U. S. Department of Commerce
National Bureau of Standards

Research Paper RP1904
Volume 41, July 1948

Part of the Journal of Research of the National Bureau of Standards

Density and Refractive Indices of Lactose Solutions

By Emma J. McDonald and Anne L. Turcotte

The density and refractive indices of lactose solutions in the range of unsaturation are reported to five decimal places at 20° and 25° C. For solutions of higher concentration up to 50-percent lactose, the corresponding values are reported at 25° C and for refractive indices alone at 15° C.

I. Introduction

Lactose, the disaccharide commonly known as milk sugar, has for many years been of commercial importance. Until recently its principal use was in the manufacture of pharmaceuticals, food products, and in the preparation of modified diets; however, the penicillium industry now also requires large quantities of this sugar.[1]

In the present investigation measurements have been made to determine the density and refractive indices of lactose solutions. The tables here presented are expected to be of use to the investigator working with pure solutions as well as to the analyst dealing with solutions in which the total solids may be calculated as lactose.

II. Preparation of Lactose

The lactose used in this investigation was carefully prepared by repeated crystallizations of the commercial product. The procedure consisted in heating a 50-percent solution in a water bath at 90° C, treating with decolorizing carbon, and filtering. The filtrate was then seeded with α-lactose hydrate crystals and stirred continuously while cooling in an ice bath. The resulting crystals, which were uniformly small and well formed, were purged on a centrifugal machine and washed consecutively with cold water and alcohol. After three crystallizations, the ash content had decreased to 0.002 percent and remained constant. The specific rotation of α-lactose hydrate in equilibrium solution is +50.53°. Thus the direct reading of a 10-percent solution would amount to +30.35°. The effect of the last traces of impurities on this reading is beyond the accuracy of the observations. Analysis by the method of Munson and Walker showed the product to be unchanged within the accuracy of the method. Here, as in the case of specific rotation, small traces of impurities would not affect the results noticeably. We, therefore, believe that ash content is a more reliable criterion for purity. The recrystallized product was dried in the air and stored in a desiccator over commercial α-lactose hydrate. Under these conditions the vapor pressure was so maintained that loss of water of crystallization was prevented.

Lactose hydrate contains 1 molecule of water of crystallization, which amounts to 5 percent of the total weight. Any change in the water of hydration would be reflected as an error in the percentage composition of the prepared solutions and hence in the reported physical constants; we, therefore, carefully investigated the composition of the crystals as to moisture content and were assured that we were dealing with a hydrate of uniform composition. Many moisture determinations were run in order to determine the most favorable conditions for this analysis.

The results obtained when the hydrate was dried in a vacuum at 85°, 120°, and 130° C are shown in figure 1. In all cases the loss in water was accompanied by some decomposition of the sugar as indicated by a yellowing of the sample. This discoloration was more pronounced in the recrystallized sugar than in the original commercial product. Because the ash content decreased on recrystallization from 0.0135 to 0.002 percent, it is suggested that a buffering material is present in the commercial product and that this retards decomposition. The minimum discolora-

[1] E. O. Whittier, J. Dairy Sci. **27**, 505 (1944); a review, Lactose and its utilization, contains many references to work on lactose.

789520—48——5

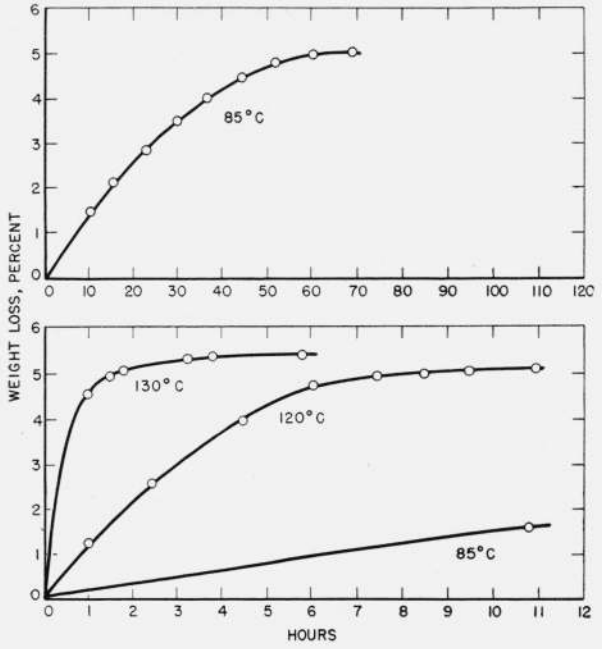

FIGURE 1. *Dehydration of α-lactose hydrate at 85°, 120°, and 130° C in vacuum.*

tion during loss of water appeared to take place at 120° C, and, therefore, this temperature was subsequently used in determining the moisture content. The drying curves shown in figure 2 are representative of those obtained during the progress of the investigation. Inspection of these curves shows the danger of using an arbitrary time when determining the moisture content of any given sample of α-lactose hydrate.

III. Physical Measurements

The range in concentration where accurate density and refractive index measurements can be made is limited by the relatively low solubility of lactose and by the readiness with which supersaturated solutions tend to deposit crystals. At 20° C, a saturated solution of lactose contains 16 percent of sugar, a low value as compared with 49, 67, and 70, the respective percentages of saturated dextrose, sucrose, and levulose solutions at this temperature. Supersaturated solutions deposit lactose hydrate crystals with great ease and thus limit the reliability of measurements made upon them. We have, therefore, carried out density and refractive index measurements with high precision in the range of unsaturation. The values reported for supersaturated solutions, although not obtained with the same precision, are suitable for many purposes and thus are included in this investigation.

1. Density of Lactose Solutions

Density measurements were made in a carefully calibrated flask. When filled to the lowest graduation at 20° C, this flask contained 126.333 ml. Its neck, 6-mm inside diameter, was graduated in 10 divisions at intervals of 0.02 ml. The volume reading could be estimated to one-tenth of one of these divisions, thus permitting a precision slightly greater than two units in the fifth place of density. The flask was recalibrated from time to time during the progress of the investigation, and the volume was found to be constant within the error of reading.

A quantity of α-lactose hydrate was introduced

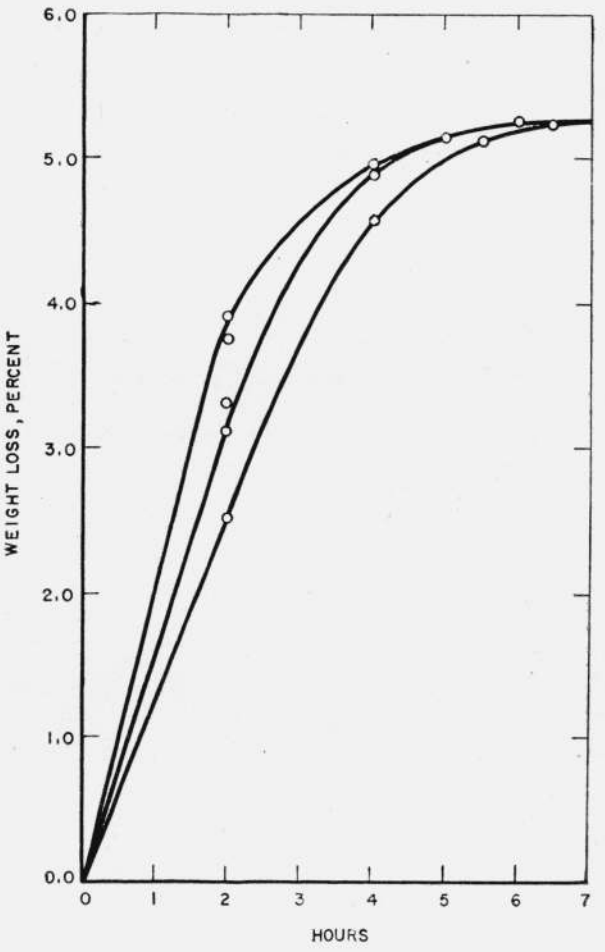

FIGURE 2. *Dehydration of α-lactose hydrate at 120° C in vacuum.*

into the weighed flask and accurately weighed. The sugar was dissolved in distilled water and air removed from the solution. The latter was accomplished by placing the slightly warmed flask in an environment of reduced pressure, care being taken to avoid spattering during this procedure. Water was then added to some point within the graduation, and the flask was rotated in such a way that the solution did not wet the upper part of the neck of the flask. After removing the water adhering to the flask above the solution by means of a current of dry air, the flask was placed in a thermostat and the final volume taken when the solution had come to temperature. As the manipulation was time-consuming, mutarotation was completed before the final observation of volume.[2]

At the time of each weighing, the density of the air in the balance case was determined by weighing a glass bulb, whose true mass had been determined at this Bureau. All weights were reduced to vacuum, the value for the density of solid α-lactose hydrate being taken as 1.540 in accordance with the determinations recorded below.

The experimental data are assembled in tables 1 and 2. From these observed values the following equations were calculated by the method of least squares. Here the density of a lactose solution is expressed as a function of the percentage, (p), of α-lactose present.

$$D_4^{20}=0.99823+0.003739p+0.00001281p^2. \quad (1)$$

$$D_4^{25}=0.99707+0.003717p+0.00001263p^2. \quad (2)$$

The deviation of the observed values from those calculated by formula are given in the last column of each table. The observed values show a mean deviation of less than 3 in the fifth place at 20° C, and less than 2 at 25° C, from those calculated by formula. We, therefore, believe that the formulas are valid to some units in the fifth decimal.

The values now given in the literature for the density of lactose solutions have been determined in connection with specific rotation studies. The work of Schmoeger,[3] as well as that of Fleischmann and Weigner,[4] will be considered because these

[2] The procedure used in making the density determinations was essentially that described by Jackson and Matthews in their work on levulose; BS J. Research 8, 405 (1932) RP426.

[3] M. Schmoeger, Ber. deut. chem. Ges. 13, 1922 (1880).

[4] W. Fleischmann and G. Weigner, J. Landw. 58, 45 (1910).

TABLE 1. *Density of lactose solutions at 20.0° C*

Weight of lactose [1] (air brass wt.)	Weight of lactose (vacuum)	Weight of solution (vacuum)	Volume of solution	Concentration of lactose	D_4^{20} observed	D_4^{20} calculated by formula [2]	Deviation from formula
g	*g*	*g*	*ml*	*Percent*			
2.2413	2.2427	126.9997	126.381	1.766	1.00490	1.00487	+0.00003
2.7596	2.7613	127.1586	126.344	2.172	1.00644	1.00641	+.00003
5.8969	5.9004	128.3891	126.405	4.596	1.01570	1.01569	+.00001
10.9203	10.9268	130.2569	126.404	8.389	1.03048	1.03050	−.00002
15.8408	15.8502	132.1234	126.444	11.996	1.04492	1.04493	−.00001
17.1718	17.1903	132.6147	126.442	12.963	1.04882	1.04885	−.00003
23.5294	23.5441	134.9147	126.398	17.451	1.06738	1.06738	0
24.0298	24.0450	135.1012	126.402	17.798	1.06884	1.06884	0
25.3294	25.3444	135.6730	126.493	18.681	1.07357	1.07255	+.00002

[1] α-lactose hydrate.

[2] $D_4^{20}=0.99823+0.003739p+0.00001281p^2$.

TABLE 2. *Density of lactose solutions at 25.0° C*

Weight of lactose [1] (air brass wt.)	Weight of lactose (vacuum)	Weight of solution (vacuum)	Volume of solution	Concentration of lactose	D_4^{25} observed	D_4^{25} calculated by formula [2]	Deviation from formula
g	*g*	*g*	*ml*	*Percent*			
1.0643	1.0650	126.4207	126.390	0.843	1.00024	1.00021	+0.00003
1.8585	1.8596	126.7549	126.432	1.467	1.00255	1.00255	0
5.2778	5.2810	128.0341	126.436	4.125	1.01264	1.01262	+.00002
8.1789	8.1838	129.1437	126.470	6.337	1.02114	1.02113	+.00001
10.2912	10.2974	129.9499	126.496	7.924	1.02730	1.02732	−.00002
15.1632	15.1723	131.7102	126.457	11.519	1.04154	1.04157	−.00003
20.1185	20.1306	133.5434	126.463	15.074	1.05599	1.05598	+.00001

[1] α-lactose hydrate.

[2] $D_4^{25}=0.99707+0.003717p+0.00001263p^2$.

investigators extended their observations to supersaturated solutions. Schmoeger reported values for the density of lactose solutions containing as much as 36 percent of the sugar. His values are given in the fourth place. Fleischmann and Weigner obtained data on solutions containing as much as 69 percent of sugar. The latter authors report that great difficulty was encountered in preparing solutions of the higher concentrations, and hence only few observations were made. We believe that the inclusion of density values on supersaturated solutions, although they cannot be obtained with great precision, may be of value to investigators working with this sugar. Therefore, we have applied the method of least squares to the data presented by these early investigators on supersaturated solutions for concentrations as high as 50 percent and obtained the following

equation relating the density to the percentage concentration.

$$D_4^{20}=0.9982+0.00370p+0.0000150p^2. \quad (3)$$

Tables 3 and 4 are working tables containing density values for unsaturated solutions and for saturated solutions of concentrations up to 50 percent. The values for unsaturated solutions are based on eq 1 and 2, and those for the supersaturated solutions were calculated by use of eq 3.

TABLE 3. *Density of lactose solutions at 20° and 25° C*

α-Lactose hydrate	D_4^{20}[1]	D_4^{25}[2]	α-Lactose hydrate	D_4^{20}[3]
Percent			*Percent*	
0	0.99823	0.99707	20	1.0782
1	1.00198	1.00080	22	1.0869
2	1.00576	1.00456	24	1.0956
3	1.00956	1.00834	26	1.1045
4	1.01339	1.01214	28	1.1136
5	1.01725	1.01597	30	1.1227
6	1.02113	1.01983	32	1.1320
7	1.02503	1.02371	34	1.1414
8	1.02896	1.02762	36	1.1509
9	1.03292	1.03155	38	1.1605
10	1.03690	1.03551	40	1.1702
11	1.04091	1.03949	42	1.1801
12	1.04494	1.04350	44	1.1900
13	1.04900	1.04753	46	1.2001
14	1.05309	1.05159	48	1.2104
15	1.05720	1.05568	50	1.2207
16	1.06135	1.05978	------	------
17	1.06550	1.06392	------	------
18	1.06968	------	------	------

[1] $D_4^{20}=0.99823+0.003739p+0.00001281p^2$.
[2] $D_4^{25}=0.99707+0.0037175p+0.00001263p^2$.
[3] $D_4^{20}=0.9982+0.00370p+0.0000150p^2$.

2. Density of Crystalline Lactose

The density of the crystalline α-lactose hydrate [5] was required in order to convert the weight in air to weight in vacuum.

The lactose was weighed in a calibrated flask, and the flask was filled with dry toluene saturated with lactose at 20° C. The trapped air was removed from the crystals by applying gentle suction while rotating the flask. All weights were reduced to the vacuum standard. The results of these measurements are given in table 5. The density of lactose hydrate was found to be 1.540. The density of commercial β-lactose, an anhydrous form that has found extensive commercial use because of its greater solubility, was found to be 1.589.

[5] Previous values (E. O. von Lippmann, Chemie Der Zuckerarten, p. 1526 (von Friedrick Vieweg und Sohn (1904)); Lichtenstein 1.543, Boedeker 1.5384, Filhol 1.534, Joule and Playfair 1.530, and Pionchon 1.525 (C. S. Hudson and F. C. Brown, J. Am. Chem. Soc. **30**, 960 (1908)), Hudson and Brown 1.54 (sp gr α-lactose hydrate) 1.59 (sp gr β-lactose).

TABLE 4. *Density of lactose solutions at 20° C*

Anhydrous lactose	D_4^{20}	Anhdyrous lactose	D_4^{20}
Percent		*Percent*	
1	1.00218	18	1.07367
2	1.00616	20	1.0827
3	1.01016	22	1.0919
4	1.01420	24	1.1012
5	1.01824	26	1.1107
6	1.02236	28	1.1203
7	1.02647	30	1.1300
8	1.03062	32	1.1399
9	1.03480	34	1.1498
10	1.03901	36	1.1600
11	1.04324	38	1.1702
12	1.04750	40	1.1806
13	1.05179	42	1.1911
14	1.05611	44	1.2017
15	1.06046	46	1.2125
16	1.06484	48	1.2234
17	1.06924	50	1.2345

3. Refractive Indices

The refractive indices of unsaturated solutions of lactose at 20° and 25° C were determined by use of a carefully calibrated immersion refractometer under accurately controlled conditions of temperature. All measurements were made in a constant-temperature room whose temperature was maintained within 2 degrees of that at which the water in the bath surrounding the instrument was held.

The instrument was inserted in a large glass tube, 83 mm in diameter and 350 mm in length. This contained sufficient water to surround the instrument cup containing the sugar solution. The glass tube with the suspended instrument was placed in a constant-temperature water bath, so that only a small part of it extended above the surface of the water. A thermometer was placed beside the instrument. Illumination was supplied by an electric bulb submerged in a glass tube similar to that containing the refractometer. The floor of the bath reflected the light into the instrument. Under these conditions a very sharp line characteristic of total refraction was observed,

TABLE 5. *Density of crystalline lactose*

Weight of pure toluene (vacuum)	Volume toluene	D_4^{20} toluene	Weight α-lactose hydrate (vacuum)	Weight toluene (vacuum)	Total volume	Volume toluene	Volume α-lactose hydrate	D_4^{20} α-lactose hydrate
g	*ml*		*g*	*g*	*ml*	*ml*	*ml*	
86.6441	100.2355	0.86441	19.1054	98.5649	126.4265	114.0243	12.4022	1.5405
86.5196	100.0915	.86441	19.1054	98.6121	126.4838	114.0789	12.4049	1.5401
86.6413	100.2285	.86444	19.1054	98.5252	126.3883	113.9784	12.4099	1.5395
86.5947	100.1765	.86442	20.0613	97.9627	126.3597	113.3277	13.0320	1.5394
86.5650	100.1425	.86442	----------	---------	---------	---------	----------	---------
	avg.....	0.86442						
86.6176	100.1685	.86472	10.2736	103.5757	126.4532	119.7836	6.6696	1.5404
86.6241	100.1805	.86468	10.2736	103.5025	126.3692	119.6989	6.6703	1.5402
86.5739	100.1235	.86467	----------	---------	---------	---------	----------	---------
	avg.....	0.86469					avg.....	1.5400
			Weight β-lactose (vacuum)				Volume β-lactose	D_4^{20} β-lactose
-------	---------	0.86469	22.8752	96.8837	126.4704	112.0794	14.3910	1.5895
-------	---------	.86469	22.8752	96.7750	126.3501	111.9537	14.3964	1.5890
-------	---------	.86469	22.8752	96.8459	126.4265	112.0357	14.3908	1.5896
							avg.....	1.5893

[1] Samples of toluene from two different lots were used for these experiments. The density of toluene recorded in the determinations on β-lactose is the average value for the second lot of reagent.

permitting a precision of a few hundredths and an estimated accuracy of 0.1 to 0.2 of a scale division in the reading of the instrument.

The conversion tables supplied by the manufacturer are based on readings for distilled water. In accordance with these tables, the instrument was set at 14.50 at 20° C and at 13.25 at 25° C. These readings are equivalent to indices of 1.33299 at 20° C and 1.33252 at 25° C. Readings on distilled water were made frequently, thus insuring the setting of the instrument. The arbitrary scale readings were converted to refractive indices by use of the manufacturer's tables.

Equations relating the refractive index to the concentration were prepared by subjecting the observed data to the method of least squares.

$$n_D^{20}=1.33299+0.001409p+0.00000498p^2 \quad (4)$$

$$n_D^{25}=1.33251+0.001405p+0.000004805p^2. \quad (5)$$

Tables 6 and 7 contain the observed data as well as those calculated by formula.

TABLE 6. *Refractive indices of lactose solutions at 20.0° C*

Weight of lactose [1] (air brass wt)	Weight of lactose (vacuum)	Weight of solution (vacuum)	Concentration of lactose	Zeiss immersion scale reading $t=20.0°C$	n_D^{20} observed	n_D^{20} calculated by formula [2]	Deviation from formula
g	*g*	*g*	*Percent*				
2.2413	2.2427	126.9997	1.766	21.00	1.33551	1.33549	+0.00002
3.3207	3.3227	127.3975	2.608	24.12	1.33671	1.33669	+.00002
5.2365	5.2397	101.7278	5.151	33.74	1.34038	1.34038	0
7.3119	7.3162	102.5546	7.134	41.47	1.34330	1.34329	+.00001
15.1730	15.1819	131.8051	11.518	59.10	1.34988	1.34988	0
15.2437	15.2525	131.8654	11.567	59.30	1.34995	1.34996	−.00001
22.2235	22.2367	134.4964	16.533	80.41	1.35765	1.35764	+.00001

[1] α-lactose hydrate.

[2] $n_D^{20}=1.33299+0.001409p+0.0000498p^2$.

The refractive indices of supersaturated solutions at 25° C, as well as those of all concentrations at 15° C, were determined with an Abbe refractometer. The scale of the instrument used permitted readings to a few units in the fourth decimal place. This was the limiting factor in the precision of the readings, since they were all made

TABLE 7. *Refractive indices of lactose solutions at 25.0° C*

Weight of lactose [1] (air brass wt)	Weight of lactose (vacuum)	Weight of solution (vacuum)	Concentration of lactose	Zeiss immersion scale reading $t=20.0°C$	n_D^{25} observed	n_D^{25} calculated by formula [2]	Deviation from formula
g	*g*	*g*	*Percent*				
1.0643	1.0650	126.4207	0.843	16.32	1.33371	1.33370	+0.00001
2.5473	2.5498	127.0832	2.006	20.54	1.33534	1.33535	−.00001
5.2778	5.2810	128.0341	4.125	28.54	1.33841	1.33839	+.00002
8.1789	8.1838	129.1437	6.337	36.90	1.34158	1.34160	−.00002
10.2912	10.2974	129.9499	7.924	43.20	1.34396	1.34395	+.00001
15.1632	15.1723	131.7102	11.519	57.63	1.34933	1.34933	0
20.1185	20.1306	133.5434	15.074	72.46	1.35478	1.35478	0

[1] α-lactose hydrate.
[2] $n_D^{25}=1.33251+0.001405p+0.000004805p^2$.

under carefully controlled conditions. Equations 6 and 7 are based upon these data.

$$n_D^{25}=1.3325+0.001384p+0.00000624p^2 \quad (6)$$

$$n_D^{15}=1.3334+0.001412p+0.00000537p^2. \quad (7)$$

The observed data for indices of refraction and also the values calculated by the formulas are given in tables 8 and 9. Table 10 is included as a working table. It has been prepared by use of the proper formula for each temperature.

TABLE 8. *Refractive indices of lactose solutions at 25° C*

Weight of lactose [1] (air brass wt)	Weight of lactose (vacuum)	Weight of solution (vacuum)	Concentration of lactose	n_4^{25} observed Abbé	n_4^{25} calculated by formula [2]	Deviation from formula
g	*g*	*g*	*Percent*			
10.9856	10.9923	53.7058	20.468	1.3634	1.3634	0.0000
14.0010	14.0095	54.9307	25.504	1.3720	1.3719	+.0001
17.1285	17.1389	56.6435	30.257	1.3800	1.3800	.0000
20.2416	20.3139	56.8220	35.750	1.3899	1.3900	−.0001

[1] α-lactose hydrate.
[2] $n_D^{25}=1.3325+0.001384p+0.00000624p^2$.

TABLE 9. *Refractive indices of lactose solutions at 15° C*

Weight of lactose [1] (air brass wt.)	Weight of lactose (vacuum)	Weight of solution (vacuum)	Concentration of lactose	n_4^{15} observed Abbé	n_4^{15} calculated by formula [2]	Deviation from formula
g	*g*	*g*	*Percent*			
2.6839	2.6855	50.7500	5.292	1.3410	1.3410	0.0000
13.1331	13.1412	131.1152	10.023	1.3480	1.3480	.0000
26.3086	26.3248	136.0419	19.351	1.3629	1.3627	+.0002
33.1227	33.1431	138.4840	23.933	1.3701	1.3703	−.0002
39.6613	39.6857	141.0027	28.145	1.3772	1.3774	−.0002
52.8430	52.8755	145.7118	36.288	1.3919	1.3917	+.0002

[1] α-lactose hydrate.
[2] $n_D^{15}=1.3334+0.001412p+0.00000537p^2$.

TABLE 10. *Refractive indices of lactose solutions*

α-Lactose hydrate	n_D^{25}	n_D^{20}	n_D^{15}	$\Delta n/\Delta t$
Percent				
1	1.33392	1.33440	1.3348	0.000096
2	1.33534	1.33583	1.3362	.000098
3	1.33677	1.33726	1.3376	.000098
4	1.33821	1.33871	1.3391	.000100
5	1.33966	1.34016	1.3406	.000100
6	1.34111	1.34162	1.3421	.000102
7	1.34258	1.34310	1.3436	.000104
8	1.34406	1.34458	1.3451	.000104
9	1.34554	1.34607	1.3466	.000106
10	1.34704	1.34758	1.3481	.000108
11	1.34855	1.34909	1.3496	.000108
12	1.35006	1.35062	1.3511	.000112
13	1.35159	1.35215	1.3526	.000112
14	1.35312	1.35369	1.3542	.000116
15	1.35467	1.35524	1.3558	.000116
16	1.35622	1.35681	1.3573	.000118
18	1.3594	-------	1.3605	.00011
20	1.3627	-------	1.3637	.00010
22	1.3660	-------	1.3670	.00010
24	1.3693	-------	1.3703	.00010
26	1.3727	-------	1.3737	.00010
28	1.3762	-------	1.3770	.00008
30	1.3797	-------	1.3805	.00008
32	1.3832	-------	1.3839	.00007
34	1.3868	-------	1.3875	.00007
36	1.3904	-------	1.3910	.00006

WASHINGTON, January 27, 1948.