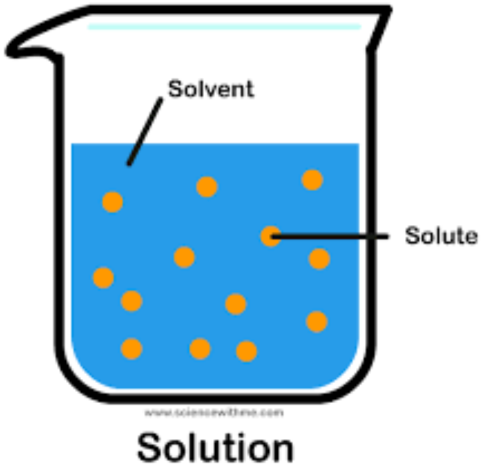
Sultan Qaboos University
College of Medicine & Health Science
Methods in Clinical Chemistry (BIOC4201)



Osmolality and Osmometry

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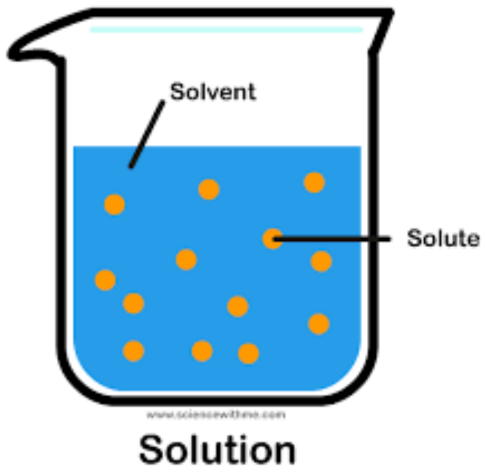
What is Osmolality?

Osmolality: is a measurement of the total number of **solutes** in a liquid solution expressed in osmoles of solute particles per kilogram of **solvent**.

(mOsmole/kg of solvent)

When any solute is dissolved in a solvent, **four** of the *colligative properties* of the solution are affected in a roughly linear response to the concentration of solute added:



Colligative Properties:

Colligative Properties:
Boiling Point
Freezing Point
Osmotic Pressure
Vapor Pressure

Effect

Change per mole solute per kg solvent:	
Boiling Point Elevation	0.52°C
Freezing Point Depression	1.86°C
Osmotic Pressure Elevation	17,000 mm Hg
Vapor Pressure Depression	0.3mm Hg

Properties of Solutions:

The resulting changes in these properties are not proportional to the weight, size or shape of the dissolved particles, but only to their molal concentration.

Osmolality therefore is an ideal measurement to estimate the total concentration of solutes in a near limitless variety of liquid sample matrices, including blood, serum, plasma, urine, milk, cell culture media and almost all forms of aqueous based solutions.

- One mole of urea will **depress** the freezing point of the solution by 1.86°C

- One mole of sodium chloride NaCl (1 mole Na^+ + 1 mole Cl^-) will **depress** the freezing point by 3.72°C

- One mole of calcium chloride $CaCl_2$ (1 mole Ca^{2+} + 2 mole Cl^-) will **depress** the freezing point by 5.58°C

- The actual mass of the particle is irrelevant, since a small molecule will exert the same effect as a large molecule

Osmolarity vs. Osmolality

- **Osmolarity:** is a measure of the osmoles of solute per liter of solution. A capital letter "**M**" is used to abbreviate units of **mol/L**. Since the volume of solution changes with the amount of solute added as well as with changes in temperature and pressure, *Osmolarity is difficult to determine*.

- **Osmolality:** is a measure of the osmoles of solute per kilogram of solvent expressed as **(mol/kg, molal, or m)**. Since the amount of solvent will remain constant regardless of changes in temperature and pressure, osmolality is easier to evaluate and is more commonly used, and often preferred, in practical osmometry. Most commercially available osmometers report results using **osmolality** units.

Types of Osmometers:

- Freezing Point Osmometers – determine the osmotic strength of solution by utilizing freezing point depression

- Vapor Pressure Osmometers – determine the concentration of osmotically active particles that reduce the vapor pressure of the solution

- Membrane Osmometers – measure the osmotic pressure of a solution separated by a semipermeable membrane

Advantages and disadvantages of the different osmometer

Osmometer Type:	*Advantages :*	*Disadvantages:*	*Comments:*
Freezing Point Osmometry (FPO)	• Performs rapid and inexpensive measurements • Simple and reliable performance • Industry preferred FP method • Small sample size (nL to μL range) • Ideal for dilute biological and aqueous solutions	• Samples must be of low viscosity • Not ideally suited for high molality or colloidal solutions	FPO provides rapid and inexpensive results with the industry preferred freezing point method. Requires small sample size and ideally suited for most biological and aqueous applications.

Advantages and disadvantages of the different osmometer

Vapor Pressure Osmometry (VPO)	• Performs rapid and inexpensive measurements • Small sample size (nL to μL range) • Ideal for dilute biological and aqueous solutions	• Less accurate than FPO • Cannot be used for volatile solutes like alcohols or other organic solvents • Not ideally for high molality or colloidal solutions	VPO provides fast an inexpensive measurements requiring a small sample size, although not as fast or reliable as FPO. Volatile solutes are not amenable to VPO limiting its utility in many applications

Advantages and disadvantages of the different osmometer

Membrane Osmometry (MO)	• Provides potentially unlimited direct measurement of osmotic pressure and solution osmolality • Good for colloidal solutions • No limitation on sample concentration • Can determine MW of macromolecules	• Time consuming and difficult to operate • Requires large sample volume • Not applicable for small molecules and aggressive solvents due to membrane porosity and compatibility • Irreproducible results due to clogging of membrane pores	MO provides a direct measurement of osmolality and is suitable for high molality and colloidal samples. Long analysis time and requires a large sample volume. Not applicable for small molecule applications.

Using a freezing point depression osmometer to measure serum osmolality

In a freezing point depression osmometer, a sample of serum/ urine is introduced to a cooling chamber where it is cooled to a temperature below its expected freezing point.

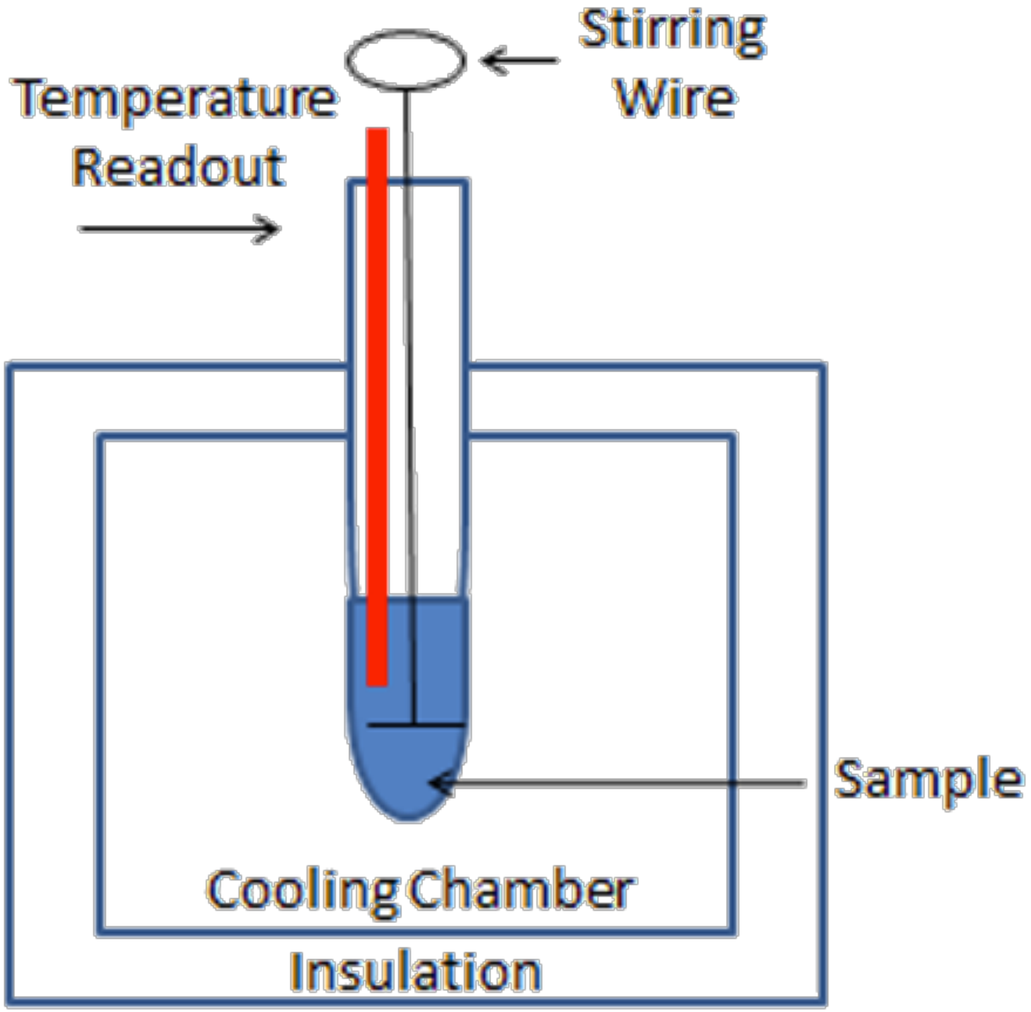
The sample is moved and mixed with a wire, which causes the sample to become partially crystallized. The heat generated from this process brings the sample to a temperature that is equal to its freezing point.

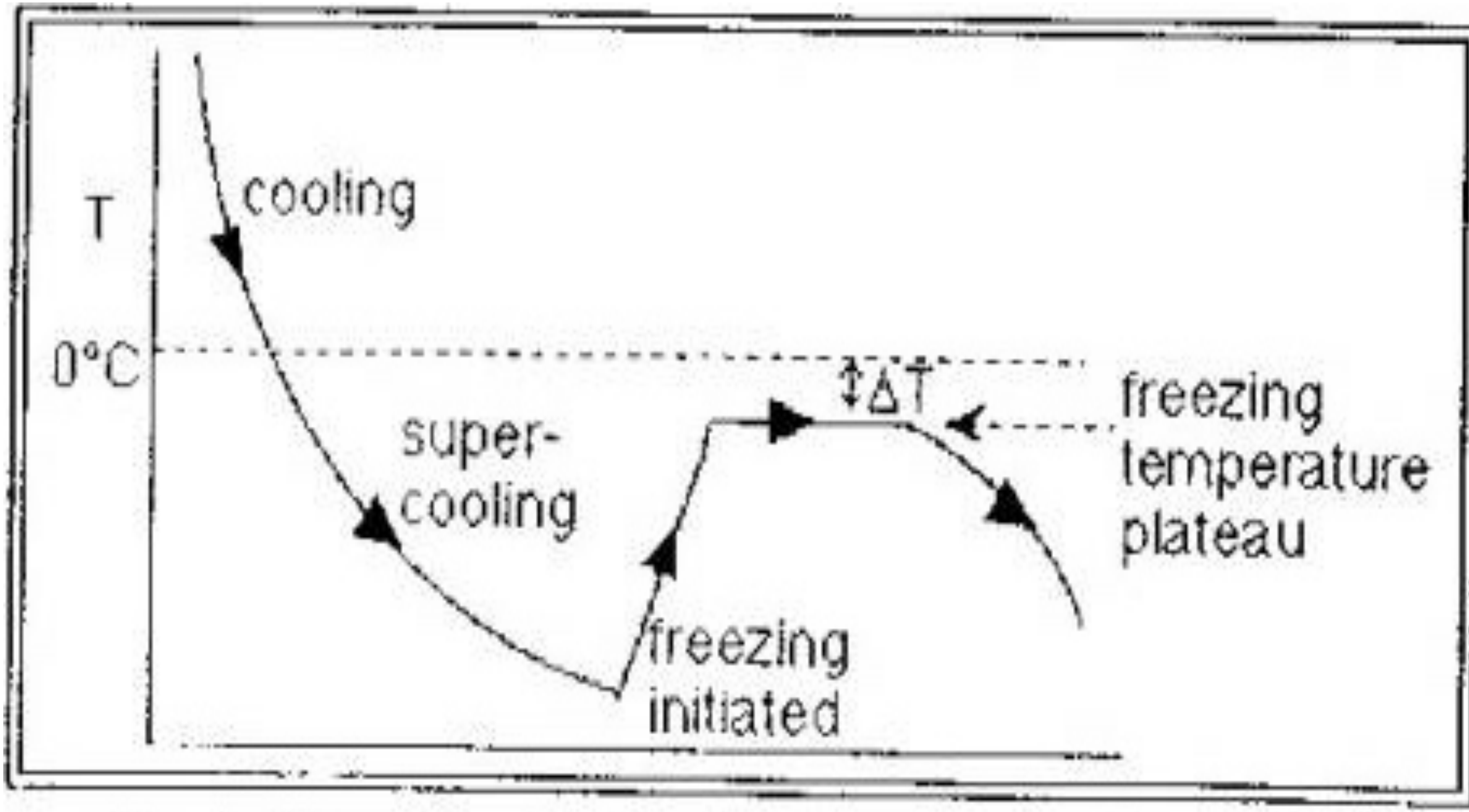
A standard freezing curve is generated, and the osmolality of the serum sample is calculated from the freezing point depression using the following equation:

$$\Delta T = kf \times osmolality\text{, where}$$

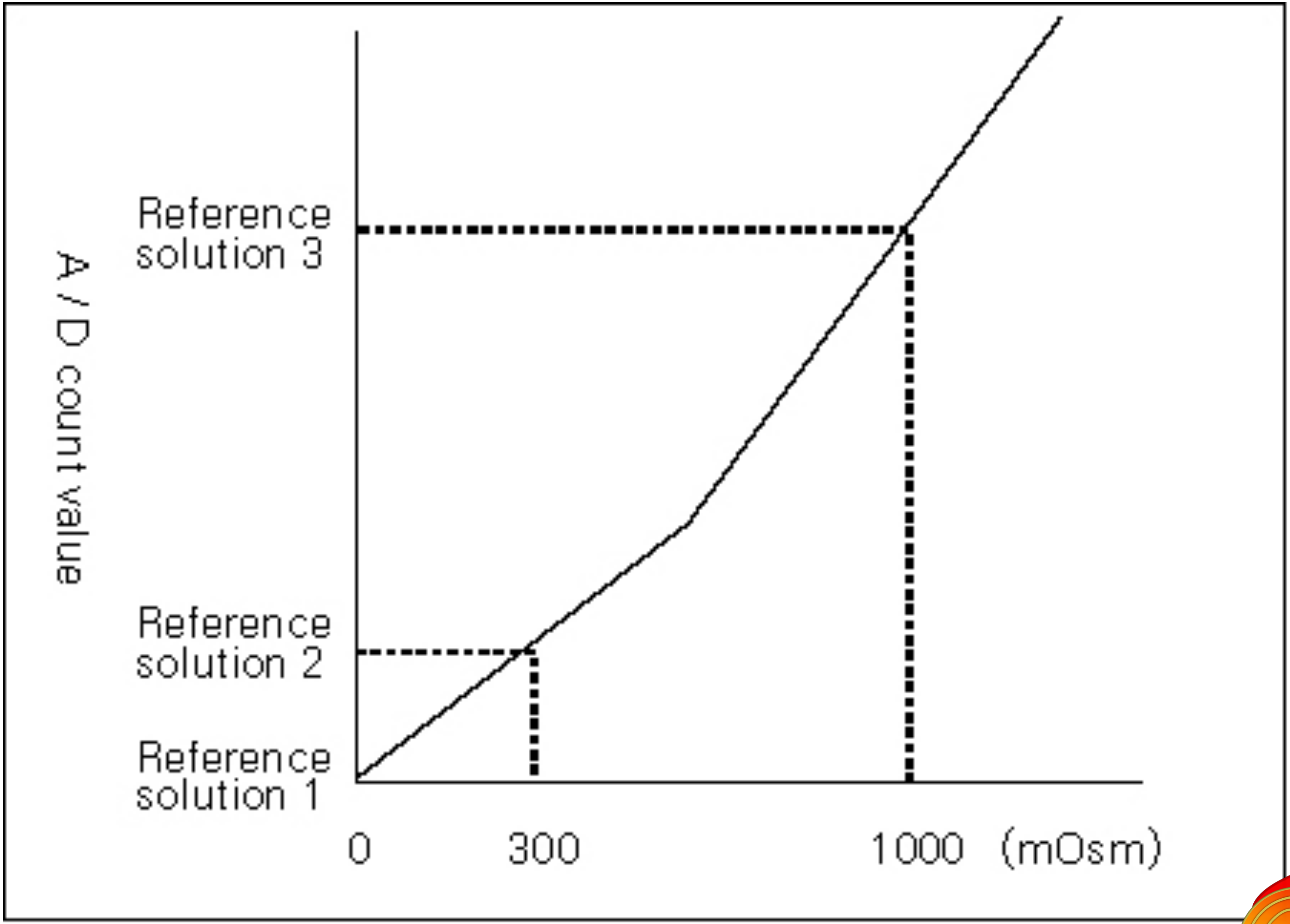
where , kf is known as the cryoscopic constant, which is equal to $1.86 K \cdot kg/mol$.

Schematic Diagram of a Freezing Point Depression Osmometer





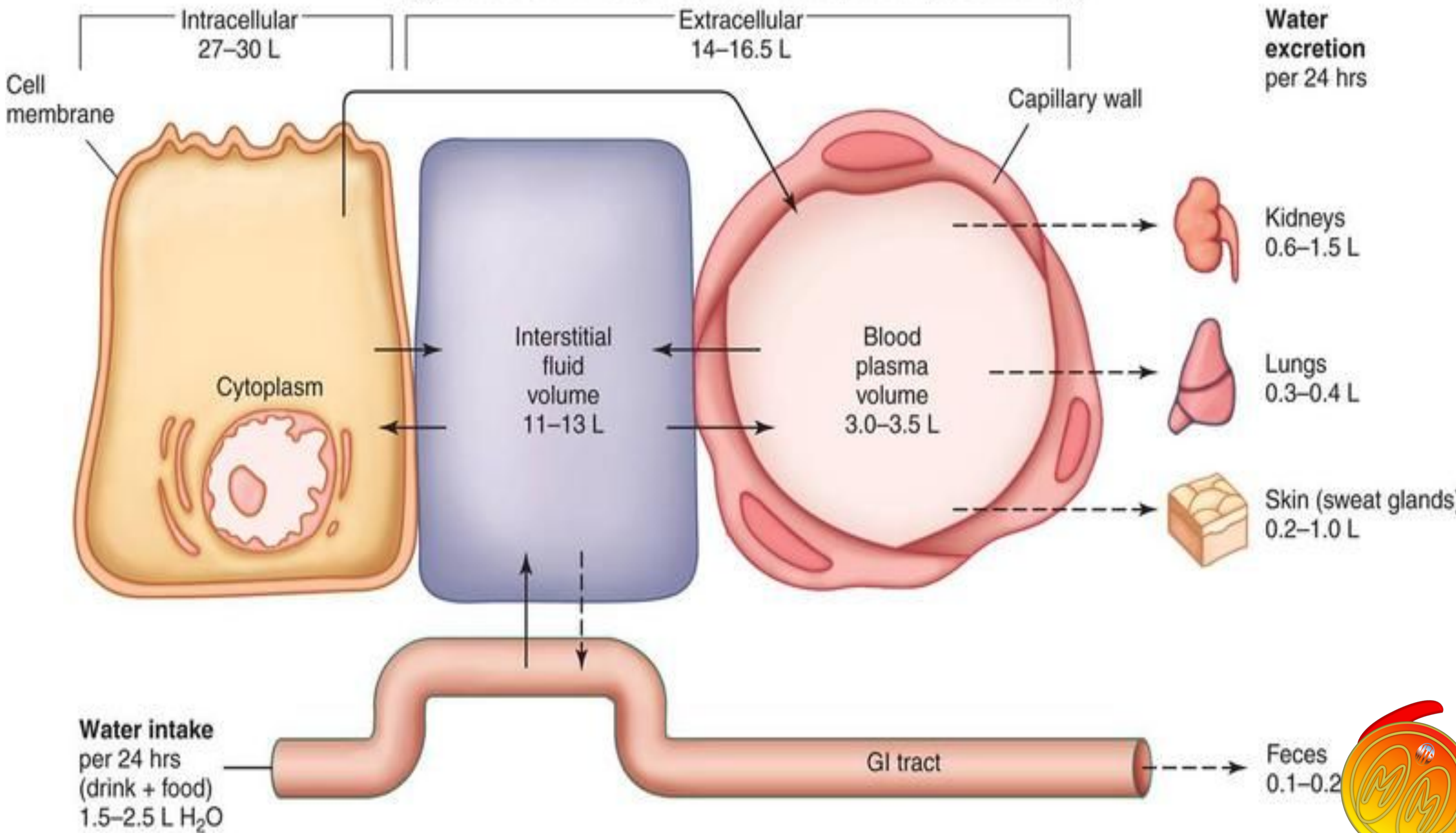
Graph illustrating the freezing point change (depression)



Analytical curve graph using 3 point calibration metho


50
mOsm/kg
Calibration Standard
1500
mOsm/kg
Calibration Standard
850
mOsm/kg
Calibration Standard
900
100
2000
500
1500

Body Compartments:



Water distribution in 70 kg man
60% of total body weight (42L)

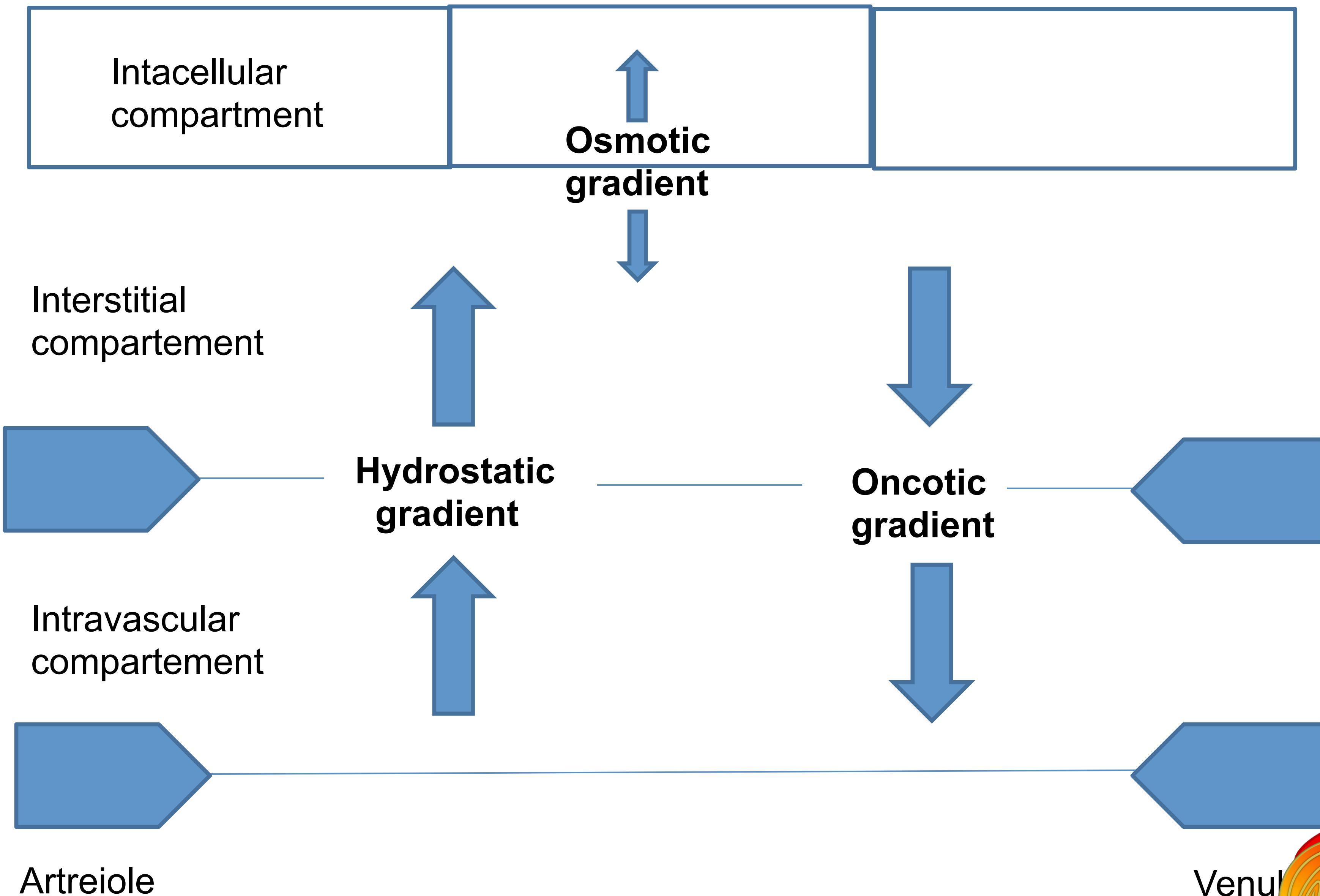
Plasma
5 L

Interstitial
Fluid
13 L

Intracellular
Fluid
24 L

<u>Water flow through compartments are influenced by</u>:

- Osmotic pressure (Osmolality)
- Oncotic pressure
- Hydrostatic blood pressure


Intacellular compartment
Osmotic gradient
Interstitial compartement
Hydrostatic gradient
Oncotic gradient
Intravascular compartement
Artreiole
Venul

Osmolality within our body

- 90% of extracellular osmolality is determined by **Na^+ and Cl^- concentrations.**
- Intracellular osmolality is mainly determined by **K^+**.
- The differential electrolytes concentration is maintained by **Na-K ATPase pumps.**

Causes of low or high serum osmolality

- The normal values of serum osmolality range from 280 to 300 mOsm/kg
- A wide array of medical conditions can lead to abnormally high or low serum osmolality
- Two endocrine diseases that can alter the serum osmolality are SIADH and Diabetes Mellitus
- Syndrome of Inappropriate Antidiuretic Hormone Hyper-secretion (SIADH) is a condition where water is abnormally retained by the kidneys. This syndrome is characterized by low serum sodium levels
- Diabetes mellitus is a condition that occurs when the pancreas no longer produces enough insulin or when the body does not respond to the insulin that is produced by the pancreas. This causes the serum glucose to remain elevated

Osmolar gap

- Calculated osmo =2[Na^+]+[K^+]+[urea]+[gluc]

- Osmolar gap = Measured osmo – calculated osmo

  – Normal range 10 – 15 mmol / L
  – Increased osmolar gap due to e.g., ethanol, methanol, ethylene glycol