Chemistry. — *On colloidally bound water.* By H. R. KRUYT and H. DE BRUYN Mzn.

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I. *Introduction.*

The determination of colloidally bound water has been tried along various ways and the results of these investigations have led to contradictory conclusions.

Leaving the viscosimetric investigations apart (on account of the difficulty they present of correct interpretation), there remain four types of investigation:

1. Determination of water which does not freeze into ice at low temperatures. As a criterion we can take the measurement of the melting heat at a rise of temperature (RUBNER 1), THOENES 2)), or the volume change (JONES and GORTNER 3)).

2. Determination of the actual concentration of a substance added to the sol; the difference between the total quantity of water and that which proves available as solvent, is the colloidally bound water. The concentration may be determined by cryoscopic measurement (NEWTON and GORTNER 4)), or polarimetrically or volumetrically (KOETS 5) in those cases when the liquid can be simply poured away from the colloid.

3. Separation of colloid and medium may also be done by means of ultrafiltration (GREENBERG and GREENBERG 6)). The criterion is, of course, the difference between the total quantity of water and that which serves as a colvent for a molecular dispersion substance (concentration of the ultrafiltrate).

4. Determination of the difference in concentration of a dissolved substance on either side of a dialysis membrane, when on the one side there is colloid, water and dissolved substance, on the other only water and dissolved substance (OAKLEY 7)); the interpretation is again as in 2 and 3.

The remarkable fact is, that methods 1, 2 and 4 have led to similar results, at least as regards the order of magnitude (water binding in the order of 1 gr of water per gram of colloid), whereas method 3 gives an absolutely negative result. This is especially striking in methods 3 (GREENBERG) and 4 (OAKLEY), which differ only in that the one is by ultrafiltration, the other by dialysis.

The calculation of the results of all these methods easily causes errors; the concentrations should be calculated per water unit, not per solution

unit. Some communications in the literature are so concise, that it is impossible to check the method of calculation. We have, therefore, thought it necessary to investigate the same objects by different methods and by calculations which are in every respect comparable. We have, however, abstained from checking method 1, because we deem its results impossible of interpretation without suppositions which cannot be verified themselves; method 2 we have left unchecked, because two widely different investigations in this laboratory (KOETS 5), KRUYT and WINKLER 8)) have already confirmed the correctness of the conclusions drawn from this method.

We have, therefore, occupied ourselves with OAKLEY's and GREENBERG's methods, which, for all their similarity, have led to such opposite conclusions. As objects of both methods we took: *sodiumarabinate* for colloid and urea for the moleculary dispersed substance, whose concentration was to be determined. We have applied OAKLEY's and GREENBERG's methods, we have modified them, made supplementary investigations where it seemed necessary, yet *we have had to fully confirm the apparently paradoxical result*: "in OAKLEY's method there is bound water, in GREENBERG's there seems to be none".

In the following experimental part a complete description is given of some of the measurements taken, so that others may check our results and get an idea of the limits of accuracy. In the treatises published on this subject this is often impossible, it has been our wish to prevent this uncertainty in the reader.

II. *Experimental part.*

A. OAKLEY's dialysis method.

1. *Material.*

The *Na arabinate* was prepared by the method of BUNGENBERG DE JONG and THEUNISSEN 9); starting from gum Arabic "Senegal, petit boule blanche I", it was precipitated four times with acetate of sodium and alcohol.

The urea was a *purissimum* preparation, the purity of it was checked by analysis, as described below.

2. *Analysis.*

The urea concentration was determined by the micro-KJELDAHL method namely, following HENWOOD and CAREY 10). This method was first tested by numerous blank determinations, with known quantities of urea.

3. *Investigation*

We made little collodion bags by fabricating four successive collodion films, one on top of the other in ERLEMEYER flasks of 25 cc. These bags were pushed over a length of vacuum tube, in which a glass tube had been

introduced. With this glass tube the dialysis membrane was secured and placed in a covered beaker containing the sol-free solution, which was renewed after 1, 2, 4, 6 and 10 days respectively; after a fortnight 10 cc of the inside and 10 cc of the outside liquid were analyzed; moreover, 5—10 cc of the inside liquid were dried in a vacuum drier over P_2O_5, in order to determine the quantities of gum arabic and urea.

Only in experiment 5 did we analyze after a month, after the outside liquid had been renewed 10 times. From the figures obtained in this way we calculated "the quantity of bound water per gram of colloid, h", according to the formula given by GREENBERG:

$$h = \frac{1}{c}\left(1 - \frac{x}{y}\right),$$

in which x is the urea concentration per gram of the total quantity of water in the colloid solution inside the dialysator, y the same concentration but in the beaker, so outside the dialysator, c the colloid concentration in grams per gram of water.

Table I shows that the quantity of bound water is about 1 gram per gram of *sodiumarabinate* and that in an *arabinate* solution of ca 5 %. In 3—8 % concentrations OAKLEY found 1.1 gram.

In principle, therefore, our results confirm those of OAKLEY.

Meanwhile the objection may be raised that possibly in OAKLEY's method the equilibrium has not yet been established after a fortnight and 5 renewals. Although experiment 5, in which, as has been remarked, 10 renewals were made in 30 days, does not point in that direction, we thought it would be well to approach the equilibrium from the other side, namely in two experiments (I and II) we placed a greater urea concentration inside the dialysator than outside, while afterwards in experiment III the concentrations were chosen so that acocrding to experiments I and II there was equilibrium in concentration from the first. In order to promote the establishment of equilibrium this series of experiments was made with somewhat thinner collodion membranes, but owing to this a slight quantity of gum arabic permeated, so that this substance had also to be determined in the outside liquid (Table II).

In this series intermediary determinations were also made. We shall not describe the results extensively, but restrict ourselves to publishing the figures (Table III).

The results of these experiments do not leave any doubt that there is bound water, namely, one to one and a half grams per gram of arabinate.

B. GREENBERG's ultrafiltration method.

1. The material used and the method of analysis were identical to those applied with OAKLEY's method.

TABLE I.

	1		2		3		4		5	
	outside	inside	outside	inside	outside	inside	outside	inside	outside	inside
g. liquid	10.0800	9.9922	10.0082	10.0480	9.9984	10.0677	10.0604	10.0437	10.0863	10.0265
g. urea	0.1210	0.1089	0.1202	0.1071	0.1196	0.1060	0.1202	0.1076	0.1209	0.1073
idem/g. liquid	0.01201	0.01090	0.01201	0.01066	0.01196	0.01053	0.01195	0.01071	0.01199	0.01070
g. liquid		5.1171		10.1044		9.9864		10.0888		8 6494
g. dry substance		0.2996		0.6585		0.6561		0.6516		0.5467
g. gum arabic		0.2438		0.5508		0.5510		0.5435		0.4541
idem/ liquid		0.04764		0.05451		0.05517		0.05387		0.05250
water/liquid	0.9880	0.9415	0.9880	0.9348	0.9880	0 9343	0.9880	0.9354	0.9880	0.9368
h.	0.93		1.06		1.17		0.92		1.05	

TABLE II.

	I		II		III	
	outside	inside	outside	inside	outside	inside
g. liquid		20.5969		20.5167		20.5559
g. urea		0.2424		0.2555		0.2158
id/g liquid	0.01120	0.01177	0.01120	0.01245	0.01197	0.01050
g. gum/g liquid		0.09882		0.09876		0.09895
water/g liquid	0.9888	0.8894	0.9888	0.8888	0.9880	0.8905
after days	12		13		10	
g. liquid	10.0295	9.7520	10.0430	10.0613	10.0390	9.5945
g. urea	0.1137	0.1064	0.1163	0.1114	0.1198	0.1081
id/g. liquid	0.01134	0 01091	0.01158	0.01107	0.01193	0.01127
g. liquid	6.0074	5.5306	6.5000	2.9325	5.3543	3.7023
g. dry subst.	0.1289	0.1937	0.1043	0.0957	0.0722	0.1307
g. gum	0.0608	0.1334	0.0290	0.0632	0.0083	0.0809
id/g. liquid	0.01012	0.02412	0.00446	0.02155	0.00155	0.02404
water/g. liquid	0.9785	0.9650	0.9840	0.9674	0.9865	0.9647
h	1.69		1.56		1.46	

TABLE III.

	After days	h
I	6	—
	12	1.69
II	11	1.03
	13	1.56
III	3	0.78
	8	1.02
	10	1.46

2. *Investigation.*

The ultrafiltration took place at diminished pressure; seeing that in such a case there is a danger of loss of water of the filtrate through evaporation, two calcium chloride tubes were placed behind the suction tube. We found that the weight of the second tube did not increase, while the water received in the first was taken into account.

The first experiments were made with "Ultrafein-Schnell" membrane filters and yielded the results stated in Table IV.

TABLE IV.

	I		II	
	Filtrate	Liquid	Filtrate	Liquid
g. liquid	7.9816		10.0017	
g. urea	0.0964		0.1317	
idem/5. liquid	0.01208	0.01088	0.01317	0.01210
g. gum/g liquid		0.09091		0.09880
water/g liquid	0.9879	0.8982	0.9868	0.8891
h	0.09		− 0.18	

These results, in which h is calculated in the same way as in the investigations according to OAKLEY's dialysis method, show that indeed, the bound water thus calculated is practically nil in GREENBERG's ultrafiltration method.

In order to make the two methods as much alike — and hence as comparable — as possible, we applied in the ultrafiltration the same collodion membrane as in the OAKLEY experiments. It was simply placed in a porous beaker (ultrafilter of BECHHOLD–KÖNIG 11)). This gave a double advantage: in the first place the material was the same in both series of experiments, secondly the collodion membrane is much more permeable, so that it is possible to filter at less diminished pressure. For the dialysis method may be conceived as ultrafiltration in which the difference in pressure is nil. In order to achieve different diminished pressures with the same water-jet pump, an adjustable regulator was placed behind the suctionflask and the working pressure was read from an open mercury manometer. It has been said that the thin collodion membranes allow a slight quantity of arabinate to pass, this was determined in the filtrates. The level of filter beaker was kept constant by adding sol and the homogeneousness of the liquid was ascertained by stirring. The results are given in Table V.

These experiments too confirm, therefore, GREENBERG's result that ultra-

TABLE V.

	I				II			
g. urea/g. liquid	original liquid			0.01192				0.01209
g. gum/g. liquid	original liquid			0.02351				0.02287
g. water/g liquid	original liquid			0.9646				0.9650
	filtrate	liquid	filtrate	liquid	filtrate	liquid	filtrate	liquid
g. liquid	8.3615		8.6531	8.2633	8.3034		7.5265	8.2450
g. urea	0.1032		0.1062	0.0988	0.1032		0.0949	0.1017
g. urea/g. liquid	0.01234	0.01216	0.01228	0.01196	0.01243	0.01230	0.01261	0.01233
g. liquid	7.3431		7.0880	7.4985	3.6588		3.8708	5.7796
g dry substance	0.0905		0.0915	0.4080	0.0556		0.0559	0.2662
g. gum	—		0.0045	0.3183	0·0101		0.0071	0.1949
idem/g. liquid	—	0.0333	0.00063	0.04245	0.00276	0.0303	0.00183	0.03372
water/g. liquid	0.9877	0.9545	0.9871	0.9456	0.9848	0.9574	0.9856	0.9540
pressure in cm Hg	60		60		20		31	
h	— 0.56		— 0·38		— 0.62		— 0.30	

filtration experiments do not lead to the conclusion that bound water is real, rather do they point to a negative effect.

III. *Discussion.*

Although we can fully confirm the experimental results of the authors mentioned, we yet think we must contradict the conclusion that GREENBERG's method indicates the absence of colloidally bound water.

As well in dialysis as in the ultrafiltration method (at least with infinitely slow ultrafiltration and uninterrupted contact of the two liquids) the potential of the water on either side of the membrane should be equal. The question whether or not the water is bound may be formulated as follows: is the potential of the water modified by the colloid or not? If we give an affirmative answer to this question on the ground of GORTNER's and OAKLEY's experiments, we may express the potential of the water inside the ultrafilter, the dialysis membrane respectively, as

$$[\mu_{H_2O}]_{in} = f(T) + p_{in}\, v_0 + f(c_t)_{in} + f(c_c)_{in} \quad . \quad . \quad . \quad . \quad (1)$$

in which c_t is the concentration of the substance in true solution, c_c that of the colloid, and v_0 the molecular volume of H_2O. Outside the ultrafilter, the dialysis membrane respectively, applies:

$$[\mu_{H_2O}]_{out} = f(T) + p_{out}\, v_0 + f(c_t)_{out} \quad . \quad . \quad . \quad . \quad . \quad (2)$$

To hold for equation:

$$[\mu_{H_2O}]_{in} = [\mu_{H_2O}]_{out} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

term $f(c_c)_{in}$, in equation (1) will have to be compensated either by a difference between p_{in} and p_{out}, or by one between $(ct)_{in}$ and $(c_t)_{out}$, In OAKLEY's method, where the pressure is the same inside and outside the membrane, there is indeed a concentration difference as equilibrium phenomenon. In GREENBERG's method there is a difference in pressure between sol and ultrafiltrate; hence it is by no means certain that a concentration difference is necessary, as long as $p_{in} - p_{out}$ is sufficiently great.

In calculating what difference in pressure agrees with the concentration difference found, we find a value of ca. $\frac{1}{4}$ atmosphere; in our experiments we were always higher than that. Moreover, it should be borne in mind that in the ordinary ultrafiltration the condition of infinitely slow ultrafiltration with permanent maintenance of the equilibrium is by no means fulfilled, owing to which an inversion of the phenomenon for higher pressure values becomes doubtful. The homogeneous liquid phase simply permeates, and as it becomes separated from the membrane, equation (3) is not held for.

GREENBERG's *method, therefore, by no means contradicts the presence of bound water,* OAKLEY's *method confirms the existence of it unequivocally.*

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VAN 'T HOFF *Laboratorium of the Rijks Universiteit.*

REFERENCES.

1) M. RUBNER: Abh. preuss. Akad. Wiss., Phys.-Math. Kl. **1922**, 3.
2) F. THOENES: Biochem. Z. **157**, 174 (1925).
3) I. D. JONES and R. A. GORTNER: J. physic. Chem. **36**, 387 (1935).
4) R. NEWTON and R. A. GORTNER: Botan. Gaz. **74**, 442 (1922).
5) P. KOETS: Proc. Kon. Akad. v. Wetensch., Amsterdam, **34**, 420 (1931).
6) D. M. GREENBERG and M. GREENBERG: J. Biol. Chem. **94**, 373 (1931).
D. GREENBERG and W. C. KOHN: J. Gen. Physiol. **16**, 559 (1932—'33); **18**, 93 (1935).
7) H. B. OAKLEY: Biochem. J. **31**, 28 (1937).
8) H. R. KRUYT and K. C. WINKLER: Z. anorg. Ch. **188**, 200 (1930).
9) H. G. BUNGENBERG DE JONG and P. H. THEUNISSEN: Koll. Beih. **47**, 254 (1938).
10) A. HENWOOD and R. M. GAREY: J. Frankl. Inst. **221**, 531 (1936).
11) H. BECHHOLD—A. KÖNIG: D. R. P. 403405 Kl. 421 (1924).