

gram susceptibility of the complex was $+3 \pm 1.5$ c.g.s. units/g. at 20° . This is sufficiently positive to indicate the undoubted paramagnetic nature of the complex, and if multiplied by the molecular weight of $\text{Ru}(\text{th})_3$ of 331, gives a molar susceptibility of 1000 ± 500 c.g.s.u./mole. Since the contribution of one unshared electron would be about 1300, and since Ru(III) in covalent compounds has one unshared electron, within the experimental error this shows that the ruthenium in the complex is in the III state. Ru(IV) must be excluded since it has an even number of electrons and would hardly be expected to have one unpaired electron.

Conclusion

Ruthenium(III) and ruthenium(IV) perchlorate react with thiourea Hth, to form two blue-green complexes, $\text{Ru}(\text{th})_1^{+2}$ and $\text{Ru}(\text{th})_3$. The complexing agent behaves as an acid and each molecule of thiourea releases a proton. At an ionic strength of 3.0, the monothiourea complex was found to have a formation constant, K_1 , equal to 16.3 ± 0.5 , while the constant K_3 for the trithiourea complex was found to equal 5.3 ± 0.1 . No evidence was found for the dithiourea complex, $\text{Ru}(\text{th})_2^+$.

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Absolute Verdet Constants for Water over a Range of Temperatures and Visible Wave Lengths

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The purpose of this paper is to present new absolute values for the Verdet Constant of water over a range of temperatures and visible wave lengths. Based upon measurements of the magnetic rotation of water and measurements of the field strength of the solenoid employed, these values have been calculated with uncertainties of $\pm 0.05\%$. The results obtained serve to define more specifically this important constant for water and to answer directly certain questions that have been raised in the literature during the past years.

I. Introduction

When a beam of plane polarized monochromatic light is passed through any transparent isotropic liquid or solid, placed in a magnetic field parallel to the lines of force of the field, the plane of vibration of the light beam is rotated through a definite angle. This phenomenon is known as the Faraday effect. If R is the total angle of rotation in minutes, l the length of the substance in centimeters through which the light beam passes, H the magnetic field strength in gauss, θ the angle between the direction of the magnetic field and the light path, and V the proportionality constant, then

$$R = V \times l \times H \times \cos \theta$$

It has been found that every substance has a characteristic value for the proportionality term, V , and this has been named the Verdet constant. V varies with wave length and temperature in a manner analogous to the refractive index of substances. A more useful term for the identification of molecular species and characteristic functional groups is the molecular Verdet constant, \mathbf{V} , which is the Verdet constant multiplied by the molecular weight and divided by the density of the substance.

For some time there has been an increasing interest in the Faraday effect from both the experimental and the theoretical standpoint. To date, unfortunately, there has not been good correlation between theory and experiment. The lack of precise, absolute values for the Verdet constants for various pure substances has been mainly responsible for this discrepancy. The need, then, for precise values of absolute Verdet constants to overcome this limitation has long been recognized.

Water is by far the most widely studied substance

from the standpoint of the Faraday effect. At present, however, there is still uncertainty in the Verdet constants for this important substance. Current values given in the literature refer mainly to a single wave length and temperature, and there is some discordancy among them. This is largely due to inaccuracies of the method of obtaining the magnetic field strength. For example, the most recently published value for the absolute Verdet constant for water² was obtained from a calculated, rather than an experimentally determined value of the field strength of the solenoid employed.

In view of this it was felt that the absolute Verdet constants for water should be redetermined over a range of temperatures and wave lengths. It is the purpose of this paper, therefore, to present new absolute Verdet constants for water using a precision Faraday effect apparatus³ and an experimentally determined magnetic field strength.

II. Experimental

Four different water samples were employed for the measurements; three were triple-distilled and the fourth was distilled once. Sample 1 was ordinary tap water distilled once by a Vigreux column. Samples 2, 3 and 4 were distilled in a Barnes Laboratory still and then distilled from alkaline permanganate in a Vigreux column. This distillate was again distilled from the Vigreux column. The criterion of purity used was the refractive index which was measured on a Bausch and Lomb precision refractometer. At 20° , four different water samples varied by only 0.00001 with the literature values⁴ at 589 μ .

Values of the magnetic field strength of the Faraday solenoid, at points over a range of 14 cm. along the axis on either side of the center, were obtained from measurements

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(3) Samuel Steingiser, George J. Rosenblit, Robert L. Custer and Chas. E. Waring, *Rev. Sci. Instruments*, **21**, 109 (1950).

(4) Dorsey, "Properties of Ordinary Water Substance," Reinhold Publishing Corp., New York, N. Y., 1940, pp. 200-202.

(1) The data presented were submitted in partial fulfillment of the requirements of the Ph.D. degree at the University of Connecticut.

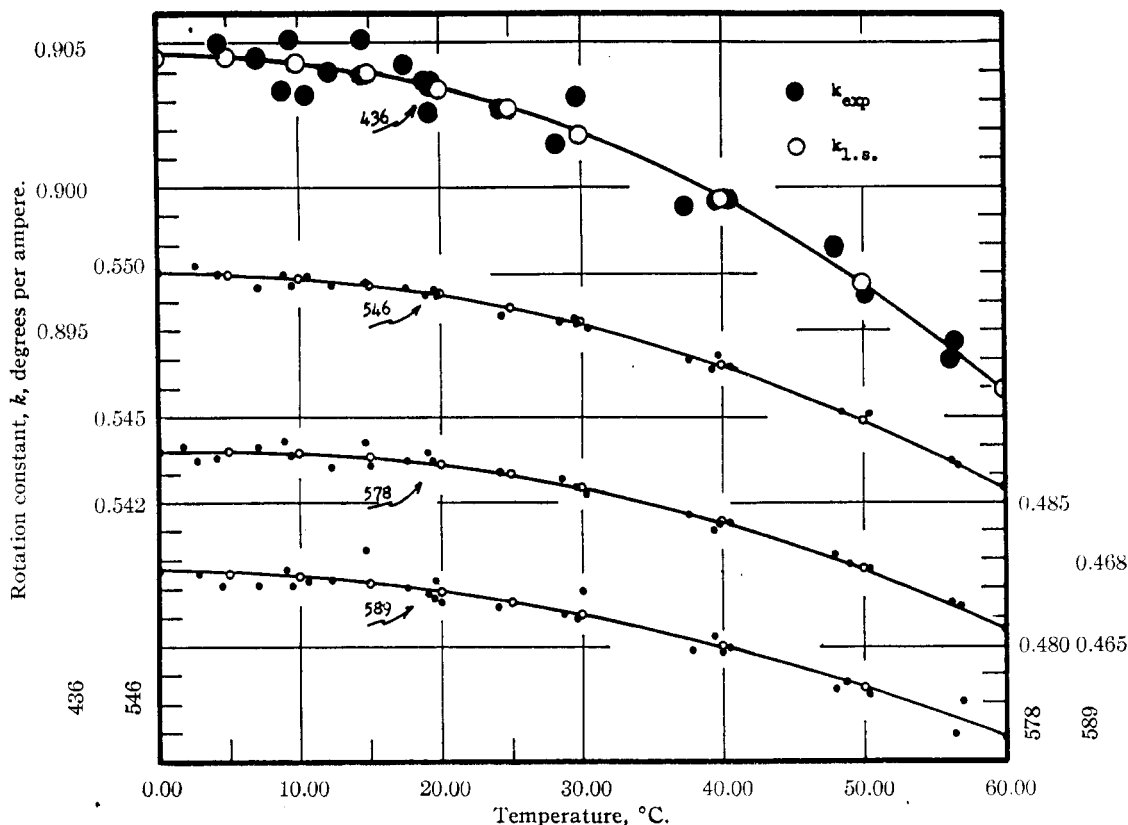


Fig. 1.—Variation of k , the rotation constant for water, with temperature.

made by a method based upon proton nuclear magnetic resonance.⁵

Four least squares equations of field strength as a function of axial position were derived from the data. The most suitable of these, according to the Gauss criterion, was extrapolated over the some 56-cm. length of the Faraday effect cell to obtain a value of the magnetic potential per ampere over this length. The value so obtained was 2152.6 gauss cm. per ampere, with an uncertainty of $\pm 0.03\%$.

The measurements of the magnetic rotation per ampere, k , of water were made in the manner previously described⁶ for some thirty points over a temperature range from near 0° to about 60° for each of the wave lengths 589, 578, 546 and 436 $m\mu$. Each value for the rotation was an average of thirty-six polarimeter readings, eighteen in the positive and eighteen in the negative direction of current flow through the magnet. Appropriate corrections were made in all the k -values for water for the rotation due to the air in the light path and to the rotation due to the end plates in the quartz cell. The complete data⁷ are presented in Fig. 1. The larger error and the scattering of the points at 436 $m\mu$ are due to the difficulty of determining the match points on the two-field polarimeter at that wave length.

III. Results

Least squares equations were derived from the experimental data represented in Fig. 1 and values of k_{H_2O} , corresponding to arbitrarily chosen values of temperature, were calculated. The plot of k versus t in Fig. 1 for both the experimental and calculated values shows the fit to be excellent.

Table I presents the equations for k , the rotation constant for water, as a function of temperature, at the four wave lengths. The dispersions, obtained

by dividing the k -values at three wave lengths by the k 's at 589 $m\mu$, were found to be essentially constant over the entire temperature range. Table II summarizes the results of calculations leading to absolute Verdet constants. Figure 2 shows these values plotted against temperature.

TABLE I
LEAST SQUARES EQUATIONS FOR k_{H_2O} AS A FUNCTION OF TEMPERATURE

λ	$k =$
(1) 589	$0.46763 - 8.7108 \times 10^{-6} t - 1.4842 \times 10^{-6} t^2$
(2) 578	$0.48675 + 2.4397 \times 10^{-6} t - 2.1264 \times 10^{-6} t^2$
(3) 546	$0.54995 + 8.8815 \times 10^{-6} t - 2.2063 \times 10^{-6} t^2$
(4) 436	$0.90450 + 1.7114 \times 10^{-6} t - 3.5007 \times 10^{-6} t^2$

IV. Discussion

The values of k , magnetic rotation per ampere, were calculated with an uncertainty of about $\pm 0.04\%$ ⁸ and the value of the magnetic potential per ampere over the length of the Faraday cell has an estimated uncertainty of $\pm 0.03\%$.⁵ Thus, the absolute Verdet constants may be calculated with an uncertainty of about $\pm 0.05\%$. This precision is better than that heretofore attributed to any absolute Verdet constants.

Four values of the absolute Verdet constant for water are given in the literature. Of three at 20° for 589 $m\mu$, Arons⁸ gives 0.01298, Rodger and Watson⁹ give 0.01311, and Siertsema¹⁰ gives

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(9) J. W. Rodger and W. Watson, *Proc. Roy. Soc. (London)*, **A58**, 234 (1895).

(10) L. H. Siertsema, *Kon. Akad. Wet. Amsterdam*, **5**, 131 (1897).

(5) ONR Contract N6 ori 216 T.O. No. 1 Technical Report No. 4, October, 1950.

(6) ONR Contract N6 ori 216 T.O. No. 1, June, 1949.

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TABLE II

$t, ^\circ\text{C.}$	$\lambda = 589$		$\lambda = 578$		$\lambda = 546$		$\lambda = 436$	
	k	$V \times 10^{20}$	k	$V \times 10^2$	k	$V \times 10^2$	k	$V \times 10^2$
0	0.46764	1.3035	0.48676	1.3568	0.54995	1.5329	0.90450	2.5211
5	.46755	1.3032	.48682	1.3569	.54993	1.5328	.90450	2.5211
10	.46740	1.3028	.48679	1.3568	.54981	1.5325	.90432	2.5206
15	.46717	1.3021	.48664	1.3564	.54958	1.5318	.90397	2.5196
20	.46687	1.3013	.48639	1.3557	.54925	1.5309	.90344	2.5182
25	.46649	1.3003	.48604	1.3548	.54879	1.5296	.90274	2.5162
30	.46604	1.2990	.48557	1.3534	.54823	1.5281	.90186	2.5138
40	.46491	1.2959	.48433	1.3500	.54678	1.5240	.89958	2.5074
50	.46349	1.2919	.48266	1.3453	.54487	1.5187	.89661	2.4991
60	.46177	1.2871	.48056	1.3395	.54254	1.5122	.89293	2.4889

^a $V = 60 k \text{ min.}/2152.6 \text{ gauss cm.}$

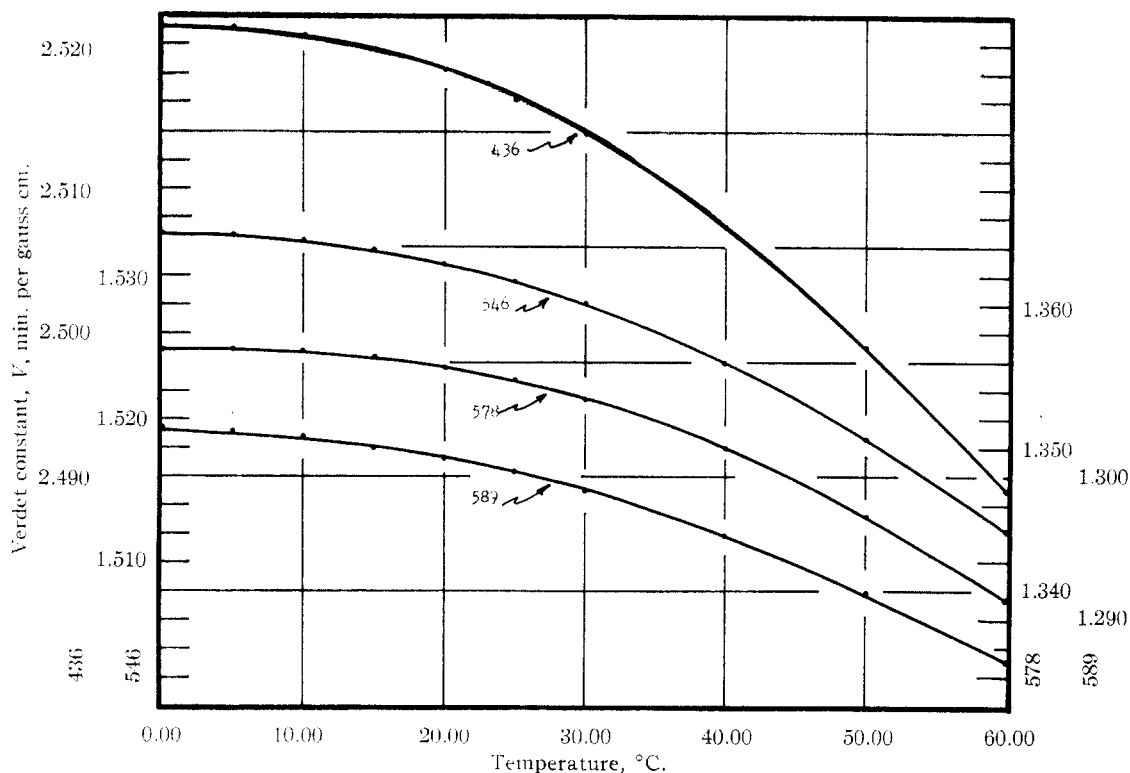


Fig. 2.—Variation of the Verdet constant for water with temperature.

0.01303. The most recent value, reported by deMalleman and co-workers,² at 11.5° for $546 \text{ m}\mu$, is 0.01543 ± 0.00001 . The value at 11.5° calculated from equation (3) Table I, is $0.015324 \pm 0.05\%$. The two latter values differ by 0.7% . These workers made extremely careful measurements at this one point. However, they did not measure the magnetic field strength but rather calculated it from the simple Ampere law. Experiences of some investigators¹¹ have shown that calculated values of the magnetic field strength of an air core solenoid can be more than 0.5% smaller than measured values. Considering this, the two values are seen to be in very good agreement. Also, in respect to the three values at 20° and $589 \text{ m}\mu$, there is good agreement; these have an average equal to 0.01304 , our value being $0.013013 \pm 0.05\%$.

The results of this work answer for the first time in the case of water, a question raised previously

by Ingersoll.¹² This concerned the dependency of the temperature coefficient of the Verdet constant on wave length. The results of a portion of his work designed to clarify this question were inconclusive. It is seen from the slopes of the curves in Fig. 2, equations (1), (2) (3) and (4), that the temperature coefficient of the Verdet constant for water increases appreciably with wave length. It is of interest to note that the temperature coefficient for $436 \text{ m}\mu$ is approximately twice that for $589 \text{ m}\mu$.

From another standpoint, the results help to answer the question as to the thermal variation of the dispersion.¹³ The results of research done by F. G. Slack¹⁴ and co-workers suggested that V/V_{589} decreases slowly with temperature. The decrease in the values above 25° concurs with the direction

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(14) F. G. Slack, R. L. Reeves and J. A. Peoples, Jr., *Phys. Rev.*, [2] **46**, 724 (1934).

(11) J. S. Campbell, Ph.D. Thesis, California Institute of Technology, 1931, p. 8.

of the variation they found but is of a much smaller magnitude. There may be some question as to whether the variation is real in light of the precision involved. However, the trend cannot be denied.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STATE UNIVERSITY OF IOWA]

Phenolic Hydroxyl Ionization in Proteins. I. Bovine Serum Albumin¹⁻³

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The ionization of the phenolic hydroxyl groups of bovine serum albumin has been studied by measuring the ultraviolet light absorption at 295 $m\mu$ as a function of pH at three temperatures. The ionization curves are steeper than theory would predict, and can be fitted with a computed curve only if the effective negative charge on the albumin molecule is assumed not to rise much above -50 . The intrinsic pK for ionization at 25° lies between 10.0 and 10.3; the heat of ionization is 11.5 kcal./mole; the intrinsic entropy of ionization is -8 e.u. The heat of ionization of di-iodo phenolic groups in iodinated albumin is 7.0 kcal./mole. These figures all suggest that the phenolic OH group in native albumin may be hydrogen-bonded; however, the hydrogen bonds are certainly not involved in maintaining the native structure of the molecule, for the ionization proceeds instantaneously and reversibly.

While numerous potentiometric studies have been made of hydrogen ion dissociation in proteins, such studies are always complicated, particularly in the alkaline range, by the fact that the ionization ranges of different groups overlap. Crammer and Neuberger,⁴ however, have shown that one of the important groups contributing to hydrogen ion dissociation in the alkaline range, the phenolic hydroxyl group, can be studied independently of other groups by utilization of the shift in the ultraviolet absorption spectrum which accompanies the dissociation of a hydrogen ion from this group. Crammer and Neuberger in this way made a study of the ionization of the phenolic hydroxyl groups of tyrosine, insulin and egg albumin. The present paper attempts a considerably more detailed investigation for bovine serum albumin. The results obtained are of rather special interest for two reasons: (1) because they throw light on the problem of hydrogen bonding in proteins, in which phenolic hydroxyl groups may be expected to play a prominent role; and (2) because the work described constitutes a precise thermodynamic study of a protein reaction at a pH very far removed from the iso-ionic point, and, hence, a very exacting test of the theory underlying the effect of electrostatic charge upon protein reactions.

Experimental

Reagents.—Armour crystalline bovine serum albumin was used in this study. Its concentration in stock solution was determined from the light absorption at 280 $m\mu$ wave length in an appropriately diluted solution. The method was standardized by measurements on a solution whose concentration was determined independently by drying at 105°. The value of $E_{1\%}^{1cm}$ was found to be 6.60, in agreement with

a value reported by Cohn, Hughes and Weare.⁵ The molecular weight has been taken to be 69,000.⁶ Stock solutions of piperidine buffers, hydrochloric acid and potassium chloride were prepared from reagent grade materials; stock solutions of potassium hydroxide were prepared carbonate-free by the method of Kolthoff.⁷ Carbon dioxide-free water was used throughout.

Solutions for measurement contained between 0.2 and 0.3% serum albumin and their pH was adjusted by the careful addition of appropriate amounts of acid or base, or by means of the piperidine buffers. Potassium chloride was used to bring the total ionic strength of each solution to 0.15.

Measurement of pH .—Measurements of pH were made on a Model G Beckman pH meter, using external electrodes and a small cell which could be lowered into any one of three constant temperature baths, maintained at 15.0, 25.0 and 35.0°. Bureau of Standards borax pH standard (pH 9.18 at 25°) was used for calibration. The instrument was checked at high pH values by means of potassium hydroxide solutions of known molality, containing sufficient potassium chloride to bring the ionic strength to 0.15. The pH of such solutions could be computed from the activity coefficients obtained from hydrogen electrode measurements by Tanford⁸ or Green.⁹ (The substitution of potassium for sodium should not alter these activity coefficients appreciably.) It was found that individual measurements with the glass electrode showed random deviations from the computed values of a few hundredths of a pH unit; average values were not significantly in error, however. It is concluded that pH measurements were identical with hydrogen electrode measurements, with an accuracy of about 0.02 pH unit, even in the neighborhood of pH 13.0.

Ultraviolet Light Absorption.—Measurements were made on a Beckman model DU spectrophotometer. 10-mm. quartz cells with 7 mm. inserts were used to give a 3 mm. light path. Each cell was always used with the same insert, and calibrated with both water and tyrosine solution. The temperature in the cell compartment was maintained at 15.0, 25.0 or 35.0° by use of four Beckman thermospacers,¹⁰ through which water from the appropriate constant temperature bath was circulated.

Except in Fig. 2, light absorption measurements are re-

(1) This work was supported by a grant from the Research Corporation.

(2) Presented at the XIIth International Congress of Pure and Applied Chemistry, New York, September, 1951.

(3) Abstracted from the thesis submitted by George L. Roberts, Jr., in partial fulfillment of the requirements for the Ph.D. degree, State University of Iowa, August, 1951.

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