V. THE HYDRATION OF GUM ARABIC AND GLYCOGEN

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THE quantitative estimation of the hydration of hydrophilic colloids has given discordant results according to the method employed. This has been well shown by Greenberg & Greenberg [1933]. The methods depending on viscosity [Kunitz, 1927] or on the comparison of particle weight determined by osmotic pressure with the particle size determined by diffusion velocity [Kunitz *et al.* 1934] rest on many somewhat uncertain assumptions. A more direct way of attacking the problem is to make use of the idea that the water of hydration no longer has the power of dissolving another substance which may be added to the solution of the colloid. Since only a part of the total water present is available for forming the solution, the actual solution will have a concentration higher than the value calculated from the amounts of water and dissolved "reference substance" added.

There are three methods available for estimating this enhanced concentration. Firstly that of Newman & Gortner [1922] who use the depression of the freezing-point produced by dissolving a known amount of reference substance in the solution of the colloid. Secondly there is the method introduced by MacBain & Jenkins [1922] and used by Greenberg & Greenberg [1933] and also by MacBain & Kistler [1929] in which a sample of solution is freed from the colloid and its bound water by ultrafiltration. The concentration of the reference substance is then estimated in this sample by chemical or physical means and compared with the concentration at which the original solution was made up. The third method is that used by Weber & Nachmannsohn [1929] in which a solution of the colloid is brought into equilibrium with a solution of the reference substance across a membrane which is permeable to the latter and to water. The concentration is then determined relative to the total water in both solutions, when it is found that the colloid solution is the more dilute in respect of the reference substance.

All the methods present experimental difficulties. Each one is sensitive to error from even slight adsorption of the reference substance by the colloid, which would lead to low or even to negative results for the bound water. In each case it is necessary to use a non-electrolyte as reference substance, especially when the colloid is an electrolyte since the distribution would be greatly affected by a Donnan membrane equilibrium. This appears to be the case even when the colloid is commonly regarded as a non-electrolyte such as glycogen, as the author and Young [1936] have shown by osmotic pressure measurements. At first sight it might appear that this is an unnecessary condition for Gortner's method but even here an unknown change in the activity coefficients of the ions could easily vitiate the results.

The chief difficulty of the cryoscopic method is the smallness of the freezing-point depressions to be measured.

With regard to the ultrafiltration method a serious objection in the author's opinion is the probability that there will be a preferential passage of the water compared with the reference substance. The results of Greenberg & Greenberg [1933] show practically no bound water for gelatin, caseinogen and glycogen, and although Bull [1933] has suggested that the results have been vitiated by adsorption of reference substance Greenberg & Cohn [1934] have presented further evidence which shows that this is very improbable. The present author's criticism of differential rates of filtration does not appear to have been made before and it seems well worth while testing whether a solution of glucose changes its concentration under the conditions of ultrafiltration used. Even this test is not quite rigid since it is quite possible for membrane characteristics to be considerably altered by adsorption of certain colloidal materials.

The third method depending on the attainment of equilibrium across a membrane is the one used in this work.

THEORY OF THE DISTRIBUTION OF A REFERENCE SUBSTANCE ACROSS A MEMBRANE

It should be noted that even if no water is bound by the colloid the partition of the reference substance relative to the water on both sides of the membrane will not be quite equal.

The problem has been treated by Donnan [1934] who gives the equation:

$$\frac{N_s'}{(N_0')^r} = \frac{N_s''}{(N_0'')^r}, \quad \text{hence } \frac{N_s'}{N_s''} = \left(\frac{N_0'}{N_0''} \right)^r \quad \dots\dots(1),$$

where N_s' and N_0' are the mol. fractions of the reference substance S_1 and of water respectively on the side of the membrane containing colloid; N_s'' and N_0'' have the same significance on the side containing no colloid; and r is the partial molar volume of s_1 divided by the partial molar volume of water in the solution considered.

Further

$$N_0' = 1 - N_{s_1}' - N_{s_2}',$$

where N_{s_2}' is the mol. fraction of the colloid S_2 .

When N_{s_1}' is small N_{s_1}'' may be taken as equal to N_{s_1}' for the purpose of calculating $N_0'' = 1 - N_{s_1}''$.

In the experiments described later acetone has been used as the reference substance and gum arabic as the colloid. From osmotic data on the sodium salt of the gum the author (not yet published) has recently deduced that it behaves as if it were 50% ionized at a concentration of 10%, and in another paper [1936] that its "molecular weight" is very high (240,000). The equivalent weight is 1200, so that the effective molar concentration of a solution containing 10 g. of gum per 100 g. of water is

$$100 \times 0.5/1200 = 0.04.$$

If the acetone concentration is taken to be 0.1 mol. per 1000 g. of water, the following mol. fractions can be calculated:

In colloid solution			In outside solution	
Acetone	Gum arabic	Water	Acetone	Water
N_{s_1}'	N_{s_2}'	N_0'	$N_{s_1}'' (\sim N_{s_1}')$	N_0''
0.0018	0.0009	0.9973	—	0.9982

The partial molar volume of acetone obtained by a graphical method from data in tables for the specific gravity of dilute solutions is 65, that of water may be taken as 18, so that $r = 65/18 = 3.6$.

Substituting these values in equation (1) we obtain for the ratio of the mol. fractions of the acetone N_{s_1}'/N_{s_1}'' the value 0.9966. To obtain the ratio of concentrations of the acetone relative to the water we have to divide the mol. fractions of acetone by the mol. fractions of water, i.e. we have to multiply the above ratio by $N_0''/N_0' = 1.0009$ which brings it to 0.9974.

It is interesting to note that if the partial mol. volume is negative as it is with ethyl alcohol in water then the exponent r is negative and the above ratio will be greater than unity giving the effect of "negative hydration" or positive adsorption of the reference substance by the colloid.

CALCULATION OF HYDRATION

If for the moment we neglect the above effect and attribute the unequal distribution of the reference substance entirely to some of the water being bound by the colloid then we may easily calculate the hydration as follows.

Let x' and x'' be the concentrations of the reference substance per g. of total water in the colloid solution and in the outside solution respectively; C = concentration of colloid as g. per g. of water; and h = bound water per g. of colloid (water of hydration).

Then in the colloidal solution the amount of free water per g. of total water is $1 - hC$ and the true concentration of the reference substance is $x'/1 - hC$. This is equated to x'' , whence

$$h = \frac{1}{C} \left(1 - \frac{x'}{x''} \right).$$

This equation has also been used by Greenberg & Greenberg [1933].

If we substitute in this formula the value for the ratio calculated above, the apparent hydration is 0.026 g. of water per g. of colloid. This small correction is mainly dependent on the mol. fraction of the colloid and it will be shown that hydration values 10–40 times as large have been obtained.

EXPERIMENTAL

As mentioned previously difficulty is experienced in obtaining a reference substance which can be estimated accurately in the presence of the colloid. Acetone can be completely and easily distilled from the colloid solutions and estimated readily by the iodoform reaction with excess of a standard iodine solution and subsequent titration with thiosulphate [Kuhn & Roth, 1932].

Distilling apparatus

The distilling apparatus consisted of a 150 ml. flask fitted with a ground glass stopper to which was sealed a delivery tube bent to form in one piece the condenser tube of a Liebig condenser. By this means it was hoped to avoid loss of acetone around the stopper which might occur in an ordinary distilling flask with a side-tube. During distillation care was taken to keep water round the rim of the flask and its stopper to prevent loss of acetone by creeping into the ground glass joint. The condenser tube dipped into about 10 ml. of water contained in a 50 ml. receiver fitted with a stopper. The receiver was well cooled with crushed ice. Even with careful boiling the solutions were inclined to foam but the foam was successfully dispersed by placing a cylindrical coil of copper gauze in the neck of the flask. After collecting about 20 ml. of distillate and rinsing out the condenser tube, 10 ml. of 2*N* NaOH were added and then excess of standard iodine solution. After standing for at least half an hour 3 ml. of concentrated HCl were added and the excess iodine titrated with standard thiosulphate ($N/10$ or $N/50$).

Membrane equilibrium apparatus

The membranes consisted of 11 ml. collodion sacs bound by thread to a piece of pressure tubing slipped over the end of the capillary tube of a glass stopcock. This tube passed through a rubber stopper situated between the tap and the sac. The sac containing the colloid was brought to equilibrium at 25° with several changes of acetone solution of the required strength. A crystal of thymol was always added and about 14 days were allowed for equilibrium to be established.

Preparation of gum and glycogen

The gum was prepared by dialysing 10% solutions against $N/2$ solutions of the chloride of the base required. The chloride was afterwards removed by dialysis in several changes of acetone solution as described above until no reaction with silver nitrate was obtained.

The glycogen was kindly supplied by Dr F. G. Young who prepared it by extracting *Mytilus edulis* with 40% KOH and precipitating several times with alcohol followed by dialysis.

Sampling technique

Before sampling the tube with its sac was well cooled with ice. The contents of the sac after rejecting the first few drops were quickly squeezed into a weighing bottle and weighed. 10 ml. were quickly pipetted out and rinsed into the cooled distilling flask containing about 5 ml. of water and immediately distilled. The weighing bottle containing about 1 ml. of residual solution, after reweighing was placed on a water-bath and finally dried *in vacuo* at 100° and weighed again in order to determine the concentration of the gum.

A similar sampling technique was adopted for the outer liquid in the boiling-tube which was kept well stoppered and cooled in ice until samples had been taken. It was found inadvisable to use samples corresponding to more than 10 ml. of $M/60$ acetone; so that solutions much more concentrated than this were diluted by weight with all precautions against evaporation and a weighed aliquot was taken for analysis. The samples of external liquid were added to the residual solution of gum in the flask left over from the previous distillation of the sample from the sac since it was felt that the conditions would then be more comparable.

In spite of successive estimations of acetone agreeing to within 0.2% when no distillation was performed the latter process seemed to increase the error to about 0.5%. This brings the error of the ratio of two such estimations to 1%

Table I. *Hydration of calcium gum*

Acetone conc.	Gum %	x'/x^*	Hydration per g. gum
<i>M</i>	9.56	0.9219 ± 0.012	0.82 ± 0.12
<i>M/10</i>	6.88	0.9379 ± 0.005	0.90 ± 0.08
<i>M/60</i>	9.78	0.9213	0.80
<i>M/60</i>	10.60	0.9290	0.67
<i>M/180</i>	8.47	0.9202	0.94
<i>M/180</i>	10.13	0.9068	0.93
<i>M/180</i>	10.13	0.9049	0.94
<i>M/180</i>	9.87	0.9210	0.80
<i>M/500</i>	10.55	0.9255	0.71
<i>M/500</i>	8.06	0.9557	0.55

* This is the ratio of concentration of acetone in the gum solution to that in the outside solution calculated relative to the water.

and this variation in a 10% solution of gum corresponds to 10% hydration calculated on the dry weight of the gum. It is thus seen that the accuracy cannot be expected to be high unless the analytical technique is improved.

From Table I which gives the results for calcium gum it will be seen that the average water of hydration is about 0.9 g. per g. of gum. The results with *M*/500 acetone are lower. Although this could be explained by a slight adsorption of the acetone by the gum this does not seem likely since the apparent hydration of the gum remains constant (within the rather large experimental error) over a large increase in concentration of acetone. The large discrepancy between the two results with *M*/500 acetone rather suggests that the deficiency is due to analytical uncertainty.

Table II. *Hydration of sodium gum*

Acetone conc.	Gum %	x'/x''	Hydration per g. gum
<i>M</i> /60	7.78	0.9132	1.12
<i>M</i> /60	8.31	0.9550	1.18
<i>M</i> /180	6.58	0.9374	0.95
<i>M</i> /180	3.10	0.9635	1.10

Table II gives results for sodium gum. The average hydration of 1.1 is about 20% greater than for calcium gum, this may, perhaps be attributed to the ionization of the former compound being about twice as great as that of the latter, though whether this acts by a swelling of the gum molecule (mol. wt. = 240,000) or by acetone molecules being unable to penetrate so far into the atmosphere of "gegenionen" is not clear. In contrast to this result Weber & Nachmannsohn [1929] found no difference in hydration between isoelectric and ionized albumin or serum globulin. These investigators used glucose as reference substance which was admittedly adsorbed and the results were calculated as "non-dissolving volume" of the colloid instead of on a weight basis.

As a comparison results have been calculated for Na and Ca gums on the volume basis using the above author's formula:

$$\text{Non-dissolving volume per g. of colloid } V = \frac{C_0 - C_i}{C_0} \cdot \frac{1}{C},$$

where C_0 and C_i are the concentrations per ml. of the reference substance in the outside solution and colloid solution respectively, and C = g. of gum per ml. of solution.

In order to calculate these concentrations the specific gravities were interpolated from data given by Briggs [1934].

Colloid	V ml. per g. gum	Hydration g. per g. gum
Albumin	1.0	—
Serum globulin	1.4	—
Ca gum arabic	1.62	0.93
Na gum arabic	1.80	1.12

It is not possible to estimate quantitatively the hydration from the non-dissolving volume of a colloid owing to the fact that the specific volumes of the colloid and of the bound water are not known. It is probable that the density of the latter is considerably greater than normal. However the much larger volume of gum arabic shows that it is more strongly hydrated than albumin.

Gortner & Gortner [1934] obtained values of 0.6–0.7 for the hydration of calcium gum by the cryoscopic method and Gortner's earlier work indicated

an amount greater than 1 so that the figures of these authors are in reasonable agreement with my own figures.

It is of interest to calculate the water of hydration assuming that each oxygen atom of the gum holds 1 mol. of water by polar adsorption. The formula of the acid gum has been given by O'Sullivan [1884; 1890] as $C_{89}H_{146}O_{74}=2394$; now $74H_2O=1340$ which makes the hydration 0.56 per g. The above molecule would contain two ionizable atoms of Na^+ . If it is assumed that these ionizing groups can bind a further 8 mol. of water each, the hydration per mol. will be increased by $16H_2O=288$ making the total hydration 0.68; this value still leaves some 0.4 g. unaccounted for. It is impossible to say whether this is due to each oxygen atom binding on the average 2 mol. of water or to each ionizing group binding as many as 24 mol. of water. It seems that the former explanation is more likely though a combination of the two hypotheses would be better able to account for the difference in hydration of sodium and calcium gums. It may, however, be objected that the existence of layers of adsorbed water many molecules thick around ions would show greater variation with the concentration of the acetone owing to an increase of the penetration of the acetone molecules into the adsorbed layer.

Particle volume of gum arabic

In a previous paper [1936] the author has shown by osmotic methods that calcium gum has a "molecular weight" of about 240,000. The volume and diameter of the particle (assuming a spherical shape) calculated from this and the figures already given for the effective volume per g. of gum are as follows. There are also added figures for an unhydrated spherical particle with an assumed density of 1.1.

Dimensions of single hydrated molecule

Gum	Volume $m\mu^3$	Diameter $m\mu$
Na	720	11.1
Ca	650	10.7
Unhydrated particle $D=1.1$	360	8.8

Measurements of the diffusion coefficients appear to give figures very much lower than those presented here even when carried out in the presence of $N/10$ electrolyte as has been shown by unpublished work of Miss S. Kronstein.

The hydration of glycogen

An experiment with glycogen gave the following results:

Acetone conc.	Glycogen %	x'/x''	Hydration per g.
$M/180$	14.15	0.9560	0.31
$M/180$	11.0	0.9749	0.23
			Mean 0.27

It will be seen that the hydration is less than a third of that of gum arabic. The author and Young [1936] have recently shown that glycogen has a very large particle weight (about 2 million). The low hydration is presumably connected with a close packing of the constituent parts of this particle whereas all the hydrophilic parts of a gum arabic particle may be accessible to the water. Greenberg & Greenberg [1933] did not detect any hydration for glycogen or for several other colloids as mentioned previously.

It is the author's opinion that, while Greenberg and Cohn [1934] are correct in maintaining that the extent of hydration of colloids has been greatly over-estimated, yet the negligible hydration shown by their work errs in the opposite direction, perhaps for the reasons already suggested.

SUMMARY

1. The distribution of acetone across a membrane between 10% solutions of gum arabic or of glycogen and water has been determined analytically through a range of concentrations from $M/1$ to $M/500$ acetone.

2. The following weights of "bound water" per g. of colloid have been calculated. Ca gum (arabic) 0.9 ± 0.05 , Na gum 1.1 ± 0.05 , glycogen 0.27 ± 0.05 . The results for gum arabic agree with those of Gortner obtained by the cryoscopic method.

3. The "non-dissolving volume" in ml. per g. of gum has been calculated as 1.6 for Ca gum and 1.8 for Na gum.

4. The diameter of each "molecule" of gum assuming a mol. wt. of 240,000 has been calculated as 11 μ .

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REFERENCES

- Briggs (1934). *J. phys. Chem.* **38**, 867.
 Bull (1933). *J. gen. Physiol.* **17**, 83.
 Donnan (1934). *Z. phys. Chem. A*, **168**, 369.
 Gortner & Gortner (1934). *J. gen. Physiol.* **17**, 327.
 Greenberg & Cohn (1934). *J. gen. Physiol.* **18**, 93.
 — & Greenberg (1933). *J. gen. Physiol.* **16**, 559.
 Kuhn & Roth (1932). *Ber. dtsh. chem. Ges. B*, **65**, 1291.
 Kunitz (1927). *J. gen. Physiol.* **10**, 311.
 — Anson & Northrup (1934). *J. gen. Physiol.* **17**, 365.
 MacBain & Kistler (1929). *J. phys. Chem.* **33**, 1806.
 — & Jenkins (1922). *J. chem. Soc.* **121**, 2325.
 Newman & Gortner (1922). *Bot. Gaz.* **74**, 442.
 Oakley (1936). *Trans. Faraday Soc.* **32**, 1360.
 — & Young (1936). *Biochem. J.* **33**, 868.
 O'Sullivan (1884). *J. chem. Soc.* **45**, 41.
 — (1890). *J. chem. Soc.* **57**, 59.
 Weber & Nachmannsohn (1929). *Biochem. Z.* **204**, 220.