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 $40.5\ 10^{-6}$, 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.

[Contribution from the Biochemic Division, Bureau of Animal Industry, U. S. Department of Agriculture.]

ARSENIOUS OXIDE AS A STANDARD SUBSTANCE IN IODIMETRY.

By Robert M. Chapin.

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Introduction.

Following the development of a reliable laboratory method[1] 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 Washburn[2] 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

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

[2] This Journal, 30, 31 (1908).

free from dust by resubliming some material purified in the manner already referred to.[1]

Pure iodine was obtained by a process abridged from that of Guichard.[2] Potassium iodide U. S. P., 100 parts, and iodine U. S. P., 150 parts, were dissolved in water, 100 parts. To the solution was added about 5 parts crystallized copper sulfate in warm concentrated solution. After thorough stirring and standing for some time the precipitate was filtered off and the filtrate distilled from a retort into a cooled flask, water being added as necessary. The iodine in the distillate was filtered with suction on a hardened paper in a Buchner funnel, washed with water, and redistilled with water from the same apparatus. After again filtering, washing and draining, it was thoroughly dried over calcium chloride, then sublimed twice with intermediate drying. The subliming apparatus consisted of a shallow beaker, on the rim of which rested a globular flask filled with cold water. During the second sublimation the substance was not collected until a liberal preliminary portion had been allowed to escape, and it was obtained in loose crystals by frequent removal from the condensing flask. It was finally ground to coarse powder in a glass mortar, and preserved over calcium chloride.

Preparation of Solutions.

The solution of arsenious oxide in dilute sulfuric acid, as recommended by Roark and McDonnell[3] appears for obvious reasons the safest method of preparation. The only question is the possible volatilization of an appreciable quantity during the necessary boiling, and therefore the following experiment was conducted.

Experiment 1.—In a side-tube distilling flask was placed 5 cc. concentrated H_2SO_4 in 300 cc. H_2O. Flask and condenser were fitted with bark corks. The distillate, collected in portions of 25 cc. and tested for arsenic in a modified Gutzeit apparatus, yielded a very low blank. To the remaining 200 cc. liquid in the flask was then added $2^1/_2$ g. As_2O_3, and distillation was resumed, successive portions of 25 cc. being taken off while the volume in the flask was maintained at 100 to 200 cc. All portions collected except the first, which contained less, owing to delayed solution of the substance, showed a constant content of 20 micromg. As_2O_3. Finally the condenser tube was filled with 50 cc. diluted H_2SO_4 and left one-half hour. No more As_2O_3 was found in this solution than in the blank.

Therefore, in the preparation of one liter of tenth-normal arsenious oxide solution it would be necessary to boil off more than 600 cc. in order to diminish the strength of the finished solution by 1 part in 10,000.[4]

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[1] The raw material comprised several grades; chiefly "technical" white arsenic.

[2] *Ann. de Chim.*, 7, 27 (1917).

[3] *J. Ind. Eng. Chem.*, 8, 327 (1916).

[4] Since hardly a fourth of the above proportion of liquid need be lost through gentle boiling in a long-necked volumetric flask, any such additional precaution as a

About 0.8 g. dry arsenious oxide was weighed out by difference from a vial into a weighed 100 cc. volumetric flask. To avoid loss of dust the vial was chosen small enough to slip easily within the neck of the flask during transference of the substance. Solution was effected by very gentle boiling in a mixture of 16 cc. of dilute sulfuric acid (1 in 10) with about 60 cc. water. Water was then added to the cooled solution up to the base of the neck of the flask and a second weighing was made.

For weighing the iodine, ampules were prepared by blowing a bulb of slightly less than 2 cm. diameter on the end of a glass tube of 5 mm. bore, which was further drawn down to about 2 mm. at 1 to 2 cm. above the bulb, and lastly cut off to a total length of about 11 cm. Into the bulb of this miniature flask was charged through a slender tube 1.5 cc. of a solution of C. P. potassium iodide (10 g. per 15 cc.) without wetting the interior of the apparatus above the constriction. After the weight, constant on the balance for 10 minutes, had been ascertained, about 0.8 g. iodine was introduced, most of which remained above the constriction. The tube was drawn out above the iodine to a capillary, which, after cooling to room temperature over calcium chloride, was sealed off by a small blow-pipe flame. The tip thus separated was gently heated to remove condensed iodine, and finally, after cooling, the weight of tube plus tip, constant for 10 minutes, was ascertained, and consequently the weight of iodine obtained. A tube treated in a similar way, except that no iodine was added, showed a variation in weights before and after sealing decidedly less than 0.1 mg.

All weights, including buret readings, were reduced to vacuum and the weights of the two substances themselves were further corrected by means of the Bureau of Standards certified corrections for the set of weights employed.

The water used was freshly collected from a large laboratory still of a modern type which delivers water substantially free from dissolved gases. All solutions were employed shortly after preparation.

Execution of the Titrations.

In the ordinary titration of arsenious oxide with iodine, sodium bicarbonate is the buffer usually employed to maintain neutrality and is entirely appropriate for the purpose. For the reverse titration here contemplated it presented obvious defects. The sodium phosphate mixture advocated by Washburn appears to react with inconvenient slowness. A borax-boric acid mixture was also stated by Washburn to be theoretically applicable but was not regarded by him as practically so useful as phosphate or bicarbonate. Barneby[1] found such a mixture useful in "differ-

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reflux condenser, which has been suggested as an absolute preventive against loss, is obviously superfluous.

[1] THIS JOURNAL, 38, 330 (1916).

ential iodimetry." It effects a rapid reaction and is otherwise entirely convenient. The proportion of boric acid necessary to prevent the formation of iodate was determined by the following experiment:

Experiment 2.—To 50 cc. of a solution containing recrystallized $Na_2B_4O_7.10H_2O$, with or without H_3BO_3, was added 50 cc. *N*/20 I. After standing 22 hours an excess of H_3BO_3 was added and the solution was titrated with $Na_2S_2O_3$ (*N*/100 at end) and starch. Then diluted HCl was added in excess and the liberated iodine titrated with *N*/100 $Na_2S_2O_3$.

TABLE I.—FORMATION OF IODATE IN PRESENCE OF BORAX AND BORIC ACID.

No.	$Na_2B_4O_7.10H_2O$. G.	H_3BO_3. G.	*N*/100 $Na_2S_2O_3$. Cc.
1	0	0	0.00
2	5	0	9.15
3	5	1	1.90
4	5	2	0.10

It appears, therefore, that a buffer solution containing borax and boric acid in the weight ratio of 2 to 1 may be used with entire safety as respects formation of iodate. The solution adopted contained 8 per cent. of crystallized borax and 4 per cent. boric acid.

With this fact established the following experiment was carried out to test the accuracy of the method:

Experiment 3.—A tube of iodine, thoroughly shaken to dissolve all the substance, was placed in a stout 500 cc. bottle of clear glass to which was also added 30 cc. of a freshly prepared 10 per cent. solution of pure potassium iodide and 125 cc. of the buffer solution. After cooling the whole to about 10° C., the tube of iodine was shattered by pressure with a stout glass rod and an arsenious oxide solution was run in from a weight buret to decoloration. After adding 2 cc. of freshly prepared starch solution[1] the final end-point was reached by cautious titration with *N*/100 iodine.

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[1] The starch solution contained 0.5% potato starch, boiled 2 hours with water, plus 3% boric acid added toward the end of the boiling period. A briefly boiled starch solution is revealed under a magnifying glass as a suspension of enormously swollen granules. When such a solution is used as an indicator the resulting color is located partly in the granules and partly in the medium; not an ideal condition if delicacy of end-point is a paramount consideration. Though still visible after a half-hour, granules can no longer be detected after 1 hour of boiling, while boiling for an additional hour appears to effect no further change.

Long boiled solutions are equally as sensitive as solutions boiled only 2 minutes. The tints afforded with minimal amounts of I are likewise entirely comparable, ranging from pure blue through violet to rose as the concentration of KI and other salts is increased. Boric acid, not previously used as a preservative for starch solutions so far as the writer is aware, appears to be very effective against molds, though not against salivary enzymes. Solutions used intermittently during several months, and closed only by a pipet or inverted beaker, appear indistinguishable from fresh solutions. In contrast solutions of soluble starch prepared by acid hydrolysis soon suffer degradation.

For the blank[1] another bottle was charged with 40 cc. potassium iodide solution, 125 cc. buffer solution, 2 cc. starch and water to equalize volumes, and the mixture cautiously titrated with the N/100 iodine solution until

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[1] The truth of the blank obtained by this procedure has been questioned. In the first place, making the justifiable assumption that NaI produced from 0.8 g. I will exert exactly the same effect upon the end-point as an equivalent amount of KI, particularly in the presence of a preponderating amount of the latter salt, it will be noted that the equivalent of 4.04 g. KI was present in the regular run against 4.0 g. in the blank. Secondly, the volumes of buffer and starch solutions were the same, while the colors were matched at equal final volumes. From previous experience assurance was felt that, provided equal concentrations of KI and approximately equivalent concentrations of total salts were maintained in order to equalize the salt effect upon the end-point, no specific effect would be produced by the minor differences in composition which existed. On the contrary it was feared that far greater risk of error would be incurred through the introduction of extraneous substances in an attempt to make the composition of the solutions exactly similar. But the possible magnitude of error in the blank has now been investigated. To a "true" blank should be added HI derived from 0.8 g. I, As_2O_5 derived from 0.312 g. As_2O_3 and 6.24 cc. of the H_2SO_4 solution. There remained in hand the identical solution of H_2SO_4 originally used, and this was diluted and boiled. A stock solution of As_2O_5 was made from the originally used As_2O_3 by oxidation with HNO_3 (Menzies and McCarthy, THIS JOURNAL, 37, 2021 (1915)). Since HI could only be introduced in the form of KI, a corresponding reduction of 1.2 g. borax and an increase of 0.78 g. H_3BO_3 is called for in the buffer. The various buffer solutions necessary were made from single bottles of the chemicals. Various mixtures of the above ingredients were titrated with N/200 I and starch in a total volume of 200 cc. to the first appearance of a plain and persistent color, whatever the tint might be. The titrations were made by allowing the I solution to drop constantly from a buret at the rate of 1 drop in 3 seconds, the drops actually entering the titration being caught at half-minute intervals. Frequent tests showed that 1 cc. was very consistently delivered in 32 drops. The following table gives the results obtained:

Run No.	KI. G.	H_3BO_3. G.	$Na_2B_4O_7.10H_2O$. G.	H_2SO_4 sol. Cc.	Oxidized As_2O_3. G.	N/200 I. Drops.
1	1	0	0	0	0	2–3
2	4	0	0	0	0	3
3	4	5	0	0	0	2–3
4	4	5	10	0	0	5–6
5	4	5	8	0	0	5–6
6	4	6	8	0	0	5
7	4	6	8	7	0	4
8	4	6	8	7	0.4	4–5

It will be noted that, although the mixtures were made up slightly to exaggerate possible effects, the original blank, represented by Run 4, falls behind the true blank, Run 8, by at most 2 drops N/200 I.

Next, to a mixture prepared as in Run 8 was added 13 drops (0.406 cc.) of N/200 I, and a mixture prepared as in Run 4 was titrated by the drop method at half-minute intervals until the colors matched. Triplicate comparisons showed between 16 and 17 drops to be necessary, and no difference between the final tints could be detected. Therefore the original blank falls behind the true blank by at most 4 drops N/200 I, or 0.125 cc. This quantity represents 0.00008 g. I or a maximum error of 1 part in 10,000 which may possibly have occurred in the blank as originally obtained.

the colors in the two bottles matched. The volume of *N*/100 iodine needed for titration of excess arsenious oxide was in no case more than 2 cc. and for the blank never more than 0.2 cc. The colors were not significantly affected by further addition of the buffer mixture, nor by longer standing. The results calculated from the titrations appear in Table II, under Tests No. 1 to 4.

TABLE II.—COMPARISON OF IODINE AND ARSENIOUS OXIDE.

No.	As_2O_3 sol.	$\frac{\text{Weight I}}{\text{Weight } As_2O_3}$
1	A	2.5664[1]
2	A	2.5657
3	B	2.5661
4	B	2.5658
5	C	2.5656
6	C	2.5661
7	D	2.5656
Average ratio determined		2.5658
Theoretical ratio by 1918 official atomic weights		2.5651
Difference		0.0007

After this work had been completed there appeared a paper by McCrosky[2] in which it was stated that perfectly dry iodine was obtained only by fusion in the weighing tube, a procedure which affected his results by more than 1 part in 700. In contrast with McCrosky the present writer had not passed a continuous current of cold water through his subliming apparatus and had noted no condensation of moisture thereon, while in the final sublimation the substance was collected only after considerable heating, possibly only after complete fusion. But the uncertainty necessitated further work. The following experiment was therefore carried out:

Experiment 4.—In a 2 cc. glass-stoppered weighing tube about 0.8 gram iodine was fused as described by McCrosky and, after weighing, the tube with loosened stopper was dropped into a 500 cc. funnel-necked glass-stoppered Erlenmeyer flask containing 7 cc. of 40 per cent. potassium iodide solution. Into the funnel around the stopper was poured 3 cc. of the potassium iodide solution, and after all iodine had been dissolved, this, followed by 125 cc. of borax buffer, was allowed to flow down the sides of the flask. The titrations were made as previously described and the results are given in Table II under Tests 6 and 7. Test 5 was made without fusion on a sample of the original iodine which had been contained in a glass-stoppered weighing bottle resting in a dark cabinet during seven months.

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[1] Excluded in computing average.

[2] THIS JOURNAL, 40, 1662 (1918).

Evidently the original iodine was perfectly dry, and pure iodine is not hygroscopic. Evidently, too, the cumbersome method of weighing employed in the first four tests offers no advantage over the use of a stoppered weighing bottle with proper precautions. Fusion before weighing, though unnecessary in the present case, is certainly a desirable precaution. Excluding the unsubstantiated result of Test 1, the greatest variation of any test (No. 3 and No. 6) from the average was therefore 1 part in 8500, while the average of the observed ratios differed from the theoretical ratio by less than 1 part in 3600. The difference is in the direction which indicates a less purity, or a greater loss during manipulation, of the iodine, and consequently is in favor of arsenious oxide as the more reliable standard in practice.

Precautions in the Preparation and Use of Standard Iodine Solutions.

Aside from correct standardization, iodine solutions intended for accurate work demand some special precautions previously known in a general way but rarely observed. The first of these is the use of potassium iodide in quantity sufficient not merely to dissolve the iodine in concentrated solution but to inhibit dissociation of potassium tri-iodide and consequent ready volatilization of iodine after dilution to standard strength. The proportion of double the weight of iodine in twentieth-normal solutions authoritatively recommended[1] is unquestionably too low, for loss of strength during a series of titrations with such a solution as ordinarily manipulated may be readily demonstrated. Since potassium iodide is a high-priced substance each worker must decide for himself what proportion the character of his work warrants him in using. The writer has adopted the proportion of 4 per cent. in both tenth-normal and twentieth-normal solutions in which a high degree of accuracy and permanence is desired.

The presence of iodate in an iodine solution may naturally cause variation in its apparent strength according to whether titrations are conducted in a neutral or acid medium. Though commercial potassium iodide appears generally to be free from iodate there are some grounds[2] for the suspicion that iodate may be formed in iodine solutions under certain conditions of storage. It may be tested for by the procedure described under Expt. 2, and if present may be removed by addition of the corresponding amount of sulfuric acid.

A final precaution is to attain the end-point with starch at a temperature not above 25° C. It is well enough known that the blue color of iodized starch disappears completely on slight heating, but in fact warming even to 30° C. is sufficient to affect distinctly the delicacy of the end-point.

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[1] *J. Assoc. Official Agr. Chemists,* 2 (1916); Report of Comm. on Edit. Meth. of Anal., p. 63.

[2] Deiss, *Chem. Ztg.,* 38, 413 (1914).

Summary.

It was found that by employing weight burets with appropriate precautions, six titrations of pure arsenious oxide against pure iodine gave highly precise results, the average of which agreed within 1 part in 3600 with the theoretical value. The evidence is in favor of arsenious oxide as the more reliable standard substance in practice. Precautions in the preparation and use of standard iodine solutions are emphasized.

WASHINGTON, D. C.

NOTES.

Retardation by Sugars of Diffusion of Acids in Gels.—In a paper under this title, E. A. and H. T. Graham[1] have occasion to refer to the power of salt and of sugar to decrease the swelling of gelatin which has been immersed in dilute acid solution and has become swollen in consequence. They state that "the decrease of swelling by sugar cannot be accounted for by Procter's ion concentration theory, but must be explained in some other way." Procter attributes the swelling of gelatin in acid solution to the fact that the anion of a highly ionizable salt formed from the acid and gelatin tends to diffuse outward, and the amount of swelling to the excess of concentration of diffusible ions in the jelly over that in the external solution. In the case of an equilibrium between a colloid jelly and a solution of electrolytes, at least one of which forms an ionizable compound with the colloid, it has been shown[2] that the concentration of diffusible ions in the jelly must always be greater than in the surrounding solution; likewise that adding to such a system an electrolyte which does not interact chemically with any of the components of the system must result in a lessening of the excess of concentration of ions in the jelly over that in the external solution and therefore in a reduction of the swelling. This explains the action of salt.

Procter and Wilson, however, dealt only with highly ionized electrolytes and assumed that the very small concentration of neutral molecules was the same in both phases. The quantitative agreement between their theoretical calculations and experimental data shows that no serious error was introduced by this assumption. But, where a mixture of electrolyte and non-electrolyte is concerned, it is necessary to take into consideration the effect of thermodynamic environment. Since the molal fugacity of neutral molecules increases very rapidly with increasing concentration of ions in a solution, the molal fugacity of sugar would be greater in the jelly than in the surrounding solution and hence, upon the establishment of equilibrium, the sugar will have become

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[1] THIS JOURNAL, 40, 1916 (1918).

[2] Procter and Wilson, *J. Chem. Soc.*, 109, 307 (1916); J. A. and W. H. Wilson, THIS JOURNAL, 40, 886 (1918).