Summary

The activity coefficients in aqueous solution at 25° of potassium chloride from $C = 0.005$ to 3.00 moles per liter, and of calcium chloride from $C = 0.002$ to 0.1 have been determined from measurements of potentials of concentration cells with transference, and accurate transference numbers.

It has been found that the simple Debye–Hückel equation $-\log f_r = \alpha\sqrt{C}/(1 + \beta\sqrt{C})$ fits the data, with high precision to $C = 0.2$ for potassium chloride and to $C = 0.03$ for calcium chloride, with distances of closest approach of 4.1 and 5.2Å., respectively.

The equation

$$-\log f_r = \frac{0.5056\sqrt{C}}{1 + 1.50\sqrt{C}} - 0.0365C\log C$$

holds for potassium chloride up to $C = 3.00$, and the expression

$$-\log f_r = \frac{1.7515\sqrt{C}}{1 + 2.814\sqrt{C}} - 0.147C$$

fits the data for calcium chloride up to $C = 0.1$.

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[CONTRIBUTION FROM THE T. JEFFERSON COOLIDGE, JR., MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

A Revision of the Atomic Weight of Carbon. II

BY GREGORY P. BAXTER AND ARTHUR H. HALE

Quantitative combustion of hydrocarbons has been continued during the past year and the results confirm the conclusion already reported[1] that the atomic weight of carbon is not far from 12.010.

Only minor changes were made in the combustion apparatus and analytical technique. The ring seal of the second bulb in the water absorption system was eliminated in order that complete equalization of pressure might be more easily secured during weighing. Furthermore, the air in this system was saturated with water during the first weighing, so that no correction was necessary on this score. In some of the analyses (71, 74, 78, 79, 80), a somewhat smaller but similar system was used for absorbing the carbon dioxide, in order to increase the accuracy of weighing.

During the earlier work preliminary experiments had shown that the "blank" corrections were very small. These and later experiments, carried out by passing air and oxygen through the empty combustion tube while it was heated as in the combustions, produced the changes in weight (positive) given in the following tables.

AIR

	Duration of run, hours	H_2O, mg.	CO_2, mg.
	44	0.26	0.38
	64	.52	.62
Total	108	.78	1.00
Gain per hour		.0072	0.0093

OXYGEN

At the close of each run the tubes were swept out with air, and a correction made for the period during which air was used.

	Duration of run, hours	H_2O, mg.	CO_2, mg.
	44	0.76	0.01
	71	.68	1.17
	69	.53	0.90
	80	.73	.81
Total	264	2.70	2.89
Gain per hour		0.0102	0.0109

On the basis of these results in each of the new experiments as well as in those already reported a negative correction based on these blanks has been applied. This correction usually amounted to about 0.1 mg. each for both water and carbon dioxide.

Purification of Hydrocarbons

Pyrene.—Although our earlier attempts to purify pyrene had apparently been unsuccessful, further efforts were made as follows. One specimen after crystallization from pure benzene was first melted in vacuum with freshly fused potassium hydroxide and then distilled from the potassium hydroxide. After another crystallization the sample was melted in nitrogen at low pressure in contact with metallic sodium, and then distilled from the sodium. Crystallization from benzene, distillation at low pressure and another crystallization followed.

Another sample was kept melted, in nitrogen at low pressure, over metallic mercury for twenty hours. This was followed by crystallization from benzene, treatment with sodium as described above, another crystallization, distillation at low pressure and a final crystallization.

The benzene used in these experiments and in the puri-

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[1] Baxter and Hale, THIS JOURNAL, **58**, 510 (1936).

TABLE I

ATOMIC WEIGHT OF CARBON

O = 16.0000 H = 1.0078

Analysis	Sample	Hydrocarbon, g.	H_2O, g.	H, g.	C, g.	CO_2, g.	O, g.	Ratio $C:O_2$	At. wt. of C
					Chrysene				
39	I	2.78052	1.31192	0.14678	2.63374	9.65237	7.01863	0.375250	12.008(0)
40	I	2.69266	1.27591	.14275	2.54991	9.34366	6.79375	.375332	12.010(6)
45	I	2.97790	1.41044	.15780	2.82010	10.33440	7.51430	.375298	12.009(5)
47	I	2.99659	1.41906	.15877	2.83782	10.39868	7.56086	.375330	12.010(6)
66	II	3.01102	1.42558	.15950	2.85152	10.44739	7.59587	.375404	12.012(9)
67	II	2.97646	1.40901	.15764	2.81882	10.32819	7.50937	.375374	12.012(0)
68	II	2.97260	1.40723	.15744	2.81516	10.31566	7.50050	.375330	12.010(6)
70	II	1.56689	0.74145	.08295	1.48394	5.43767	3.95373	.375327	12.010(5)
73	III	3.08222	1.45976	.16332	2.91890	10.69608	7.77718	.375316	12.010(1)
74	III	2.07420	0.98195	.10986	1.96434	7.19819	5.23385	.375315	12.010(1)
							Average	.375328	12.010(5)
					Triphenylbenzene				
42	I	3.00022	1.58994	0.17788	2.82234	10.34128	7.51894	0.375364	12.011(6)
44	II	2.99781	1.58711	.17757	2.82024	10.33453	7.51429	.375317	12.010(1)
48	II	2.99647	1.58580	.17742	2.81905	10.33018	7.51113	.375316	12.010(1)
60	III	3.00284	1.58874	.17775	2.82509	10.35196	7.52687	.375334	12.010(7)
61	III	6.00641	3.17865	.35563	5.65078	20.70670	15.05592	.375320	12.010(2)
63	IV	2.99682	1.58563	.17740	2.81942	10.33123	7.51181	.375332	12.010(6)
64	IV	3.00217	1.58866	.17774	2.82443	10.35028	7.52585	.375297	12.009(5)
65	IV	2.99844	1.58649	.17750	2.82094	10.33739	7.51645	.375302	12.009(7)
							Average	.375323	12.010(3)
					Anthracene				
55	I	2.99495	1.51439	0.16943	2.82552	10.35385	7.52833	0.375318	12.010(2)
56	I	2.04939	1.03659	.11597	1.93342	7.08535	5.15193	.375281	12.009(0)
62	III	2.87189	1.45078	.16231	2.70958	9.92877	7.21919	.375330	12.010(6)
71	III	2.95847	1.49521	.16729	2.79118	10.22821	7.43703	.375308	12.009(9)
72	III	2.88436	1.45742	.16306	2.72130	9.97193	7.25063	.375319	12.010(2)
76	II	6.06324	3.06430	.34284	5.72040	20.96070	15.24030	.375347	12.011(1)
77	II	5.44882	2.75414	.30814	5.14068	18.83572	13.69504	.375368	12.011(8)
80	II	2.81287	1.42365	.15928	2.65359	9.72451	7.07092	.375282	12.009(0)
81	II	5.54044	2.80123	.31340	5.22704	19.15276	13.92572	.375352	12.011(3)
							Average	.375323	12.010(3)
							Average of all results	.375325	12.010(4)
					Pyrene				
78		3.00338	1.33857	0.14976	2.85362	10.44871	7.59509	0.375719	12.023(0)
79		2.99923	1.33674	.14956	2.84967	10.43690	7.58723	.375588	12.018(8)

fications described below had been treated with concentrated sulfuric acid, distilled and three times fractionally frozen.

The two samples of pyrene, when quantitatively burned, yielded 12.023 (Analysis 78) and 12.019 (Analysis 79) for the atomic weight of carbon, results essentially like those previously obtained with pyrene. Apparently these efforts at purification were as unsuccessful as the earlier ones. Actually Dr. E. B. Hershberg was able to detect sulfur in both these samples.

Chrysene.—A portion of the chrysene used in Analyses 39, 40, 45 and 47 of the previous work was further purified by distillation in nitrogen at low pressure and by crystallization from pure benzene (Sample II). The melting point was kindly determined by Dr. Hershberg to be 254.1–254.4°, corr.[2] Before this purification the melting point was 254.5–255.0°, although the composition before the treatment described above was identical with that of this sample.

Through the kindness of Professor L. F. Fieser we received a synthetic sample of chrysene[3] built upon naphthalene as a nucleus. We subjected this sample to three crystallizations from benzene with intermediate distillations at low pressure; m. p. 254.1–254.4° (corr.), Sample III.

Triphenylbenzene.—A portion of Sample II (previous paper, m. p. 174.3–174.5°, corr.) was slowly distilled at very low pressure with rejection of about a gram of slightly yellow residue. The product was once crystallized from pure benzene; m. p. 174.3–174.5° (corr.), Sample III.

A new sample was prepared for us by Dr. M. S. Newman under the direction of Professor Fieser. Benzaldehyde

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[2] All other melting points given in this paper were determined by Dr. Hershberg.

[3] Fieser, Fieser and Hershberg, THIS JOURNAL, **57**, 1851 (1935); **58**, 1463 (1936).

from amygdalin was converted to secondary phenylethyl alcohol with methylmagnesium chloride. Oxidation with dichromate and sulfuric acid yielded acetophenone, and condensation of acetophenone in a sealed tube with hydrochloric acid at 200° yielded triphenylbenzene. The latter was crystallized from acetic acid, distilled at low pressure and crystallized four times from dioxane. We purified the sample further by two crystallizations from benzene, low pressure distillation and a final crystallization from benzene; m. p. 174.2–174.5° (corr.), Sample IV. The sample previously used melted at 174.3–174.5° (corr.).

Anthracene.—A new sample of anthracene was purified as described in our earlier paper, Sample II.

Still a third sample was prepared from phthalic acid and benzene. Phthalic acid, after crystallization from water, was dehydrated to the anhydride. From phthalic anhydride and benzene with aluminum chloride *o*-benzoylbenzoic acid was prepared, and crystallized from benzene. Treatment of the product with concentrated sulfuric acid yielded anthraquinone which was recrystallized from glacial acetic acid. Reduction of anthraquinone to anthrone was effected with tin and hydrochloric acid. After recrystallization from a mixture of benzene and petroleum ether the anthrone was reduced to anthracene with zinc dust, sodium hydroxide and ammonia, and the anthracene was three times recrystallized from benzene. To purify the anthracene further it was melted in contact with potassium hydroxide in nitrogen at low pressure, distilled from the mixture, and crystallized from benzene. These processes were then repeated. Finally the product was melted in nitrogen, distilled at low pressure and recrystallized from pure benzene; m. p. of distilled material, 216.2–216.4° (corr.). The sample previously prepared melted at 216.4–216.7° (corr.), Sample III.

In Table I the weights of hydrocarbon have been corrected to vacuum. The weights of water have been corrected to vacuum, for dissolved air (see preceding paper), and for the blank (see page 506). The weights of carbon dioxide have been corrected to vacuum, and for the blank (see page 506).

A restandardization of the weights brought to light an error in the correction of one of the weights used in the earlier experiments. Because of this, and because "blank" corrections are now available the recalculated results of the earlier experiments are included with the new ones. No experiments are omitted from the table except those abandoned because of some accident.

In most of the analyses about 3 g. of hydrocarbon was burned. This quantity seemed to ensure sufficient accuracy and since seven to nine hours were required for combustion alone of this amount the experiment was as prolonged as it could be without too much tax on the analyst. In four experiments about 6 g. was burned, Nos. 61, 76, 77 and 81. The average result of these four experiments is 12.0111, that is, slightly less carbon dioxide was recovered than in the others. But of these four, Analysis 61 yielded more than the average and the atomic weight 12.0102. In Analyses 56, 70 and 74 only 1.5–2.0 g. was burned. The average of these three is 12.0099, but the average of Analyses 70 and 74 is 12.0103.

In four experiments, Analyses 55, 60, 68 and 77 the rate of burning was less than normal, about twelve hours for 3 g., and the average result 12.0108, while in Analysis 61 6 g. was burned in ten hours, yet Analysis 61 yielded slightly more carbon dioxide than the normal.

It seems unlikely that variations in quantity of hydrocarbon burned or minor variations in speed of combustion had any important effect on the result.

The average atomic weight of carbon (excluding results with pyrene), 12.010, is 0.001 unit higher than that reported in our earlier paper, partly because of the new corrections, partly because the effect of experiments 39 and 56 is distributed over a larger number.

As we have already pointed out, because of the low percentage of hydrogen in our hydrocarbons the method is comparatively insensitive to the present uncertainty in the atomic weight of hydrogen. Aston's[4] recent value, corrected to the chemical scale and for H^2, is 1.0081. With this value for hydrogen, that of carbon is lowered only 0.00026. It seems unlikely that natural variations in isotopic proportions (H, O, C) will be observed large enough to affect present chemical accuracy.

Although in this method it is unnecessary that the hydrocarbons should be pure, but only necessary that they should be free from impurities other than hydrocarbons, the composition of each hydrocarbon seems to correspond very closely with the formula in every case except that of pyrene. In the following table calculations of the percentages of hydrogen and carbon are made from the weights of water and carbon dioxide, with the use of the values 1.0078 and 12.010, for hydrogen and carbon, respectively. For the twenty-seven analyses (those with pyrene excluded) the average net deficiency from the theoretical in hydrogen is 0.0001%, that in carbon 0.002%. This apparent deficiency in carbon necessarily disappears entirely if our average atomic weight, 12.0104, is employed.

Aston's[4] most recent value for C^{12}, converted to the chemical scale with the factor 1.00025, is

[4] Aston, *Nature*, **137**, 357 (1936).

TABLE II

Analysis	% H	% C	Total
	Chrysene, $C_{18}H_{12}$		
39	5.279	94.733	100.012
40	5.301	94.695	99.996
45	5.299	94.704	100.003
47	5.298	94.698	99.996
66	5.297	94.686	99.983
67	5.296	94.693	99.989
68	5.296	94.700	99.996
70	5.294	94.703	99.997
73	5.299	94.701	100.000
74	5.296	94.703	99.999
Average	5.296	94.701	99.997
Calculated	5.298	94.702	
	Triphenylbenzene, $C_{24}H_{18}$		
42	5.929	94.062	99.991
44	5.923	94.076	99.999
48	5.921	94.078	99.999
60	5.919	94.077	99.996
61	5.921	94.078	99.999
63	5.920	94.077	99.997
64	5.920	94.082	100.002
65	5.920	94.082	100.002
Average	5.922	94.077	99.999
Calculated	5.921	94.079	
	Anthracene, $C_{14}H_{10}$		
55	5.657	94.342	99.999
56	5.659	94.347	100.006
62	5.652	94.345	99.997
71	5.655	94.346	100.001
72	5.653	94.346	99.999
76	5.654	94.339	99.993
77	5.655	94.335	99.990
80	5.663	94.343	100.006
81	5.657	94.336	99.993
Average	5.656	94.342	99.998
Calculated	5.655	94.345	
	Pyrene, $C_{16}H_{10}$		
78	4.986	94.939	99.925
79	4.987	94.963	99.950
Calculated	4.983	95.017	

12.0005. This is 1/10,000 lower than the value reported by him a year earlier, 12.0018.[5] Bainbridge (private communication) finds 12.0012, corrected to the chemical scale. With the value 12.0012 for C^{12} and our atomic weight the calculated abundance ratio of C^{13} is 1/109. This value is lower than that computed from our earlier results. Jenkins and Ornstein[6] find 1/106; Vaughan, Williams and Tait[7] 1/92 and Aston[8] 1/138.

Summary

It seems unlikely that our final value would be altered materially by continuance of the experiments. Of the four hydrocarbons investigated, one, pyrene, proved incapable of being purified by any method we could devise. The other three yielded identical results for carbon, whether they had been initially separated from coal tar, or synthesized, from a simple coal tar product in two cases (chrysene and anthracene), from a natural product in the third (triphenylbenzene).

The average atomic weight of carbon obtained by combustion of chrysene, triphenylbenzene and anthracene, 12.010, may therefore be taken as a fair outcome of this method of attack.

CAMBRIDGE, MASS. RECEIVED DECEMBER 14, 1936

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(5) Aston, *Nature*, **135**, 541 (1935).

(6) Jenkins and Ornstein, *Proc. Acad. Sci. Amsterdam*, **35**, 1212 (1932).

(7) Vaughan, Williams and Tait, *Phys. Rev.*, **46**, 327 (1934).

(8) Aston, *Proc. Roy. Soc.* (London), **A149**, 400 (1935).

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Studies in the Physical Chemistry of the Proteins. XIV. The Amphoteric Properties of Hemoglobin*

BY EDWIN J. COHN, ARDA A. GREEN AND MURIEL H. BLANCHARD

The behavior of proteins is determined largely by the number and distribution of their electrically charged groups. An accurate knowledge of the number of dissociable groups is therefore an essential requisite for the study of their distribution by such means as dielectric constant measurements and measurements of change in activity coefficient with change in ionic strength. In extending to proteins relationships developed by the study of amino acids and peptides, we shall first consider those that can be obtained as pure chemical individuals.

From certain points of view the hemoglobin of the horse may be considered the best known of the proteins. It is readily crystallizable by a number of procedures. It contains 0.335% iron[1] and

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* For supplementary tables of data obtained in this work, order Document 1007 from Science Service, 2101 Constitution Ave., Washington, D. C., remitting 25¢ for microfilm form, or 50¢ for photocopies readable without optical aid.

(1) Zinoffsky, *Z. physiol. Chem.*, **10**, 16 (1886).