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MADISON, WISCONSIN.

[CONTRIBUTION FROM THE WOLCOTT GIBBS MEMORIAL LABORATORY OF HARVARD UNIVERSITY.]

COMPRESSIBILITY OF AQUEOUS SOLUTIONS OF CASEIN AND PEPTONE.

BY SVEN PALITZSCH.

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To understand many of the problems of physiological chemistry, a knowledge of molecular forces in solutions has become of greater and greater importance. Surface tension has, especially in the recent times, been considered as one of the forms of expression of these forces. However, in the case of the more or less colloidal solutions in question, this property is frequently very difficult to measure; in most solutions of proteins, split proteins, and similar substances, the surface tension is lowered with rising concentration, and the substance will therefore, according to Gibbs' rule, accumulate, sometimes to a very high degree, in the surface, where it furthermore often coagulates, forms films, etc.

For that reason it would be advantageous to investigate a property, which, like the surface tension, might be expected to give information as to the molecular conditions, and which could be measured without the difficulties arising from the peculiar state of the surface. Such a property is compressibility, which gives an expression of the state of all the molecules in the solution, not only those in the surface. As mentioned in a foregoing paper,¹ in such solutions the relation between the surface tension and the compressibility does not seem at first to be very plain, because of the changing nature of the solvent; but whether or not the relation between the two properties is clear and simple the compressibility is nevertheless such an important property that it may be able to furnish valuable information as to the internal structure and behavior of physiologically interesting solutions.

As regards the compressibility of colloidal solutions, very few measurements have been published; and nobody has, so far as I know, measured both the surface tension and the compressibility of identical solutions. Comparison of data from different sources, on account of the very uncertainly defined composition of the colloids employed, could hardly be of great value.

¹ Richards and Palitzsch, "Compressibility of Urethane Solutions, Etc.," *THIS JOURNAL*, 41, 59 (1919).

However, it should be noted that G. de Metz,¹ in 1890, measured the compressibility of liquids rotating polarized light. He included a non-gelatinizing glue solution and a 10% aqueous solution of gum arabic, both of which showed a compressibility less than that of water. On the other hand, 2% glue solution, immediately after dissolving, was more compressible than water; but on gelatinizing it diminished in compressibility, approaching the compressibility of water. The surface tension of these solutions is far from having been well determined. G. Quincke² found, both with a highly diluted gelatine solution and a 20% solution of gum arabic, a surface tension considerably less than that of water. On the other hand, L. Zlobicki³ obtained with gelatine solutions a less surface tension than with water, but with a 2% solution of gum arabic, one markedly greater. In 1891-1898 Barus⁴ examined the compressibility of aqueous solutions of proteins, but the method used was too primitive to furnish really quantitative results.

The first accurate measurements were carried out by L. J. Henderson and F. N. Brink,⁵ in 1908, at the Chemical Laboratory of Harvard College, by means of the method devised by Theodore W. Richards. They found that at 20.0° the absolute compressibility of a

10 per cent gelatine solution is 39.10^{-6} between 100 to 500 megabars
 2 per cent gelatine solution is 41.10^{-6} between 100 to 500 megabars
 water solution is 42.10^{-6} between 100 to 500 megabars

This is a decreasing compressibility with increasing concentration; the surface tension probably decreases at the same time.

Methods, apparatus, and calculations employed in the present research were quite the same as detailed in the previous paper already mentioned.⁶

The casein employed was prepared by C. A. F. Kahlbaum according to Hammarsten's method; such casein is entirely free from fat and has a fairly constant composition. It was weighed and dissolved in weighed quantities of water and 0.1 *N* sodium hydroxide and then filtered. The alkaline solutions contained 10 cc. and the acid solutions 4.8 cc. 0.1 *N* sodium hydroxide per gram of casein.

Not only the concentration of the casein, but also that of hydrogen ion, was varied. It is a well known fact that pure casein is almost insol-

¹ *Wied. Ann.*, **41**, 663 (1890); the original paper in Russian language is published in *Abhandl. d. meth. Abt. d. Naturforscherges. d. Univ. Odessa*, **9**, 139 (1889); the paper in *Wied. Ann.*, **35**, 497 (1888), contains nothing about compressibility, although it is often quoted as if this were the case.

² *Wied. Ann.*, **35**, 582 (1888).

³ *Bull. Acad. Sci. Cracovie*, July 1906, p. 488; quoted from Wo. Ostwald, *Kolloidchemie*, **1911**, Aufl., p. 226.

⁴ *Am. J. Sci.*, [4] **156**, 285 (1898); the data are detailed quoted in Wo. Ostwald, *Kolloidchemie*, **1911**, 2 Aufl., p. 147-148.

⁵ *Am. J. Physiol.*, **21**, 248 (1908).

⁶ Richards and Palitzsch, *Loc. cit.*

ble in water, but can be dissolved in acid and in alkaline solutions; this behavior is expressed more rationally by saying that casein is insoluble at its isoelectric point (corresponding to the hydrogen ion concentration, $2.5 \cdot 10^{-5}$, as measured by L. Michaelis and H. Pechstein),¹ and that it is soluble both at higher and at lower hydrogen ion concentration (consequently also at neutral reaction), although in somewhat different degree. Strongly alkaline casein may behave like a "true" (molecularly dispersed) solution, while with the hydrogen ion concentration approaching the isoelectric point casein may change, becoming more and more like a suspensoid until finally precipitated. The assumption of this possibility receives support from the fact that the surface tension changes with the hydrogen ion concentration.

Two series of solutions of casein were examined; the hydrogen ion concentration of the one corresponded to p_H^2 equal to about -10 and that of the other to p_H equal to about -6 . The former solutions are strongly alkaline, and color phenolphthalein deep red, while the latter are weakly acid and close to the isoelectric point.

The hydrogen ion concentration was measured electrometrically. The slight difference of p_H of the various casein and peptone concentrations are due to the dilution and the resulting increase in dissociation and not to any addition of acid or base.

As seen from Table I and II, *the compressibility, when solutions of identical concentration are compared, is very nearly the same in weakly acid as in alkaline solutions.* In other words, the compressibility is apparently independent of the hydrogen ion concentration.

TABLE I.
Compressibility, Etc., of Acid Casein Solutions.
Temperature 20.0°, Pressure Range 100 to 300 Megabars.

G. casein for 100 g. water.	G. NaOH for 100 g. water.	Density. D.	Solution- volume in solution, ³	p_H .	Weight of sol'n used. W. G.	w . ³	Compress- ibility $\times 10^6$.	Viscosity $t/t_0 \times 100$.
0	0	0.99823	43.25	100
1.481	0.029	1.00220	0.735	6.82	28.424	3.0982	42.58	145
3.053	0.059	1.00625	0.738	6.71	27.488	2.9538	42.11	211
5.522	0.107	1.01245	0.738	6.65	28.379	3.0015	41.77	430
8.597	0.166	1.01985	0.738	6.62	27.763	2.8643	41.03	1450
9.983	0.195	1.02265	0.743	6.60	28.765	2.9193	40.56	..

¹ *Biochem. Z.*, **47**, 260 (1912).

² *Compt. rend. trav. Lab. Carlsberg*, **8**, 4 (1909); *Biochem. Z.*, **21**, 131 (1909).

³ The "solution-volume" (0.72 to 0.743), recorded in Tables I and II, is the sum of the volume of casein and of sodium hydroxide; on account of the formation of sodium caseinate and of the dissociation of this salt, etc., subtraction of the volume of sodium hydroxide from this sum is without purpose.

⁴ The significance of w and the equation for calculating the result is to be found in *THIS JOURNAL*, **41**, 62 (1919).

TABLE II.
Compressibility, Etc., of Alkaline Casein Solutions.
Temperature 20°, Pressure Range 100 to 300 Megabars.

G. casein for 100 g. water.	G. NaOH for 100 g. water.	Density. D.	Specific volume in solution.	p_H .	Weight of sol'n used, W. G.	w.	Compressibility. $\times 10^6$.
5.009	0.201	1.01190	0.729	9.98	28.280	2.9884	41.71
8.006	0.322	1.01955	0.729	9.85	29.183	3.0086	41.08
10.012	0.402	1.02495	0.725	9.77	27.771	2.8128	40.51

The viscosity in alkaline solutions was also measured, in a separate series; the data, recorded in Table III, show that the viscosity rises at first slowly and then, at 6 to 10%, with extraordinary rapidity.¹ No similar behavior was found with the compressibility, as the data obtained (see Tables I and II) showed a compressibility decreasing continuously, and (at least as regards the more concentrated solutions) approximately linearly.

TABLE III.
Viscosity of Alkaline Casein Solutions.

G. casein for 100 g. water.	p_H .	Time of flow. Sec.	$t/t_0 \times 100$.
0.0	...	331.5	100
2.0	10.08	551.6	166
4.0	10.01	1010	305
6.0	9.94	2212	667
8.0	9.85	7200	about 2200
		7400	
10.0	9.77	30000	about 8400
		28200	
		26400	

Further, it may be noted that the viscosity of the acid solutions increased considerably more slowly than is the case with the alkaline solutions; this has indeed already been pointed out by E. Laqueur.² As above mentioned, the compressibility was nevertheless the same.

The viscosity of the most concentrated casein solutions changed very rapidly. The three values, recorded in Table III, of the 10% alkaline solution, were observed in immediately successive samples. The time between the preparation of the casein solution and the measurement of its viscosity not having been observed and not having been exactly the same in every case, the values obtained are not very exact, but are still sufficiently good for the comparison here made. The latter remark also applies to the peptone solutions described below, the viscosity of which was measured merely by a Traube stalagmometer.

Since the specific volume of casein in solution (including the small amount of sodium hydroxide present) is in the neighborhood of 0.73 or

¹ A similar result obtained by H. Chick and C. J. Martin, *Kolloid. Z.*, 11, 102 (1912). The hydrogen ion concentration of the solutions used was not measured.

² Hofmeister's, *Beitr. chem. Phys. Path.*, 7, 279 (1906).

0.74, and the specific volume of casein in the solid state is $1/1.318 = 0.759$ cc.,¹ a slight contraction may take place during solution.

As representing hydrolyzed proteins, "pepton Roche," prepared from silk fibrin, was also examined. Probably it is a tetrapeptide consisting of 2 molecules of glyocoll, 1 molecule of alanin, and 1 molecule of tyrosin. According to Fischer and Abderhalden,² "pepton Roche" contains 15.30% N, 6.05% H, and 52.43% C. The preparation employed for the present research was from F. Hoffmann-LaRoche et Cie., Basel Greuzbach; it contained 14.90% of nitrogen, and was hydrolyzed to such an extent that 59% of the total nitrogen could be determined by formol titration³ as amino-acid nitrogen. The measurements of the several properties follow:

TABLE IV.
Compressibility of Peptone Solutions.
At 20.0° under a Pressure of 100 to 300 Megabars.

G. peptone for 100 g. water.	Density. D.	Weight of sol'n used. W. G.	<i>w.</i>	Compressibility times $\times 10^6$.
0	0.99823	43.25
3.986	1.01125	29.174	3.0487	41.29
7.978	1.02325	29.329	2.9059	39.71
11.970	1.03470	29.730	2.8054	38.33
16.032	1.04555	29.524	2.7088	37.67

TABLE V.
Hydrogen Ion Concentration, Specific Volume, Surface Tension and Viscosity of
Peptone Solutions.

G. peptone for 100 g. water.	p_H .	Specific volume in solution.	Surface tension.		Viscosity.	
			Drop number.	Mg./mm.	Time of flow. Sec.	$t/t_0 \times 100$.
0	52.8	7.20	200	100
3.986	6.34	0.665	67.0	5.74	226	113
7.978	6.40	0.670	69.0	5.64	246	123
11.970	6.43	0.671	72.0	5.46	280	140
16.032	6.45	0.673	74.0	5.37	295	148

The results in Table IV show that the compressibility decreases continuously with rising concentration, and much more rapidly than in the case with casein solutions. From Table V, the "solution-volume" is seen to be considerably less than that of casein. The surface tension decreases at first rapidly and then very slowly; consequently the compressibility and the surface tension change with increasing concentration in the same direction; not—as is the case for inorganic salts—in opposite directions. The viscosity increases continuously with increasing concentration, as would be expected.

¹ H. Chick and C. L. Martin, *Kolloid. Z.*, 12, 69 (1913).

² *Sitzb. kgl. preuss. Acad. Wiss.*, 30, 574 (1907).

³ *Compt. rend. trav. Lab. Carlsberg*, 7, 1 (1907); E. Abderhalden, *Handbuch der biochemischen Arbeitsmethoden*, 6, 262 (1912).

Summary.

The compressibility of casein solutions decreases with rising concentration and very nearly to the same extent in weakly acid and in strongly alkaline solutions. The compressibility of peptone solutions also decreases, even more markedly, with rising concentration. The concentration being 10 g. for 100 g. water, the compressibility of acid casein solution is $40.6 \cdot 10^{-6}$, of alkaline casein solution $40.5 \cdot 10^{-6}$, and of a peptone solution $39.0 \cdot 10^{-6}$. The hydrogen ion concentration, the specific volume in solution (or "solution-volume"), and the viscosity were also measured; likewise the surface tension in the case of peptone.

In concluding, it gives me great pleasure to acknowledge my indebtedness to Professor Theodore W. Richards for the use of the facilities of the Wolcott Gibbs Memorial Laboratory and for his kind interest during the work; and through him to the Carnegie Institution of Washington for the use of apparatus belonging to that Institution.

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ARSENIOUS OXIDE AS A STANDARD SUBSTANCE IN IODIMETRY.

BY ROBERT M. CHAPIN.

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Introduction.

Following the development of a reliable laboratory method¹ for the preparation of pure arsenious oxide, an investigation has been made of the accuracy possible when the substance is employed in iodimetry. Though the process has long been in common use it seems never to have received the rigorous testing demanded by modern standards. The work of Washburn² extended merely to a study of the precision possible, that is, the closeness of agreement among titrations. The work here presented involves the direct comparison, by titration with weight burets, of known amounts of pure arsenious oxide and pure iodine. The object was to establish the reliability of properly purified arsenious oxide as a standard to replace the less convenient iodine which has been the final recourse in work calling for the highest possible degree of accuracy. This substance is the simple oxide of an element of very accurately determined atomic weight; it is non-hygroscopic, permanent in the solid state and also highly permanent in a properly acidified solution.

Preparation of Pure Substances.

Arsenious oxide was obtained in the form of a coarse powder nearly

¹ *J. Ind. Eng. Chem.*, 10, 522 (1918).

² *THIS JOURNAL*, 30, 31 (1908).