poured onto 50 g. of ice. The precipitated product was collected by filtration and recrystallized from Barnsdall-6 or ethanol.

Procedure for the Preparation of 2-(3-Diethylaminopropylamino)-quinoxalines.—The 2-chloroquinoxaline (2–3 g.) was heated with 2 equivalents of 3-diethylaminopropylamine on the steam-bath for three hours. The reaction mixture was treated with 20 ml. of 2 *N* hydrochloric acid and filtered. The filtrate was cooled in an ice-bath and made basic by the slow addition of aqueous 20% sodium hydroxide. The oil which separated was extracted with 50 ml. of ether. The ether solution was washed with five 10-ml. portions of water, dried with magnesium sulfate and the ether evaporated. The oil thus obtained was treated with slightly more than one equivalent of methyl iodide in the cold, and warmed carefully on the steam-bath if no reaction occurred initially. The methyl iodide derivatives were recrystallized from absolute ethanol.

Procedure for the Preparation of 2-(2-Dimethylaminoethoxy)-quinoxalines.—The 2-chloroquinoxaline (0.0075 mole) was added to 10 ml. of 2-dimethylaminoethanol containing a slight excess of sodium. The mixture was allowed to stand twenty-four hours at room temperature and then heated one hour on the steam-bath. The reaction mixture was poured into 50 ml. of ice-water. 2-(2-Dimethylaminoethoxy)-7-chloroquinoxaline separated as a solid and was collected by filtration and recrystallized from Barnsdall-6. 2-(2-Dimethylaminoethoxy)-7-methoxyquinoxaline was obtained as an oil and was isolated in the same manner as described in the previous procedure.

2-(2-Diethylaminoethyl)-quinoxaline (IX).—2-Methylquinoxaline was prepared from *o*-phenylenediamine and isonitrosoacetone[7] by the procedure of Borsche and Doeller.[8] 2-Methylquinoxaline (7.2 g.) was added to 7.7 g. of diethylamine hydrochloride and 10 ml. of formalin. The mixture was refluxed and stirred twenty hours. It was diluted with 50 ml. of water, treated with charcoal and filtered. The filtrate was extracted with two 50-ml. portions of ether. The aqueous layer was made basic by the addition of 50–60 ml. of aqueous 10% sodium hydroxide. The mixture was extracted with three 50-ml. portions of ether, the ether solution dried with magnesium sulfate, treated with charcoal, filtered and the ether evaporated. The residue was distilled at reduced pressure. A yellow oil was obtained, distilling at 168–172° (9 mm.). The yield was 3.6 g. (31%). For analysis, a monopicrate was prepared in ethanol. After recrystallization from ethanol, bright yellow needles were obtained melting at 141–143°.

Anal. Calcd. for $C_{14}H_{19}N_3 \cdot C_6H_3O_7N_3$: C, 52.40; H. 4.84. Found: C, 52.69; H, 4.88.

Summary

1. The synthesis of various 2-piperidino, 2-(3-diethylaminopropylamino)- and 2-(2-dimethylaminoethoxy)-quinoxalines, as well as the intermediates used in preparing these compounds, has been described.

2. 2-(2-Diethylaminoethyl)-quinoxaline has been prepared by the utilization of 2-methylquinoxaline in a Mannich reaction.

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[7] Freon, *Ann. chim.*, **11**, 460 (1939).

[8] Borsche and Doeller, *Ann.*, **537**, 42 (1938).

LINCOLN, NEBRASKA RECEIVED JANUARY 16, 1950

[CONTRIBUTION FROM THE PHILADELPHIA QUARTZ COMPANY]

Spectral Changes of Pinacyanol Chloride in Sodium Silicate–Salt Solutions

BY R. C. MERRILL AND R. W. SPENCER

Pinacyanol chloride is a cationic dye which is blue in water but becomes purple in the presence of colloidal electrolytes such as the siliceous soluble silicates.[1,2] This change in the color of an adsorbed dye depending on the nature of the stainable substrate is the phenomenon which P. Ehrlich called metachromasy. It is attributable to interaction, probably involving both electrostatic, van der Waals, and hydrogen bonding forces of a dimeric or polymeric form of the dye with oppositely charged colloidal electrolyte micelles.[2] Dyes which show metachromasy disobey the usual form of Beers law for a single absorbing species in aqueous solution, due to the formation of molecular aggregates of the dye ion with increasing concentration.[3] The characteristic absorption bands of such dyes are frequently attributed to monomeric, dimeric and polymeric forms,[4,5,6,7,8] and regarded as characteristic of these species. However, these same absorption bands are generally apparent in organic solvents where there is no evidence of dimerization or polymerization, so that the bands generally attributed to dimers or polymers may be due to vibrationally coupled transitions proper to the monomeric ions which are, however, enhanced in the dimer or trimer.[9]

In a previous publication[2] we have shown that the addition of sodium chloride, sulfate or trisodium phosphate decreases the effect of a sodium silicate ($Na_2O \cdot 3.3SiO_2$) on the absorption spectra of pinacyanol chloride, and that the effects of these salts are different at the same ionic strength. This paper reports the effects of fourteen electrolytes, three of which form micelles in aqueous solution, on the absorption spectra of 1×10^{-5} *M* pinacyanol chloride in water and in a 0.01 *M* solution of the sodium silicate with a silica to alkali (Na_2O) molecular ratio of 3.3.

Experimental

The sodium silicate used was the commercially

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[1] R. C. Merrill, R. W. Spencer and R. Getty, THIS JOURNAL, **70**, 2460 (1948).

[2] R. C. Merrill and R. W. Spencer, *ibid.*, **70**, 3683 (1948).

[3] L. Michaelis and S. Granick, *ibid.*, **67**, 1212 (1945).

[4] G. Scheibe, *Kolloid Z.*, **82**, 1 (1938).

[5] W. L. Lewschin, *Acta Physicochem. U. S. S. R.*, **1**, 685 (1934).

[6] S. M. Solov'ev, *J. Gen. Chem.* (*U. S. S. R.*), **16**, 1405 (1946); *C. A.*, **41**, 4716 (1947).

[7] H. O. Dickinson, *Trans. Faraday Soc.*, **43**, 486 (1947).

[8] E. Rabinowitch and L. R. Epstein, THIS JOURNAL, **63**, 69 (1941).

[9] S. E. Sheppard and A. L. Geddes, *ibid.*, **66**, 2003 (1944).

available clarified "E" silicate solution of the Philadelphia Quartz Co. which contains 8.72% Na_2O (determined by titration with standard acid) and 28.13% SiO_2 (determined gravimetrically). The inorganic salts were J. T. Baker reagent grade chemicals. Cetyltrimethylammonium bromide, cetyldimethylethylammonium bromide, and pinacyanol chloride were obtained from the Eastman Kodak Co. Sodium lauryl sulfate was purchased from the Eimer and Amend Co.

Absorption curves were obtained at room temperature (~25°) with a General Electric Co. recording spectrophotometer. Molar extinction coefficients, ϵ_m, were calculated from the equation, $\epsilon_m = (1/cd) \log_{10} I_0/I$ where C is the molar concentration of the dye (1×10^{-5} *M*), *d* the width of the cell (1.00 cm.), and I_0 and I the intensity of the incident and transmitted light. Stock solutions of the dye, and salt or salt-silicate solutions were mixed immediately before the transmission curve was determined.

Results

Figure 1 shows the absorption spectra of 1.0×10^{-5} *M* pinacyanol chloride in water and in 0.1 and 0.5 *M* sodium chloride. The molar extinction coefficient of the α band of the dye at 600 mμ is reduced 30% and that of the β band at 548 mμ about 25% by the addition of 0.1 *M* salt. In 0.5 *M* sodium chloride the intensity of the α band is reduced 39%, that of the β band 56%, and a new γ band appears at 474 mμ as well as a "shoulder" at around 640 mμ indicating the presence of an α' band.

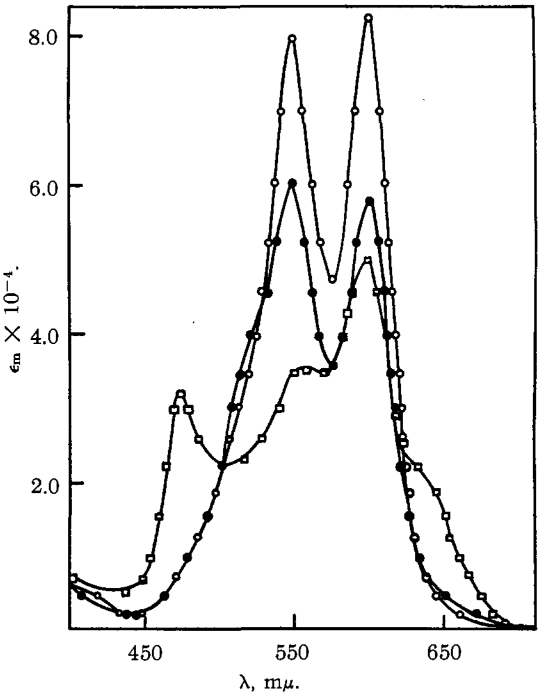

Fig. 1.—Molar extinction coefficients of 1.0×10^{-5} *M* pinacyanol chloride in water ○; 0.10 *M* NaCl ●; and 0.50 *M* NaCl □.

The absorption spectrum of 1.0×10^{-5} *M* pinacyanol chloride in 0.01 *M* $Na_2O{\cdot}3.3SiO_2$ (Fig. 2) shows a well-defined γ band at 499 mμ with but an indication of the α band. The addition of 0.1 *M* sodium chloride decreases the intensity of the γ band and both α and β bands become apparent; the addition of 0.5 *M* salt produces a similar but larger effect. The effect of 0.75 *M* sodium chloride could not be studied because the dye and silicate were not completely soluble in the salt solution.

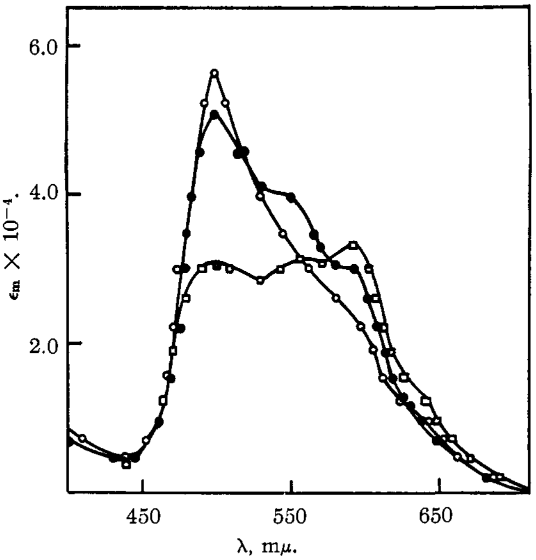

Fig. 2.—Molar extinction coefficients of 1.0×10^{-5} *M* pinacyanol chloride in 0.010 *M* $Na_2O{\cdot}3.3SiO_2$ ○; 0.010 *M* $Na_2O{\cdot}3.3SiO_2$ + 0.10 *M* NaCl ●; 0.010 *M* $Na_2O{\cdot}3.3SiO_2$ + 0.50 *M* NaCl □.

The effects of 0.1 *M* solutions of seven additional sodium salts with different anions on the absorption spectra of 1×10^{-5} *M* pinacyanol chloride in water and in 0.01 *M* $Na_2O{\cdot}3.3SiO_2$ are shown in Figs. 3–6. The absorption spectra vary widely, even in the absence of the silicate, and the effect on each absorption band differs somewhat. The effects of the pyrophosphate, carbonate, bromide and iodide are particularly large and varied. Each produces a well defined γ band whose wave length at the band maximum varies from 478 to 498 mμ and the molar extinction coefficient from 26,000 to 43,000.

The effect of the 0.1 *M* sodium salts on the absorption spectra of the dye in 0.010 *M* $Na_2O{\cdot}3.3SiO_2$ solutions in general increased in the order, chloride, fluoride, iodide, sulfate, carbonate, bromide, phosphate, and pyrophosphate although the exact order depends on the particular band or method of making comparisons. This order of effectiveness in changing the spectra is different from that obtained for the dye in water. Added

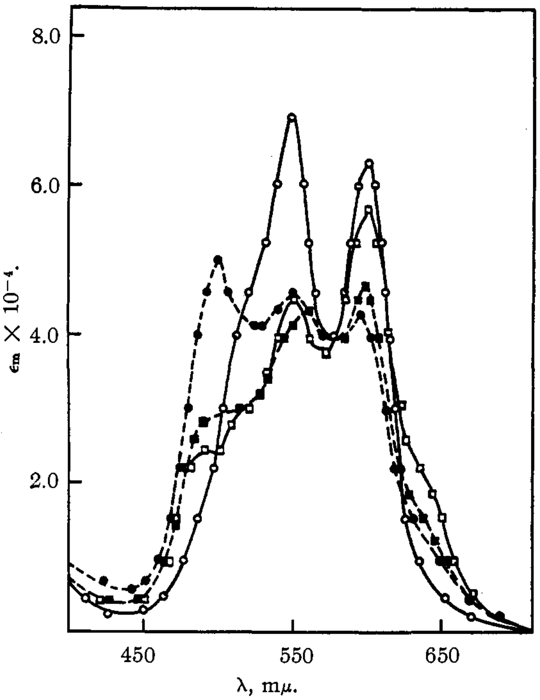

Fig. 3.—Molar extinction coefficients of 1.0 × 10^{-5} *M* pinacyanol chloride in 0.10 *M* Na_2SO_4 ○—○; 0.10 *M* Na_2SO_4 + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ●---●; 0.10 *M* Na_3PO_4 □—□, and 0.10 *M* Na_3PO_4 + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ■---■.

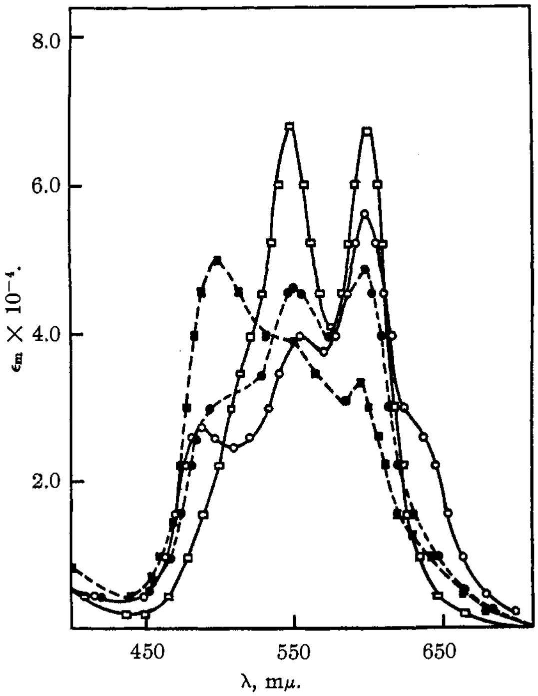

Fig. 4.—Molar extinction coefficients of 1.0 × 10^{-5} *M* pinacyanol chloride in 0.10 *M* $Na_4P_2O_7$ ○—○; 0.10 *M* $Na_4P_2O_7$ + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ●---●; 0.10 *M* NaF □—□, and 0.10 *M* NaF + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ■---■.

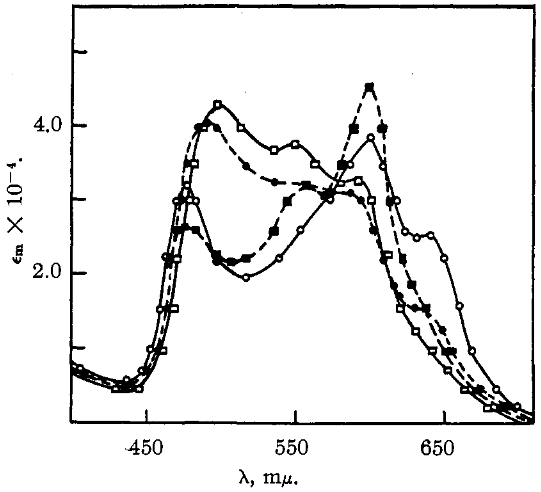

Fig. 5.—Molar extinction coefficients of 1.0 × 10^{-5} *M* pinacyanol chloride in 0.10 *M* NaI ○—○; 0.10 *M* NaI + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ●---●; 0.10 *M* NaBr □—□, and 0.10 *M* NaBr + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ■---■.

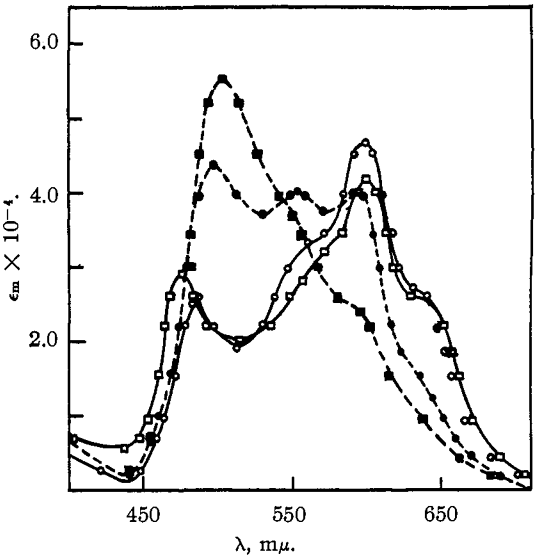

Fig. 6.—Molar extinction coefficients of 1.0 × 10^{-5} *M* pinacyanol chloride in 0.10 *M* Na_2CO_3 ○—○; 0.10 *M* Na_2CO_3 + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ●---●; 0.025 *M* $(C_4H_9)_4NI$ □—□; 0.025 *M* $(C_4H_9)_4NI$ + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ■---■.

salt decreased the effect of the silicate on the absorption spectrum of the dye, although the type and extent of the change varied greatly.

There is little difference in the spectra of 1.0 × 10^{-5} *M* pinacyanol chloride in 0.1 *M* lithium, sodium, or potassium chloride solutions (*e. g.*, *cf.* Figs. 1 and 7) but definite differences in the effect of the same concentration of these salts on the dye's spectrum in 0.01 *M* $Na_2O \cdot 3.3SiO_2$ (Figs. 2 and 7). Their effect in the silicate solution increases in the order sodium, lithium and potassium chloride. The influence of bivalent cations

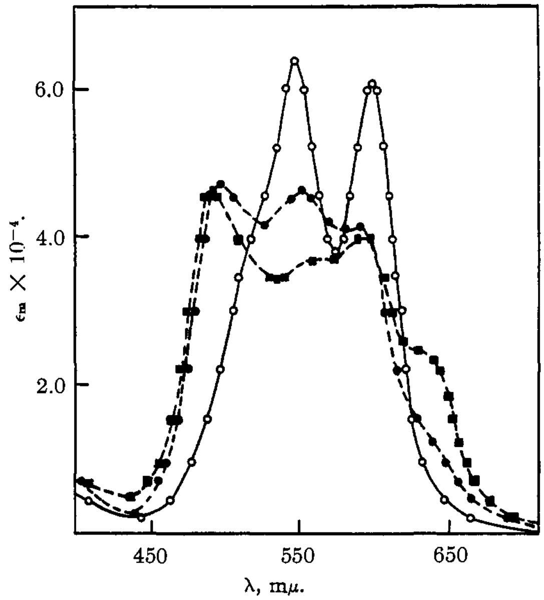

Fig. 7.—Molar extinction coefficients of 1.0×10^{-5} *M* pinacyanol chloride in 0.10 *M* KCl ○—○; 0.10 *M* KCl + 0.010 $Na_2O \cdot 3.3SiO_2$ ●---●; 0.10 *M* LiCl + 0.010 *M* $Na_2O \cdot 3.3SiO_2$ ■---■.

such as calcium and magnesium, and the trivalent aluminum ion could not be studied because they formed a precipitate with the silicate at these concentrations.

Absorption spectra of 1×10^{-5} *M* pinacyanol chloride in water and cetyltrimethylammonium bromide (CTAB) solutions are compared in Fig. 8. (The band maxima of the dye in water are just slightly less than those in Fig. 1 because a different stock solution of dye was used with the organic salts.) A 0.05 *M* solution of cetylethyldimethylammonium bromide produced about the same effect as the corresponding concentration of CTAB. The intensity of the α band at 600 mμ is progressively *increased* and the location of the maxima shifted toward longer wave lengths by the addition of the CTAB. The intensity of the β band at 548 mμ is not greatly affected by the CTAB but it also is shifted toward longer wave lengths. The influence of CTAB on the absorption spectra of the dye in 0.01 *M* $Na_2O \cdot 3.3SiO_2$ could not be studied because a precipitate forms when the silicate and quaternary ammonium salt are mixed.

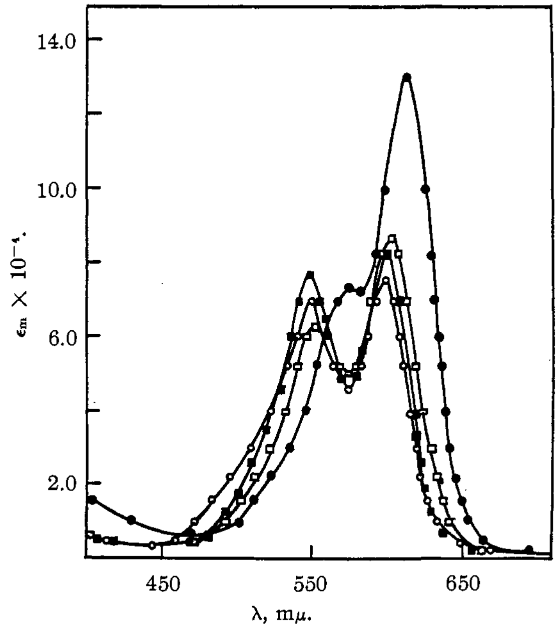

Fig. 8.—Molar extinction coefficients of 1.0×10^{-5} *M* pinacyanol chloride in water ○; 0.0010 *M* cetyltrimethylammonium bromide (CTAB) ■; 0.010 *M* CTAB □---□; and 0.050 *M* CTAB ●.

A 0.001 *M* solution of sodium lauryl sulfate (NaLS) decreases the intensity of the α and β bands of pinacyanol chloride as compared with that in water, shifts these maxima about 10 mμ toward longer wave lengths, and causes the appearance of a γ band at 480 mμ (Fig. 9).

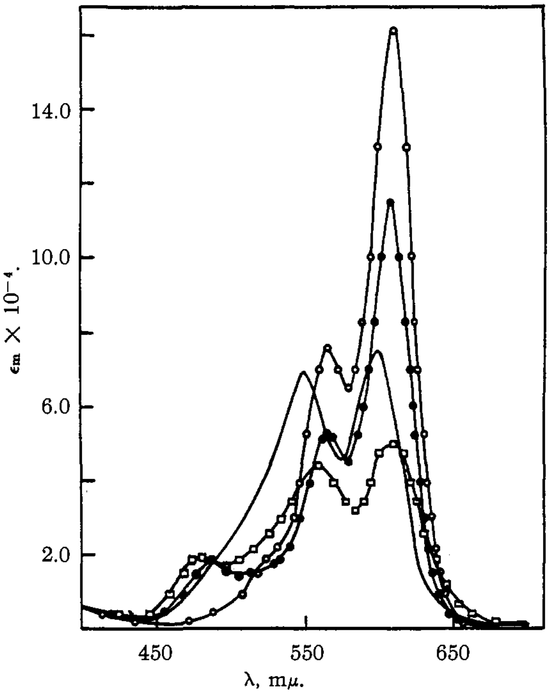

Fig. 9.—Molar extinction coefficients of 1.0×10^{-5} *M* pinacyanol chloride in water — (solid line); 0.001 *M* sodium lauryl sulfate (NaLS) □; 0.01 *M* NaLS ●; 0.05 *M* NaLS ○.

The intensity of the α band is greatly increased in 0.01 *M* NaLS, and in 0.05 *M* NaLS is twice that in water. The intensity of the β band is not so much affected by the higher concentrations but all bands occur at longer wave lengths than in water. Sodium lauryl sulfate also produces large effects on the absorption spectrum of 1.0

$\times 10^{-5}$ *M* pinacyanol chloride in 0.01 *M* $Na_2O \cdot 3.3SiO_2$, even in concentrations as low as 0.001 *M* (Fig. 10). The absorption spectrum of the dye in 0.05 *M* NaLS is practically unaffected by the addition of 0.01 *M* $Na_2O \cdot 3.3SiO_2$ even though this silicate concentration produces large changes in the absence of the organic salt.

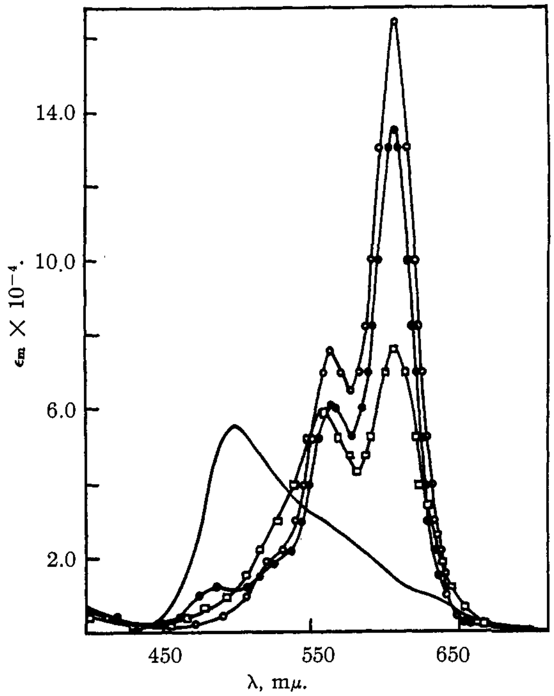

Fig. 10.—Molar extinction coefficients of 1.0×10^{-5} *M* pinacyanol chloride in 0.01 *M* $Na_2O \cdot 3.3SiO_2$ — (solid line); 0.01 *M* $Na_2O \cdot 3.3SiO_2$ + 0.001 *M* NaLS □; 0.01 *M* $Na_2O \cdot 3.3SiO_2$ + 0.01 *M* NaLS ●; 0.01 *M* $Na_2O \cdot 3.3SiO_2$ + 0.05 *M* NaLS ○.

Discussion

The order of increasing effect of the various cations used as chlorides and the anions as their sodium salts on the absorption spectrum of 1.0×10^{-5} *M* pinacyanol chloride in water resembles in general Hofmeister or lyotropic series, although there are differences from the order usually observed perhaps due to specific interactions. The much larger effects of simple ions such as iodide or bromide as compared with metasilicate do not agree with the suggestion[2] that it contained ion aggregates because of its greater effect on the dye than chloride, sulfate, or orthophosphate. The colloidal electrolytes, CTAB and NaLS, greatly increase the intensity of the α band of the dye even though the micelles they form are of opposite charge. Their effect is in contrast to the decreased intensity observed with inorganic or non-micelle forming salts or colloidal electrolytes below the critical concentration for micelle formation.

The differences between the order of increasing effect of the salts on the spectra of pinacyanol chloride in water as compared with that in 0.01 *M* $Na_2O \cdot 3.3SiO_2$ indicates a specific interaction of some of the salts with the silicate. While such interactions with silicates are not unexpected for lithium, sodium, and potassium ions, they are somewhat surprising for anions. Further evidence is needed. Results on the influence of NaLS and $Na_2O \cdot 3.3SiO_2$ mixtures on the spectra of the dye can be interpreted in terms of competition between the two negative micelles for the cationic dye aggregate. The tendency of the organic detergent to combine with the dye is evidently much larger than that of the silicate.

The hypothesis that the α, β and γ bands of pinacyanol chloride are due to monomers, dimers and polymers, respectively, whose concentration is indicated to explain our results. The ratios of the intensities of the α band to that of the β are larger in about half the salt solutions than in water. The contrary should be true if the above hypothesis were correct since salts ordinarily increase the extent of association of large organic ions. Other results not readily explainable on this hypothesis are the inability to deduce equilibrium constants valid over a range of concentrations for the dimerization and polymerization,[3] the similar large effect of positive and negative micelles different from that of ordinary salts, and the specific effects of ions. Salts probably affect the probabilities of transition between the ground and excited states of the molecule directly as well as by influencing the extent of aggregation of the dye cation. Changes in the wave length of the band maxima show that electrolytes, positive and negative detergent micelles, and soluble silicates affect the energies of the ground and excited states of the dye.

Summary

Fourteen electrolytes produced large and different effects on the absorption spectra of 1.0×10^{-5} *M* pinacyanol chloride in water and in 0.01 *M* $Na_2O \cdot 3.3SiO_2$ solution. Changes produced by 0.1 *M* solutions of sodium salts on the spectrum of the dye in water in general increased in the order fluoride, sulfate, chloride, orthophosphate, pyrophosphate, carbonate, bromide and iodide. Added salts decrease the influence of 0.01 *M* $Na_2O \cdot 3.3SiO_2$ on the spectrum of the dye. The order of increasing effect in the silicate was chloride, fluoride, iodide, sulfate, carbonate, bromide, phosphate and pyrophosphate. Lithium, sodium, and potassium chlorides affect the spectrum of the dye in water similarly, but their effects on the dye in silicate differ. The differences in the effect indicate interaction of salts with the sodium silicate.

Cetyltrimethylammonium bromide which forms cationic micelles and sodium lauryl sulfate which forms anionic micelles produced large effects different from those obtained with inorganic or non-micelle forming organic salts. Both types of micelles increased greatly the intensity of the α band of the dye without a large effect on the

intensity of β and γ bands. Sodium silicate and lauryl sulfate micelles compete for dye ions with the organic detergent having the greater tendency to interact with pinacyanol chloride.

The results indicate that salts affect the absorption spectra of the dye directly as well as by influencing aggregation.

PHILADELPHIA 6, PA. RECEIVED SEPTEMBER 1, 1949

[CONTRIBUTION NO. 1353 FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA]

Interatomic Distances and Bond Angles in the Polypeptide Chain of Proteins[1,2]

BY ROBERT B. COREY AND JERRY DONOHUE

In many problems of protein structure, it has long been necessary to assume probable distances between the atoms which make up a polypeptide chain. The magnitude assigned to these interatomic bonds and to the angles which are associated with them, have undergone progressive revisions corresponding to advancing knowledge of the structures of molecules in general. Recent X-ray determinations of the structures of amino acids, peptides, and related compounds now provide direct experimental data regarding the dimensions of the peptide linkage and other atomic groupings characteristic of protein molecules.

Some time ago, data from the structures of diketopiperazine[3] and glycine[4] were used to derive probable dimensions of the polypeptide chain,[5] and more recently the data obtained from an X-ray examination of DL-alanine[6] were incorporated in a similar discussion.[7] No data from a linear peptide were then available, so that bond angles were still in doubt by as much as 5°, and some of the distances required the support of additional data before they could be accepted with complete confidence. In particular, no explanation could be advanced for the short αC–N distance (about 1.43 Å.) found in alanine (about 1.40 Å. in the less precisely determined glycine structure). Further X-ray studies have recently provided satisfactory answers to these questions. They include determinations of the crystal structures of L-threonine[8] and N-acetylglycine[9] and a revision of the parameters of DL-alanine,[10] in all of which the refinement of atomic positions was carried out by means of three-dimensional Fourier syntheses involving the simultaneous use of all intensity data. Also a complete structure determination for crystals of β-glycylglycine[11] now supplies the first direct structural data for a linear peptide.

Practical methods for the rapid calculation of three-dimensional Fourier summations have been devised only within the last few years. The power of this technique is well illustrated by the case of DL-alanine, for which two-dimensional Fourier projections were inadequate to satisfactorily resolve any atom of the structure whereas a three-dimensional Fourier plot calculated from the data originally published completely resolved all C, N and O atoms.[10]

Interatomic distances and bond angles found in these recent structure determinations are compared in Table I. Figure 1 represents the probable dimensions of the fully-extended polypeptide

TABLE I

A COMPARISON OF CERTAIN DIMENSIONS FOUND IN SOME CRYSTALS OF AMINO ACIDS AND PEPTIDES

	L-Threonine[8]	DL-Alanine[10]	N-Acetylglycine[9]	β-Glycylglycine[11]
A. Interatomic Distances, Å.				
Carboxyl C–O	1.24	1.21	1.19	1.21
	1.25	1.27	1.31	1.27
Carboxyl C–αC	1.52	1.54	1.51	1.53
αC–N	1.49	1.50	1.45	1.48
αC–βC	1.54	1.51	....	....
βC–C	1.50	....	....	....
N–Carbonyl C′	....	....	1.32	1.29
Carbonyl C′–O′	....	....	1.24	1.23
Carbonyl C′–αC′	....	....	1.50	1.53
αC′–N′	....	....	....	1.51
B. Bond Angles				
Carboxyl O–C–O	127°	125°	124°	124.5°
Carboxyl O–C–αC	117	121	124	123
	116	113	112	112
Carboxyl C–αC–N	110	108	110	110.5
Carboxyl C–αC–βC	113	111	....	....
N–αC–βC	108	110	....	....
αC–N–Carbonyl C′	....	....	120	122
N–Carbonyl C′–O′	....	....	121	125
N–Carbonyl C′–αC′	....	....	118	121
αC′–Carbonyl C′–O′	....	....	121	121
Carbonyl C′–αC′–N′	....	....	....	110

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[1] Aided by a grant from the National Foundation for Infantile Paralysis.

[2] Presented at the Symposium on the Chemistry of Proteins, held at the One-hundred and Sixteenth meeting of the American Chemical Society in Atlantic City, New Jersey, September 1949, under the auspices of the Division of Biological Chemistry of the American Chemical Society.

[3] R. B. Corey, THIS JOURNAL, **60**, 1598 (1938).

[4] G. Albrecht and R. B. Corey, *ibid.*, **61**, 1087 (1939).

[5] R. B. Corey, *Chem. Revs.*, **26**, 227 (1940).

[6] H. A. Levy and R. B. Corey, THIS JOURNAL, **63**, 2095 (1941).

[7] R. B. Corey, *Adv. in Protein Chem.*, **4**, 385 (1948).

[8] D. P. Shoemaker, J. Donohue, V. Schomaker and R. B. Corey, THIS JOURNAL, **72**, 2328 (1950).

[9] G. B. Carpenter and J. Donohue, *ibid.*, **72**, 2315 (1950).

[10] J. Donohue, *ibid.*, **72**, 949 (1950).

[11] E. W. Hughes and W. J. Moore, *ibid.*, **71**, 2618 (1949).