[CONTRIBUTION FROM THE PHARMACOLOGICAL LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE REFRACTOMETRIC DETERMINATION OF ALCOHOLS AND ESTERS IN AQUEOUS AND IN COTTONSEED OIL SOLUTIONS[1]

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In order to determine the partition coefficients[2] between oil and water for a number of aliphatic alcohols and alkyl esters, a method of accurately and rapidly determining their concentrations was needed. Search of the literature failed to reveal any method which seemed suitable for this purpose. Specific-gravity determinations could not be used because of the necessity of making a great many determinations in a comparatively short time. Attention was then directed to the possibility of making quantitative determinations by measuring refractive index values. This method proved to be perfectly satisfactory.

The refractometer has been used for the quantitative determination of various substances since 1842, when Deville[3] reported the refractive indices of aqueous solutions of methyl and ethyl alcohols and of acetic acid. Increasing accuracy of this index, due to improvements in apparatus and refinements in operation, has increased the value of these determinations.[4,5,6,7,8] Refractive indices have been determined for solutions of various chemicals,[4,8,9,10,11,12,13,14,15,16,16a] for food products,[17,18] for biochemical

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[1] This report is abstracted from one portion of the dissertation submitted to the George Washington University, Washington, D. C., in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] This problem was suggested by Dr. E. W. Schwartze, Pharmacologist in Charge of this Laboratory, because of the necessity of ascertaining partition coefficients for the interpretation of the pharmacological action of anesthetics which we are now studying.

[3] Deville, *Ann. chim. phys.*, [3] **5**, 129 (1842).

[4] Chenevau, *Ann. chim. phys.*, **8**, 145, 289 (1907).

[5] Eisenlohr, "Spektrochemie organischer verbindungen," F. Enke, Stuttgart, **1912**.

[6] Hess, *Ann. phys.*, [4] **27**, 598 (1908).

[7] Perry and Roberts, *J. Sci. Instruments*, **1**, 87 (1923).

[8] Sidersky, "La refractometrie et ses applications pratiques," Gauthier-Villars, Paris, **1909**.

[9] Becka, *Z. physik. Chem.*, **121**, 288 (1922).

[10] Beythien and Hennicke, *Pharm. Zentralhalle*, **48**, 1005 (1907).

[11] Clemens, *J. Ind. Eng. Chem.*, **13**, 813 (1921).

[12] deCrinis, *Z. physiol. Chem.*, **110**, 254 (1920).

[13] Getman and Wilson, *Am. Chem. J.*, **40**, 468 (1908).

[14] Goldsmith, *J. Oil Colour Chem. Assoc.*, **4**, 57 (1921).

[15] Goldsmith, "Optical Methods in Research Laboratories," Section on Refractometry, Adam Hilger, London, **1923**.

[16] Simmonds, "Alcohol: its Production, Properties and Applications," MacMillan and Co., London, **1919**.

materials (blood, urine, etc.[19]), and for aqueous solutions of methyl[20,21] and ethyl alcohols.[20,21,22,23,24,25] Simmonds[16] gives Zeiss values at 15.6° for aqueous solutions of the higher alcohols; no other available data upon these solutions were found.

Water from a constant-temperature tank at 25.00 ± 0.01° constantly passed through chambers surrounding the prisms of the Abbé refractometer, which had a refractive index range from 1.2800 to 1.6000. Distilled water gave a reading of 1.3325 at the time of each test (the value found by Baxter, Burgess and Daudt,[26] n_D^{25} against air, was 1.33248). The Zeiss refractometer[27] was immersed in the constant-temperature bath until no further change in index of a solution was observed. This usually required about five minutes.[28] Zeiss scale readings from —5 to 105 were found to be perfectly linearly correlated with refractive indices from 1.32540 to 1.36639 ($r = 1.00 \pm 0.00$), so Zeiss readings have been used without translation into refractive indices. Readings of 13.25 were consistently obtained with distilled water.

Physical Properties of Solvents and Solutes

The distilled water used was obtained by the condensation of steam in block-tin condensers and was stored in large, closed copper tanks. Each morning a quantity deemed sufficient for the day's use was drawn, actively boiled for at least 15 minutes in a porcelain evaporating dish to remove dissolved gases, placed in stoppered Erlenmeyer flasks, and held at approximately 25° until used. Immersion refractometer readings on each day's supply agreed in Zeiss index, which was 13.25 at 25°, corresponding to a refractive index of 1.3325.

Thirty-nine one-quart (approximately 900 cc.) cans of a special purified

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[16a] Wagner, "Tabellen zur eintauchrefraktometer," Dissert. Sondershausen, **1907.**

[17] Kupelweiser, *Biochem. Z.*, **131,** 413 (1922).

[18] Thurston, "Pharmaceutical and Food Analysis," D. van Nostrand Co., New York, **1922,** pp. 22-23.

[19] Abderhalden, "Handbuch der biochemischen Arbeitsmethoden," Urban and Schwarzenburg, **1910-1919,** Berlin, vol. 1, pp. 568-583; vol. 8, pp. 84-119.

[20] Leach, "Food Analysis," John Wiley and Co., New York, **1914,** pp. 439-441.

[21] St. John, *Bur. Chem. Bull.*, No. **162,** 221 (1912).

[22] Andrews, This Journal, **30,** 353 (1908).

[23] Randall, *J. Ind. Eng. Chem.*, **10,** 629 (1918).

[24] St. John, In "Official and Tentative Methods of Analysis of the A. O. A. C." Washington, **1920,** pp. 300-387.

[25] Wagner and Schultze, *Z. anal. Chem.*, **46,** 508 (1907).

[26] Baxter, Burgess and Daudt, This Journal, **33,** 893 (1911).

[27] Kupelweiser (Ref. 17) gives an exhaustive description of the instrument and of various tests for accuracy, etc.

[28] Working at 20°, Randall (Ref. 23) reported that the temperature of a solution within the metallic cup differed from that of the bath. A number of tests during this investigation failed to show any such differences.

winter-pressed cottonseed oil, all filled from the same lot, were obtained from H. S. Bailey, Savannah, Georgia. Upon receipt, the oil was stored in a refrigerator at 1–2°. Individual cans were removed and opened as needed. Analysis of a sample of the oil by the Oil, Fat and Wax Laboratory of the Bureau of Chemistry gave the following results: d_{25}^{25}, 0.9181; n^{20}, 1.4725; I No. (Hanus), 110.3; sapon. val., 193.8; acid val., 0.04.

In a personal communication, Bailey stated that unpublished experiments conducted in his Laboratory indicated that the oil was insoluble in water, and that water dissolved in the oil to the extent of 0.03% at 25°.

The alcohols and esters used in this investigation were bought from commercial firms as special C. P. products, or were prepared by the Analytical Reagent Investigations Laboratory of the Bureau of Chemistry, which made determinations of the boiling point and density on all samples, and of the saponification values of some of the esters. The boiling points obtained have been computed to 760 mm. pressure by Young's formula[29] and are reported in Table I. The densities at 25° are reported in Table II.

TABLE I

ALIPHATIC ALCOHOLS AND ALKYL ESTERS: BOILING POINTS

Corrected to 760 mm. pressure

	Methyl	Ethyl	*n*-Propyl	*iso*-Propyl	*n*-Butyl	*iso*-Butyl	*sec.*-Butyl	*tert.*-Butyl	*iso*-Amyl	*sec.*-Amyl	*tert.*-Amyl
Alcohol	66.4	77.8	97–98	80.7	116.6	105.9	98.0	82.0	130.0	119.0	101.6
Formate	31.7	53.4	.....	...	105.6	96.6	..	..	121.5	...	.....
Acetate	55.9	76.5	101–103	88–90	124–126	115.7	..	..	139.8	...	124–125
Propionate	79.0	97.6	121.0	...	.....	134.5	..	..	158.5	...	.....
Butyrate	101.6	120.3	141.5	...	163.6	156.6	..	..	...	...	.....
iso-Valerate	115.0	133.8	154.6	141.8	175.7	168.1	..	..	188.4	...	.....

TABLE II

DENSITIES OF ALIPHATIC ALCOHOLS AND ALKYL ESTERS AT 25°

	Methyl	Ethyl	*n*-Propyl	*iso*-Propyl	*n*-Butyl	*iso*-Butyl	*sec.*-Butyl	*tert.*-Butyl	*iso*-Amyl	*sec.*-Amyl	*tert.*-Amyl
Alcohol	0.7977	0.7851	0.8056	0.7976	0.8089	0.8016	0.8095	0.7859	0.8095	0.8091	0.8098
Formate	.9636	.9236	....	....	.8848	.8810	....	....	.8770	....	....
Acetate	.9282	.8970	.8845	.8690	.8761	.8892	.9096	....	.8699	....	....
Propionate	.9107	.8850	.8780	....	....	.8640	....	....	.8650	....	....
Butyrate	.8940	.8751	.8710	....	.8680	.8600	....	....	....	....	....
*iso*Valerate	.8785	.8720	.8700	.8461	.8584	.8617	....	....	.8541	....	....

The refractive indices, read on an Abbé refractometer by the author, are given in Table III. These data indicate that most of the products are reasonably pure. Saponification results indicated that, although a few of the esters contained a slight excess of alcohol or acid, most of them were 99 to 100% pure.

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[29] Young, *J. Chem. Soc.*, **80**, 777 (1902). Young says, "In order to correct a boiling point from observed to normal pressure, Δ_t is to be added, where

$$\Delta_t = (760 - p)\,(273 + t).C \quad \times \quad \times \quad \times \quad \times \quad \times \quad \times\ ;$$

p is the observed pressure and t is the boiling point as observed or, better, roughly corrected." He gives data on four aliphatic alcohols showing C to be approximately 100×10^{-6}; on 29 alkyl esters, 114×10^{-6}.

TABLE III

INDICES OF REFRACTION OF ALIPHATIC ALCOHOLS AND ALKYL ESTERS (n_D^{25})

Abbé Refractometer

	Methyl	Ethyl	*n*-Propyl	*iso*-Propyl	*n*-Butyl	*iso*-Butyl	*sec.*-Butyl	*tert.*-Butyl	*iso*-Amyl	*sec.*-Amyl	*tert.*-Amyl
Alcohol	1.3275	1.3595	1.3820	1.3768	1.3970	1.3936	1.3942	1.3840	1.4042	1.4050	1.4020
Formate	1.3415	1.3575	....	....	1.3874	1.3835	....	....	1.3940	....	....
Acetate	1.3588	1.3700	1.3828	1.3740	1.3914	1.3880	1.3840	....	1.3978	....	1.3995
Propionate	1.3738	1.3900	1.3930	....	....	1.3942	....	....	1.4036	....	....
Butyrate	1.3870	1.3895	1.3980	....	1.4045	1.4005	....	....	....	....	....
*iso*Valerate	1.3900	1.3975	1.4035	1.3938	1.4058	1.4056	....	....	1.4100	....	....

Water—1.3325, Cottonseed oil—1.4075.

Values for the refractive indices of many of these products are not reported in the literature. The ease of determination and the accuracy of reading recommend the refractive index as another criterion of purity of alcohols and esters.

The hydrolysis of the alkyl esters during the course of three or four hours was found to be negligible, with the single exception of the formates. The amounts hydrolyzed are so slight as not to affect the refractometric determinations.

Refractive Increments of Aqueous Solutions

Solutions of various known (volume) concentrations were obtained by diluting measured volumes of an alcohol or ester in volumetric flasks to volume with water or with oil at 25°. The water or oil was added from a water-jacketed buret, kept at 25° by a current of water from the constant-temperature tank.

The values for the aqueous solutions fell within the range of the Zeiss refractometer, which was used to determine their indices. Subtracting the Zeiss value for water (13.25) from the index of the aqueous solution, leaves a figure indicating the change in index produced by the presence of the solute. Dividing this value by the concentration of substance present, the "Refractive Increment," n (the change in Zeiss reading produced by passing from distilled water to a unit concentration of solute in water) was obtained. Obviously, the value of the increment depends upon the unit chosen; the increment for a 1% solution is designated $n_{1\%}$; for a molar solution, n_m.

In preference to a series of charts, plots, or extensive tables showing the

TABLE IV

REFRACTIVE INCREMENTS FOR 1 PER CENT. AQUEOUS SOLUTIONS OF ALIPHATIC ALCOHOLS AND ALKYL ESTERS AT 25°: $n_{1\%}$

	Methyl	Ethyl	*n*-Propyl	*iso*-Propyl	*n*-Butyl	*iso*-Butyl	*sec.*-Butyl	*tert.*-Butyl	*iso*-Amyl	*sec.*-Amyl	*tert.*-Amyl
Alcohol	0.40	1.40	1.90	1.82	2.17	2.20	2.27	1.98	2.43	2.40	2.50
Formate	1.12	1.55	..	..	1.80	2.00	..	..	2.20	..	..
Acetate	1.52	1.79	1.90	1.80	2.10	2.10	1.90	..	2.00	..	1.60
Propionate	1.82	2.05	2.30	..	...	2.50	..	..	2.00	..	..
Butyrate	2.20	2.00	2.75	..	(1.33?)	(1.25?)	..	..	..	..	..
*iso*Valerate	2.10	(1.50?)	3.00	..	2.50	...	..	..	..	..	..

Zeiss values corresponding to various concentrations the values for N for aqueous solutions are given in compact, serviceable form in Tables IV and V. Plotting concentration against Zeiss value, curves are obtained from 0 up to 75 or 100% concentrations.[13,14,22,24,25,30] Within the limits of 0 to 25 or 30% by volume, however, the deviations from linear values are very small, and these data are fitted by a straight line as closely as by a curve.[8,14,25,30] Lack of sufficient data prevented calculations of correlation coefficients. Similar linear applications have been made to solutions of inorganic compounds,[4,9,11,12,13,30] to protein solutions, and to blood.[17,19,31] In the general linear equation, $y = ax + b$, n is the slope of the line connecting concentration (x) with Zeiss index (y):

$$y = nx + 13.25 \qquad (1)$$

Table IV contains the values of $n_{1\%}$ read from the curves. From these data, the values for n_m for molar solutions have been calculated by multiplying $n_{1\%}$ by the molecular volume (the molecular weight divided by the density), and then multiplying by 100/1000 (since $n_{1\%}$ is on a percentage basis or parts in a hundred, and molar solutions are on the basis of parts per thousand). The equation for conversion of $n_{1\%}$ to n_m is:

$$n_m = n_{1\%} \times \frac{\text{Molecular weight}}{\text{Density}} \times \frac{100}{1000} \qquad (2)$$

These values are given in Table V.

TABLE V

REFRACTIVE INCREMENTS FOR MOLAR AQUEOUS SOLUTIONS OF ALIPHATIC ALCOHOLS AND ALKYL ESTERS AT 25°: n_m

	Methyl	Ethyl	*n*-Propyl	*iso*-Propyl	*n*-Butyl	*iso*-Butyl	*sec.*-Butyl	*tert.*-Butyl	*iso*-Amyl	*sec.*-Amyl	*tert.*-Amyl
Alcohol	1.61	8.21	14.17	13.71	19.88	20.34	20.78	18.67	26.45	26.14	27.20
Formate	6.98	12.43	...	...	20.77	23.18	...	...	29.13	...	...
Acetate	12.13	17.58	21.93	21.15	27.84	28.06	...	...	29.92	...	...
Propionate	17.60	23.65	30.42	...	...	37.66	...	...	33.33	...	...
Butyrate	25.13	26.54	41.09	...	(22.09)	(20.95)	...	...	...	...	...
*iso*Valerate	27.76	(22.39)	49.71	...	46.07	...	...	...	...	...	...

TABLE VI

ZEISS INDICES OF AQUEOUS SOLUTIONS OF METHYL ALCOHOL

Vol. concn., %	Zeiss index	Obs. $n_{1\%}$	Av. $n_{1\%}$
0.0	13.25	..	
1.0	13.55	0.30	
2.0	14.05	.40	
5.0	15.25	.40	
10.0	17.80	.45	
25.0	23.15	.40	0.40
40.0	32.65	.48	
50.0	35.20	.44	
100.0	0.3	—	

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[30] Faust, *Z. anal. Chem.*, **58**, 145 (1919).

[31] Robertson: "The Physical Chemistry of the Proteins," Longmans, Green and Co., New York, **1918**, pp. 359–368.

An example may help to make this procedure clear. The preceding data were obtained for methyl alcohol.

While the observed $n_{1\%}$ for a 1.0% solution was 0.30, the general trend for the slightly higher concentrations (through 25%) seems to approach the value 0.40. The values for 40 and 50% are quite different. The plots of weight concentration against Zeiss index[24,25,30] indicate an inflexion point at about 45% by weight, but indicate that the relationships up to approximately 25% may be considered as linear. Accordingly, it was felt that the results on solutions up to 25% warranted the selection of 0.40 as the value for $n_{1\%}$. n_m was calculated by Equation 2.

$$n_m = 0.40 \times \frac{32.037}{0.7977} \times \frac{100}{1000} = 1.61$$

Substituting these values in Equation 1:

For a 1% solution, the Zeiss index, y, is $0.40 + 13.25 = 13.65$
For a M solution, the Zeiss index, y, is $1.61 + 13.25 = 14.86$

Refractive Increments of Cottonseed Oil Solutions

As the values for the oil solutions were above the range of the Zeiss instrument, readings were made on an Abbé refractometer to 0.0001.

TABLE VII

REFRACTIVE INCREMENTS FOR 1 PER CENT. COTTONSEED OIL SOLUTIONS ($\times 10^{-4}$) OF ALIPHATIC ALCOHOLS AND ALKYL ESTERS AT 25°: $n_{1\%}$

	Methyl	Ethyl	*n*-Propyl	*iso*-Propyl	*n*-Butyl	*iso*-Butyl	*sec.*-Butyl	*tert.*-Butyl	*iso*-Amyl	*sec.*-Amyl	*tert.*-Amyl
Alcohol	13.9	10.8	8.9	9.7	7.6	7.9	8.0	8.6	6.6	6.9	6.9
Formate	10.5	10.4	...	...	8.2	8.5	...	...	7.5	...	...
Acetate	10.2	9.8	8.5	9.1	7.8	7.5	8.3	...	7.3	...	...
Propionate	9.0	8.1	7.8	...	...	7.6	...	...	6.5	...	...
Butyrate	8.4	8.0	7.2	...	6.5	6.8	...	...	...	...	...
*iso*Valerate	8.2	7.4	6.8	7.4	6.0	5.9	...	...	6.1	...	...

TABLE VIII

REFRACTIVE INCREMENTS FOR MOLAR COTTONSEED OIL SOLUTIONS ($\times 10^{-4}$) OF ALIPHATIC ALCOHOLS AND ALKYL ESTERS AT 25°: n_m

	Methyl	Ethyl	*n*-Propyl	*iso*-Propyl	*n*-Butyl	*iso*-Butyl	*sec.*-Butyl	*tert.*-Butyl	*iso*-Amyl	*sec.*-Amyl	*tert.*-Amyl
Alcohol	55.8	63.4	66.4	73.1	69.6	73.0	73.2	81.1	71.8	75.1	75.1
Formate	65.4	83.4	...	...	94.6	98.5	...	..	99.3	..	..
Acetate	81.4	96.2	98.1	106.9	103.4	100.2	106.0	..	109.2	..	..
Propionate	87.0	93.5	103.2	...	...	114.5	...	..	108.3	..	..
Butyrate	95.9	106.2	107.6	...	108.0	114.0	...	..	...	..	..
*iso*Valerate	108.4	110.4	112.7	126.1	110.6	108.3	...	..	123.0	..	..

TABLE IX

REFRACTIVE INDICES OF OIL SOLUTIONS OF METHYL ALCOHOL

Vol. concn., %	Refr. index	Obs. $n_{1\%}$ ($\times 10^{-4}$)	Av. $n_{1\%}$ ($\times 10^{-4}$)
0.0	1.4705	..	
2.8	1.4666	13.9	
3.2	1.4660	14.1	
3.8	1.4650	14.5	
4.9	1.4640	13.2	13.9

Values for $n_{1\%}$ and n_m are reported in Tables VII and VIII. For methyl alcohol, the preceding data were obtained:

While not as good agreement was found in the individual values for $N_{1\%}$ as in the case of the aqueous solutions of methyl alcohol, more weight was put on the value 13.9, the average of the four individual values, and it was accordingly selected as $n_{1\%}$. To calculate n_m

$$n_m = 13.9 \times \frac{32.037}{0.7977} \times \frac{100}{1000} = 55.8\ (\times\ 10^{-4})$$

Since the refractive indices of the alcohols and esters are lower than those of the oil, solutions will have a lower value than the pure solvent, in accordance with the equation

$$y = 1.4705 - nx \tag{3}$$

Substituting the calculated values for $n_{1\%}$ and n_m

For a 1% solution, the refractive index, $y = 1.4705 - 13.9\ (\times\ 10^{-4}) = 1.4691$
For a M solution, the refractive index, $y = 1.4705 - 55.8\ (\times\ 10^{-4}) = 1.4649$

To illustrate the application of Equation 3, the observed values for the refractive index of various concentrations of ethyl acetate in oil are given in the second column of the following table, and the corresponding values, calculated from the equation $y = 1.4705 - 0.00098x$ are given in the third column. The differences between observed and calculated results are small, (except for the pure (100%) ester). (Table X.)

TABLE X

REFRACTIVE INDICES OF OIL SOLUTIONS OF ETHYL ACETATE

Vol. concn. of ester, x, %	Obs. index, y	Calcd. ($1.4705-0.00098x$)	Calcd. minus obs.
100	1.3700	1.3725	0.0025
50	1.4210	1.4215	.0005
37.5	1.4340	1.4338	− .0002
28.6	1.4430	1.4425	− .0005
23	1.4480	1.4480	.0000
16.7	1.4540	1.4541	.0001
13	1.4580	1.4578	− .0002
0	1.4705	1.4705	.0000

The close agreement between observed results and values calculated from linear equations is striking. While the aqueous solutions tended to correspond to curves, the oil solution-concentration: refractive-index results fit linear expressions very closely.

The Zeiss $n_{1\%}$ for aqueous solutions and the refractive index $n_{1\%}$ for oil solutions were correlated. Rather unexpectedly, a high degree of correlation was found: $r = -0.78 \pm 0.04$. The negative sign indicates inverse correlation, that is, one value increases as the other decreases. Since the refractive indices of the alcohols and esters are lower than the value for the pure solvent, this inverse relationship would be expected. The value for r, 0.78, is not overly high, but the fact that it is about 20 times its probable error indicates that it is very probably significant.

The equation of the regression of y on x is

$$y = 13.08 - 2.44x \tag{4}$$

where y is the $n_{1\%}$ of the oil solution and x the $n_{1\%}$ of the aqueous solution. By applying this equation, it is possible to determine the value of $n_{1\%}$ for an oil solution, if the corresponding $n_{1\%}$ for an aqueous solution is known, or *vice versa*. The $n_{1\%}$ for aqueous solutions can be determined with a Zeiss refractometer with an error less than 0.05 Zeiss unit, or approximately 0.00002 in refractive index. As the direct readings of an Abbé instrument do not go closer than 0.00010, the error in the value for the n of an oil solution might be five times as large when determined directly as when determined from the regression equation. Several applications of the regression equation are given in Table XI.

TABLE XI

REFRACTIVE INCREMENTS OF AQUEOUS SOLUTIONS

	$n_{1\%}$ = 13.08 − 2.44 times	Calcd. ($\times 10^{-4}$)	Found ($\times 10^{-4}$)
Ethyl alcohol	1.40 =	9.8	10.8
sec.-Butyl alcohol	2.27 =	7.5	8.0
tert.-Butyl alcohol	1.98 =	8.75	8.6
sec.-Amyl Alcohol	2.40 =	7.2	6.9
tert.-Amyl alcohol	2.50 =	7.0	6.9
Methyl formate	1.12 =	10.35	10.5
Ethyl butyrate	2.00 =	8.2	8.0
*iso*Butyl formate	2.00 =	8.2	8.5
iso-Amyl acetate	2.00 =	8.2	7.3

While the agreement between calculated and observed values is not very exact in some instances, the trend of agreement is fairly close, most of the calculated values falling within 5 to 10% of observed values.

Similarly, the correlation coefficient for n_m for aqueous and oil solutions was found to be 0.71 ± 0.05. Being positive, the values for one n_m increase directly with the values for the other. The absolute value of r, 0.71, is lower than in the case of the $n_{1\%}$ results. Nevertheless, it is about 14 times its probable error, indicating that it is probably significant. The scattering of individual observations is rather large, so the results calculated by the equation for the regression of y on x, $y = 1.18x(\times 10^{-4}) + 62.4(\times 10^{-4})$, do not agree as closely with observed values as they did

TABLE XII

REFRACTIVE INCREMENTS OF OIL SOLUTIONS

	n_m = 1.18 times	Calcd. ($\times 10^{-4}$)	Found ($\times 10^{-4}$)
Ethyl alcohol	8.21 + 62.4 =	72.0	63.4
Methyl formate	6.98 + 62.4 =	70.6	65.4
Ethyl butyrate	26.54 + 62.4 =	93.8	106.2
*iso*Butyl formate	23.18 + 62.4 =	89.8	98.5
iso-Amyl acetate	29.92 + 62.4 =	97.8	109.2

in the $n_{1\%}$ calculations. Some results calculated by this equation and the corresponding observed values are given in Table XII.

The results given fall within about 10% of the observed values, which may be close enough in many instances.

Application of Results

The quantity of an alcohol or an ester present in an aqueous or a cottonseed oil solution may be readily and accurately determined from the refractive index of the solution. If but one alcohol or ester is present, distillates from medicines, beverages, perfumes and essences could be tested directly. In case of a mixture of several of these products in the distillate, the refractive index will be of material assistance, but additional information will be required in order to determine the quantities of the various constituents in the mixture. This procedure has already been applied to mixtures of methyl and ethyl alcohol by Leach and Lythgoe, who determine the refractive index and the specific gravity of the mixture as bases for calculating the proportions present. Beythien and Hennicke[10] determined the refractive indices of several mixtures containing varying proportions of the substances sought as a basis for quantitative determinations of the composition of mixtures containing two refractive constituents.

The refractometry of oil solutions has an apparent value in analyzing oils for the presence of some adulterants or added medicaments as, for example camphor in oil, or phenol in glycerol. Knowing the refractive increment of camphor or phenol, and the refractive indices of the solution and of the pure solvent, the composition may be readily determined.

The hydrolysis of the alkyl esters in aqueous solutions has been followed by reading refractive indices from time to time, in a study of the rate and degree of hydrolysis.

A number of attempts were made to connect change in n in aqueous solutions with possible changes in the degree of association of water, but without success. The significant correlations between the alterations in indices of water or of oil solutions and the changes in concentration of the alcohols and esters studied suggested the possibility of developing a mathematically sound and rigid proof that solubility in water and in oil are similar physical phenomena, but more data are required before this can be done.

Summary

1. Refractive increments of aliphatic alcohols and alkyl esters in aqueous and in cottonseed oil solutions have been determined.
2. Quantitative determinations of these products may be readily and accurately made by the use of these increments.
3. There is significant correlation between the refractive increments of aqueous and of oil solutions: ($r = —0.78 \pm 0.04$). Consequently it is

possible to calculate the increment for either solution if the value for the other is known.

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THE IONIZATION CONSTANT OF CREATININE[1]

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Introduction

In view of the fact that the values for the ionization constant of creatinine, $NH{=}\underline{CNHCOCH_2N}CH_3$, appearing in the literature are few and in very bad agreement, it was decided to attempt to measure this constant more exactly.

The values appearing in the literature at present are 1.85×10^{-10} at 17°, and 3.57×10^{-11} at 40.2°. The first is that of Hahn and Barkan,[2] obtained from hydrogen-ion measurements in solutions of the hydrochloride. The second is that of J. K. Wood,[3] obtained by measuring the degree of hydrolysis of the hydrochloride by its catalytic effect on the hydrolysis of methyl acetate. It can be seen that a more exact determination of this constant is needed.

Many different methods, such as conductivity measurements, hydrogen-ion determinations, catalytic methods, distribution, solubility and colorimetric determinations, have been used for determining the ionization constants of weak organic bases of this type. In this set of investigations it was decided to use several of the above-mentioned methods, so as to obtain a check on our results, as well as to compare the various methods used as to their ease of application and probable accuracy. Conductivity measurements and hydrogen-ion determinations were decided on. It was also considered best to apply two of the less generally used methods, and distribution and solubility were selected. Results should thus be obtained representing with a good degree of accuracy the true ionization constant of creatinine.

The creatinine used throughout was prepared by the method of Edgar and Hinegardner,[4] from purified creatine. A white, finely crystalline odorless compound was obtained.

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[1] This investigation completes the series on the physicochemical properties of creatine and creatinine undertaken in this Laboratory. It was outlined and begun under the direction of Dr. Graham Edgar and finished under the direction of Dr. Robert N. Pease.

[2] Hahn and Barkan, *Z. Biol.*, **72**, 25 (1920).

[3] Wood, *J. Chem. Soc.*, **83**, 568 (1903).

[4] Edgar and Hinegardner, *J. Biol. Chem.*, **56**, 886 (1923).