NOTES

The Effect of Ions on Sedimentation of Colloidal Particles by Means of the Centrifuge.—It is sometimes assumed that the ease of removal of colloidal particles by means of the centrifuge is a measure of the size and density of the colloidal particle, but with ionizing solvents this may not be the case. We have noticed that the Sharples turbine-driven Laboratory Centrifuge with a centrifugal force of 40,000 times gravity will sometimes remove much dissolved hemoglobin (even from alkaline solutions) if the sample is centrifuged for twelve hours, while at other times the same treatment will not even remove a heavy opacity due to relatively large particles suspended in water.

This difficulty was at first believed to be due to convection currents, for Svedberg[1] ran his "ultracentrifuge" for sedimenting dissolved proteins (giving 90,000 times gravity) in an atmosphere of hydrogen under greatly reduced pressure to avoid convection currents. We reduced this difficulty by running the bowl at about 4°, where the density of water does not change with the temperature. For this purpose the shielding jacket and upper "cover" of the centrifuge are cooled with brine at about −10°. The 14° temperature difference is maintained by friction in the air surrounding the bowl. This air is at the same temperature as the bowl, so that the temperature gradient appears to be entirely in the iron shielding jacket and at its surfaces. For such purposes the jacket should be thinner, or even better, of copper rather than cast iron.

The maximum density of water is not much affected by salts. For example, 0.5 N sodium chloride lowers the temperature of the maximum density of water by 6.6°, this being a typical value, and the effect being for a particular salt nearly proportional to the concentration of that salt.[2]

A simple calculation shows that the difficulty could well be electrical. Suppose that at a distance h from the axis of the centrifuge the force is L times the force of gravity, g, that the concn. of ion No. 1 is c_1 moles per liter, that the ion has molecular weight M_1, partial molal volume[3] $\overline{V}_1$ and n_1 equivalents of positive electricity per mole (n_1 being either positive or negative). Let the electrical potential be E volts; R is the gas constant and ρ the density of the *solution* (not of the solvent); T the absolute temperature, and ln means "natural logarithm." We have, using absolute c. g. s. units and the usual differential notation, and assuming activity to be equal to concentration[4]

$$RT\,\mathrm{d}\ln c_1/\mathrm{d}h = gL(M_1 - \rho\overline{V}_1) - 96{,}494 \times 10^7\, n_1\,\mathrm{d}E/\mathrm{d}h \qquad (1)$$

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[1] Svedberg, *Z. physik. Chem.*, **121,** 65 (1926); **127,** 51 (1927).

[2] Landolt-Börnstein, "Physikalisch-chemische Tabellen," **1923,** Vol. I, p. 439.

[3] Lewis and Randall, "Thermodynamics and the Free Energy of Chemical Substances," McGraw-Hill Book Co., New York, **1923,** p. 204.

[4] Compare Lewis and Randall, Ref. 3, p. 244.

We define ρ_1 as $M_1/\overline{V}_1$ and define the "buoyancy coefficient," r_1 by

$$M_1 - \rho\overline{V}_1 = M_1(1 - \rho/\rho_1) = M_1r_1 \qquad (2)$$

We have for the various ions present the quantities c_2, c_3, c_4, etc., respectively, each ion giving an Equation 1 and an Equation 2. Also we must have for all the ions together, for any value of h whatever

$$n_1c_1 + n_2c_2 + n_3c_3 + \ldots = \Sigma(nc) = 0 \qquad (3)$$

Combining (1) and (2) for each ion with the derivative of (3) with respect to h, and remembering that $\mathrm{d}\ln x/\mathrm{d}x = 1/x$, we solve for E and find

$$RT\,\mathrm{d}\ln c_1/\mathrm{d}h = gL[M_1r_1 - n_1\Sigma(Mrnc)/\Sigma(n^2c)] \qquad (4)$$

the summations extending over all the ions. We write for each separate ion an equation of the type $I_1 = n_1{}^2c_1/2$, where I_1 is the "ionic strength" of that ion.[5] (4) may now be written

$$RT\,\mathrm{d}\ln c_1/gL\,\mathrm{d}h = M_1r_1(\Sigma I - I_1)/\Sigma I - n_1[\Sigma(Mrnc) - M_1r_1n_1c_1]/2\Sigma I \qquad (5)$$

The equivalent of this formula has been given[6] for the case where there are but two kinds of charged ions (including the charged colloid). Svedberg's formula takes account of the sedimenting effect of heavy ions only when the effect of those on the density of his original un-ionized colloid is considered. Colby, Des Coudres and Tolman[7] have each given theoretical and experimental treatment of centrifugal potentials in electrolytes. They assume no concentration gradient, and accordingly their potential differs from ours by a concentration cell potential. As the latter involves transference numbers,[8] we see how these three observers could obtain transference numbers, although our potential of Equation 1 does not involve transference numbers.

The first term on the right of (5) involves the pull on ion No. 1 only, while the second term involves the centrifugal pull on all the other ions. For an uncharged substance the second term vanishes as n_1 is 0.

An efficient centrifugal process may use either or both of the two terms. Consider the first only, for a protein of density about 1.25,[9] whence $r_1 = (1.25 - 1)/1.25 = 0.2$ approx. (This should not presumably be affected by "swelling," although the *time* of reaching equilibrium may be enormously increased thereby.) Assume a 1% solution of this protein with molecular weight 40,000 and an equivalent weight of 1000 toward sodium hydroxide and assume the sodium salt to have been obtained by adding sufficient sodium hydroxide. We have $c_1 = 10/40{,}000 = 0.00025$ mole per liter, and $n_1 = 40$, whence $I_1 = {}^1/_2 \times 40^2 \times 0.00025 = 0.2$. But the ionic strength due to sodium ion is only $40 \times 0.00025/2 = 0.005$. Hence the

[5] Lewis and Randall, Ref. 3, p. 373.

[6] Svedberg, *Kolloid-Z.*, Ergänzungsband zu, **36,** 63 (1925).

[7] Tolman, THIS JOURNAL, **33,** 121 (1911).

[8] See Lewis and Randall, Ref. 3, p. 338.

[9] See Svedberg, *Z. physik. Chem.*, **121,** 65 (1926).

concentration gradient attainable will be only 0.005/0.205 or 1/41 (*i. e.*, $1/(n_1 + 1)$ that attainable by also adding salt to the solution). We may remark that association in this alkaline solution is of no avail. Supposing groups of 10 molecules of protein to associate, giving a molecular weight of 400,000, we have now a factor of 1/401 by which this is to be multiplied, for the association does not presumably change the equivalent weight toward sodium hydroxide. But association will naturally decrease the *time* of attaining equilibrium. In this particular case salt (or excess sodium hydroxide) might be added to an additional ionic strength of somewhat more than 0.2, for maximum efficiency. It is to be noted that the removal of the protein has the same effect on the efficiency as the addition of salt, so that if enough salt is present for the particles to really start to be sedimented, the process might be expected to continue with increasing efficiency.

To illustrate the second right-hand term of (5) we may suppose that to the above alkaline 1% protein solution is added 0.1 mole of thallous sulfate per liter of solution, giving an additional ionic strength of 0.3. Owing to its low weight and density we neglect the sedimenting effect of the SO_4^{--}, and take for Tl^+ the approximate atomic weight 200, also $r = 1$ approximately for this heavy ion. We have then neglecting the Na^+ present

$$RT\,d\ln c_1/dh = gL[40{,}000 \times 0.2\ (0.5 - 0.2)/0.5 - (-40) \times 200 \times 0.2/2 \times 0.5] = gL\ (4800 + 1600)$$

Evidently simple ions like Tl^+ do not enormously affect the second term, the increase due to the weight of the Tl^+ (as against an equivalent amount of, say sodium or ammonium sulfate) being here about $33^1/_3$%. The total effect is 6400/(40,000 × 0.2/41) or 32.8 times that where no salt whatever is added, but is 6400/40,000 × 0.2 or 0.8 times that attainable at the isoelectric point. We may note that at the isoelectric point the effect of ions must entirely disappear, and speaking roughly is proportionately greater the farther the protein is from its isoelectric point.

Some improvement is obtained in the second term by using a *monovalent* negative ion with the Tl^+. There are available certain heavy *negative* ions such as phosphotungstate and phosphomolybdate ions, *e. g.*, as one with 24 gram-atoms or 2304 g. of molybdenum metal per mole, and if intelligently used such an ion might be equivalent to a ten-fold increase in the force of the centrifuge. The virtues of such ions are well known. These ions will be a disadvantage for a negatively charged colloid, unless enough should be added to cause the colloid to *rise* rather than sink.

Summary

It is shown how the intelligent use of salts may enormously affect the efficiency of centrifugal processes in ionizing solvents, apart from any

possible agglutination, association or chemical combination caused by the added salt.

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Note on the Magnetic Susceptibility of the Manganocyanide Ion.—We had occasion to make a rough determination of the susceptibility of the manganocyanide ion. The method employed was the Gouy[1] method, so modified as to eliminate most of the pull due to the water. The upper compartment was kept filled with a solution of manganous sulfate 0.058 molal, and the lower, in the first instance, open to the atmosphere. In the second series of runs the lower compartment was filled with a solution of the same concentration of manganous ion in the presence of 2.3 *M* potassium cyanide.

The solution was so prepared as to avoid the oxidation of the manganocyanide by the oxygen of the air. This was done by using xylene for covering.

The diamagnetic susceptibility of the potassium cyanide was determined in a separate run, thus making our determination of the susceptibility of the manganocyanide independent of the impurities in the potassium cyanide. The densities of the solutions were determined to 0.1%. The sample of manganous sulfate was of high purity (containing 0.001% of iron).

Employing the values of the magnetic susceptibility for air, water and Cabrera's[2] value for manganous sulfate, we have calculated the susceptibility of the manganocyanide ion. This value, corrected for the total cyanide is 1650×10^{-6}. The presence of the sulfate was also corrected for; -37×10^{-6} was taken for its molal susceptibility.

It is to be noted that this value (1650×10^{-6}) lies between one (1300×10^{-6}) and two (3300×10^{-6}) Bohr magnetons if S levels are assumed. This is to be expected since this ion is iso-electronic with ferricyanide ion, which has a susceptibility of about 1700×10^{-6}. According to the Welo-Baudisch[3] rule, the manganocyanide $Mn(CN)_6^{----}$ (as well as the ferricyanide $Fe(CN)_6^{---}$) would have a susceptibility of one Bohr magneton. The deviation from one Bohr magneton in this case is in the same direction and same order of magnitude as in the case of the ferricyanide. However, it is possible to ascribe the deviation in our case as due to bivalent manganese

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[1] Shaffer and Taylor, THIS JOURNAL, **48**, 843 (1926).

[2] Cabrera, Moles and Marquinas, *J. chim. phys.*, **16**, 11 (1918).

[3] Welo-Baudisch, *Nature*, **116**, 606 (1925).