

0.003 sec. and to the extent of 99% in 0.3 sec. In only three experiments did we attempt a measurement until at least twenty minutes after stirring. As Doss has emphasized,^{10,13} here the adsorption is delayed and therefore the diffusion "activated."

It is clear from all the phenomena referred to that the surfaces of ordinary solutions are far from being the simple structures commonly taken for granted. As was recently pointed out¹⁰ the Gibbs equation is sometimes incompatible with their being monomolecular; for solutions of the simpler soluble fatty acids the Gibbs predicted adsorption remains constant with increase in concentration while the surface tension continues to drop. Much remains to be discovered about

(13) Doss, *Kolloid-Z.*, **86**, 205 (1939).

the depth, electrification and organization of surfaces of solutions.

Summary

1. Even after efficient stirring, the experimentally determined adsorption of β -phenylpropionic acid in the surface of its aqueous solution as measured by the microtome method is not completed immediately, but may require several hours, whereas diffusion processes would require only about a tenth of a second.

2. The surface tension is not finally established until after much longer periods of time.

3. The final measured absolute adsorption seems to be slightly less than that calculated by the Gibbs theorem from surface tensions of comparable age and closest approach to equilibrium.

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[CONTRIBUTION FROM THE GEOPHYSICAL LABORATORY, CARNEGIE INSTITUTION OF WASHINGTON]

The Effects of Pressure, Temperature and Chemical Composition on the Absorption of Light by Mixtures of Aromatic Amines and Nitro Compounds

BY R. E. GIBSON AND O. H. LOEFFLER

Introduction

It has been known for a long time that, when pure aniline and pure nitrobenzene are mixed, the solution immediately develops a deep orange color.¹ This is an example of a very general phenomenon, similar color changes being observed whenever primary, secondary or tertiary aromatic amines are mixed with aromatic nitro compounds,² aliphatic nitro compounds such as tetranitromethane,^{2,3} nitroso compounds, quinones⁴ or liquid sulfur dioxide,⁵ etc. These color changes are due neither to adventitious impurities nor to irreversible reactions; addition of dilute hydrochloric acid to aniline-nitrobenzene mixtures discharges the color immediately, and the deep orange or red solutions of aniline or dimethylaniline in nitrobenzene freeze to very pale yellow solids which are immediately stained red when melting begins.⁶ Furthermore, in the solutions we have studied, phototropic effects are not ob-

vious although the mixtures do not differ greatly from some phototropic compounds.⁷

A satisfactory discussion of the changes in light absorption which occur when amines are mixed with *mono-nitro* compounds does not seem to have been given. It has been assumed that the colors observed are due to the formation of small amounts of intermolecular compounds in the solutions⁸ and this has received some support from the known existence of a wide variety of stable, colored, solid intermolecular complexes between polynitro compounds and amines or hydrocarbons, substances which have been studied extensively.⁹

(7) L. Chalkley, *Chem. Rev.*, **6**, 217 (1929).

(8) See P. Pfeiffer, *ref. 4*, pp. 358 and 384; G. M. Bennett and R. L. Wain, *J. Chem. Soc.*, 1108 (1936). Further references to articles supporting this view are given by D. L. Hammick and G. Sixsmith, *ibid.*, 972 (1939). In this paper we shall follow the customary practice and use the term "compound formation in solution" to mean the formation of covalent bonds between atoms in unlike molecules of the original components, as contrasted with general intermolecular attractions that result from dipole interaction or other polarization effects. A definite criterion, *vis.*, a measurable reaction velocity, has been proposed for this type of "compound formation" by D. L. Hammick and G. Sixsmith, *ibid.*, 580 (1935). Unless such a restriction is placed on the term "compound formation," it becomes too broad to be useful in the present state of our knowledge.

(1) I. Ostromisslensky, *Ber.*, **44**, 268 (1911). The phenomenon must have been noticed much earlier than this by such workers as Hepp (1882) or Kremann, but we have not been able to find any specific discussion of it in the literature.

(2) C. K. Tinkler, *J. Chem. Soc.*, **103**, 2171 (1913).

(3) D. L. Hammick and R. P. Young, *ibid.*, 1463 (1936).

(4) P. Pfeiffer, "Organische Molekulverbindungen," F. Enke, Stuttgart, 1927, p. 283.

(5) H. W. Foote and J. Fleischer, *THIS JOURNAL*, **56**, 870 (1934).

(6) For other examples see *ref. 2*.

(9) (a) See P. Pfeiffer, *ref. 4*; (b) P. Pfeiffer, *Chem. Ztg.*, **59**, 205 (1935); (c) J. W. Baker and G. M. Bennett, *Ann. Rept. Chem. Soc., London*, **28**, 128 (1931); (d) D. L. Hammick and colleagues, papers in *J. Chem. Soc.* from 1935 to date; (e) G. Briegleb, *Z. physik. Chem.*, **B31**, 58 (1935); G. Briegleb and J. Kambeitz, *ibid.*, **B32**, 305 (1936); G. Briegleb, "Zwischenmolekulare Krafte und Molekulstruktur," F. Enke, Stuttgart, 1937.

Recent work, however, has shown that the stability of these solid addition compounds may be accounted for by the interaction energy arising from the mutual polarization of the molecules,¹⁰ and their significance in supporting a theory of compound formation in the solution has consequently depreciated.

Furthermore, important differences in behavior indicate that the potential energy due to attractive forces between unlike molecules is less in systems containing mononitro compounds than in systems containing polynitro compounds, and that the former systems actually show positive deviations from ideality. For example, the freezing point curves of mixtures of aniline with (a) trinitrobenzene, (b) *m*-dinitrobenzene, (c) nitrobenzene indicate the formation of a congruently melting solid compound in case (a), of an incongruently melting compound in case (b) and no solid compound in case (c).¹¹ Linard's conclusion that a compound is formed in system (c) does not seem to be supported by his own diagram,¹² or by the disappearance of color when these solutions freeze. Tinkler² found a simple eutectic in the system *p*-chloronitrobenzene-diphenylamine, and, indeed, it has been stated categorically that no crystallized addition compounds of mononitro compounds are known.¹³ The measurements of partition coefficients made by Moore, Shepherd, and Goodall¹⁴ lead to the same conclusion. The fluidity-composition-curves for mixtures of mononitro compounds and amines¹⁵ show positive deviations from ideal behavior.¹⁶ A small amount of heat is *absorbed* on the formation of aniline-nitrobenzene solutions¹⁷ (132 cal./mole for a 50 mole per cent. solution at 14°) and we have already indicated that the volume changes on mixing at different

pressures and temperatures make it unlikely that compound formation takes place in these solutions, and that if it did, the compound would be destroyed by rise of pressure.¹⁸

In this paper we give the results of some observations of the effects of pressure changes from 1 to 1500 bars and of temperature changes between 25 and 85° on the absorption of light by solutions of aniline, dimethylaniline, diphenylamine and triphenylamine in nitrobenzene, and by a number of solutions of related substances. The bearing of these observations on the problem of molecular interaction in solution, on the mechanism of the color changes to which we have referred, and on the role of intermolecular action in determining the color of organic compounds will be discussed.

Experimental

The effects of pressure and temperature on the absorption of light by the various solutions were measured in the same bomb and thermostat and with the same absorption cells that were used in our work on the determination of refractive indices under pressure.¹⁹ Light from an incandescent tungsten strip filament entered the pressure bomb by one window, passed through the solution which was contained over mercury in the glass cell, and after emerging through the other window, impinged on the slit of a Hilger Type D2 constant deviation wave length spectrometer. The character of the incident light was varied to meet our requirements by adjustment of the filament temperature or by the use of suitable glass filters. The spectra of the transmitted light were photographed on Wratten panchromatic plates. An approximate scale of wave lengths was provided by similar photographs of the spectra of mercury and helium arcs, a spectrum of the mercury arc being superimposed on the transmission spectrum of each solution. The results of these experiments will be given in the form of microphotometer tracings of the photographs of the transmission spectra which were made on a Moll recording microphotometer.²⁰

In these diagrams the abscissas give the approximate wave lengths and the ordinates are proportional to the logarithms of the intensity of the transmitted light over the ranges of intensity indicated by the vertical arrows in the right-hand margins.

The chief part of the experimental work consisted of comparing the transmission spectra at different pressures and temperatures with those at 25° and 1 bar, and care was taken to make the first and last photographs of any series with the solution under these standard conditions. In any pressure experiment the cell was filled with freshly prepared solution and the transmitted light was photographed

(10) L. Pauling, *Proc. Nat. Acad. Sci.*, **25**, 577 (1939); G. Briegleb, ref. 9e; B. R. Hamilton and D. L. Hammick, *J. Chem. Soc.*, 1352 (1938).

(11) R. Kremann, *Monatsh.*, **25**, 1271 (1904); R. Kremann and O. Rodinis, *ibid.*, **27**, 125 (1906). With *o*- and *p*-dinitrobenzene, aniline forms no solid compound.

(12) J. Linard, *Bull. soc. chim. Belg.*, **34**, 381 (1925).

(13) P. Pfeiffer, ref. 4, p. 358. Recently, however, G. P. Luchinskii and A. I. Likhacheva, *J. Phys. Chem. (U. S. S. R.)*, **7**, 723 (1936), have reported two eutectics in the system nitrobenzene-dimethylaniline.

(14) T. S. Moore, F. Shepherd and E. Goodall, *J. Chem. Soc.*, 1447 (1931).

(15) Computed from the data of R. Kremann, F. Gugl and R. Meingast, *Monatsh.*, **35**, 1365 (1914); R. Kremann and R. Ehrlich, *Sitaber. Akad. Wiss. Wien*, **116**, 786 (1907); D. E. Tsakalotos, *Bull. soc. chim.*, **IV**, **11**, 284 (1912).

(16) R. H. Ewell, *J. Chem. Phys.*, **5**, 967 (1937).

(17) R. Kremann, R. Meingast and F. Gugl, *Monatsh.*, **35**, 1235 (1914).

(18) R. E. Gibson and O. H. Loeffler, *THIS JOURNAL*, **61**, 2877 (1939).

(19) R. E. Gibson and J. F. Kincaid, *ibid.*, **60**, 511 (1938).

(20) We take this opportunity of thanking Dr. J. H. Hibben, formerly a member of this Laboratory, for his generous assistance in the use of this apparatus.

at different pressures in the following order, 1, 500, 1000, 1500 (in a few cases), 500 and 1 bar. All exposures to be compared were made on the same plate, the cell being left undisturbed whenever possible, and the exposure times were made long enough (30 to 60 sec.) to ensure adequate reproducibility. In the same way, when the effects of temperature changes were being studied, the exposures taken at the higher temperatures were bracketed between initial and final exposures at 25°. Except in rare cases when obvious causes were present, the photometer curves for exposures taken under the same conditions coincided within 2 mm. on a photographic record where the total possible excursion was 100 mm.

The Effect of Pressure on the Absorption of Light by Solutions of Amines in Nitrobenzene

The following observations were made on solutions of aniline, dimethylaniline, diphenylamine and triphenylamine in nitrobenzene, but it seems probable that conclusions drawn from them may be applied to solutions of any amine in a nitro compound. When the pressure is raised, the absorption of visible light by these solutions is increased much more than can be accounted for by the increase in the density of the solution. The effect is quite reversible and shows no time lag. Typical features of our results are to be seen in Fig. 1, which illustrates the results obtained with a 50% solution of dimethylaniline in nitrobenzene.

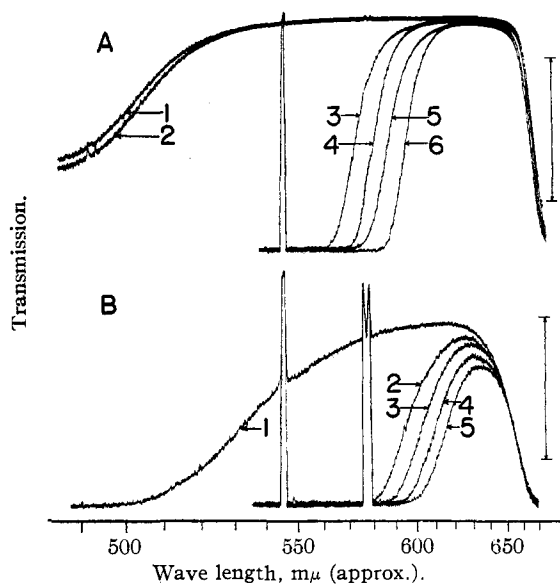


Fig. 1.—Transmission curves for a 50% solution of dimethylaniline in nitrobenzene at 45°: A1, aniline in 0.9-cm. cell; A4, solution in 0.9-cm. cell at 1 bar; A6, solution in 0.9-cm. cell at 1000 bars; A2, aniline in 0.5-cm. cell; A3, solution in 0.5-cm. cell at 1 bar; A5, solution in 0.5-cm. cell at 1000 bars; B1, same as A1; B2, solution at 1 bar; B3, solution at 500 bars; B4, solution at 1000 bars; B5, solution at 1500 bars.

Figure 1A shows the transmission spectra at 1 and 1000 bars with two different absorption cells: cell (a) 0.9 cm. thick and cell (b) 0.5 cm. thick. Curves 1, 4 and 6 refer to cell (a) and curves 2, 3 and 5 to cell (b). Curves 1 and 2 were taken with the cells full of pure aniline, no filter was used, the source of light being held at a low temperature in this series. Curves 3 and 4 were obtained at 1 bar and curves 5 and 6 at 1000 bars. The magnitude of the effect of pressure on the absorption of light by this solution may be estimated from a comparison of curves 3, 4 and 5, due allowance being made for the separation of curves 1 and 2. It will be seen that a 1.8-fold increase in the thickness of the absorbing layer does not increase the absorption by any means as much as an increase of pressure of 1000 bars even though the change in density due to the rise of pressure is less than 4%. In Fig. 1B, a systematic study of the effect of pressure on this system is given; cell (a) was used in this and in all the following experiments, and curves 2, 3, 4 and 5 give the transmission spectra at 1, 500, 1000 and 1500 bars, respectively. It will be noticed that at wave lengths where the solution is quite transparent at 1 bar, its absorption is considerable at 1500 bars, and that in the strongly absorbing regions on the short wave side of the transmission spectrum the logarithm of the intensity decreases linearly with rise of pressure.

Transmission curves for a 50% solution of aniline in nitrobenzene are given in Fig. 2A. The incident light (curve 1) passed through a filter of Corning Daylight Glass (5 mm.). Curves 2 and 3 represent different exposures at 25° and 1 bar and their reproducibility is typical. Curve 5 was obtained at 1000 bars. In one set of experiments with a photocell and galvanometer, it was found that the amount of light of wave length 546 mμ (mercury green line) transmitted by an aniline-nitrobenzene solution fell to 40% of its value at 1 bar when the pressure was raised to 1000 bars. From an examination of a series of mixtures of aniline and nitrobenzene over the whole range of concentration, it was concluded that there was no obvious influence of concentration on the effect of pressure on the absorption of light by these solutions.

The behavior under pressure of an 8.5% solution of triphenylamine in nitrobenzene is shown in Fig. 3A and that of an 8.5% solution of diphenylamine in Fig. 3B. The legend for the

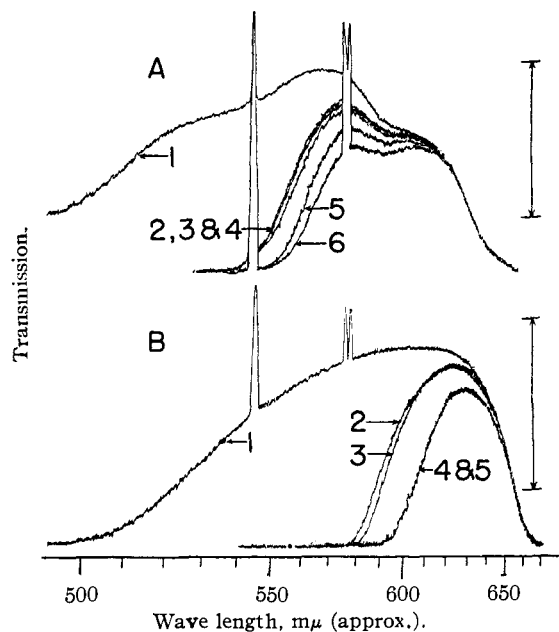


Fig. 2.—Transmission curves A for a 50% solution of aniline in nitrobenzene, B for a 49% solution of dimethylaniline in nitrobenzene: A1 and B1, aniline in cell; A2 and A3, solution at 25° and 1 bar; A4, solution at 85° and 1 bar; A5, solution at 25° and 1000 bars; A6, solution at 85° and 1000 bars; B2, solution at 85° and 1 bar; B3, solution at 25° and 1 bar; B4 and B5 (indistinguishable), solution at 25 or 85° and 1000 bars.

figure identifies the curves. It will be noticed that as regards the effect of pressure on the absorption of light these solutions resemble those of the other amines in nitrobenzene. The transmission curve (A4) for the triphenylamine solution at 85° and 1000 bars is, however, anomalous in that it converges on the curve for 85° and 1 bar (A2) at a wave length of 580 mμ.

The Effect of Temperature on the Absorption of Light by Solutions of Amines in Nitrobenzene

We examined the transmission spectra of the solutions described in the previous section at 25, 55 and 85°. As the results at 55° were always intermediate between those at 25 and 85°, they are omitted from the published diagrams for the sake of clarity. The results are so diversified that each solution must be described separately. Figure 2A illustrates the results found with the aniline-nitrobenzene solution. At atmospheric pressure a rise of temperature from 25 to 85° produces a very small but reproducible increase in the absorption of light by this solution (curves 2, 3 and 4) which gains in significance when it is noticed that at 1000 bars the same rise of tem-

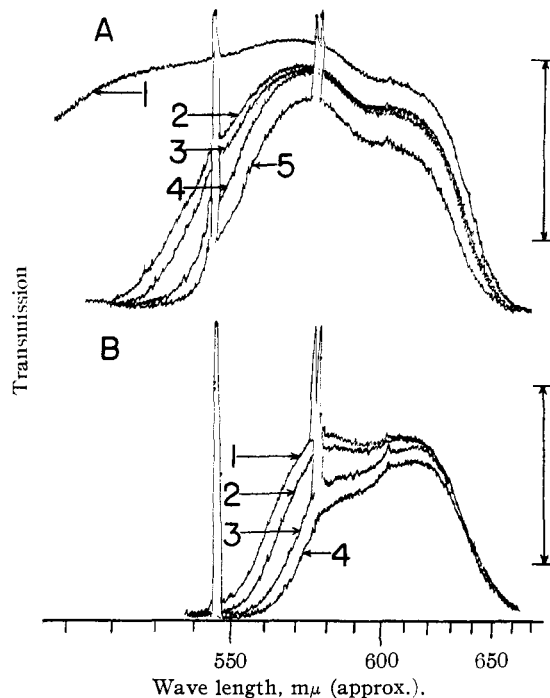


Fig. 3.—Transmission curves for 8.5% solutions of A triphenylamine in nitrobenzene, B diphenylamine in nitrobenzene: A1, aniline in cell; A2, solution at 85° and 1 bar; A3, solution at 25° and 1 bar; A4, solution at 85° and 1000 bars; A5, solution at 25° and 1000 bars; B1, solution at 85° and 1 bar; B2, solution at 25° and 1 bar; B3, solution at 85° and 1000 bars; B4, solution at 25° and 1000 bars.

perature causes a pronounced increase in the absorption of light by this solution, as a comparison of curves 5 and 6 will show. These observations apply to all the nitrobenzene-aniline solutions we have studied. The behavior of a 50% solution of dimethylaniline in nitrobenzene is shown in Fig. 2B. At 1 bar a rise of temperature from 25 to 85° causes a slight decrease in the light absorption particularly on the short wave edge of the transmission spectrum (curves 2 and 3). This change is reproducible and seems to be real. At 1000 bars the same rise of temperature produces no observable change in the absorption of light by the solution. As in the case of aniline-nitrobenzene mixtures, the effect of raising the pressure is to make the temperature coefficient of the absorption more positive. The curves given in Fig. 3 show that the absorption of light by the solutions of triphenylamine and of diphenylamine in nitrobenzene decreases quite significantly when the temperature is raised from 25 to 85° at both 1 and 1000 bars, and that there is no appreciable effect of pressure on the temperature coefficient.

At 85° and 1000 bars the specific volumes of all the solutions discussed in this section are approximately the same as at 25° and 1 bar. It will be seen from the diagrams, therefore, that rise of temperature at constant volume causes an increase in the absorption of light by all the solutions.

Miscellaneous Observations

In order to satisfy ourselves that the pressure effects just described were not due to some unexpected characteristic of our apparatus, we made similar observations on an 11% aqueous solution of potassium bichromate. No significant effect of pressure on the absorption of light by this solution was found (Fig. 4B). We also observed that the transmission of light by the apparatus, when the cell was filled with pure aniline, was not affected by pressure.

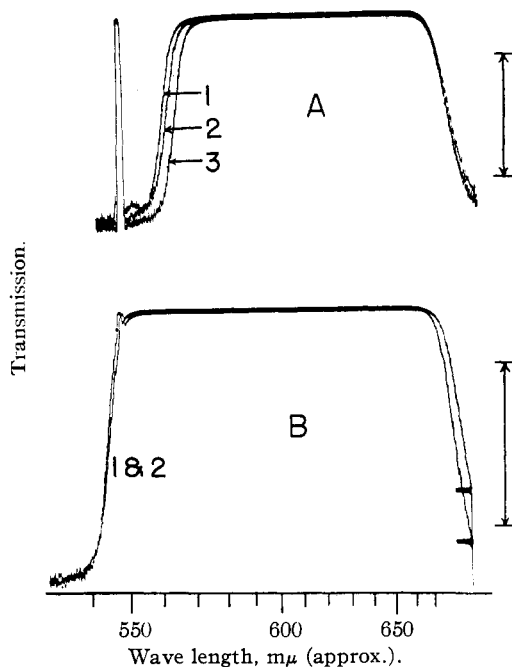


Fig. 4.—A. The transmission of light by a 3.1% solution of dimethylaminoazobenzene in benzene at 1 bar (1), 500 bars (2), and at 1000 bars (3). B. The transmission spectra of an 11% solution of potassium dichromate in water at 1 and at 1000 bars.

Solutions of *p*-nitrophenol in aniline absorb light less strongly in the visible than nitrobenzene-aniline solutions of the same molal composition, but their behavior under pressure changes is the same. On the other hand, the absorption of light by the *p*-nitrophenol solutions is notably increased by rise of temperature. The transmis-

sion curves at 25° (1) and 85° (2) for a 44% solution are given in Fig. 5, where it will be seen that the positive temperature coefficient is much larger than any other we have described.

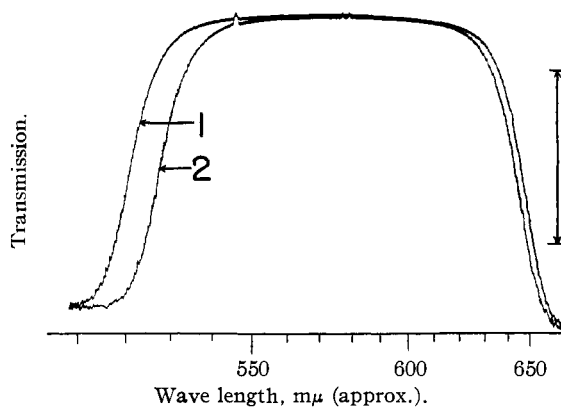


Fig. 5.—The effect of temperature on the transmission of light by a 44% solution of *p*-nitrophenol in aniline. Curve 1 was obtained at 25° and curve 2 at 85°, the pressure being 1 bar.

Solutions of nitrosobenzene in aniline and other amines are much more highly colored than the corresponding solutions of nitrobenzene. Figure 6A shows that rise of pressure increases the absorption of light by a dilute (1.1%) solution of nitrosobenzene in aniline. Similar results

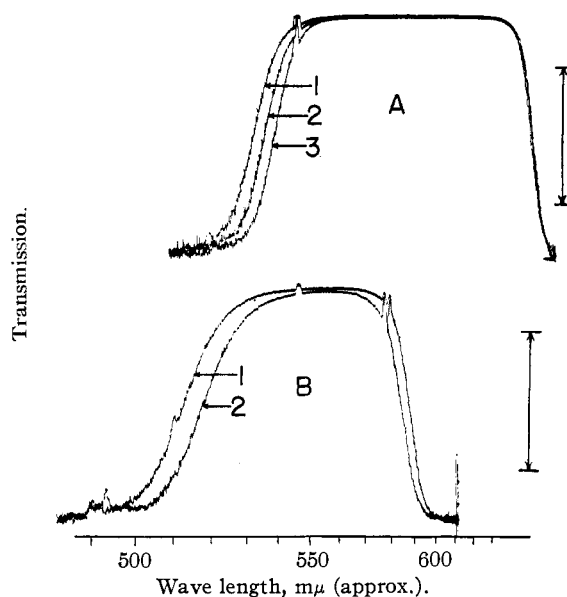


Fig. 6.—A. The transmission spectra of a 1.1% solution of nitrosobenzene in aniline at 1 bar (1), at 500 bars (2), and at 1000 bars (3). B. The effect of pressure on the transmission of light by an 18% solution of nitrosobenzene in benzene. Curve 1 was obtained at 1 bar and curve 2 at 1000 bars.

were obtained with solutions of nitrosobenzene in dimethylaniline. The absorption of light which gives rise to the well-known green color of solutions of **nitrosobenzene in benzene** also is influenced significantly by pressure changes (Fig. 6B).

We have observed an effect of pressure on the transmission curves of dilute solutions of **dimethylaminoazobenzene in benzene** (Fig. 4A) and of solutions of *o*- or *p*-nitroaniline in acetone (not illustrated). The pressure coefficients of the light absorption by these solutions are, however, not as large as those of solutions in which the NO₂ and NH₂ groups are on entirely separate molecules.

Solutions of *s*-Trinitrobenzene in Aniline.—

These solutions absorb light much more strongly than any others we have discussed and it is known that a 1:1 solid addition compound is formed in this system.²¹ Figure 7 shows that a 2.5% solution of trinitrobenzene in aniline behaves in the same way as mixtures of mononitrobenzene with amines under pressure changes and that the absorption of light is diminished by rise of temperature more rapidly than in the other cases we have studied. At constant volume, however, increase of temperature enhances the absorption of visible light by this solution. A comparison of Figs. 2, 3 and 7 shows that the stronger the absorption of visible light by the solution, the more negative is its temperature coefficient at atmospheric pressure.

Thermodynamic Discussion of Results

The experiments just described contribute strong evidence against the hypothesis that the colors of these solutions arise from the presence of small amounts of compounds that have been formed by the rearrangement of covalent bonds. A consideration of the temperature coefficients of the absorption of light by aniline-nitrobenzene solutions at different pressures, of the absence of time lag, of the data summarized in the introduction, and of the fact that the solutions all absorb more strongly when the temperature is raised at constant volume will show the improbability of such an assumption. On the other hand, the hypothesis that the colors of the solutions arise from the mutual polarizations of the unlike molecules when they are separated by distances which

(21) B. R. Hamilton and D. L. Hammick [*J. Chem. Soc.*, 1350 (1938)] have found that the absorption coefficients of solutions of aniline and *s*-trinitrobenzene in carbon tetrachloride diminish with rise of temperature.

are short compared with the mean distance of separation, *i. e.*, during a collision, furnishes the basis for a fairly detailed account of our results.

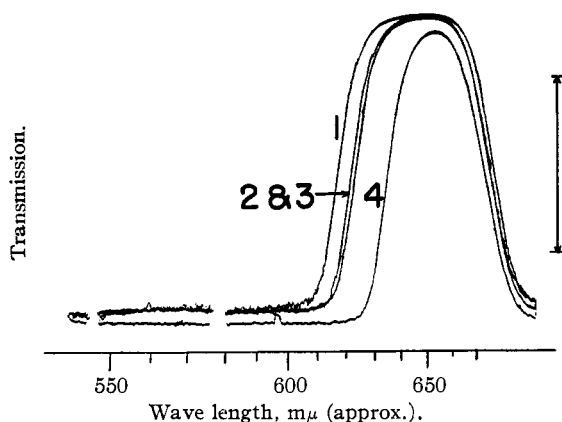


Fig. 7.—The effects of pressure and temperature on the transmission spectra of a 2.5% solution of *s*-trinitrobenzene in aniline. Curves 2 and 3 are from different photographs at 25° and 1 bar. Curve 1 gives the transmission at 85° and 1 bar, and curve 4 refers to 25° and 1000 bars.

We shall assume, therefore, that the absorption of visible light by the solution is proportional to the average time the active groups on unlike molecules spend very close to each other, *i. e.*, to the probability of finding two unlike molecules at very short distances apart and suitably oriented in the solution. The average time spent by the molecules at close distances may be correlated qualitatively with the pressure and temperature through the familiar model for liquids in which the thermal motion of the molecules is expressed in terms of the oscillations of a given molecule in a free volume, v_f , bounded by other molecules which are held stationary in their mean positions.²² On the assumption that the potential energy of the molecule changes only during collisions, a formula for the oscillation frequency ν in such a system has been derived by Bradley and is essentially

$$\nu = \text{Const. } T^{1/2} / M^{1/2} V_f^{1/3} \quad (1)$$

where T is the absolute temperature, M the molecular weight, and V_f the free volume per mole. From our thermodynamic data on aniline, nitrobenzene and their mixtures,²³ we can estimate V_f by means of a relation whose validity

(22) (a) J. O. Hirschfelder, D. Stevenson and H. Eyring, *J. Chem. Phys.*, **5**, 896 (1937); (b) R. S. Bradley, *Trans. Faraday Soc.*, **33**, 1185 (1937); *J. Chem. Soc.*, 1910 (1934); (c) N. F. Mott and R. W. Gurney, "Repts. on Progress of Physics," Vol. V, The University Press, Cambridge, 1939, p. 46; (d) J. Frenkel, *Trans. Faraday Soc.*, **33**, 58 (1937).

(23) R. E. Gibson and O. H. Loeffler, *THIS JOURNAL*, **61**, 2515, 2877 (1939).

has been made probable by Kincaid and Eyring²⁴ and which may be written in the form

$$V_f^{1/3} = 2R/V^{2/3} (\partial P/\partial T)_V \quad (2)$$

At constant temperature we have found empirically that $V_f^{1/3}$ is inversely proportional to the pressure over a range of 1000 bars. By equation (1), therefore, the oscillation frequency of the molecules which determines the average time they spend in close proximity under these conditions should be directly proportional to the pressure, and, according to our hypothesis, the absorption of light of a given wave length should also vary directly with the pressure. This conclusion is in accord with the results given in Fig. 1A.

At constant volume we found empirically that the product $T^{1/2}(\partial P/\partial T)_V$ was constant over a range of 60° at least, whence it follows that $V_f^{1/3}$ is directly proportional to $T^{1/2}$ or to the mean square velocity of the molecules at their mean positions, and that the oscillation frequency is independent of the temperature (eqn. 1). This increase of free volume with temperature is due to the compressibility of the molecules,²⁵ their distance of closest approach during a collision depending on the velocity with which they collide, and on consequent changes in molecular distribution. As a result of this increasing interpenetration the molecules spend on the average a larger fraction of the time at very close distances as the temperature rises, even though the oscillation frequency computed from equations (1) and (2) remains constant, and the absorption of light of longer wave lengths increases. All the solutions we have studied show a marked increase in light absorption as the temperature is raised at constant volume.

The effect of increasing temperature at **constant pressure** is not so easy to predict, since the rapid increase in free volume reduces the probability that two unlike molecules will be close together, while the increase in the kinetic energy of the molecules and their closer approach on collision influences this probability in the opposite direction. It will be noticed that the influence of temperature at constant pressure on the absorption of light by all the solutions of amines in nitrobenzene is quite small, as might be expected when two opposing effects are at work. Calculations with equations (1) and (2) show that the oscillation frequency in aniline-nitrobenzene mix-

tures decreases when the temperature is raised at constant pressure, and that the decrease is slightly less rapid at 1000 bars than at 1 bar, owing to the fact that the thermal expansion of the solution diminishes with pressure. As the interpenetration effect may be presumed to be independent of pressure, the effects of pressure on the temperature coefficient of the light absorption shown in Figs. 2A and 2B are correlated with the thermodynamic properties of the solutions.

Another factor in determining the probability that two active groups on unlike molecules shall be in contact is the existence of short-range, specific attractive forces between groups in the respective molecules which will cause a temporary "sticking" when the molecule in our model collides with an appropriate spot on the walls of the "cage" of surrounding molecules. This effect is to be expected in systems showing negative deviations from ideality, especially those in which solid addition compounds are formed, and its importance decreases with rise of temperature according to the Boltzmann law.²⁶ In solutions where the mutually attracting groups are those intimately connected with the absorption of visible light this "sticking" effect will increase the absorption at low temperatures and produce a relatively large negative temperature coefficient of the light absorption. We consider this to be the state of affairs in solutions of *s*-trinitrobenzene in aniline and we shall refer later to the mechanism of the "sticking" effect in this system.

If, however, one or both of the mutually attracting groups is not directly connected with the absorption process, the probability of color-producing collisions will fall, the solution will absorb light to a less extent at low temperature (other things being equal) and the temperature coefficient of absorption will have a relatively large positive value. Solutions of *p*-nitrophenol in aniline provide, we think, an example of this effect, the strong specific attraction being between the OH and NH₂ groups. The behavior of these solutions suggests that a phenomenon akin to complex isomerism in some solid addition compounds²⁷ is appearing in the solutions. We recognize two separate intermolecular actions in these solutions: (a) interaction between the NO₂ and NH₂ groups giving rise to the color, and (b)

(26) The short-range attractions between groups on like molecules must not be overlooked in this discussion.

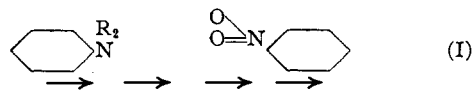
(24) J. F. Kincaid and H. Eyring, *J. Chem. Physics*, **6**, 620 (1938).
 (25) For a definition of this term see R. E. Gibson and O. H. Loeffler, *THIS JOURNAL*, **61**, 2520 (1939), or ref. 22c.

(27) E. Hertel and K. Schneider, *Z. physik. Chem.*, **A151**, 413 (1930); **B13**, 387 (1931).

interaction between the NH_2 and OH groups tending to inhibit (a) at lower temperatures and giving rise to solid addition compounds. It is well known that mixtures of phenol and aniline²⁸ and of *p*-nitrophenol and aniline²⁹ have relatively high viscosities, show large negative departures from Raoult's law, and deposit solid addition compounds at lower temperatures, but that these deviations from ideal behavior diminish rapidly as the temperature is raised.³⁰

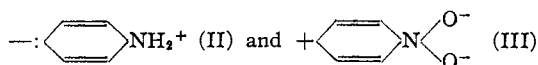
The Effect of Composition on the Absorption of Light by the Solutions

In the systems we have studied, reactions involving a transfer of electrons from the amino group of an *aromatic* amine³¹ to the nitro or nitroso group of the other component (*e. g.*, oxidation-reduction or acid-base reactions) are possible, and, although they do not actually proceed under the conditions of our experiments, we suggest that the primary inductomeric or electromeric polarizations³² associated with these reactions do occur and are responsible for the color changes. Kinetic studies of the reduction of nitrobenzene derivatives³³ have shown that the rate of reduction increases when the inductive and resonance³⁴ effects of the nuclear substituents tend to diminish the negative charge on the nitro group and hence to increase its attraction for external electrons. We shall assume, therefore, that a drift of electronic charge from the NR_2 group to the oxygens of the NO_2 group with consequent displacements of negative charge in the $\text{N}:\text{O}$ bond and in the polarizable aromatic nuclei in the direction indicated by the arrows in formula I takes place when an aromatic amino group and a nitro group come very close together in the solution.



Hammick³⁵ and his collaborators already have suggested the same polarization mechanism in their assumption that the primary activation in the formation of solid addition complexes between polynitro compounds and aromatic hydrocarbons is a driving of electronic charge from the oxygen to the nitrogen of the NO_2 group.

In demonstrating from qualitative considerations of the inductive and resonance effects of substituents in either compound that the absorptions of the solutions are pushed to longer wave lengths (a) as the electron attraction power of the oxygen of the NO_2 group increases and (b) as the electron mobility in the aromatic amine increases, we should first note that the configurations



which, together with the orthoquinonoid forms, contribute significantly to the structure of aniline and of nitrobenzene,³⁶ actually oppose the polarization we have suggested. We should emphasize, therefore, that the electron drift in formula I is presumed to occur in aniline-nitrobenzene solutions only when the molecules are brought into very close contact by their thermal motion (*cf.* the discussion of the temperature coefficient).

Electron-attracting groups such as NO_2 , Cl or CO_2CH_3 in the ring of the nitro compound promote the electronic drift by reducing the negative charge on the NO_2 group. This gives rise to short-range attractions and affords an explanation of the differences in behavior between solutions of polynitro compounds and those of mononitro compounds in amines or hydrocarbons mentioned in the introduction and accounts for the greater absorptions of the polynitro solutions. It will be recalled that dilute alkalis cause color changes in polynitrobenzenes but not in pure nitrobenzene.³⁷

Electron-repelling groups such as NH_2 or CH_3 in the ring of the nitro compound reduce its ability to polarize the amino compound both by their inductive effects and, in *o*- and *p*-positions, by their resonance effects. We found from

(35) D. L. Hammick and G. Sixsmith, *J. Chem. Soc.*, 580 (1935); D. L. Hammick and A. Helliwell, *ibid.*, 761 (1938); T. T. Davies and D. L. Hammick, *ibid.*, 763 (1938).

(36) L. Pauling, *ref. 34*, p. 192.

(37) See N. V. Sidgwick, T. W. J. Taylor, and W. Baker, *ref. 33*, p. 259.

(28) F. A. H. Schreinemakers, *Z. physik. Chem.*, **29**, 577 (1899). R. Kremann, *Monatsh.*, **25**, 1234 (1904); *ibid.*, **27**, 91 (1906).

(29) R. Kremann and O. Rodinis, *ibid.*, **27**, 125 (1906).

(30) See, for example, the effect of temperature on the viscosity-composition curves of mixtures of phenol with amino compounds studied by A. Bramley, *J. Chem. Soc.*, **109**, 10 (1916).

(31) We have found that no significant color change occurs when α -phenylethylamine, a stronger base than aniline, is mixed with nitrobenzene. This observation suggests strongly that the amino group must be attached to the ring either directly or through a conjugated bond system, if a color change is to be produced.

(32) See J. R. Johnson, "Organic Chemistry," H. Gilman, Editor, J. Wiley and Sons, Inc., New York, N. Y., 1938, Chap. 19; C. K. Ingold, *Chem. Rev.*, **15**, 225 (1934).

(33) See N. V. Sidgwick, T. W. J. Taylor, and W. Baker, "The Organic Chemistry of Nitrogen," Clarendon Press, Oxford, 1937, p. 255.

(34) See J. R. Johnson, *ref. 32*; G. W. Wheland and L. Pauling, *THIS JOURNAL*, **57**, 2086 (1935); L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1939, p. 141. The "mesomeric" effect of Ingold (*ref. 32*) is now generally referred to as the resonance effect.

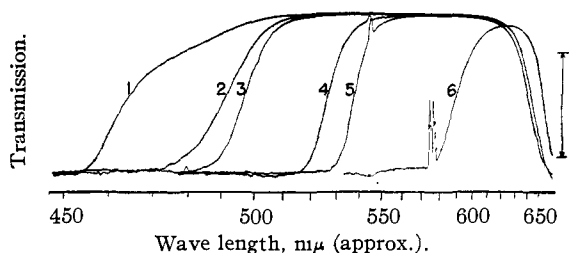


Fig. 8.—A comparison of the transmission of light by equimolecular solutions of (1) *p*-nitroaniline in nitrobenzene, (2) aniline in nitrobenzene, (3) *p*-nitroaniline in aniline, (4) diphenylamine in nitrobenzene, (5) dimethylaniline in nitrobenzene, and (6) *p*-phenylenediamine in nitrobenzene.

curves similar to those in Fig. 9 that the absorption of visible light by 0.31 molar solutions in aniline increased in the order *p*-nitroaniline < nitrobenzene < *m*-nitroaniline. Quinonoid structures being impossible in *m*-nitroaniline,³⁸ this result shows that the inductive effect of the NH₂ group does not compensate for the loss of the resonance effect when nitrobenzene is changed to *m*-nitroaniline and that the resonance in nitrobenzene does diminish its interaction with aniline.³⁹

As regards the effects of substituents in the amine nucleus, Fig. 9 shows that 0.22 molar solutions of the nitroanilines in nitrobenzene increase in light-absorbing power in the order *p*-nitroaniline ~ *o*-nitroaniline < aniline ≪ *m*-nitroaniline. The effect of an absence in the meta compounds of possible quinonoid configurations is again striking and indicates that resonance involving these configurations does reduce the interaction of aniline with nitro compounds as formula I suggests. A comparison of the transmission curves of equimolecular solutions of aniline, *m*-nitroaniline and *p*-nitroaniline in nitrobenzene, on the one hand, and of nitrobenzene, *m*-nitroaniline and *p*-nitroaniline dissolved in aniline, on the other, showed that absorptions of light are more influenced by the resonance effect of the NO₂ group than by that of the NH₂ group, a conclusion which is supported by the interaction dipole moments of aniline and nitrobenzene.⁴⁰ A transmission curve of a solution of *p*-nitroaniline in nitrobenzene is shown also in Fig. 8, curve 1. The sharp bend in this curve about 465 mμ is a feature of interest, because the absorption below

(38) N. V. Sidgwick, T. W. J. Taylor, and W. Baker, ref. 33, p. 74.

(39) In alkaline solutions *m*-nitroaniline is reduced much more rapidly than the *o* and *p* isomers. H. Goldschmidt and M. Eckardt, *Z. physik. Chem.*, **56**, 424 (1906).

(40) L. Pauling, ref. 34, p. 146.

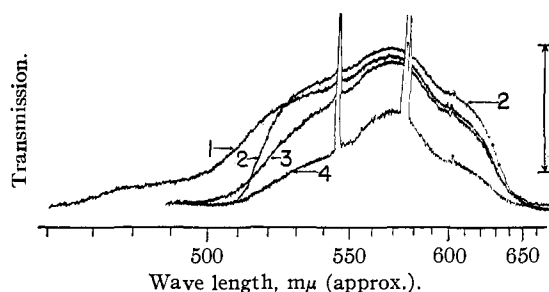


Fig. 9.—A comparison of the transmission curves of 0.22 molar solutions of *p*-nitroaniline (1), *o*-nitroaniline (2), aniline (3), and *m*-nitroaniline (4) in nitrobenzene.

this wave length is sensitive to pressure changes and hence of intermolecular origin, whereas the absorption at longer wave lengths is not sensitive to pressure changes, being due presumably to the nitroaniline itself. By their inductive effects, groups such as NH₂ or CH₃⁴¹ increase the potential concentration of electronic charge on the amino group. This effect on the absorption of light is strikingly shown by the curve for a dilute solution of *p*-phenylenediamine (a much stronger reducing agent than aniline) in nitrobenzene in Fig. 8 (curve 6). The exposure from which this curve was made was four times as long as that of any of the other amines in this diagram.

In acetanilide and *p*-nitroacetanilide the configurations C₆H₅NHC(CH₃)=O and C₆H₅⁺NH=C(CH₃)O⁻ contribute to the structure,⁴² and the resonance introduced lowers the possibility of the formation of a double bond between the nitrogen and the ring and renders the NH group less polarizable. The interaction of the NH group with external or internal NO₂ groups is, therefore, reduced by the attachment of the acetyl group. We have observed, on the one hand, that acetanilide and *p*-nitroacetanilide (a white solid) dissolve in nitrobenzene without any appreciable color change, and, on the other, that the transmission spectra of *p*-nitroacetanilide-aniline solutions resemble closely those of nitrobenzene-aniline solutions, being quite different from the spectra of *p*-nitroaniline-aniline solutions of the same molar concentration.

The electron mobility in the N-substituted amines, as measured, for instance, by the interaction dipole moments (resonance contribution to the dipole moment) of their *p*-substituted

(41) B. R. Hamilton and D. L. Hammick (*op. cit.*) showed that the absorption coefficients of carbon tetrachloride solutions of *s*-trinitrobenzene and different amines decreased in the order *o*-toluidine > *m*-toluidine > *p*-toluidine > aniline > chloroaniline.

(42) See L. Pauling, ref. 34, p. 192.

derivatives, increases as the number of methyl groups on the nitrogen increases.⁴³ Our results show that the absorption of light by nitrobenzene solutions of these N-substituted amines is pushed to longer wave lengths as the solute changes from aniline to dimethylaniline (curves 2 and 5, Fig. 8). It is of interest to note that the heats absorbed on the formation of 50 mole per cent. solutions of nitrobenzene in aniline, ethylaniline and diethylaniline are, respectively, 132, 27 and -15 cal./mole.¹⁷

In accord with the well-known fact that nitrosobenzene is much more readily reduced than nitrobenzene,³³ we have observed that the colors of liquid mixtures of amines and nitrosobenzene are much more intense than those of the corresponding mixtures with nitrobenzene. Both these facts may be explained by the recognized existence of the NO₂ group itself as a resonating structure⁴⁴ to which several configurations stabilizing the electronic charge on the oxygen contribute. We conclude from our observations of the effect of pressure on the absorption of light by solutions of nitrosobenzene in benzene that part, at least, of this absorption is due to intermolecular action. The interaction of a polarizable benzene molecule with a nitroso group is apparently large enough to bring the light absorption into the visible region, whereas the action of benzene on a nitro group is apparently not large enough. The role of the amino group seems to be that of a liaison between the polarizable aromatic nuclei and the oxygen atoms of the NO₂, NO, SO₂, etc., groups, the amino group focussing the polarization, and the difference between the interactions of aromatic hydrocarbons and of aromatic amines with these oxygens is, therefore, only one of degree.

Without a quantum mechanical treatment, methods of which are, however, rapidly developing,⁴⁵ it is difficult to locate exactly the electron system responsible for the absorption of visible light by these solutions. The behavior of tetranitromethane shows that the aromatic part of the nitro compound is not essential to the phenome-

non. Sklar⁴⁶ has shown theoretically that the weak 2500 Å. band in benzene is intensified and shifted toward the red when the symmetry of the ring is distorted by the resonance effects of substituents. It seems improbable, however, that any intermolecular polarization of the type we have discussed can cause distortions in the aromatic ring as large as those produced intramolecularly in nitrobenzene or nitroaniline, whose absorptions in the green or yellow are very much less than those of the solutions. Furthermore, the behavior of hydrocarbons suggests that the amino group is not absolutely essential, so that we are left with the N:O group as the most probable seat of the absorption. It will be seen from our picture in formula I that the proximity of the polarizable amine or hydrocarbon lowers the energy of a state in which an ionic binding such as N^-O^+ exists in the N:O group.⁴⁷

Our hypothesis that the colors of the solutions discussed in this paper arise from preliminary polarizations in oxidation-reduction reactions suggests comparison with other labile intermediate oxidation-reduction products such as quinhydrone, Wurster's salts, or semiquinones in general,⁴⁸ whose strong absorptions have been ascribed to charge-resonance involving odd electrons.⁴⁹ Nitrosobenzene and *p*-nitrosodimethylaniline are also relatively unstable, deeply colored intermediate steps in oxidation-reduction series, but they differ from the semiquinones in that they are diamagnetic⁵⁰ and have, presumably, no odd electrons. The apparent similarities in origin and behavior of the semiquinones, the nitroso compounds and the colored solutions are, however, sufficiently striking to merit further investigation, even though a theoretical picture embracing all these types of systems is not immediately obvious.

If our assumption that the changes in the absorption of light by these solutions are due to pre-reaction polarizations is substantiated, interesting possibilities of investigating reaction mixtures under different thermodynamic conditions by ultraviolet and visible absorption spectroscopy arise. Indeed, the large accelerating effect of pressure on reactions such as the addition of isopropyl iodide to dimethylaniline⁵¹ is exactly

(43) G. M. Bennett and S. Glasstone, *Proc. Roy. Soc. (London)*, **A145**, 71 (1934). R. J. B. Marsden and L. E. Sutton [*J. Chem. Soc.*, 599 (1936)] give 0.95 and 1.86×10^{-18} e. s. u. for the interaction dipole moments of *p*-nitroaniline and *p*-nitrodimethylaniline, respectively.

(44) L. Pauling, ref. 34, p. 186.

(45) L. Pauling, *Proc. Nat. Acad. Sci.*, **25**, 577 (1939); R. S. Mulliken, *J. Chem. Phys.*, **7**, 570 (1939), the latest of a series; A. L. Sklar, *ibid.*, **7**, 984 (1939). G. N. Lewis and M. Calvin [*Chem. Rev.*, **25**, 273 (1939)] give a comprehensive survey of the data and the theories of the color of organic compounds, and advance a theory of color based on empirical and quantum considerations.

(46) A. L. Sklar, ref. 45.

(47) R. S. Mulliken, *J. Chem. Phys.*, **7**, 123 (1939).

(48) L. Michaelis, *Chem. Rev.*, **16**, 243 (1935).

(49) L. Pauling, ref. 34, p. 259.

(50) E. B. Wilson, *THIS JOURNAL*, **56**, 747 (1934).

(51) M. W. Perrin and E. G. Williams, *Proc. Roy. Soc. (London)*, **A159**, 162 (1937).

paralleled by the effects of pressure on the absorption of light by similar solutions.

Summary

We have found that the absorption of visible light by solutions of aromatic amines in nitro or nitroso compounds is pushed very significantly toward the longer wave lengths when the hydrostatic pressure over the solutions is raised at constant temperature, and also when the temperature is raised at constant volume. The absorption of light by these solutions may increase, decrease or remain constant as the temperature is raised at constant pressure. These results have been correlated into a consistent theory which avoids any assumption of the formation of colored compounds by the hypothesis that the colors of the solutions arise from the mutual polarizations of the molecules when appropriate groups are in

close proximity, account being taken of the influence of pressure and temperature on the collision frequency, the distance of closest approach and the effect of short-range attractions between groups.

We have also shown from orienting experiments that substituents in the nitro compound and in the amine influence the absorption of light by the solutions in a way that parallels closely their effects on the reducibility of the NO₂ or NO group on the one hand, and the electron mobility in the amino compound on the other. It is suggested that the polarizations which give rise to the colors of the solutions may be regarded as primary steps in possible reactions, such as oxidation and reduction, involving transfer of electrons from the aromatic amine or hydrocarbon to the oxygen of the nitro or nitroso group.

WASHINGTON, D. C.

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[CONTRIBUTION FROM THE MULTIPLE FELLOWSHIP OF THE COTTON RESEARCH FOUNDATION, MELLON INSTITUTE]

Composition of Cottonseeds. III. Solubility of Proteins in Alkaline Solutions of Neutral Salts¹

By H. S. OLCOTT AND T. D. FONTAINE

Oil-free cottonseed meats contain approximately 8.5% nitrogen (50–55% protein). Water will extract 25–30% of the nitrogen compounds. Dilute solutions of neutral salts extract 75–85% of the total. The materials not dissolved by water but extracted by salt are the cottonseed globulins. Osborne and Vorhees² and Jones and Csonka³ have recorded some of the properties of these proteins. Their extraction by salt and alkaline solutions has been described more recently.⁴

Methods were sought for isolating the globulin fraction on a large scale. Extraction with salt solutions was efficient but means of recovery therefrom did not appear to be practical. Dilution required relatively large amounts of water, and precipitation with acid, though easily effected, yielded an insoluble product. The cottonseed globulins, like several other vegetable globulins, are very readily denatured in acid solution.

Extraction with alkali at pH 10.0 and precipita-

tion by the addition of acid to the isoelectric point (pH 6.5–7.0) was found to be a satisfactory procedure but suffered from the disadvantage that cottonseed pigments are easily oxidized in alkaline solution, yielding brown products which affect the color of the protein.

A consideration of the theories of protein solubility suggested that the globulin might be extracted at lower pH levels in the presence of low concentrations of salt; that is, that the two methods, salt peptization and alkali solution, would be mutually additive. Experimentally, however, it was found that the opposite was true; in the presence of low concentrations of salt, the solubility of the protein at a pH below the maximum for extraction was actually depressed. The phenomena observed were of sufficient interest to invite further investigation.

Experimental

Data were obtained in the following manner. Ether-extracted cottonseed meats^{4,5} were used. A sample was

(1) Presented in part before the Division of Biological Chemistry of the American Chemical Society at Boston, Mass., Sept. 11–15, 1939.

(2) Osborne and Vorhees, *THIS JOURNAL*, **16**, 778 (1894).

(3) Jones and Csonka, *J. Biol. Chem.*, **64**, 673 (1925).

(4) Olcott and Fontaine, *THIS JOURNAL*, **61**, 2037 (1939).

(5) The amount of nitrogen extracted under a given set of conditions varied for different preparations of cottonseed meal. With the two meals used in the present investigation, alkali at pH 9.0 extracted 83% of the nitrogen from one, 77% from the other.