Determination of Isosmolar Blood Anticoagulant Solutions by the Freezing-Point Method

Harold M. Kaplan and Edward F. Stephens

Although suitable concentrations of many anticoagulants have been determined for mammalian blood, isosmolar concentrations are not generally available among vertebrates. In some instances the correct concentrations may be computed from known equations (1) or from known freezing-point depressions (ΔTs) at specific concentrations (2). The data are still fragmentary. In the present study, ΔTs covering a wide range of useful concentrations of 7 anticoagulants which are currently of importance and can work in vitro have been determined.

MATERIALS AND METHODS

ΔTs in degrees C. were determined with a Beckmann thermometer, calibrated from +1° to −5°, and surrounded by a crushed ice-saline mixture. ΔT of any solution was found by obtaining the period of constant temperature after the heat of fusion was dissipated. Each value was determined 30 times.

The anticoagulants studied included potassium oxalate, lithium oxalate, sodium oxalate, ammonium potassium oxalate, sodium citrate, disodium ethylenediamine tetraacetate (Sequestrene), and dipotassium ethylenediamine tetraacetate (Versene). All solutions were prepared in molar concentrations for biologic preference.

“G” values (Van't Hoff i; isotonic coefficients) were computed.

The G value indicates that a given concentration of an electrolyte has a ΔT a certain number of times greater than that of a nonelectrolyte. If, for example, ΔT for turtle blood (3) is minus 0.522°, and if ΔT for a one molal concentration of any ideal nonelectrolyte is minus 1.86°,

From the Department of Physiology, Southern Illinois University, Carbondale, Ill.
Received for publication November 16, 1957.
then 0.522/1.86, or 0.28, gives the G constant for that blood. To illustrate its usage, if ΔT of 0.25 M lithium oxalate is 1.192, then 1.192/(1.86×0.25) equals 2.56 or G. The ratio G1/G2, or 0.28/2.56 gives a molarity, 0.109 of the solution that has a ΔT equal to minus 0.522°, which is isosmolar in this case with turtle blood. Isosmolar solutions can be prepared for any species where ΔT for its blood is determined or known (4). A negligible error is introduced by using the molal value 1.86° above as a molar value.

Though G values are correct only at 0°, and our results are for use at blood temperatures, still the differences are not significant in dilute solutions (5).

**EXPERIMENTAL DATA**

Table 1 shows the ΔT, and the corresponding ΔT/N, or molar freezing point constant, where N is the molar concentration used.

**DISCUSSION**

Isosmolar and isotonic concentrations are not identical. The latter have been determined for anticoagulants by other means (6).

The use of molar rather than molal solutions produces no significant variations. We tested this with lithium oxalate. No difference should exist for ordinary salts (5).

Errors occur in the higher concentrations from supercooling if the solvent freezes out. This can be minimized by adding an ice crystal to hasten freezing.

Freezing-point determinations are not useful for anticoagulants of high molecular weight because the solute settles out, or perhaps forms a transition hydrate; in either case the concentration changes.

The freezing-point constant is introduced here because it may be used to determine other colligative properties of the solution. These constants are very accurate only with large samples.

Isosmolar solutions can be approximated, knowing the valence and molecular weight of the solute, the ΔT of the blood with which it is compared, and assuming total dissociation. Thus for turtle blood, using sodium oxalate where three ions form:

\[
(0.522/1.86) \times (134.01/3) = 12.54 \text{ Gm./L.}
\]

The anticoagulants used exemplify different modes of action; the oxalates remove calcium as calcium oxalate; sodium citrate forms a nonionized citrate-calcium complex; and Versene and Sequestrene are chelating agents that bind calcium ions.
<table>
<thead>
<tr>
<th>Molar concentration</th>
<th>Lithium oxalate</th>
<th>Sodium oxalate</th>
<th>Potassium oxalate</th>
<th>Potassium ammonium oxalate</th>
<th>Sodium citrate</th>
<th>Disodium Esequitrate</th>
<th>Dipotassium Verenate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\Delta T)</td>
<td>(\Delta T/N)</td>
<td>(\Delta T)</td>
<td>(\Delta T/N)</td>
<td>(\Delta T)</td>
<td>(\Delta T/N)</td>
<td>(\Delta T)</td>
</tr>
<tr>
<td>.05</td>
<td>.270</td>
<td>5.40</td>
<td>.222</td>
<td>4.44</td>
<td>.245</td>
<td>4.90</td>
<td>.191</td>
</tr>
<tr>
<td>.065</td>
<td>.320</td>
<td>4.93</td>
<td>.301</td>
<td>4.63</td>
<td>.358</td>
<td>5.50</td>
<td>.361</td>
</tr>
<tr>
<td>.08</td>
<td>.392</td>
<td>4.90</td>
<td>.343</td>
<td>4.28</td>
<td>.371</td>
<td>4.64</td>
<td>.310</td>
</tr>
<tr>
<td>.09</td>
<td></td>
<td></td>
<td>.385</td>
<td>4.28</td>
<td></td>
<td></td>
<td>.624</td>
</tr>
<tr>
<td>.15</td>
<td>.763</td>
<td>5.09</td>
<td></td>
<td></td>
<td>.441</td>
<td>4.41</td>
<td>.755</td>
</tr>
<tr>
<td>.25</td>
<td>.921</td>
<td>4.60</td>
<td></td>
<td></td>
<td>.918</td>
<td>4.91</td>
<td>.971</td>
</tr>
<tr>
<td>.3</td>
<td>1.192</td>
<td>4.77</td>
<td></td>
<td></td>
<td>.986</td>
<td>4.98</td>
<td>1.001</td>
</tr>
<tr>
<td>.35</td>
<td>1.627</td>
<td>4.65</td>
<td></td>
<td></td>
<td>.647</td>
<td>4.82</td>
<td>1.231</td>
</tr>
<tr>
<td>.4</td>
<td>1.856</td>
<td>4.64</td>
<td></td>
<td></td>
<td>.699</td>
<td>4.25</td>
<td>1.730</td>
</tr>
<tr>
<td>.5</td>
<td></td>
<td></td>
<td>2.255</td>
<td>4.51</td>
<td></td>
<td></td>
<td>2.153</td>
</tr>
</tbody>
</table>
SUMMARY

Freezing-point depressions of several anticoagulants were determined from which concentrations isosmolar with the blood of any species may be computed. The method is not applicable to solutes of high molecular weight.

REFERENCES