Applications of Freezing Point Osmometry

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1 Introduction and Basic Principles

The measurement of the freezing point of a solution is related to the “osmotic concentration” of that solution. Osmotic concentration can be thought of as the concentration of particles of solute per unit amount of solvent. Sometimes this is called an “apparent concentration” since the manner in which the solute reacts with the solvent affects the number of particles that are formed.

The manner in which the solute and solvent interact is called “activity.” This refers not only to the degree of dissociation of the solute (if the solute is an ionic salt), but also to a number of other factors including the degree to which the water molecules are free to enter the solution.

If you know:	and you measure:
1 a. The normal or original freezing point of a solution b. That contamination has taken place	The freezing point of the solution
2 a. The weight of solute A b. The freezing point of solvent B	The freezing point of A plus B
3 The specific gravity of a solution	The freezing point of the solution
4 The freezing point of a solution at time A	The freezing point of solution at time B
5 a. The type of dissolved molecule b. The weight of solute c. The weight of solvent	The freezing point of the solution
6 a. The molecular weight of the dissolved solute molecule b. The weight of solute c. The weight of solvent	The freezing point of the solution

Some physical chemists prefer to interpret the freezing point of a solution as the *concentration of water* in that solution. Others relate freezing point to the *water* “potential” of the solution.

For the purposes of this discussion, it is most convenient to describe freezing point as the *concentration of particles* in solution. Freezing point won’t tell you how big these particles are, or what shape they have, or if they are charged. It only tells you how many.

At relatively low concentrations, freezing point is linear with the number of dissolved particles. Twice as many particles will depress the freezing point twice as much, three times as many, three times as much, and so forth.

There are six ways in which the knowledge of freezing point can be applied. These are summarized in the table below.

you can determine:	the application is:
The percentage dilution or change in concentration.	Concentration of body fluids. Quality control of standards and solutions.
The volume of the container occupied by B	Measuring volumes of containers, tanks, kettles, pipets, human body, etc.
The approximate size and number of dissolved molecules.	Measuring the dialyzability of solute.
The change in number of molecules due to diastasis or other factors. If freezing point is measured over a number of known time intervals, then the rate of reaction can be measured.	Measurement of enzyme activity.
The molecular weight of the solute.	Molecular weight determinations.
The osmotic coefficient of the solute in the solvent.	Determination of water binding, activity, etc.

2 Biological Applications

The fluid compartments within the human body as well as most other animals are generally iso-osmotic. The freezing point of the serum is very nearly the same as that of the cerebro-spinal fluid. Intra-cellular and extra-cellular fluid are very close in concentration. Milk serum is in “osmotic equilibrium” with blood serum. And so forth.

Urine, on the other hand, varies widely in concentration. One of the major functions of the kidney is to remove solute or water (depending on whether there is too much of one or the other) from the blood in order to control the concentration of the blood. Sweat glands and the “thirst mechanism” perform a similar function.

Bar graph “Gamblegram” in mEq/l serum (plus mg% of urea (BUN) & glucose)

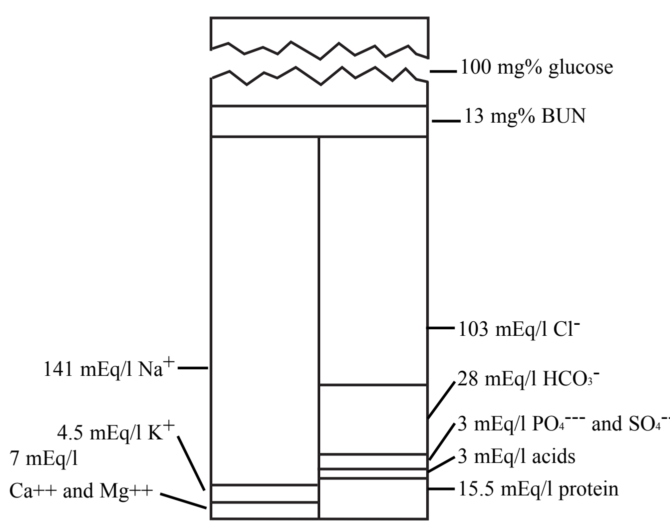

Figure 1: Analysis of solute make-up in normal human serum

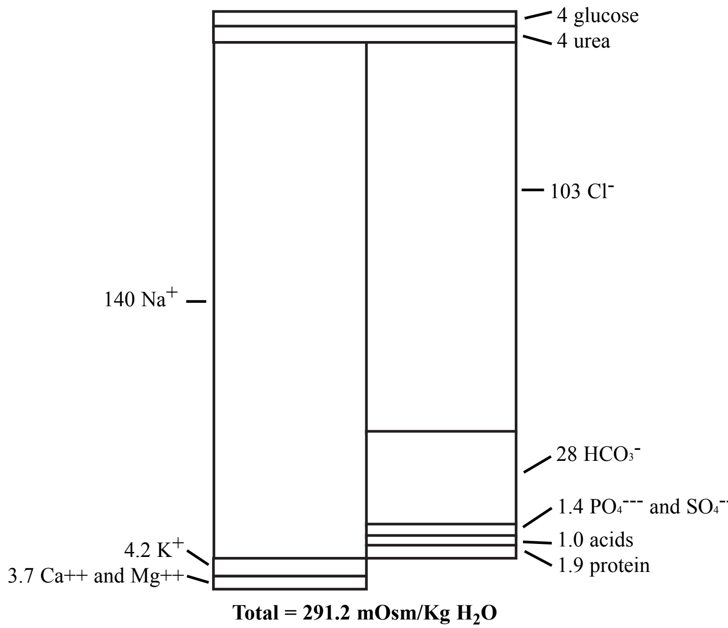

Figure 2: The same components as in Figure 1 expressed in mOsm/Kg H_2O

Although the various body "compartments" are relatively iso-osmotic - contain the same number of particles - the particles which make up the total are different. Serum is one of the most important body "compartments." The following is an analysis of the osmotic components of serum and what high and low values mean clinically.

Normal value for human serum is 285 to 290 mOsm
(=-0.532 to 0.539°C).

2.1 Range of, and reason for, abnormal serum values.

1. What is abnormal - osmolality or osmotic concentration is one of the most highly regulated body variables. Generally a deviation of ±10 from the normal is considered significant, but obviously a knowledge of the patient history and other bio-chemical information must be taken into account before any diagnosis can be made.
2. Low osmolality - an extremely low osmolality would be 230 mOsm (this has been reported). The cause of low osmolality is too little sodium (hyponatremia) or too much water. There are no other possibilities. In either case the sodium is low.
3. High osmolality - serum osmolality has been reported as high as 400 mOsm in adults, sometimes higher in children. This can be caused by:
   a. High sodium - hypernatremia
   b. High glucose - hyperglycemia
   c. High urea - uremia
   d. The presence of an unknown molecule
   e. A combination of the above

2.2 Osmolality and sodium concentration

Sodium *and* osmolality together are helpful in distinguishing among: dilution or water intoxication, true sodium depletion, progressive renal disease, primary water loss, and false hyponatremia due to liquids or artifacts occupying serum space.

When used in conjunction with a flame photometer, an osmometer is useful in distinguishing between various types of low sodium---hyponatremia. Since an osmometer measures the concentration of solute per total *water* rather than per total *volume* (as a flame photometer), it will not be confused by the presence of something which changes the water content of the solution. Consider the following:

1. Low sodium and *normal mOsm* means high lipids or other fatty artifact.
2. Low sodium and *high mOsm* means that the particle which is increasing the osmolality is occupying volume and thus lowering the "apparent" concentration of sodium. This would be a high sugar (hyperglycemia), or high urea (uremia).
3. Low sodium and *low mOsm* occur only when the sodium concentration per total water is low. This is either too little sodium or true dilutional hyponatremia---too much water.

2.3 Urine osmolality

For accuracy and speed, the Advanced® Osmometer is the best way to measure the concentrating power of the kidney. Certain conditions, artifacts, or drugs will falsify the specific gravity.

1. Urine varies widely in concentration in order to control the concentration of the blood. The kidney acts as a selective filter to pass water and hold solute or vice versa.

2. Urine osmolality runs from 250-1200 in a normal adult. In a 24-hour sample, it usually runs between 400 and 600 mOsm. Most adult humans have a concentration capability of from 50-1400 mOsm. Dogs can concentrate up to about 2000. The Australian Kangaroo Rat can concentrate beyond 5000 mOsm.

3. Urine/serum ratio: The ratio of urine to serum osmolality can be an important diagnostic tool.

   a. Greater than 1
   On a 24-hour urine sample, the urine/serum ratio should always be greater than 1 (for example, 600/290=2.1).

   b. Equal to 1
   If the urine/serum ratio is only slightly greater than 1 or equal to 1 (for example, 330/320=1.05), then the patient may have severe renal shutdown. The kidney is not acting as a sieve. The closer to 1, the more acute the problem.

c. Less than 1
In some cases the urine/serum ratio may be less than 1 (for example, 200/300=.67). This occurs when the patient may have diabetes insipidus.

2.4 Unknown blood solutes

Some unknown substances in blood raise the osmolality markedly. Other things being known, the Advanced Osmometer is very sensitive to some drug poisonings - salicylate and barbiturates as well as alcohol. Use of the Advanced Osmometer with a refractometer can indicate if the unknown solute can be dialyzed.

2.5 Clearance studies

1. Osmolal clearance: Osmolal clearance is defined as “the number of cc’s of plasma water which have lost all of their dissolved particles and ions in some measured period of time.” If a 24-hour urine sample is available, the procedure for determining osmolal clearance would be as follows:
   a. Measure the volume of the urine.
   b. Determine the osmolality of the urine.
   c. Determine the osmolality of the serum.
   d. Calculate the urinary output in milliosmoles per day by multiplying the liters of urine by the milliosmolality.
   e. Divide the total output in milliosmoles by the serum osmolality expressed in milliosmoles per cc.
   f. Your answer will be osmolal clearance in cc/day.

Example
Osmolal Clearance:
Assume the 24-hour vol. of urine = 2000 cc (2.000 liters)
Assume the urine osmolality = 500 mOsm/Kg
Then the osmolal output = 2.000 x 500 = 1000 mOsm/day

Assume the serum osmolality = 290 mOsm/Kg (0.29 mOsm/cc)
Then the osmolal clearance = $\frac{1000}{0.29}$ = 3448 cc's per day

2. Urea Free Osmolal Clearance: When osmolal clearance values are needed for diagnosis of salt retention or abnormal salt loss, the results are usually of more value if the urine area concentration is determined, converted to millimoles per day (milliosmoles) and this figure then subtracted from the total milliosmoles excreted. The serum osmolality (in mOsm/cc) is divided into this figure and the answer is the urea free osmolal clearance.

Example
Urea Free Osmolal Clearance
Assume the 24-hour vol. of urine = 2000 cc (2.000 liters)
Assume the urine osmolality = 500 mOsm/liter
Then the osmolal output = 2.000 x 500 = 1000 mOsm/day
Assume the area output = 30 grams/day
30 grams of urea = $\frac{30}{60}$ mole = $\frac{1}{2}$ mole = 500 millimoles =
about 500 milliosmoles
Then the urea free osmolal output = 1000 - 500 = 500 mOsm/day
Assume the serum osmolality = 290 mOsm/Kg (0.29 mOsm/cc)
Then the urea free osmolal clearance = $\frac{500}{0.29}$ = 1724 cc's day

3. Normal values:
The osmolal clearance = 2000-4000 cc's/day (quite variable)
The urea free osmolal clearance of an average person on a mixed diet varies from 1600-2200 cc's/day. A patient with congestive failure will have an osmolal clearance (urea free) as low as 600 cc's. Diuretics such as Diuril or Diamox cause dramatic increases in osmolal clearance of patients with salt retention.

2.6 Monitoring electrolyte balance

Since the osmolality of serum and most other body fluids is primarily affected by electrolytes, the Advanced Osmometer provides an excellent means of monitoring electrolyte balance in burn cases, shock or traumatic surgery. An electrolyte series can be run initially (including osmolality), then the osmolality can be measured as often as necessary as a check on the change in patient condition.

In some serious acid-base disturbances (resulting from a variety of clinical conditions) the measured osmolality is sometimes remarkedly higher than the calculated osmolality (the sum of the individual electrolytes). Findings indicate that this usually may be fatal. Measured osmolality, using an osmometer, is the prime index.

2.7 Total body water

Total body water (TBW) can be determined in a number of ways, but freezing point offers a relatively simple approach. Volume measurement by dilution is the technique. The solute is D_2O---"heavy water." It quickly diffuses through all the membranes that water does, yet it changes the freezing point markedly. Serum water and the D_2O are vacuum distilled from serum for the analysis. Pure D_2O freezes at 3.82°C (above zero). A one percent mixture of D_2O in water will freeze at 0.038°C (one percent of 3.82).

One mole of D_2O, or 20 grams, dissolved in 1000 grams of water will elevate the freezing point about 0.076°C.

With a patient whose TBW was 40,000 grams, an injection of 400 grams (1%) of D_2O would elevate the freezing point 0.038°C. The Advanced Osmometer measures with a precision of ±0.0004°C.

Therefore, the 400 grams dose would yield an accuracy of ±1%.
80 grams would yield ±5%.

2.8 Dehydration

Infant diarrhea is a major cause of pediatric death. Both in children and in adults, an Advanced Osmometer can be used to accurately indicate the extent of dehydration, to monitor the fluids which are administered, and to monitor the patient's response to the treatment.

Particularly in infant diarrhea, but also in many kinds of adult dehydration, continuous investigations of the electrolyte-water ratios in serum and urine are important. The flame photometer tells only part of the story.

2.9 ADH

Osmolality can be used for monitoring ADH suppression or secretion through the use of free water clearances.

2.10 Fluid therapy

Osmolality is a clinical aid in the regulation of fluid therapy, particularly in post-surgical or trauma patients. Lactic acid may play an important role in this particular application using the results of the formula for calculated osmolality versus measured osmolality.

$$1.86\ \text{Na} + \frac{\text{Glucose}}{18} + \frac{\text{BUN}}{2.8} + 5$$

2.11 Pediatrics

Pediatric applications include water-loss or water excess syndrome, and diarrhea and vomiting.

2.12 Blood alcohol

There are new research correlations between osmolality and blood alcohol levels.

3 Other Applications

Since osmometers measure only dissolved particles in solution (1000 milliosmols = Avogadro's number [6.02 x 10^{23}] of particles), the number of applications is almost unlimited.

3.1 Various body fluids

Osmolality can be quickly determined on saliva, CSF, sweat, gastric juices, or any other body fluid of 0.2ml volume. The sample is not destroyed and can be used for other tests.

3.2 With an artificial kidney or dialyzer

Osmolality can be used as a check on the patient's fluids and on the dialyzing media.

3.3 Molecular weight and chemical purity

If a certain weight of chemical is made up of small molecules, there will be a much greater change in freezing point than the same weight of large molecules. Thus, the Advanced Cryoscope/Osmometer is a very sensitive tool for low molecular weights and number average molecular weights of mixed substances.

The degree of chemical purity can be determined with great precision from the slope of the apparent freezing point versus supercooling curve.

3.4 Fetal maturity

Osmolality can be used to determine fetal maturity by measuring the concentration of the amniotic fluid.

3.5 Chromatographic eluates

Osmometers can monitor eluates from column chromatographic separations.

3.6 Quality control

Laboratory reagents can be checked.

3.7 Fixing solutions

The proper osmotic concentrations of a fixing solution can increase the resolution and clarity in cell microscopy, preserve enzyme activity, and prevent membrane distortion. For many biologists and anatomical pathologists, the Advanced Osmometer is becoming a necessary aid in the preparation and application of fixatives.

3.8 General concentration tests

For dialyzing media, parenteral fluids, fluids administered after surgery, etc., the osmometer provides a fast, simple and accurate check. It can prevent the costly - often fatal - mistake of the administration of a fluid with the wrong concentration.

Some hospitals now require the checking of all solutions to be administered after surgery.

In addition to checking biological solutions, the osmometer can also be used to check standards for flame photometers and other instruments as well as a quality control check in the preparation of other reagents.

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