot V = n \cdot R \cdot T \quad \text{Ideal Gas Law}$$

$$P = \frac{n}{V} \cdot R \cdot T$$

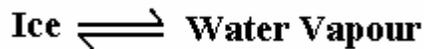
$$\text{but Conc} = \frac{n}{V} \quad \text{moles/Liter}$$

$$P = \text{Conc} \cdot R \cdot T \quad \text{by substitution}$$

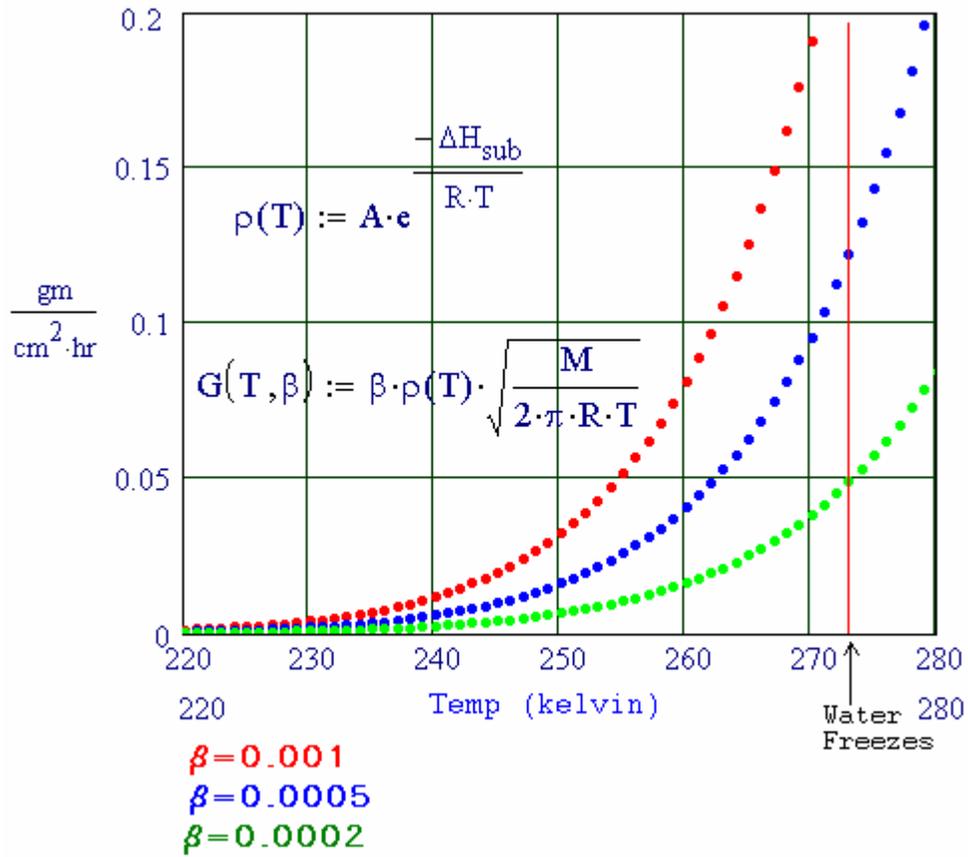
$$\text{Conc}(P, T) := \frac{P}{R \cdot T} \quad \text{and algebra}$$

### ***Classical Theory of Sublimation***

Martin Hans Christian Knudsen derived from kinetic theory an equation for the absolute rate of sublimation of ice (see Figure 4). In this case, rate implies mass removal per unit time and since an assumption is made that the mass must disappear only from the ice surface, the units are mass per unit time times surface area. Pressure ( $p[T]$ ) refers to the saturated water vapor pressure over the ice.  $M$  is the molecular weight of water.  $R$  and  $T$  are the gas constant and absolute temperature respectively.  $\beta$  is a coefficient of evaporation and may range from zero to one. In practical commercial lyophilizers, it is no larger than about 0.001. Two assumptions are paramount to this equation. First, there exists an equilibrium between the surface of the ice and the water vapor.

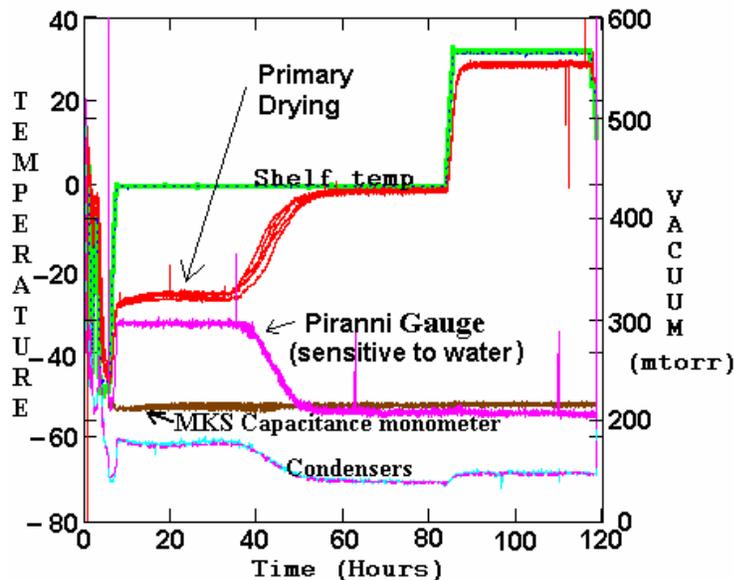


For  $\beta$  to be large, the equilibrium must greatly favor the forward rate. Second, the number of vapor molecules leaving the ice depends exclusively on the temperature of the ice. The ice molecules are considered to have vibration energy only since within a crystalline structure, their translation and rotation energies may be assumed to be close to zero. Meanwhile, the number of molecules returning to the ice surface must be a function of the total pressure, partial pressure of water vapor, and temperature of the vapor. In deriving the equation, Knudsen assumed that all pressure arose from water vapor, thus equating total pressure and partial pressure.



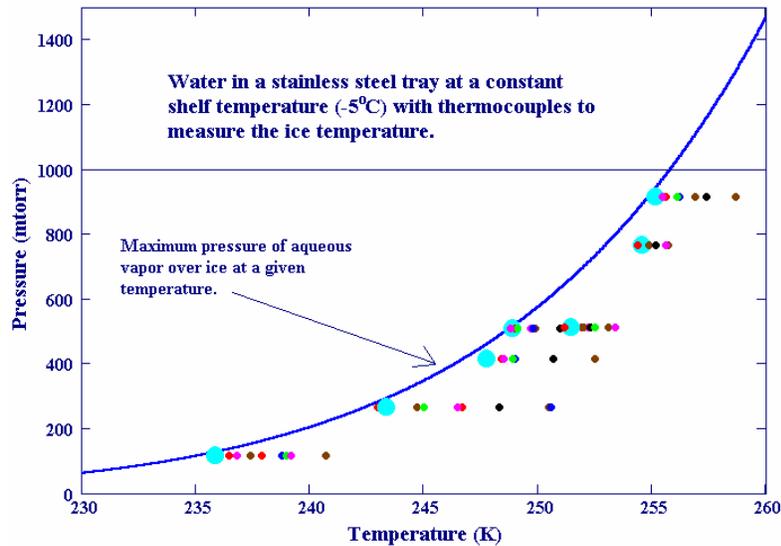
## Practical Sublimation

Figure 5 shows thermocouple data from an actual lyophilization of protein. There is an initial thermal treatment to freeze the water and coalesce (anneal) the ice crystals. That data is compressed into the first few hours of this lengthy lyophilization. Focus on the thermocouples pointed to by the arrow leading to primary



drying. They all come to the same temperature (between  $-20$  and  $-30^{\circ}\text{C}$ ) during the primary stage at constant pressure, and they do so despite a shelf temperature at  $0$  degrees C. Notice that the piranni gauge, known to have a water sensitive pressure output, suggests that water vapor dominates the partial pressure during primary and then as the primary phase ends, the water vapor very much decreases. During the entire period the MKS vacuum reading (capacitance manometer) is constant at a total pressure of about  $200$  mtorr. If one were to stop the lyophilization and remove the contents at the point that the piranni gauge has completed its apparent pressure change, then the contents (lyophile) would appear dry. At that point sublimation (primary drying) would be complete. The message is that the ice temperature during primary drying at constant partial pressure is constant and not equal to the shelf temperature, but rather a function of the partial pressure of water vapor.

In order to clarify the relationship of ice temperature to the partial pressure of water vapor and to the total pressure within the lyophilizer, an experiment was performed in which a tray of water was placed into the lyophilizer and held at different constant pressures until equilibrium could be attained. The different thermocouples are at different depths within the ice and that results in different temperature readings for a single pressure. The closer a thermocouple is to a subliming surface, the colder it will be. Sublimation and evaporation cause cooling. Warmer thermocouples at any given pressure are deeper into the ice. As is seen in figure 6 there is a general relationship between the temperature of the ice and the total pressure of the lyophilizer that approximates the theoretical curve for saturated vapor pressure of water over ice.



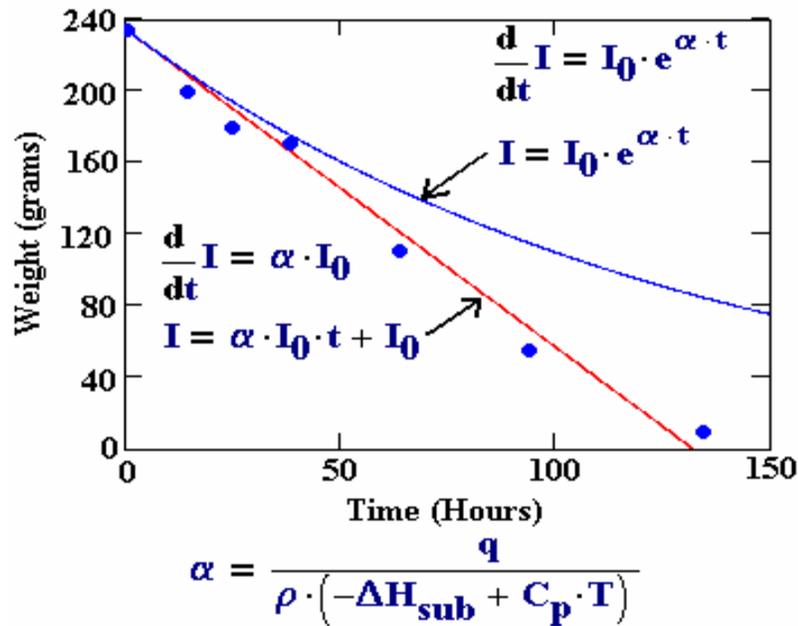
This is an experiment to do with a few vials of pharmaceutical product if you want to know what the product ice temperature will be at a fixed shelf temperature and pressure. For example, if a collapse temperature is known to be  $-13^{\circ}\text{C}$  (260 K), then a shelf temperature can be chosen for the purpose of putting in energy (for example pick  $0^{\circ}\text{C}$ ) and then the ice temperature can be observed at various constant pressures, until one finds a pressure that will hold the ice several degrees below the collapse temperature.

### Energy Model for Lyophilization

Energy models of sublimation can be written from a simple understanding that  $E_{\text{in}} - E_{\text{out}} = E_{\text{st}}$ , that is, energy in – energy out = energy at steady state.

$$\begin{aligned}
 E_{\text{in}} &= q \cdot I_0 \\
 E_{\text{out}} &= -\rho \cdot \Delta H_{\text{sub}} \cdot \frac{dI}{dt} \\
 E_{\text{st}} &= \rho \cdot C_p \cdot T \cdot \frac{dI}{dt}
 \end{aligned}
 \quad
 \begin{pmatrix} q \\ \rho \\ \Delta H_{\text{sub}} \\ C_p \\ T \\ I_0 \end{pmatrix}
 =
 \begin{pmatrix} 4 \cdot 10^5 \cdot \frac{\text{W}}{\text{m}^3} \\ 920 \cdot \frac{\text{kg}}{\text{m}^3} \\ 2839000 \cdot \frac{\text{J}}{\text{kg}} \\ 2040 \cdot \frac{\text{J}}{\text{kg} \cdot \text{K}} \\ 250 \cdot \text{K} \\ 4 \cdot 10^{-6} \cdot \text{m}^3 \end{pmatrix}
 \begin{matrix} \text{Energy input} \\ \text{Ice density} \\ \text{Enthalpy of sublimation} \\ \text{Heat capacity of water} \\ \\ \text{Initial Volume of the Ice} \end{matrix}$$

Far more complex models can be derived but this one is surprisingly accurate considering its simplicity. Also, it might seem to be common sense that the energy input would be proportional to the amount of ice on the shelf, ( $q \cdot I$  rather than  $q \cdot I_0$ ), however, that leads to a first order rate equation, which does not match with the zero order kinetics typically observed for phase changes at constant temperature. In figure 7, data is presented for the sublimation of water from an Erlenmeyer flask. At each data point, the flask was removed from the lyophilizer, weighed, and returned to continue sublimation. Conditions were  $-5^\circ\text{C}$  (shelf) and 150 mtorr pressure. The straight line is the solution to the above equations where  $E_{in} = q \cdot I_0$ , while the curved line is the solution found when  $E_{in} = q \cdot I$ , where  $I_0$  and  $I$  are the initial and actual weight of ice respectively.



### Zero Order Kinetics

Most common in biological and simple chemical systems are first and second order kinetic mechanisms.

By “order”, it is meant that the time rate of change for concentration of a reactant (or product) is directly proportional to either the existing concentration of reactant, or the second power of the existing reactant concentration. In symbols:



However, there are certain instances where the “reaction” does not change the concentration of reactant at all. Such is the case for simple sublimation.



$$\frac{d}{dt} \text{H}_2\text{O}_{\text{ice}} = \text{const}$$

In the case for sublimation, the rate equation is

This is to say, there is no change in the ice concentration as it sublimates. Although a solution to the above equation is easy enough, it isn't useful without guidance to obtain a value for the constant. Using the energy model from the prior section, we can arrange a solution as shown below that yields an equation from which it is possible to determine a constant.

$$E_{\text{in}} - E_{\text{out}} = E_{\text{st}}$$

$$E_{\text{in}} = q \cdot l_0$$

$$E_{\text{out}} = -\rho \cdot \Delta H_{\text{sub}} \cdot l' \quad E_{\text{out}} \text{ is negative.}$$

$$E_{\text{st}} = \rho \cdot C_p \cdot T \cdot l' \quad E_{\text{st}} \text{ is the steady state energy}$$

$$q \cdot l_0 - \rho \cdot \Delta H_{\text{sub}} \cdot l' = \rho \cdot C_p \cdot T \cdot l'$$

$$\frac{d}{dt} l = \frac{q \cdot l_0}{\rho \cdot (-\Delta H_{\text{sub}} + C_p \cdot T)} \quad \Leftarrow \text{Algebra to get } l'$$

$$\text{let } \alpha = \frac{q}{\rho \cdot (-\Delta H_{\text{sub}} + C_p \cdot T)} \quad \alpha := \frac{q}{\rho \cdot (-\Delta H_{\text{sub}} + C_p \cdot T)}$$

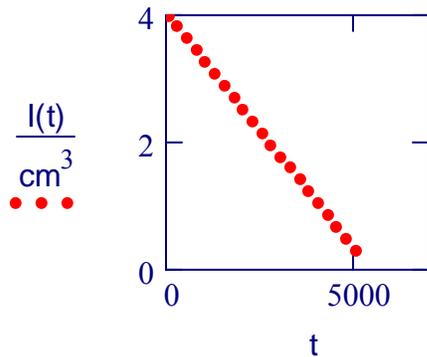
$$\frac{d}{dt} l = \alpha \cdot l_0$$

$$\alpha = -1.851 \times 10^{-4} \text{ s}^{-1}$$

$$\int_{l_0}^l l \, dl = \int_0^t \alpha \cdot l_0 \, dt$$

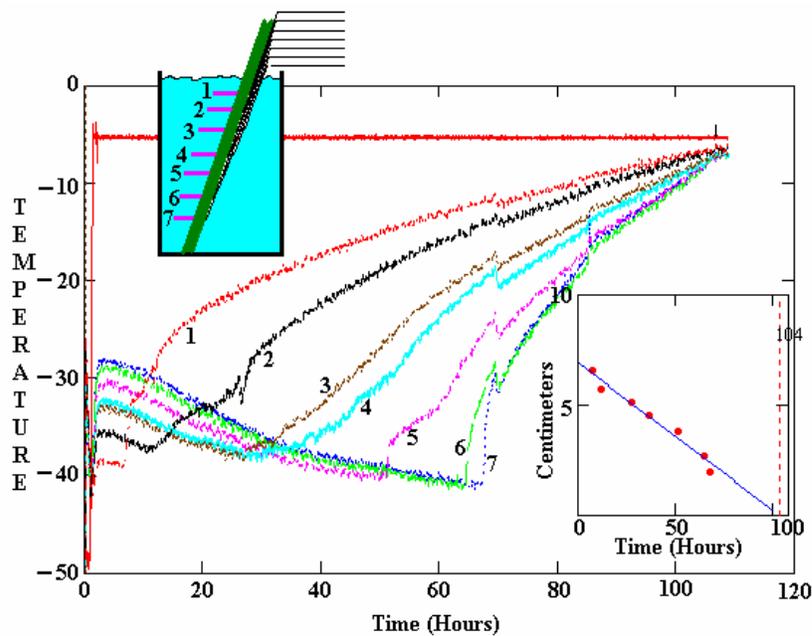
$$l - l_0 = \alpha \cdot l_0 \cdot t$$

$$l = l_0 + \alpha \cdot l_0 \cdot t \quad l(t) := l_0 \cdot (1 + \alpha \cdot t) \quad t := 0s, 250s.. 5000s$$



### Lyophilization Rate Measurements

Figure 8 displays the results obtained from lyophilizing a cylinder of ice that contains multiple thermocouples at a known height within the cylinder.



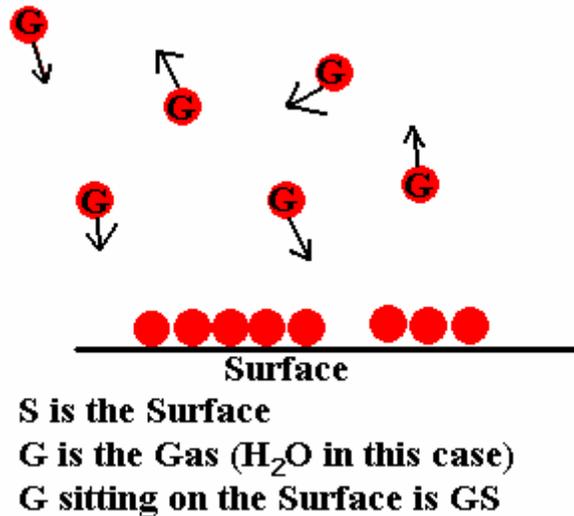
A beaker, containing 219.2 gm of water was placed into the lyophilizer and a cycle was initiated to freeze to  $-50^{\circ}\text{C}$ , evacuate to 150 mtorr and then raise the shelf temperature to  $-5^{\circ}\text{C}$ . As the sublimation front passed each thermocouple the “temperature : time” profile began to rise, thus marking the “extent of sublimation” at

a point in time. As can be seen in the insert to the figure, the fall of the subliming front is linear in time, thus demonstrating zero order kinetics for the phase change.

As an aside, at the start of this experiment, all of the thermocouples are embedded in the same block of ice, yet they all have different temperatures. Furthermore, thermocouple 1 that is closest to the subliming surface is the coldest, followed sequentially by TCs 2 through 7 in order. As each thermocouple comes closer to the subliming front due to ongoing sublimation, its reported temperature decreases to about  $-40^{\circ}\text{C}$  representative of the partial pressure of water vapor in the atmosphere immediately above the ice.

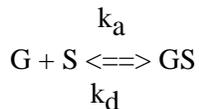
## Langmuir Model for Desorption – Secondary Drying

Irving Langmuir (1881-1957) developed a simple model for adsorption/desorption that is beneficial in understanding secondary drying. It is useful to know that after primary drying sublimation is finished and were the lyophilizer to be opened, the lyophile would appear dry. It is likely that the moisture content would be in the range of 2 to 20% and more likely 5 to 15%. The remaining water molecules are not crystalline but are rather adsorbed to the surface of the solutes left behind after sublimation. Langmuir depicted the situation as shown below.



In the model, G represents a gas molecule and S represents a solute surface. Each gas molecule independently finds a site on the surface. There is no overlap, and all sites are equal for both arrival and departure with no influence on each other. The maximum number of sites is equal to one complete monolayer of molecules.

Derivation of the Langmuir Isotherm



At equilibrium

$$K_{eq} = \frac{[GS]}{[G] \cdot [S]}$$

$\theta$  = Fraction of surface sites occupied ( $0 < \theta < 1$ )

Note that

[GS] is proportional to the surface coverage of adsorbed molecules, or proportional to  $\theta$

[S] is proportional to the number of vacant sites,  $(1 - \theta)$

[G] is proportional to the pressure of gas, P

Thus the equilibrium can be defined and is traditionally called b

$$b = \frac{\theta}{(1-\theta) \cdot P} \quad b = \frac{k_a}{k_d}$$

or, solving for  $\theta(P)$ :

$$\theta(P) := \frac{b \cdot P}{b \cdot P + 1}$$

← Langmuir Isotherm describing the fraction of sites occupied as a function of an equilibrium constant and pressure.

By use of the word “isotherm” we mean “at a single temperature” while varying the pressure what will be the fraction of sites occupied. In the case of secondary drying the goal is to reduce the number of sites occupied, or in plain terms, “make the water go away”. If anything, the above derivation is too simple to be useful. For example, pressure,  $P$ , comes in units but the denominator has a Pressure term and the term “1” with no units. A logical and correct conclusion is that “ $b \cdot P$ ” cancels out the units and since  $\theta$  is without units by definition, “ $b$ ” must come in units of inverse pressure. However, of course  $b$  is also defined as  $k_a/k_b$  which means it has units of  $M^{-1} \text{sec}^{-1} / \text{sec}^{-1} = M^{-1}$ .

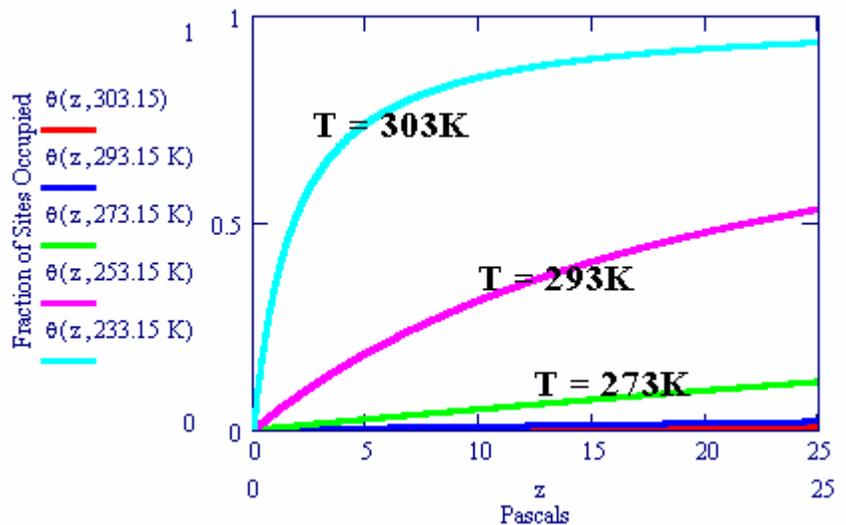
Thus  $b$  is defined in units of both pressure and concentration. Since  $P \cdot V = n \cdot R \cdot T$ , at constant temperature,  $P = (n/V) \cdot R \cdot T$  and  $n/V$  is the definition of concentration.

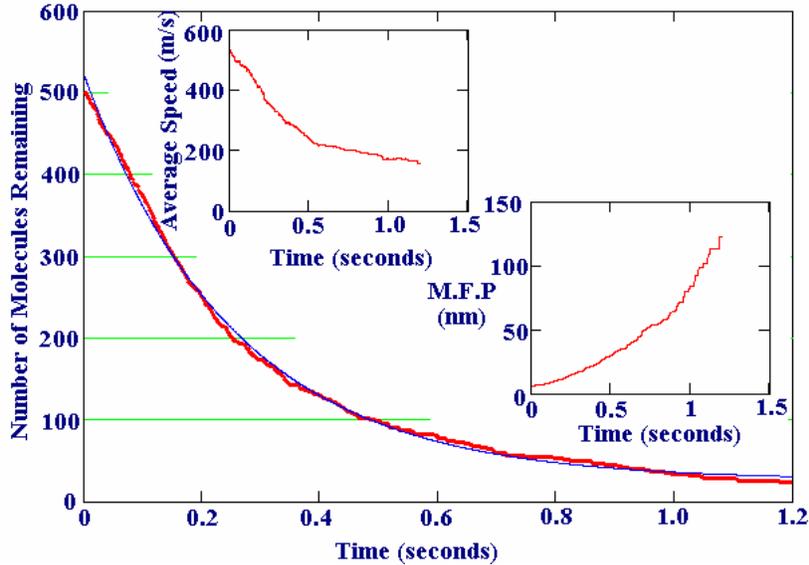
In order to put meaningful numbers into the equations, we can define the following parameters and functions.

$$R := 8.314510 \frac{\text{joule}}{\text{mole} \cdot \text{K}} \quad kJ := 1000J \quad E_a := 60 \frac{\text{kJ}}{\text{mol}} \quad A := 10^{13} \frac{1}{\text{sec}} \quad M := \frac{\text{mol}}{\text{liter}}$$

$$k_a := 400000 M^{-1} \cdot s^{-1} \quad k_d(T) := A \cdot e^{\frac{-E_a}{R \cdot T}} \quad b(T) := \frac{k_a}{k_d(T)}$$

$$\theta(p, T) := \frac{\frac{b(T)}{R \cdot T} \cdot p}{\frac{b(T)}{R \cdot T} \cdot p + 1}$$





### Vapor Escape by Diffusion

Finally, upon clearing the adsorbed surfaces, a water molecule is free to find its way to the condenser by virtue of diffusion. Two things get in the way of a speedy departure. First, fully half of the molecules at any given instance are going the wrong way and second, they continuously bump in to each other. The later reason is the most important thing that is keeping them there. Molecular collisions limit the rate of most phase changes and desorptions. You can greatly speed up vaporization by blowing a strong fan over an evaporating ice or drying cotton shirt. Lick your finger. Then blow on it. Feel that it is significantly cooler. Why, because you have greatly increased the leaving rate by removing those molecules from the surface that bump into molecules trying to leave and cause them to just go back again.

Looking at a Monte Carlo model for diffusion starting with 500 molecules having the Maxwell distribution we observe the result shown in Figure 11.

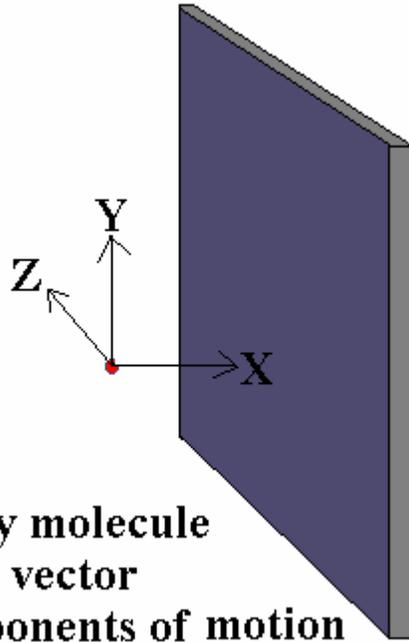
Simple diffusion is first order. The average speed of the molecules slowly decreases because the fastest ones escape and stick to the condenser. The mean free path increases with time (assuming  $N_2$  is not added) since molecules are removed from the box.

### Summary

- Lyophilization is composed of 3 very different steps.
- Sublimation and desorption are mathematically described from kinetic theory.
- Ice temperature is controlled by pressure.
- Sublimation is linear in time, and finished when the thermocouples begin to rise.
- Desorption rates are highly temperature dependent.

## Appendix I

Derivation of an expression for Pressure (from P.W. Atkins)



$m$  is the mass of a molecule  
 $v_x$  is velocity in the x direction  
 $mv_x$  is momentum in the x direction  
 $-mv_x$  is momentum after wall collision  
 $2 \cdot m \cdot |v_x|$  is total change in momentum from one collision.

The number of collisions in  $\Delta t$  depends on how many molecules can reach the wall and thus both how many there are total and how fast they are travelling.

$|v_x| \cdot \Delta t$  is the distance that one molecule can travel in  $\Delta t$ . Of course it may be going the other way!

$A$  is the area of the wall.

$A \cdot |v_x| \cdot \Delta t$  is the volume in which molecules reside that might reach the wall

$N''$  is the number of molecules per unit volume

$N'' \cdot A \cdot |v_x| \cdot \Delta t$  is the number of molecules in the volume that might make impact.

On average, half are going right and half are going left so

$\frac{1}{2} \cdot (N'' \cdot A \cdot |v_x| \cdot \Delta t)$  Average number of collisions in  $\Delta t$ .

Change in Momentum = [Number of collisions] x [momentum change per collision]

$$\Delta \text{Momentum} = \left[ \frac{1}{2} \cdot (N'' \cdot A \cdot |v_x| \cdot \Delta t) \cdot (2 \cdot m \cdot |v_x|) \right] = m \cdot N' \cdot A \cdot v_x^2 \cdot \Delta t$$

The change in momentum per unit time or

Rate of change of momentum is  $m \cdot N' \cdot A \cdot v_x^2$  which according to Newton is force.

$$\text{Force} = m \cdot N'' \cdot A \cdot v_x^2$$

Pressure = Force/Area

$P = m \cdot N'' \cdot v_x^2$  Accurate, but not useful since the group of molecules going in the x direction is hard to know.

Also, there is a distribution of molecular speeds from "go fast" to "go slow" so we would want to use the average  $v_x$  written  $\langle v_x \rangle$ . Without definite flow, the average velocity is the same in all directions.  $\langle v_x^2 \rangle = \langle v_y^2 \rangle = \langle v_z^2 \rangle$ .

So the average velocity  $\langle v^2 \rangle = \langle v_x^2 \rangle + \langle v_y^2 \rangle + \langle v_z^2 \rangle = 3\langle v_x^2 \rangle$

Let  $c^2 = \langle v^2 \rangle$

$c^2$  is the mean square speed

$$P = \frac{1}{3} \cdot N'' \cdot m \cdot c^2 \quad \text{From this we can derive the Ideal Gas Law}$$

Replace  $N''$ , the number of molecules per unit volume, with  $N/V$  where  $N$  is the actual number of molecules and  $V$  is system volume.

$$P = \frac{1}{3} \cdot \frac{N}{V} \cdot m \cdot c^2$$

Further, replace  $N$  with  $n$  (moles)  $\times N_0$  (Avogadro's Number)

$$P = \frac{1}{3} \cdot \frac{n \cdot N_0}{V} \cdot m \cdot c^2 \quad \{\text{EQ 1}\}$$

From classical physics, Kinetic Energy =  $\frac{1}{2} \cdot m \cdot v^2$  or  $\frac{1}{2} \cdot m \cdot v^2 \cdot N_0$  for 1 mole.

Also from classical physics, mean K.E. =  $\frac{3}{2} \cdot R \cdot T$

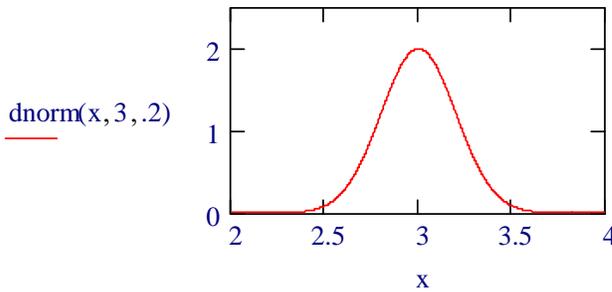
$$\frac{1}{2} \cdot m \cdot c^2 \cdot N_0 = \frac{3}{2} \cdot R \cdot T \quad \text{i.e.} \quad R \cdot T = \frac{1}{3} \cdot m \cdot c^2 \cdot N_0$$

Rearranging EQ1,  $P \cdot V = n \cdot \frac{1}{3} \cdot m \cdot c^2 \cdot N_0 = n \cdot R \cdot T$

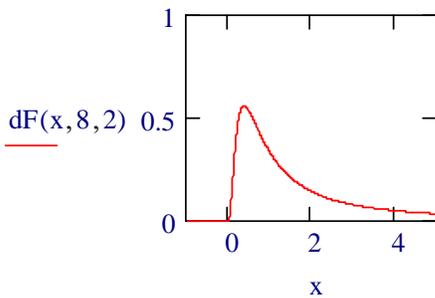
## Appendix II

### Derivation of The Velocity Distribution Function

A distribution function of the property,  $X$ , has as a definition that the property lies somewhere in the range  $X$  to  $X+\Delta X$ .



For example, this is a normal distribution of the property,  $x$ , with a mean of 3 and one standard deviation of 0.2.



This is a statistical F distribution with 8 and 2 degrees of freedom

Distribution functions show the probability,  $P(X)$ , of  $X$  in the range.

$$P(X) = f(X) \Delta X$$

The probability holds whether the range is discrete,  $\Delta X$  or continuous,  $dx$ .

$$P(X) = f(X) dx$$

We know that the probability of two independent events is the product of the probability of each event alone.

$$P(X, Y) = f(X) f(Y) \cdot dx \cdot dy$$

The velocity of a molecule is the vector sum of the  $v_x$ ,  $v_y$ ,  $v_z$  velocity components.

$$\left| \begin{pmatrix} v_x \\ v_y \\ v_z \end{pmatrix} \right| \rightarrow \left[ (|v_x|)^2 + (|v_y|)^2 + (|v_z|)^2 \right]^{\frac{1}{2}} \quad \text{i.e. magnitude of the 3d vector.}$$

$$v^2 = v_x^2 + v_y^2 + v_z^2$$

Since the distribution function,  $F(v_x, v_y, v_z)$  depends only on velocities (not direction), it can be written as  $F(v_x^2 + v_y^2 + v_z^2) = f(v_x) \cdot f(v_y) \cdot f(v_z)$  The right side, due to the probability of independent events.

Because  $e^{a+b} = e^a \cdot e^b$ , only an exponential can be a solution.

$f(v_x) \cdot dv_x = K \cdot e^{-\zeta \cdot v_x^2} \cdot dv_x$  An appropriate trial solution with K and  $\zeta$  as constants. The negative exponent is used to limit extreme velocities.

To establish the value of K and  $\zeta$ , we have two observations.

First)  $\int_{-\infty}^{\infty} f(v_x) dx = 1$  The Probability of velocity has to be somewhere!

Trying out our GUESS solution for  $f(v_x)$ ,

$$\int_{-\infty}^{\infty} f(v_x) dx = K \cdot \int_{-\infty}^{\infty} e^{-\zeta \cdot v_x^2} dv_x = K \cdot \sqrt{\frac{\pi}{\zeta}} \quad \leftarrow \text{From table of integrals}$$

Thus  $K = \sqrt{\frac{\zeta}{\pi}}$

Second) Zeta,  $\zeta$ , is obtained by finding the mean square speed of the molecules and substituting into a form of the ideal gas law, namely,  $P \cdot V = \frac{1}{3} \cdot n \cdot N_0 \cdot m \cdot c^2$  {EQU 3}

where  $n$  = moles,  $N_0$  = Avogadro,  $m$ =mass, and  $c^2$ ="mean square speed".

Aside: From Statistics, a general expression for computation of a mean is

$$\text{mean}(X) = \int X \cdot f(X) dX$$

where  $X = v_x^2$   $\text{mean}(v_x^2) = \int_{-\infty}^{\infty} v_x^2 \cdot f(v_x) dv_x$  and substituting in the proposed

solution for  $f(v_x)$ :

$$\text{mean}(v_x^2) = \sqrt{\frac{\zeta}{\pi}} \cdot \int_{-\infty}^{\infty} v_x^2 \cdot e^{-\zeta \cdot v_x^2} dv_x = \frac{1}{2} \cdot \sqrt{\frac{\zeta}{\pi}} \cdot \sqrt{\frac{\pi}{\zeta^3}} = \frac{1}{2 \cdot \zeta} \quad \leftarrow \text{Integral from Tables}$$

$$\text{mean}(v_x^2) = \frac{1}{2 \cdot \zeta} \quad \text{Now Generalize to 3d}$$

$$\text{mean}(v^2) = c^2 = v_x^2 + v_y^2 + v_z^2 = \frac{3}{2\zeta} \quad \text{Since } v_x^2 = v_y^2 = v_z^2$$

Now substitute  $\frac{3}{2\zeta}$  for  $c^2$  in {EQU 1}.

$$P \cdot V = \frac{1}{3} \cdot n \cdot N_0 \cdot m \cdot \frac{3}{2\zeta}$$

$P \cdot V = n \cdot R \cdot T = n \cdot N_0 \cdot k \cdot T$  another form of the Ideal Gas Law where  $k = \text{boltzman const}$

$$\frac{1}{3} \cdot n \cdot N_0 \cdot m \cdot \frac{3}{2\zeta} = n \cdot N_0 \cdot k \cdot T \quad \text{Equating the two equations}$$

$$\frac{m}{2\zeta} = k \cdot T \quad \& \quad \zeta = \frac{m}{2 \cdot k \cdot T} \quad \text{Now plug } \zeta \text{ and } K \text{ into the proposed solution}$$

$$f(v_x) = K \cdot e^{-\zeta \cdot v_x^2} \quad \leftarrow \text{Proposed Solution}$$

$$f(v_x) = \sqrt{\frac{m}{2 \cdot k \cdot T}} \cdot \frac{1}{\pi} \cdot e^{-\frac{m}{2 \cdot k \cdot T} \cdot v_x^2} \quad \text{and simplify}$$

$$f(v_x) = \sqrt{\frac{m}{2 \cdot \pi \cdot k \cdot T}} \cdot e^{\frac{-1}{2} \cdot \frac{m \cdot v_x^2}{k \cdot T}} \quad \text{One Dimensional Solution to the Velocity Distribution}$$

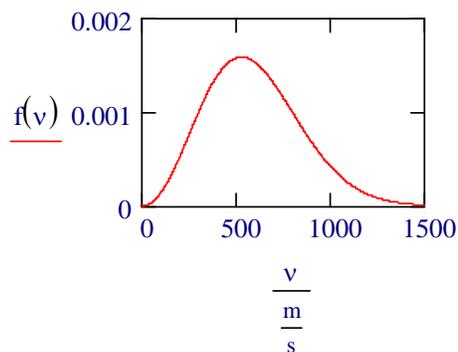
By extension, to a 3d solution:

$$df(v_x, v_y, v_z) = df(v) = \left( \frac{m}{2 \cdot \pi \cdot k \cdot T} \right)^{\frac{3}{2}} \cdot e^{\frac{-m \cdot (v_x^2 + v_y^2 + v_z^2)}{2 \cdot k \cdot T}} \cdot dv_x \cdot dv_y \cdot dv_z$$

It would be nice to convert this expression over to  $v$ , molecular velocity, rather than the individual components. Note:  $v \neq v_x + v_y + v_z$

The volume element,  $dv_x \cdot dv_y \cdot dv_z$ , may be written as  $v^2 dv \cdot d\omega$  where  $d\omega$  is the infinitesimal solid angle and then integrate over the full sphere, i.e. 0 to  $4\pi$ .

$$\int_0^{4\pi} \left( \frac{m}{2 \cdot \pi \cdot k \cdot T} \right)^{\frac{3}{2}} \cdot v^2 \cdot e^{\frac{-m \cdot (v)^2}{2 \cdot k \cdot T}} \cdot dv d\omega = 4 \cdot \pi \cdot \left( \frac{m}{2 \cdot \pi \cdot k \cdot T} \right)^{\frac{3}{2}} \cdot v^2 \cdot \exp\left(\frac{-1}{2} \cdot \frac{m \cdot v^2}{k \cdot T}\right) \cdot dv$$



## Appendix III

### Clausius Clapeyron Derivation

Consider any equilibrium reaction involving solid s and gas g. For a closed system, we have, from the definition of the Gibbs free energy:

$$dG_s = V_s dP - S_s dT \quad (1) \text{ and}$$

$$dG_g = V_g dP - S_g dT \quad (2)$$

We also know that the change in Gibbs free energy between two coexisting phases is the same.

$$V_s dP - S_s dT = V_g dP - S_g dT \quad (3) \text{ rearranging we have}$$

$$(V_s - V_g) dP = (S_s - S_g) dT \quad (4) \text{ and}$$

$$\frac{\Delta V}{dT} = \frac{\Delta S}{dP} \quad (5) \text{ Clapeyron}$$

This is the formal form of the Clapeyron equation, which gives the P-T trajectory of equilibrium states for a reaction as a function of the entropy and volume changes. Remembering the definition of entropy, it is easily shown that, at any specific pressure:

$$\Delta S = \frac{\Delta H}{T} \quad (6) \text{ Definition of entropy}$$

$$\frac{\Delta V}{dT} = \frac{\Delta H}{T \cdot dP} \text{ or } \frac{d}{dT} P = \frac{\Delta H}{T \cdot \Delta V} \quad (7) \text{ Clausius Clapeyron General}$$

$$\int_0^{P1} 1 dP = \frac{\Delta H}{\Delta V} \cdot \int_{T1}^{T2} \frac{1}{T} dT$$

This is true if  $\Delta H$  and  $\Delta V$  are approximately constant with changes in Temp. For example if we are going from a solid to a liquid. But we know that  $\Delta V$  varies a lot as we go from solid to gas, so read on.

$$P = \frac{\Delta H}{\Delta V} \cdot \ln\left(\frac{T2}{T1}\right) \quad \Leftarrow \text{OK for a liquid to solid transition.}$$

The Clausius variation:

A major approximation comes from noting that the volume of a given amount of gas is much larger than the volume of an equivalent amount of solid or liquid. Thus, we can approximate  $\Delta V$  by the volume of the gas.

$$\Delta V = V_g - V_s \gg V_g \quad \text{so replace } \Delta V \text{ with } V_g \text{ in } \frac{d}{dT}P = \frac{\Delta H}{T \cdot \Delta V}$$

and we know that  $V_g$  is  $V_g = nRT/P$  from the ideal gas law (also an approximation, since gasses aren't necessarily ideal).

$$\frac{1}{P} \cdot \frac{d}{dT}P = \frac{\Delta H}{T \cdot n \cdot R \cdot T} = \frac{\Delta H'}{R \cdot T^2} \quad \text{the prime means that } \Delta H \text{ is a molar enthalpy}$$

$$\int_{p1}^{p2} \frac{1}{P} dP = \frac{\Delta H'}{R} \cdot \int_{T1}^{T2} \frac{1}{T^2} dT \quad \int_{T1}^{T2} \frac{1}{T^2} dT \rightarrow \frac{-1}{T2} + \frac{1}{T1} \quad \int_{p1}^{p2} \frac{1}{P} dP \rightarrow \ln(p2) - \ln(p1)$$

$$\ln\left(\frac{P2}{P1}\right) = \frac{\Delta H'}{R} \cdot \left(\frac{-1}{T2} + \frac{1}{T1}\right)$$

$$\frac{P2}{P1} = e^{\frac{-\Delta H'}{R} \cdot \left(\frac{1}{T2} - \frac{1}{T1}\right)}$$

where  $\Delta T = T2 - T1$

$$P = P1 \cdot e^{\frac{-\Delta H'}{R} \cdot \left(\frac{1}{T} - \frac{1}{T1}\right)}$$

Now if we know one point (P1, T1) and  $\Delta H'$ , then we can solve for the curve.

From the table, at  $T = -15^\circ\text{C}$   $P = 1.241$  torr, and  $\Delta H_{\text{sub}} := 51102 \frac{\text{J}}{\text{mol}}$

$$R := 8.314510 \cdot \frac{\text{J}}{\text{mol} \cdot \text{K}}$$

$$P = 1.241 \text{ torr} \cdot e^{\frac{-\Delta H_{\text{sub}}}{R \cdot T}} \cdot e^{\frac{\Delta H_{\text{sub}}}{R(-15+273.15)\text{K}}}$$

$$1.241 \text{ torr} \cdot e^{\frac{\Delta H_{\text{sub}}}{R(-15+273.15)\text{K}}} = 2.714 \times 10^{10} \text{ torr}$$

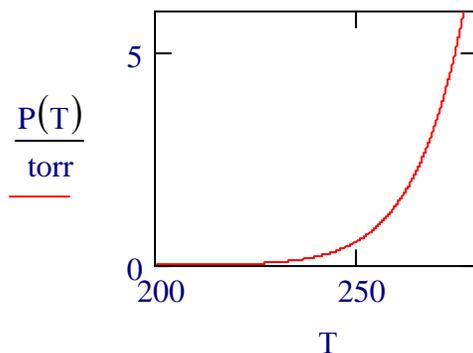
$$A := 2.714 \cdot 10^{10} \cdot \text{torr} \quad \text{derived from 1 point.}$$

$$P(T) := A \cdot e^{\frac{-\Delta H_{\text{sub}}}{R \cdot T}} \quad \text{General form for the Pressure Temperature Curve of Sublimation.}$$

Instead of using 1 point (P,T) to find the constant A, given the inherent error associated with measurement. We can use all of the measured points

and note that a graph of  $\ln(P)$  vs  $e^{\frac{-\Delta H_{\text{sub}}}{R \cdot T}}$  would have slope = A.  
Thus a more precise value for A is

$$A := 2.710 \cdot 10^{10} \text{ torr}$$



## Problem 1.

Suppose a lyo chamber has been backfilled with filtered room air to 13 psia and then the stoppers set. The product mass without water calculated to 40mg. On the day of the backfill, the room RH was 45% and the room Temp was 21C. Could enough moisture have gotten into the vial to raise the usual 0.75% moisture to 4%? The vial dimensions are ID = 24.5mm and height := 52mm.

Solution:

**Step 1.** Use an equation for Relative Humidity along with the saturated vapor pressure from the Clausius Clapeyron to obtain the absolute humidity in the vial, and thus the amount of water in the vial.

$$RH = 100 \cdot \frac{e_e}{e_s}$$

$e_e$  is the absolute vapor pressure

$e_s$  is the saturated vapor pressure and is

. temp dependent. It is the number given by .  
. the Clausius Clapeyron.

**Step 2.** Look up in a table and find the saturated vapor pressure of water at 21 °C.

$e_s := 18.65 \text{ torr} \Leftarrow$  Saturated Vapor Pressure at 21 °C. *Note: One can't use the Clausius Expression derived in this presentation because it is for the saturated vapor pressure of water above Ice.*

**Step 3.** Determine " $e_e$ ", the absolute vapor pressure.

$$45 = \frac{100 \cdot e_e}{e_s}$$

$$e_e := \frac{45}{100} \cdot e_s$$

$e_e = 8.393 \text{ torr} \Leftarrow$  Absolute pressure of water vapor in the room and in the vial.

**Step 4.** Use Concentration =  $n/V = P/RT$  to get Concentration Units.

$8.393 \text{ torr} = 1.119 \times 10^3 \text{ Pa} \Leftarrow$  It helps a lot to use a consistent set of units.

$$\frac{1.119 \cdot 10^3 \text{ Pa}}{8.314472 \cdot \frac{\text{J}}{\text{mol} \cdot \text{K}} \cdot (21\text{K} + 273.16\text{K})} = 457.522 \times 10^{-6} \frac{\text{mol}}{\text{liter}} \Leftarrow \text{water conc in vial}$$

**Step 5.** Estimate the maximum possible volume of the vial.

$$\text{Vol}_{\text{vial}} := \left( \frac{24.5\text{mm}}{2} \right)^2 \cdot \pi \cdot \text{height}$$
$$\text{Vol}_{\text{vial}} = 24.515 \text{ cm}^3$$

**Step 6.** From the concentration and volume get the amount of water in the vial.

$$457.522 \cdot 10^{-6} \cdot \frac{\text{mol}}{\text{liter}} \cdot 24.515 \cdot 10^{-3} \text{ liter} = 11.216 \times 10^{-6} \text{ mol} \leq \text{mols of water in vial.}$$

$$11.22 \cdot 10^{-6} \text{ mol} \cdot 18 \frac{\text{gm}}{\text{mol}} = 201.96 \times 10^{-6} \text{ gm} \leq \text{grams of water in vial.}$$

**Step 7.** By % mass, how much could the water from the air contribute?

$$\frac{201.96 \cdot 10^{-6} \cdot \text{gm}}{40 \cdot 10^{-3} \text{ gm}} \cdot 100 = 0.505 \leq \text{Answer. Water from air could add 0.5\%}$$

Problem #2 A Pinhole Leak

If a lyophilizer chamber had a volume of 1312 liters, already excluding the shelves, hoses, etc., and it had a measured leak rate of 1.5 mTorr/min, what size would the hole be if it were a single simple pinhole through the wall?

Solution:

We will address the question through a series of steps as follows.

**Step 1.** Obtain an expression  $\nu$  for the number of  $\frac{\text{collisions}}{\text{m}^2 \cdot \text{sec}}$  with a wall, just as if we knew the Area of the hole - which of course we don't.

The expression will be  $\nu = \frac{n' \cdot v_m}{4}$  where  $n'$  is number of  $\frac{\text{molecules}}{\text{m}^3}$  and  $v_m$  is the mean velocity from kinetic theory.

This Step is a problem all by itself, and it isn't shown here.

**Step 2.** Separately, determine the mass,  $w$ , of  $\frac{\text{collisions}}{\text{m}^2 \cdot \text{sec}}$ .  $w = \frac{\text{mass}}{\text{Area} \cdot \text{sec}}$

$w = \frac{v \cdot M}{N_a}$  where  $\nu$  is from step 1 and  $M$  is molecular mass and  $N_a$  is Avogadro's number.

$$w = \frac{\text{kg}}{\text{m}^2 \cdot \text{sec}} = \frac{\text{mass}}{\text{Area} \cdot \text{sec}}$$

**Step 3.** Substitute  $\nu$  from EQN 1 into EQN 2 and solve for  $n'$ .

$$w = \frac{n' \cdot v_m}{4} \cdot \frac{M}{N_a} \quad \text{solving for } n', \quad n' = \frac{4 \cdot w \cdot N_a}{v_m \cdot M}$$

**Step 4.** Introduce a form of the Ideal Gas Law that contains  $n'$

$$P = \frac{n'}{N_a} \cdot R \cdot T \quad \text{comment:} \quad \frac{n}{V} = \frac{n'}{N_a} = \frac{\frac{\text{molecules}}{\text{m}^3}}{\frac{\text{molecules}}{\text{mole}}} = \frac{\text{mol}}{\text{m}^3}$$

**Step 5.** Substitute n' from step 3 into the gas law from step 4.

$$P = \frac{4 \cdot w \cdot N_a}{v_m \cdot M} \cdot R \cdot T$$

$$P = \frac{4 \cdot w \cdot R \cdot T}{v_m \cdot M}$$

**Step 6.** Now Substitute in the kinetic theory expression for  $v_m$

$$v_m = \sqrt{\frac{8 \cdot R \cdot T}{\pi \cdot M}} \quad P = \frac{4 \cdot w \cdot R \cdot T}{\sqrt{\frac{8 \cdot R \cdot T}{\pi \cdot M}} \cdot M}$$

which, with a little algebra simplifies to  $P = w \cdot \sqrt{\frac{2 \cdot \pi \cdot R \cdot T}{M}}$

**Step 7.** From the data of the problem, we can obtain a value for w, the mass of molecules per unit area per sec. If we assume that all of the molecules that try to get in succeed (a pretty good assumption, considering the pressure differential inside and out), then find a number for moles/sec.

$$\Delta P := 1.5 \cdot 10^{-3} \text{ torr} = (0.200 \text{ Pa})$$

$$n = \frac{P \cdot V}{R \cdot T} \quad \text{and} \quad \frac{\Delta n}{\text{sec}} = \frac{\Delta P}{R \cdot T} \cdot V = \frac{0.2 \frac{\text{Pa}}{60\text{s}} \cdot 1312 \text{ liter}}{8.314472 \frac{\text{J}}{\text{mol} \cdot \text{K}} \cdot (25\text{K} + 273.16\text{K})} = 1.764 \times 10^{-6} \frac{\text{mol}}{\text{s}}$$

**Step 8.** Substitute into the equation of Step 6 and then solve for Area.

$P = 1 \text{ atm}$  The pressure presented to the pin hole.

$w = 1.76 \cdot 10^{-6} \frac{\text{mol}}{\text{Area} \cdot \text{sec}} \cdot 28.9 \frac{\text{gm}}{\text{mol}}$  where Area is the Area of the pinhole and 28.9 is an average molecular weight for air.

$$P = w \cdot \sqrt{\frac{2 \cdot \pi \cdot R \cdot T}{M}}$$

$$1.013 \cdot 10^5 \text{ Pa} = 1.76 \cdot 10^{-6} \cdot \frac{\text{mol}}{\text{Area} \cdot \text{sec}} \cdot 0.0289 \cdot \frac{\text{kg}}{\text{mol}} \cdot \sqrt{\frac{2 \cdot \pi \cdot 8.314472 \cdot \frac{\text{J}}{\text{mol} \cdot \text{K}}}{0.0289 \cdot \frac{\text{kg}}{\text{mol}}}}$$

solving for Area

$$\text{Area} = \frac{n}{s} \cdot \frac{M}{P} \cdot \sqrt{\frac{2 \cdot \pi \cdot R \cdot T}{M}}$$

$$\text{Area} := 1.76 \cdot 10^{-6} \frac{\text{mol}}{\text{s}} \cdot \frac{0.0289 \cdot \frac{\text{kg}}{\text{mol}}}{1.013 \cdot 10^5 \cdot \text{Pa}} \cdot \sqrt{\frac{2 \cdot \pi \cdot 8.314472 \cdot \frac{\text{J}}{\text{mol} \cdot \text{K}} \cdot (25\text{K} + 273.16\text{K})}{0.0289 \cdot \frac{\text{kg}}{\text{mol}}}}$$

$$\text{Area} = 3.686 \times 10^{-10} \text{ m}^2 \quad \text{Area} = 3.7 \text{ sq Angstrom!!}$$

$$\text{Area} = \pi \cdot r^2 \quad r := \sqrt{\frac{\text{Area}}{\pi}}$$

$$r = 10.832 \times 10^{-6} \text{ m} \quad \text{about } 21.7 \text{ } \mu\text{m} \text{ for a hole diameter}$$

Note that the calculation for the hole size doesn't depend on the chamber volume, but does require an assumption that it is essentially a perfect vacuum by comparison to atmospheric conditions.