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A Quantitative Method for the Estimation of the Arsenoso Group

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Arsenoso compounds derived by the usual synthetic procedures are frequently of dubious homogeneity. It has been noted that samples of this class of compounds are most frequently contaminated with the corresponding arsonic acid, the dearsenated organic residue, arsenic trioxide or mixtures of these substances. While the determination of total arsenic by wet combustion will detect gross contamination, considerable quantities of impurities may be present without changing the total arsenic content to such an extent as to be greatly different from the tolerance limits usually prescribed for such analytical procedures. This is particularly true if more than one impurity is present, since certain combinations, such as the dearsenated residue and arsenic trioxide or the arsonic acid and arsenic trioxide, result in compensatory errors. In addition, most if not all arsenoso compounds are isolated as the arsonous acids and the dehydration of such acids may not proceed analytically to the anhydrous form. In such instances the determination of total arsenic alone may result in an erroneous impression of the essential purity of the product.

Several tests have been devised for the detection of the aforementioned impurities but they are relatively insensitive and subject to considerable interference. The reaction of arsenicals with hypophosphorous acid has been used to detect inorganic arsenic but this reaction, which converts inorganic arsenic to elemental arsenic and organic arsonic and arsonous acids to arseno compounds, is quite complex when both inorganic and organic forms are present. Unless the amount of inorganic arsenic is quite large, no arsenic metal is formed, but rather complex polyarseno compounds.

Similarly, Blicke, *et al.*,[1] have described a qualitative test for arylarsonic acids, using concentrated hydriodic acid to produce a red color due to free iodine when pentavalent arsenic is present. While the test will detect gross contamination, it is not particularly sensitive and frequently is misleading due to the color interference caused by highly colored diiodoarsines. In addition, it gives no indication of the quantitative degree of purity. Since this problem has been encountered frequently in our researches, the chemical reactions of the arsenoso group were examined to determine if any of these reactions were sufficiently quantitative for the purpose of differential analysis.

While the arsenoso group will undergo a number of addition reactions, none of those investigated was sufficiently quantitative for use. The arsenoso group will also oxidize and reduce. Examination of the literature indicated that the reduction methods were not quantitative but that it might be possible to obtain a quantitative oxidation.

Ehrlich and Bertheim[2] utilized the reaction of iodine with arsenoso groups to estimate the degree of purity obtained when arsonic acids were reduced. Others have utilized iodine to estimate arsphenamines,[3,4,5,6,7] a procedure which required the use of an arbitrary factor which was variously determined as 7.55 to 7.75 (theory would require an iodine factor of 8 per mole of arsphenamine). Recently, McChesney[8] used iodine to follow the decomposition rate of neoarsphenamine and dichlorophenarsine hydrochloride solutions. It was noted, however, that the iodine values tended to be high and were used only for comparative purposes. To our knowledge, the general applicability of the iodine ti-

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(1) Blicke, Powers and Webster, THIS JOURNAL, **54**, 2945 (1932).

(2) Ehrlich and Bertheim, *Ber.*, **45**, 756 (1912).

(3) Gaebel, *Arch. Pharm.*, **249**, 241 (1911).

(4) Myers and Du Mez, *U. S. Public Health Repts.*, **33**, 1003 (1918).

(5) Elvove, *ibid.*, **39**, 750 (1924).

(6) Maccalu, THIS JOURNAL, **44**, 2578 (1922).

(7) Wright, *Proc. Soc. Exp. Biol. Med.*, **31**, 170 (1933).

(8) McChesney, *J. Pharmacol.*, **84**, 222 (1945).

tration of arsenoso groups has not been investigated.

Theoretically, an arsenoso compound or arsonous acid should react with iodine according to equation (1). Of the common impurities, only

$$R{-}As(OH)_2 + I_2 + H_2O \underset{b}{\overset{a}{\rightleftharpoons}} R{-}AsO_3H_2 + 2HI \quad (1)$$

arsenic trioxide might be expected to react with iodine. Also, nuclear iodination of the arsonic acid or dearsenated organic residue might conceivably interfere with the stoichiometric uptake of iodine. While arsines and arseno compounds will also react with iodine, the first cannot exist in the presence of arsenoso compounds and the second has never been known to be present as an impurity, although the reverse situation, that of small quantities of arsenoso compounds present in arseno samples, is frequently true.

In order to test the stoichiometric qualities of the arsenoso-iodine reaction, highly purified samples of 3-amino-4-hydroxybenzenearsonous acid and 4-[2′,4′-diamino-6′-triazinylamino]-benzenearsonous acid hydrate were titrated in acidified aqueous solution with standard iodine solution. As indicated in the experimental section, the reaction was found to be both stoichiometric and of adequate precision. The effect of interfering substances was also investigated. From previous unpublished researches[9] it was known that in the presence of an excess of the iodide ion, most benzenearsonic acids do not reduce above *p*H 1.1 and none of those examined reduced above *p*H 2. On the other hand, arsenic acid showed evidence of reduction at all *p*H values below 4.8. Since the effect of the hydrogen ion concentration seemed marked, the reaction of iodine with varying amounts of arsonous acid, arsonic acid and arsenic trioxide between *p*H 2 and 6.8 was investigated. It was found that the reaction between the arsonous acid and iodine remained stoichiometric between these *p*H values and that the presence of the arsonic acid had no effect on the titration. Arsenic trioxide interfered above *p*H 5 when it constituted more than 4% of the arsonous acid but between *p*H 2 and 3 the reaction was within analytical limits with as much as 8% arsenic trioxide present. With 11% arsenic trioxide the endpoint of the reaction was not definite and the iodine uptake was no longer stoichiometric for the arsonous acid but did not approach the value required for the combined organic and inorganic trivalent arsenic. The amount of arsenic trioxide which failed to interfere with the stoichiometric properties of the arsonous acid-iodine reaction appeared to be a function of the amount of arsonous acid present.

In order to determine why small quantities of arsenic trioxide failed to influence the titration appreciably, the effect of each constituent present in the reaction was investigated. *p*H alone, while influencing the arsenic trioxide-iodine reaction, did not explain the failure of the inorganic arsenic to titrate. Similarly, the presence of the amount of iodide ion equivalent to that produced during the titration of approximately 0.1 g. of arsenoso compound failed to greatly influence the arsenite reaction. The amount of *p*-arsanilic acid which would be produced under such conditions did significantly alter the reaction and a combination of *p*-arsanilic acid and iodide ion sufficed to reduce the iodine value of 1.5 mg. of arsenic trioxide from 0.570 to 0.008 ml. Similarly, the value for 12 mg. of arsenic trioxide was reduced from 4.560 to 0.024 ml. Since the titration of 0.1 g. of most arsenoso compounds or arsonous acid involves about 20 ml. of such an iodine solution, 0.008 ml. would be insignificant and 0.024 ml. barely noticeable. With lesser amounts of arsonic acid and iodide, the amount of arsenic trioxide not affecting the titration was correspondingly smaller. This phenomenon was not unique for *p*-arsanilic acid but also noted with over twenty other benzenearsonic acids.

A number of arsenoso compounds and arsonous acids were titrated under what appeared to be optimum conditions and the values were all found to compare favorably with the values obtained by combustion methods and with those required by theory.

Experimental

1. **Reaction of Iodine with Arsonous Acids.**—Approximately 0.2-g. samples of 3-amino-4-hydroxybenzenearsonous acid and 4-[2′,4′-diamino-6′-triazinylamino]-benzenearsonous acid hydrate were weighed accurately to four decimal places and then suspended in approximately 40 ml. of water and sufficient hydrochloric acid added to effect solution. Four analytical assistants titrated two samples of each compound using standardized iodine solution and the customary starch-iodide color end-point.

TABLE I

Operator	3-Amino-4-hydroxybenzenearsonous acid, As(theory) = 34.51% % As found		4-[2′,4′-Diamino-6′-triazinylamino]-benzenearsonous acid hydrate, As(theory) = 22.88% % As found	
1	34.50	34.50	22.90	22.84
2	34.47	34.54	22.87	22.85
3	34.52	34.48	22.91	22.87
4	34.51	34.49	22.93	22.88
Average	34.50		22.88	

2. **Effect of Arsonic Acid and Arsenic Trioxide on the Reaction of Arsonous Acids with Iodine.**—Highly purified 3-amino-4-hydroxybenzenearsonous acid and 3-amino-4-hydroxybenzenearsonic acid were prepared. Samples of 3-amino-4-hydroxybenzenearsonous acid weighing exactly 0.1085 g. were transferred to iodine flasks. Neutral standard solutions of arsenic trioxide (0.0500 *N*) and 3-amino-4-hydroxybenzenearsonic acid (0.1 *M*) were prepared. Varying quantities of these solutions were added to the samples of 3-amino-4-hydroxybenzenearsonous acid and water and sufficient hydrochloric acid added to give the desired *p*H and about 150 ml. of solution. The solutions were titrated with standard iodine solution using starch solution as the indicator. All titrations were carried out in duplicate. The results, calculated on the basis of total solids added, are given in Table II.

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[9] L. A. Sweet, private communication. The data were taken from the unpublished work of L. A. Sweet and E. W. Tillitson.

TABLE II

3-Amino-4-hydroxybenzenearsonous acid, mg.	Arsenic trioxide, mg.	Arsenic trioxide, %	3-Amino-4-hydroxybenzenearsonic acid, mg.	3-Amino-4-hydroxybenzenearsonic acid, %	Total impurities, %	% As calcd. Total	% As calcd. Trivalent	% As calcd. Org. trivalent	% org. trivalent As found when initial *p*H was 2	3	5	6.8
0.1085					0.0	34.51	34.51	34.51	34.54	34.47	34.51	34.47
			0.1165	100.0	100.0	31.14	0.00	0.00	0.00	0.00	0.00	0.00
.1085	0.00110	1.0			1.0	34.85	34.85	34.16	34.18	34.19	34.12	34.20
.1085	.00495	4.4			4.4	36.29	36.29	33.00	33.05	32.98	33.00	34.01^a
.1085	.00989	8.0			8.0	37.95	37.95	31.62	31.65	31.71	31.72^a	33.45^a
.1085	.01384	11.3			11.3	39.81	39.81	30.62	30.59	30.82^a	30.87^a	
.1085	.02273	17.4			17.4	41.71	41.71	28.54	28.72^a	28.98^a	29.54^a	
.1085	.04546	29.5			29.5	46.75	46.75	24.31	26.22^a	26.65^a		
.1085			.0146	11.9	11.9	34.21	30.42	30.42	30.43	30.41	30.45	30.42
.1085			.0291	21.1	21.1	33.91	27.21	27.21	27.16	27.18	27.24	27.19
.1085			.0583	35.0	35.0	33.67	22.45	22.45	22.41	22.42	22.41	22.48
.1085			.1165	51.8	51.8	33.28	16.64	16.64	16.60	16.61	16.70	16.71
.1085	.00495	3.9	.0146	11.2	15.1	35.80	32.15	29.23	29.27	29.18	29.24	
.1085	.00495	3.5	.0291	20.4	23.9	35.43	28.23	26.26	26.23	26.31	26.29	
.1085	.00495	2.9	.0583	33.9	36.8	34.87	23.98	21.79	21.84	21.77	21.75	
.1085	.00250	1.8	.0291	20.8	22.6	34.72	28.06	26.73	26.71	26.69	26.74	
.1085	.00250	1.5	.0583	34.4	35.9	34.28	23.22	22.12	22.10	22.16	22.16	
.0543					0.0	34.51	34.51	34.51	34.53	34.48	34.54	
.0543	.00495	8.0			8.0	37.95	37.95	31.62	31.59	31.64^a.	31.65^a	
.0543	.01137	17.0			17.0	41.71	41.71	28.54	28.65^a	28.73^a	28.82^a	
.0543	.02273	29.5			29.5	46.75	46.75	24.31	25.20^a	25.58^a		

[a] End-point easily recognized but not as permanent as that of blank.

3. Effect of Iodide and *p*-Arsanilic Acid on the Titration of Arsenic Trioxide at pH 2.5.—Standard solutions of arsenic trioxide (3 g./liter), potassium iodide (50 g./liter) and *p*-arsanilic acid (100 g./liter) were prepared. Approximately 0.05 *N* iodine was standardized against the arsenic trioxide solution in bicarbonate buffer. Varying quantities of the solutions were then mixed, made up to 20 ml. with the appropriate amounts of 0.1 *M* potassium acid phthalate and 0.1 *N* hydrochloric acid and titrated with the standard iodine solution, using a 5 ml. standardized microburet having 0.01 ml. subdivisions. The starch-iodide end-point was classified as follows: s, endpoint as permanent as blank; f, end-point faded slowly but first detectable color still visible after one minute; i, first end-point faded rapidly, titration continued until conditions equalled those under f. All titrations were made in duplicate.

TABLE III

*p*H	Substances, mg. As_2O_3	KI	*p*-Arsanilic acid	I_2, ml.	End-point
$NaHCO_3$	12	. .	. . .	4.560	s
2.5	12	. .	. . .	4.176	i
2.5	1.5	. .	. . .	0.518	i
2.5	12	50	. . .	4.087	i
2.5	12	. .	100	0.246	f
2.5	1.5	50	100	.008	s
2.5	3	50	100	.012	s
2.5	6	50	100	.018	f
2.5	9	50	100	.020	f
2.5	12	50	100	.024	i
2.5	18	50	100	.042	i
2.5	1.5	25	50	.006	s
2.5	3	25	50	.008	s
2.5	6	25	50	.036	i
2.5	9	25	50	.050	i
	Blank			.003	s

4. Iodine Titration of Miscellaneous Arsenicals.—Samples of approximately 0.15 to 0.2 g. were weighed accurately to four decimal places. When possible the substance was dissolved in 40 ml. of water or water containing a few drops of concd. hydrochloric acid. When not soluble under these conditions the compound was dissolved in 15 ml. of propylene glycol by warming gently and then diluting with water. The *p*H was adjusted to approximately 3.5 and the titration performed with standard iodine solution (*ca.* 0.05 *N*) using the starch end-point. The results of the titrations are given in Table IV.

Discussion

From a theoretical standpoint, some of the foregoing data are anomalous. Washburn[10,11] has studied the equilibrium indicated in equation (2) extensively. From his data it was calculated that reaction

$$AsO_3^{=} + I_2 + H_2O \underset{(b)}{\overset{(a)}{\rightleftarrows}} AsO_4^{=} + 2I^- + 2H^+ \qquad (2)$$

2b would be negligible when $[H^+] < 10^{-4}$. However, reaction 2a would not be insignificant unless $[H^+] > 1$. Also, McChesney has reported the E_0 value for the half-cell 3-amino-4-hydroxybenzenearsonic acid/3-amino-4-hydroxybenzenearsonous acid to be +0.580 v. Since E_0 $[AsO_4^{=}/AsO_3^{=}]$ is +0.574 v. and since the hydrogen ion concentration should have the same effect on both half-cells, inorganic arsenite should be oxidized under the same conditions as 3-amino-4-hydroxybenzenearsonous acid. The titration data definitely indicate the reverse. In examining the method used by McChesney to determine the organic half-cell potential, it was noted that he measured the potential when $[RAsO_3H_2] = [RAsO_2H_2]$ in *N* acid, assuming unit activity of

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[10] E. W. Washburn, THIS JOURNAL, **30**, 31 (1908).

[11] E. W. Washburn and E. K. Stachan, *ibid.*, **35**, 681 (1913).

TABLE IV

ESTIMATION OF ORGANICALLY-BOUND TRIVALENT ARSENIC IN ARSENOSO COMPOUNDS

	Compound	Calcd. % As	Total arsenic,[a] %	Trivalent arsenic, %	Diff.
1	3-Amino-4-hydroxybenzenearsonous acid	34.51	34.51	34.50	−0.01
2	5-Amino-2-hydroxybenzenearsonous acid	34.51	34.48	34.49	+ .01
3	4-Amino-2-hydroxybenzenearsonous acid	34.51	34.58	34.47	− .09
4	2-Amino-4-arsenosophenol hydrochloride	31.81	31.84	31.79	− .05
5	2-Amino-4-dichloroarsinophenol hydrochloride	26.14	26.12	26.15	+ .03
6	2,4-Diamino-6-(4′-arsenosoanilino)-*s*-triazine	25.66	25.60	25.63	+ .03
7	2-(4′-Arsenosoanilino)-4,6-di-(β-hydroxyethylamino)-*s*-triazine	19.63	19.78	19.52	− .26
8	2-(4′-Arsenosoanilino)-4,6-di-(β-hydroxy-*n*-propylamino)-*s*-triazine	18.36	18.49	18.45	− .04
9	4-Arsenosophenylurea	33.16	33.23	33.18	− .05
10	3-Amino-4-β-hydroxypropoxybenzenearsonous acid	27.22	27.26	27.25	− .01
11	1-(4′-Arsenosophenoxy)-propanol-2	31.10	31.12	31.16	+ .04
12	4-Acetoxybenzenearsonic acid	29.30	29.36	29.28	− .08
13	3-Amino-4-hydroxyethoxybenzenearsonous acid	28.74	28.72	28.70	− .02
14	β-(4-Arsenosophenoxy)-ethanol	32.84	32.80	32.75	− .05
15	2-Acetamino-4-arsenosophenol	28.95	28.85	28.80	− .05
16	4-Carbamidobenzenearsonous acid	32.71	32.65	32.74	+ .09

[a] Determined by a modification of the method of Cislak and Hamilton, THIS JOURNAL, **52**, 638 (1930).

the hydrogen ion and equal activity coefficients for the arsonic and arsonous acids. Since the degree of ionization and other necessary data are unknown, such assumptions are dangerous, particularly since arsonic acids are ionized to a greater degree than the corresponding arsonous acids. Another factor to be considered is that arsonous acids probably exist in solution in some form besides $RAs(OH)_2$ and $RAsO_2^-$.[12] Exact measurement of E_0 values of organic arsenical half-cells is not possible without further knowledge of the ion forms in solution but the limiting conditions established for reactions Ia and Ib suggest that E_0 (I^-/I_2) and E_0 $(RAsO_3^=/RAsO_3H_3^-)$ are practically identical.

The failure of the arsenite ion to titrate in the presence of *p*-arsanilic acid is unusual. Since other arsonic acids behaved in the same fashion, the arsonate ion must influence the arsenate–arsenite equilibrium in some fashion. A study of this factor is now in progress. The evidence definitely indicates that the larger the quantity of arsonate present, the smaller the titration value for equal amounts of arsenite.

It has been found advisable in practice to determine both the trivalent organic and total arsenic values for samples. As an arbitrary standard, if both values are within ±0.1% of theory and each other, the compound is considered as being pure within the limits of analysis. If theory and the two assays agree within 0.3%, the compound is generally considered sufficiently pure for pharmacological purposes (over 99%). Occasionally samples are obtained for which the two arsenic assays agree within +0.1% but the values do not agree with the theoretical value for either the arsonous acid or arsenoso compound. While this may be due to the presence of non-arsenic-containing organic or inorganic compounds, it is frequently caused by incomplete dehydration of the forms $RAsO_3H_4$ or $RAsO_2H_2$.

The iodine solution should be standardized against Bureau of Standards arsenic trioxide and acidified standard thiosulfate. Deiss[13] has shown that iodine solutions may acquire a small amount of iodate on standing. While this is not important in assay procedures where the iodine solution is standardized under the same conditions as are used in the assay, it is a source of appreciable error in this determination. In acid solution the iodate is available but it does not react under the conditions used to standardize against arsenic trioxide. Iodine solutions showing detectable iodate concentrations should not be used to titrate arsonous acids.

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Summary

1. The reactions of iodine with aromatic arsonous acids and arsenoso compounds were shown to be stoichiometric between the *p*H values 2 and 6.8.

2. The titration of aromatic arsenoso compounds with iodine was found to be independent of the amount of aromatic arsonic acid present and not appreciably affected by the presence of arsenic trioxide at *p*H 2–3 provided the arsenic trioxide did not constitute more than 8% of the sample.

3. The reaction of arsenic trioxide with iodine at *p*H 2.5 was inhibited by *p*-arsanilic acid.

DETROIT, MICHIGAN RECEIVED MAY 9, 1946

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[12] Banks, *et al.*, THIS JOURNAL, **69**, 5 (1947).

[13] E. Deiss, *Chem. Ztg.*, **38**, 413 (1944).