oxygen at liquid-air temperature are of the same order of magnitude as those which we have here obtained, although he deduced a much more rapid change of the dissociation constant with the temperature.

Summary

The magnetic properties of oxygen show that it is a mixture of O_2 and O_4. The molecule of O_2 is paramagnetic, that of O_4 is not. The paramagnetism of unassociated O_2 obeys Curie's law. At the temperature of liquid air about one-half (by weight) is in the associated form. The thermodynamic consequences of this theory with respect to the influence of temperature and of concentration are in accord with the experiments of Perrier and Kamerlingh Onnes. The heat of dissociation is about 128 calories per mole of O_4. This heat of dissociation is responsible for the diminution in the specific heat of liquid oxygen with rise of temperature. Gaseous oxygen, at room temperature and atmospheric pressure, is associated to the extent of only a few hundredths of a per cent.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

OPTICALLY ACTIVE DYES
II. ADSORPTION, ABSORPTION SPECTRA AND ROTATION

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In a previous article by Ingersoll with Adams[2] a very convenient method was described for the preparation of pairs of enantiomorphic dyes. The research had as its ultimate object the determination of whether the mechanism of dyeing of fibers was a physical process, a chemical process, or both. With the pure *d* and *l* forms of a dye, a comparison of the two equivalent solutions as regards adsorption by the fiber could be made. By means of a colorimeter or, perhaps, polarimeter, the concentration of the partially exhausted *d* and *l* solutions could be compared with each other and with the original solutions. The assumption was that compounds that are mirror images would have the same dyeing properties provided the adsorption by the fiber was a purely physical process. On the other hand, if a chemical reaction of any sort took place when the dye was adsorbed, the degrees or rates of adsorption of the *d* and *l* forms might be different on account of the optical activity of the substance of which the fiber was composed.

In order to be sure that, if such results as have been mentioned were

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[1] This communication is an abstract of a portion of a thesis submitted by Wallace R. Brode in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry at the University of Illinois.

[2] Ingersoll with Adams, THIS JOURNAL, **44**, 2930 (1922).

obtained, it would be proper to draw the above conclusions, certain assumptions are necessary. First, the physical adsorption of the *d* and *l* forms would be equal under like conditions. Second, the absorption spectra of the *d* and *l* forms are the same, otherwise it would be impossible by means of a colorimeter to determine the amount of dye adsorbed from the solution of the two dyes. Third, the change in the hydrogen-ion concentration as a result of the addition of an adsorption medium to the dye solution would not affect the rate of adsorption by the fiber or the color of the solution within experimental error. If an appreciable change in the hydrogen-ion value of the solution took place during treatment of the solution by the fiber, it would complicate the quantitative determination of the amount of dye in solution and necessitate the control of the hydrogen-ion concentration in the solution when the polarimeter and colorimeter readings were made.

Before preparing a number of pairs of optically active dyes and studying their adsorption by fibers, it seemed advisable to study one pair and determine whether experiment would substantiate these assumptions and warrant a detailed study of the mechanism of dyeing by the general method planned. In this communication the β-naphthol derivatives of the diazotized *d*, *l* and *dl*-phenyl(*p*-aminobenzoylamino)acetic acid, $HO_2C—CH—(C_6H_5)NHCOC_6H_4N{=}NC_{10}H_6OH$, were chosen for study, since this set of dyes is easily prepared and purified. The work was divided into four parts: (1) the adsorption of these dyes by various inactive physical adsorbing reagents; (2) the determination of the absorption spectra; (3) the effect of hydrogen-ion concentration upon the absorption spectra; and (4) the determination of the optical rotation of these dyes.

Adsorption

The adsorption of a dye by charcoal or an inorganic adsorbing medium has been conceded by many[3] to be of a physical nature although the point has not been generally decided. If such adsorption were chemical, the fact that the charcoal adsorbs equal amounts of the *d* and *l* does not conflict with the deduction that the action of the fiber in adsorbing different amounts of the *d* and *l* dyes is chemical. Equal adsorption by charcoal and inorganic adsorbing agents even if chemical in nature, would be a question of adsorption by a symmetrical substance, whereas fibers are unsymmetrical substances.

A number of different adsorbing media were tested, including Tennessee ball clay, kaolin, silica, alumina, aluminum hydroxide, Sil-O-Cel, fuller's

[3] Wood, "Chemistry of Dyeing," D. Van Nostrand Co., 1913. Dreaper, *Trans. Faraday Soc.*, **6**, 31 (1910). W. C. McC. Lewis, *Phil. Mag.*, **15**, 499 (1908). Liddell, "Chemical Engineering Handbook," McGraw-Hill Book Co., **1923**, Vol. II, p. 780 (Jerome Alexander). Gee and Harrison, *Trans. Faraday Soc.*, **6**, 42 (1910). Freundlich, *Z. physik. Chem.*, **57**, 385 (1906); **59**, 204 (1907).

earth and charcoal. Silica and alumina adsorbed the dyes only slightly, not enough to give good results. Sil-O-Cel and fuller's earth adsorbed satisfactorily, but difficulty was experienced in the centrifuging of the material. The adsorption by Tennessee ball clay, charcoal and kaolin was not complicated by these difficulties and gave satisfactory results, the first two of these adsorbing agents being used for the adsorption experiments.

For the absorption experiments, the *d, l* and *dl* forms of the dye were dissolved in molecular equivalents of alkali solution; the hydrogen-ion concentrations of these solutions were determined, to be certain that they were the same. Although a change in hydrogen-ion concentration of the solutions by the addition of acid or alkali was claimed by Abderhalden[4] to have no effect on the adsorbing power of charcoal, the solutions of the dyes in this investigation were prepared with the same hydrogen-ion concentration to remove any possibility of change of color or solubility which would thus affect the quantitative determination with a colorimeter of the amount of dye adsorbed.

Adsorption by Tennessee Ball Clay.[5]—Dilute solutions of the *d, l* and *dl* dyes, each containing 0.01428 g. of the dye in 50 cc. of water containing a molecular amount of alkali, were well shaken with 0.0304 g. of clay. The mixtures were allowed to stand for three weeks in tightly corked containers; then the clear supernatant liquids were pipetted off and centrifuged for half an hour to remove any fine suspended particles. The original solutions and the exhausted solutions were then compared in a Duboscq colorimeter in all possible combinations. The actual difference in any solutions before and after treatment could be determined indirectly through fifteen sets of readings, thus giving a very close check on the readings obtained. The errors in the readings were much less when solutions of approximately the same concentration were compared, such as the original *d* and the original *l*, or the exhausted *d* with exhausted *l*. There was considerably more variance when the original *d, l* or *dl* was compared with the exhausted *d, l* or *dl*. The color intensity of these solutions was a measure of the quantity of dye present.

Adsorption by Charcoal and by a Mixture of Charcoal with Silica.—Since preliminary experiments showed that the amount of dye adsorbed by powdered silica was negligible, the presence of this material did not affect the adsorption value of the charcoal, but merely diluted the dye and made possible more accurate determination of the amount of charcoal used. The ordinary animal charcoal was employed. The mixture

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[4] Abderhalden and Foder, *Fermentforschung*, **2**, 74 (1917).

[5] Clay used for this experiment was obtained from the Ceramics Department of the University of Illinois. It gave an approximate analysis of silicon dioxide, 47%; aluminum oxide, 38%; titanium oxide, 1%; iron oxide, 1%; calcium and other oxides 0.5%; moisture, 12%; organic matter, 0.5%; 98% of this clay passed through a 200-mesh sieve.

with silica consisted of 1 g. of silica with 0.1 g. of charcoal. From this mixture weighed portions were used for each experiment. In each case, the weighed amount of adsorbing agent was added to the solutions which were then thoroughly shaken and allowed to stand at room temperature for a period of five hours in the case of pure charcoal and ten hours in the case of the silica-charcoal mixture. The liquid was then pipetted off and centrifuged for one hour at high speed. The amount of dye in the clear, supernatant liquid was then determined as previously described under the experiments with Tennessee ball clay.

Determination of the Colloidal Nature of the Dye.—Fifty cc. each of the *d*, *l* and *dl* solutions as used in the adsorption experiments was filtered through a collodion filter such as is used in ultrafiltration. Filter paper of a quantitative grade was soaked in a 3–4% collodion solution in glacial acetic acid and then immersed in water and kept under water until used. The dye solutions were filtered under an air pressure of about four atmospheres and the percentage of color change in the two solutions was determined by a colorimeter as in the preceding experiments. The amount of dye not retained by the membrane was thus determined. The experiments showed that more of the *dl* form existed in a colloidal state than of the *d* or *l* solutions, a fact which would be expected from their solubility.

TABLE I

ADSORPTION OF DYES

	Tennessee ball clay		Charcoal		Charcoal and silica		Contained colloids	
	0.0304 g. used 0.01428 g. of dye 50 cc. of soln. P_H 8.4		0.0200 g. used 0.00625 g. of dye 25 cc. of soln. P_H 7.2		0.0150 g. used 0.00625 g. of dye 25 cc. of soln. P_H 7.2		Colloids 0.0125 g. of dye 50 cc. of soln.	
	Adsorbed %	Error ±	Adsorbed %	Error ±	Adsorbed %	Error ±	Colloids %	Error[6] ±
d	35.9	0.59	41.96	0.45	19.05	0.61	73.50	0.29
l	35.8	.94	41.93	.27	19.09	.61	74.66	.14
dl	41.7	.48	46.25	.22	27.05	.49	84.71	.12

Results

The above results bring out two very important points. In the first place, they show that the active forms are adsorbed to the same extent in the case of inactive adsorbing media where there is no possibility for a true chemical action. This does not mean, however, that the adsorption by these media is necessarily physical in character, since equal adsorption might be expected in the case of chemical combination with a symmetrical adsorbing agent. The conclusion can be drawn that if any difference in the adsorption of the dyes by symmetrical and asymmetrical adsorption agents could be detected, it would be due to chemical reaction.

In the second place, there is shown the necessity of having absolutely pure dyes with which to carry out these experiments, as the racemic form has a higher adsorption. This would naturally be expected from the fact

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[6] The probable error in this experiment and with others following was calculated from the formula $\frac{0.8453\,\Sigma\,(+v)}{n\sqrt{n-1}}$ in which $\Sigma\,(+v)$ is the sum of the deviations of all individuals from the mean, without regard to the sign and n is the number of individuals. Mellor, *Means and Average Errors* in "Higher Mathematics for Students of Chemistry and Physics," Longmans, Green and Co., 1913, pp. 524–531.

that it has a much lower solubility and a higher percentage in a colloidal state, than the active forms do. The presence of any racemic form in one of the active dyes would interfere seriously with adsorption experiments with fibers.

Absorption Spectra

If the enantiomorphic forms did not have the same absorption spectra, it would be impossible to make accurate quantitative determinations of the amount of dye in the solutions by means of a colorimeter. The identity of the absorption spectra of various stereo-isomers has been investigated by a number of writers[7] and the general results conform with the rule given by Kayser[8] that "substances with similarly related structure give similar molecular vibration curves, whereas substances with different structures give different curves. This applies without exception to aromatic compounds as well as to alkaloids, dyes and colored substances." The results obtained in this paper agree with this general rule and with the facts obtained by various other workers, that the optical enantiomorphs show the same frequency of vibration. The previous work, however, as a rule has included only two of the possible three forms, usually the *d* and *dl* as with corydaline;[7a] or else compounds were used in which the color ion was separate from the group containing the asymmetric carbon atom as in the case of copper tartrate.[7c,7d] The effects of color and rotation in compounds such as the latter are not necessarily connected.

The solutions of the *d*, *l* and *dl* β-naphthol dyes for the determination of the absorption spectra in the visible contained 0.01 g. of dye per 1000 cc. and in the ultraviolet contained 0.025 g. per 1000 cc. Whenever the three dye solutions were compared, care was taken that the hydrogen-ion concentration in each of the three was the same. The solutions used had a Sörensen value (P_{H}) of 7.8.

The method for plotting the curves showing the transmission for different wave lengths was similar to that used by the Bureau of Standards[9] in which $-\log_{10}$ of the transmittancy was plotted against the wave length or frequency, depending upon whether the measurements were in the visible or in the ultraviolet. The extinction coefficient in this case was k, where $k = \frac{1}{bc}\,(-\log T)$, in which b is the length of the tube, c is the con-

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[7] (a) Dobbie and Lauder, *J. Chem. Soc.*, **83**, 605 (1903). (b) Gadamer, *Arch. Pharm.*, **239**, 648 (1901). (c) Stewart, "Stereochemistry," Longmans, Green and Co., **1919**, p. 34; *J. Chem. Soc.*, 91, 199, 1537 (1907). (d) Cotton, *Compt. rend.*, **120**, 989, 1044 (1895). (e) Hartley and Huntington, *Proc. Roy. Soc.*, **31**, 1 (1881). (f) Magini, *J. chim. phys.*, **2**, 407 (1904).

[8] Kayser, "Handbuch der Spectroscopie," Hirzel, Leipzig, **1905**, Vol. III, p. 213.

[9] "Spectral Transmission of Dyes," Gibson and others, *Bur. Standards Bull.*, **440**, Vol. 18, p. 128 (1922).

centration, T the transmittancy which equals I/I' where I and I' represent the intensity of light entering and leaving the cell.

The absorption spectra in the ultraviolet was determined at the Bureau of Standards on a Hilger sector photometer.[10] The apparatus used is described in detail in Bull. 440 of the Bureau of Standards.[9] Photographic exposures were made at various transmittancies and from these plates the curve was determined. The absorption bands in the visible were determined in part by the Koenig-Martens spectrophotometer at the Bureau of Standards,[9,11] and in part by the Keuffel and Esser spectrophotometer at the University of Illinois. The Koenig-Martens instrument employs a Wollaston prism as a polarizer and uses Nicol prisms to measure the intensity of the polarized light coming through the sample and the solvent. The Keuffel and Esser spectrophotometer employs sector disks as a means of controlling the light passing through the solution containing the dye.

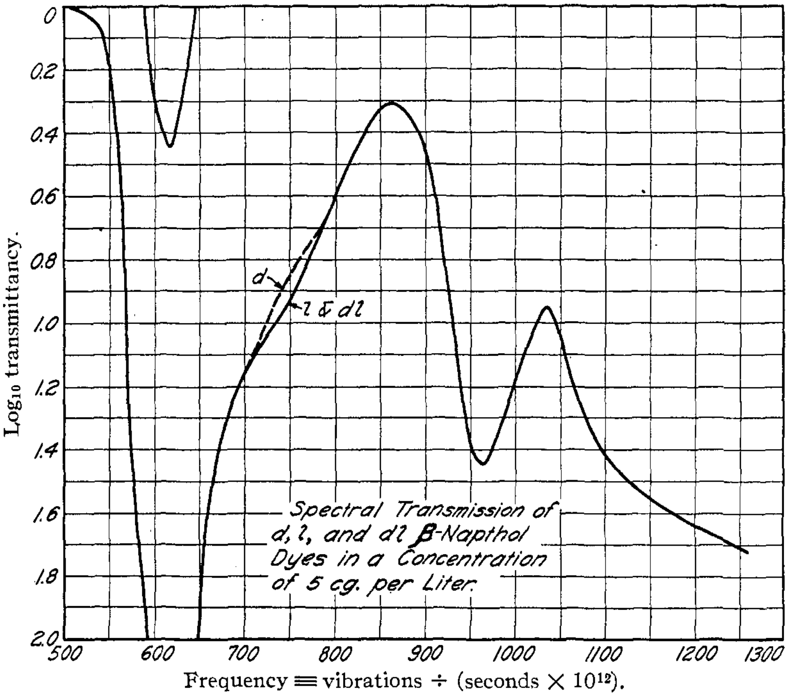

Fig. 1.

All three dyes gave bands of the same frequency both in the visible and the ultraviolet. In the case of the d dye a small difference was observed

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[10] The authors are indebted to the colorimetric section of the Bureau of Standards for help and suggestions in carrying out these determinations.

[11] *Ann. Physik* [4] **12,** 985 (1903).

in the height of one of the bands, which is indicated by a dotted line on the graph (Fig. 1). This difference was so small that it did not affect the shade of the color sufficiently to be detected in the Duboscq colorimeter since this colorimeter does not employ spectroscopic dispersion by the use of monochromatic light of wave length of the maximum absorption as the means of determining the color intensity. It is not out of the question that a slight impurity was the cause of this difference in one of the bands.

The band in the visible was of a wave length of 486 mμ or a frequency of 625. The bands in the ultraviolet appeared at frequencies of 788, 956 and 1108. These bands are approximate multiples of the definite fundamental frequency of 158, the first band in the spectrum corresponding to the fourth period and the others to the fifth, sixth and seventh periods. The light transmission of these dilute solutions of the dyes were shown to vary in accordance with Beer's law, the logarithm of the transmission being directly proportional to the concentration (Fig. 2) and the thickness (Fig. 3). In determining the transmission of various concentrations, solutions of 1, 2, 4, 10, 100 and 1000 cg. per liter were used. Transmission at various thicknesses of the solutions was determined by using tubes of 1, 2, 4, 7 and 10 cm. in length.

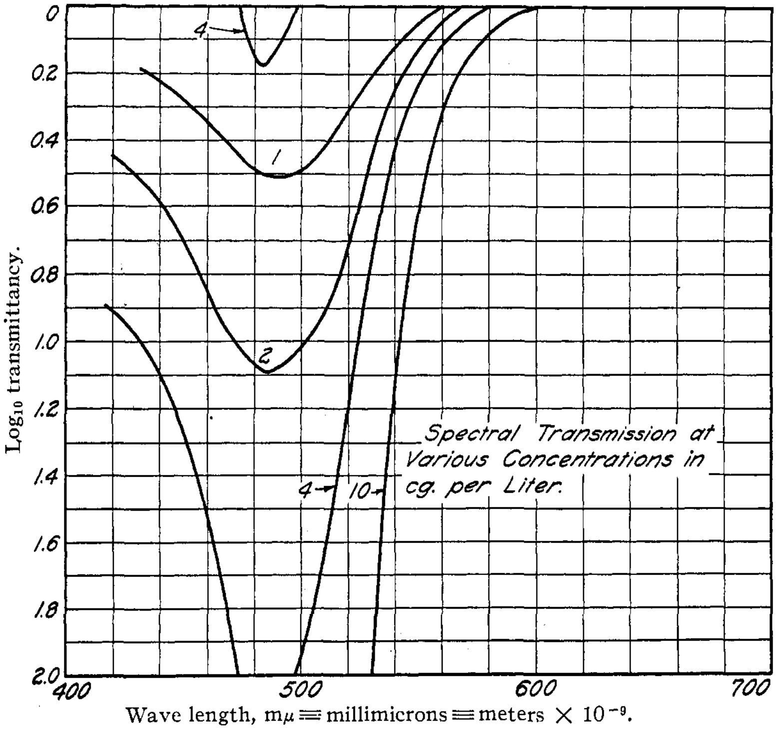

Fig. 2.

If the optical isomers, especially the two active forms, had not had the same frequency of absorption, the comparison of the two by means of a colorimeter would have been impossible; a difference in the intensity of the two bands would also complicate the determination of the dyes in the solutions by the colorimetric method. As has been shown in these results, however, the three enantiomorphic forms had bands of the same intensity throughout the visible spectrum. In a quantitative determination of the absorption of the dye by adsorbing media, the validity of Beer's law was assumed. This law was shown to hold for the solutions of the strength used in these experiments.

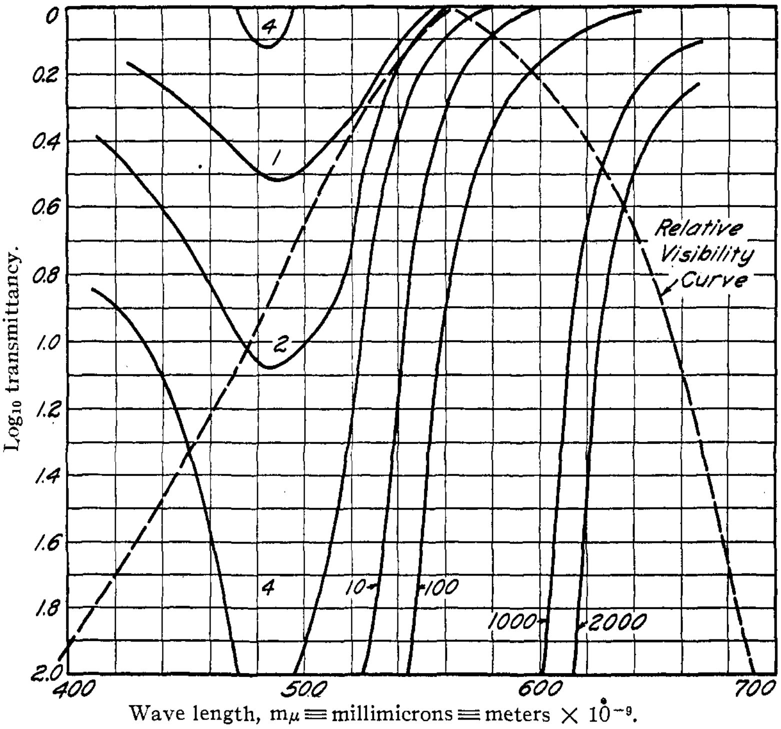

Fig. 3.—Visible spectra transmission at various thicknesses in cm.

The Effect of Change in Hydrogen-Ion Concentration upon the Absorption Spectra

The addition of an adsorbing agent to a solution almost invariably changes the hydrogen-ion concentration of the solution, the character of the change depending upon whether the adsorbing medium is of a negative or positive character.[12] It was, therefore, essential to determine the color change which is effected by the change in hydrogen-ion concentra-

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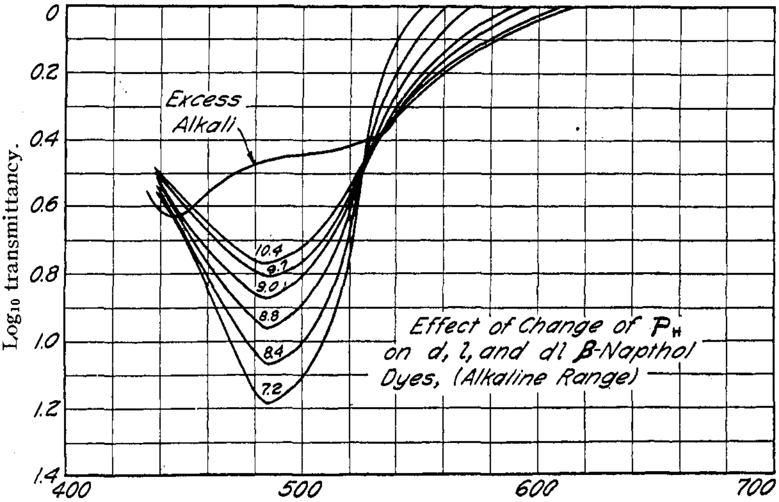
[12] Bancroft, "Applied Colloid Chemistry," McGraw-Hill Book Co., 1921, p. 155.

tion. The strength of the solutions used for this experiment was the same as those used in the determination of the absorption spectra in the visible. The hydrogen-ion concentration of the solution was determined electrolytically. The change in color with the change in hydrogen-ion concentration in the case of the β-naphthol dyes is very slight between *P*H 3 and 8.5, but beyond 8.5 a sharp drop in the band takes place and secondary bands appear at 443 and 516 mμ (Fig. 4). A similar effect is shown when the solution is made strongly acid. At *P*H 1.2 there is a sharp change with the appearance of two bands at 522 and 553 mμ (Fig. 5). This curve does not change in shape but simply drops in height, due possibly to the formation of larger colloidal particles.



Wave length, mμ ≡ millimicrons ≡ meters × 10^{-9}.
Fig. 4.

It has been shown in the experiments described above that the absorption band does not change in wave length but rather in intensity with a change in hydrogen-ion concentration. This does not lead to the conclusions of Smith and Boord,[13] that the band shifts with an increasing alkalinity. The change in intensity without change in wave length has been applied to the study of indicators. A method has been developed by which hydrogen-ion concentration could be determined by taking this fact into account.[14]

From the results obtained, it is seen that the band reaches approximately its maximum height at *P*H 7 to 8 and a change of 1 or 2 in the Sörensen value on either side does not change the color intensity of the solutions sufficiently to be detected by a colorimeter of the type used in this in-

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[13] Smith and Boord, THIS JOURNAL, **44**, 1449 (1922).

[14] Brode, *ibid.*, **46**, 581 (1924).

vestigation. The hydrogen-ion concentration in all these experiments did not vary within the experimental error and did not complicate the data in this research. The solutions used in the adsorption experiments had a Sörensen value of 7 to 8 and the actual hydrogen-ion change, after treatment, did not exceed 0.5 *P*H. It was permissible, therefore, to assume that the color change due to the change in hydrogen-ion concentration by adsorption was negligible.

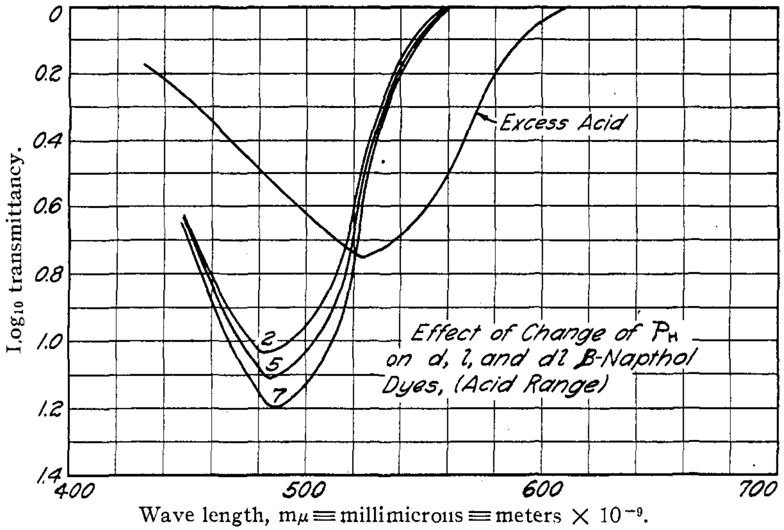

Fig. 5.

Rotation

Rotation of colored substances at different wave lengths presents a characteristic curve due to the proximity of the absorption band. This relation between the rotation and absorption bands has been studied by Lowry, Akermann, Drude, and others,[15] and the curves presented in this paper are of the same type as those obtained by previous workers.

The rotation of these dyes was very difficult to obtain, due to their physical properties, several of the difficulties being almost impossible to overcome. The dyes being red, monochromatic light sources such as sodium and mercury were eliminated for the determination of the rotations. Previous workers have described apparatus for the determination of rotations at various wave lengths other than those furnished by monochromatic light sources.[16] The best results for these dyes were

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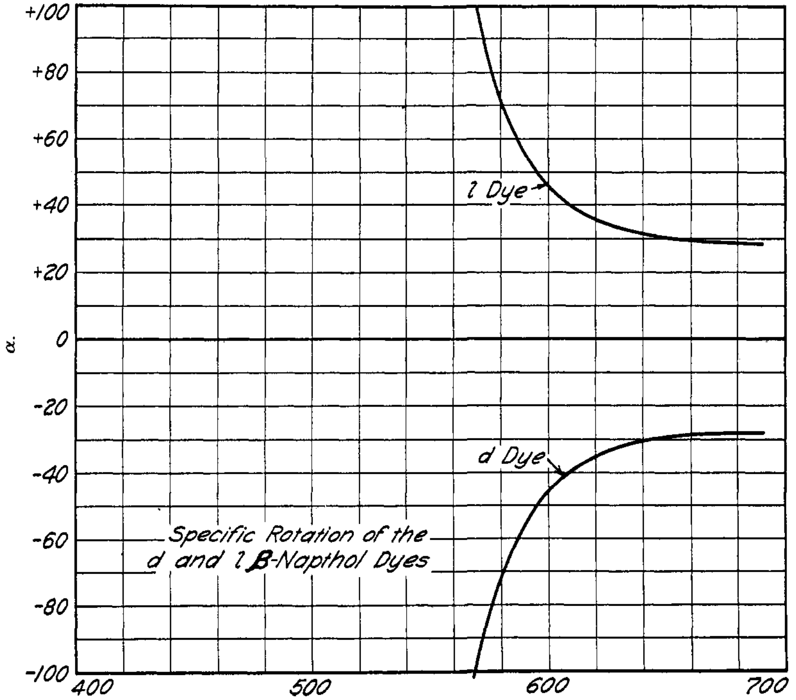
[15] Lowry, *Trans. Faraday Soc.*, **10,** 106 (1914); *J. Chem. Soc.*, **107,** 1196 (1915). Lowry and Austin, *Trans. Roy. Soc.* (London), **222A,** 272 (1922). Pickard, *J. Chem. Soc.*, **123,** 435 (1923). Akermann, *Ann.*, **420,** 1 (1920).

[16] *Bur. Standards Bull.*, **44,** 17 (1918). Bates, *Astrophys. J.*, **8,** 214 (1898); *Phil. Mag.*, **18,** 320 (1909). Patterson, *J. Chem. Soc.*, **109,** 1143 (1916).

obtained by the use of a 7-ampere, 20-volt straight condensed filament lamp. The filament was about 16 mm. long and, on overloading, gave about 200 candle power. The filament image was projected on the slit of a spectroscope and by making both the entrance and exit slits as narrow as possible, nearly homogeneous light of a mono-chromatic nature was obtained. The spectroscope used was one attached to a polarimeter which was a large Franz Schmidt and Haensch instrument capable of reading to 0.0002°. Due to the construction of the spectroscope, however, as the far red end of the spectrum was approached, the dispersion became less so that the readings obtained beyond a wave length of 650 mμ are of no great quantitative value. The rotation of the *d* dye (that is, the one obtained by diazotization and coupling of *d*-phenylamino-benzoyl-amino-acetic acid) in this case was negative and that of the *l* dye was positive (Fig. 6). These dyes act similarly to amino acids in that on long standing with sodium hydroxide, racemization takes place, and in the case of saturated solutions of the active forms the *dl*, which is less soluble, precipitates. The probable reason for the failure to obtain rotations of the dimethylaniline dye in sunlight[17] was that the rotation of the dye approached zero



Wave length, mμ ≡ millimicrons ≡ meters × 10^{-9}.
Fig. 6.

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[17] Ingersoll with Adams, This Journal, **44**, 2930 (1922).

at the wave length where the absorption curve crossed the maximum sensitivity curve of the eye. At other wave lengths, however, rotations could be observed, the rotation being positive on one side and negative on the other. The rotations by sunlight involve various wave lengths and since the rotation is dependent on the wave length, the results obtained by sunlight, or any heterogeneous source which is not treated so as to obtain a light of monochromatic nature, are of no great quantitative value.

Summary

1. The adsorption of enantiomorphic forms of dyes by charcoal, clay and other inactive adsorbing agents is the same.
2. The adsorption of the racemic form of these optically active dyes is different from that of the active.
3. The active forms exist more nearly in the form of true solutions than does the racemic modification.
4. The absorption spectra of the optical enantiomorphs give bands of the same frequency.
5. The change in hydrogen-ion concentration of the dye solutions does not change the wave length of the band but rather the intensity of the various bands.
6. Within the range of hydrogen-ion concentration change involved in the use of common adsorbing agents, the change of color of these particular dyes is negligible and the possible difference in the intensity of the two forms is negligible as far as the use of colorimetric determinations is concerned.
7. Beer's law is applicable to the dilute solutions used in these experiments.
8. As far as studied, the rotation of these dyes and intermediates is normal.

From these statements it is apparent that these dyes are suitable for future experiments in connection with the possible mechanism in the dyeing of wool and other fibers containing active constituents.

URBANA, ILLINOIS