

(usually less than 10 cm.^{-1}). In several cases other assignments nearly as good as those in Table XVII can be made, but we have not listed them for various reasons, on grounds either of excessive anharmonicity or of expected low intensity or for some reason pertinent to the particular assignment.

The bands above 2000 cm.^{-1} must be overtones

except for the hydrogen and deuterium stretching vibrations, and thus do not concern us as possible fundamentals. In view of the fact that most of them can be explained in a variety of ways, we have not bothered to list the explanations in Table XVII.

CAMBRIDGE, MASS.

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[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF SOUTHERN CALIFORNIA]

Spectral Study of Long Chain Quaternary Ammonium Salts in Brom Phenol Blue Solutions

BY EUGENE L. COLICHMAN¹

The absorption spectra of the acidic and basic forms of brom phenol blue have been determined in the visible and also in the ultraviolet region of the spectra, previously unreported. Changes in these spectra, observed when long chain quaternary ammonium salts are added to the solutions, permit evaluation of the "quaternary-dye" micellar phenomenon.

The reaction between long chain quaternary ammonium salts and brom phenol blue has received considerable attention.²⁻⁷ The influence of colloidal agents on the color of indicators was reported only qualitatively in the first report.² Empirical analytical methods^{3,4,5} for quantitative analyses of surface active agents then followed based on these initial observations. The nature of this reaction has been investigated by conductance⁶ and surface and interfacial tension methods.⁷ These later results indicate ion-pair formation in aqueous solutions followed by micellization. Considerably lower critical micelle concentrations, for the quaternary ammonium salts when in the presence of either acidic or basic forms of brom phenol blue, have been indicated, but quantitative proof has been lacking. The present spectrophotometric investigation was undertaken with this in mind.

The spectral change method of locating critical micelle concentrations, proposed by Corrin, Klevens and Harkins^{8,9} and later studied in detail by two of these investigators,^{10,11} is used in the present study.

Results and Discussion

Spectrophotometric data in the visible and ultraviolet regions of the spectra were obtained with a Beckman quartz spectrophotometer, model DU, using 1-cm. quartz cells. Temperature was $30 \pm 2^\circ$ in all measurements. The quaternary ammonium salts used were described previously.^{6,7,12} Brom phenol blue samples from both National Aniline Co. and Eastman Kodak Co. gave similar results. pH 1.00 solutions were obtained by using the appropriate dilution of hydrochloric acid. The

pH 6.50 solutions were Sorenson's phosphate buffer.¹³ The measurements in the visible region were made according to accepted procedure.

The spectrum of brom phenol blue in the presence of cetyltrimethylammonium bromide was studied, also, in the ultraviolet region. The blank solutions, set automatically at one hundred per cent. transmission, were cetyltrimethylammonium bromide-buffer solutions. Therefore, the spectrum under these conditions is that of the brom phenol blue alone, but influenced by the quaternary ammonium salt present. The effect of the other quaternary ammonium salts could not be investigated, similarly, in this region due to their much higher absorption.

Brode's work^{13,14} and a later investigation¹⁵ prove that two and only two colored forms can be responsible for the tautomeric equilibrium in the pH range investigated due to the sharp isosbestic point exhibited. The yellow or acidic form $\text{C}_6\text{H}_4(\text{SO}_3^-)^+\text{C} \begin{matrix} \text{C}_6\text{H}_4\text{OH} \\ \text{C}_6\text{H}_4\text{O}^- \end{matrix}$, present at pH 1.00 in ordinary aqueous solutions, and the purple or basic form, $\text{C}_6\text{H}_4(\text{SO}_3^-)^+\text{C} \begin{matrix} \text{C}_6\text{H}_4\text{O}^- \\ \text{C}_6\text{H}_4\text{O}^- \end{matrix}$, found at pH 6.50, are symbolized here simply as HD^- and D^- .

The spectral change method⁸⁻¹¹ of locating critical micelle concentrations (C.M.C.) in the brom phenol blue (B.P.B.)-quaternary ammonium salt systems is illustrated in Fig. 1. The abrupt change in the spectrum occurs at the C.M.C. The minimum concentration of quaternary ammonium salt which yields a constant value for the extinction coefficient is defined here as the stable micelle point (S.M.P.). This leveling off point corresponds to complete extraction of the dye into the micelle. These results are presented in Table I. Only cetyltrimethylammonium bromide gave clear solutions at concentrations as low as the C.M.C. values. The C.M.C. values for the other quaternary ammonium salts could not be determined accurately and are not given.

(13) Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Md., 1928.

(14) Brode, *THIS JOURNAL*, **46**, 581 (1924)

(15) Haring and Heller, *ibid.*, **63**, 1024 (1941).

(1) Department of Chemistry, University of Portland, Portland, Oregon.

(2) Hartley, *Trans. Faraday Soc.*, **30**, 444 (1934).

(3) Hartley and Runnicles, *Proc. Roy. Soc. (London)*, **168A**, 420 (1938).

(4) (a) Auerbach, *Anal. Chem.*, **15**, 492 (1943); (b) **16**, 739 (1944).

(5) Colichman, *ibid.*, **19**, 430 (1947).

(6) Colichman, *THIS JOURNAL*, **72**, 1834 (1950).

(7) Colichman, *ibid.*, **73**, 1795 (1951).

(8) Corrin, Klevens and Harkins, *J. Chem. Phys.*, **14**, 216 (1946).

(9) Corrin, Klevens and Harkins, *ibid.*, **14**, 480 (1946).

(10) Corrin and Harkins, *THIS JOURNAL*, **69**, 679 (1947).

(11) Corrin and Harkins, *ibid.*, **69**, 683 (1947).

(12) Colichman, *ibid.*, **72**, 4036 (1950).

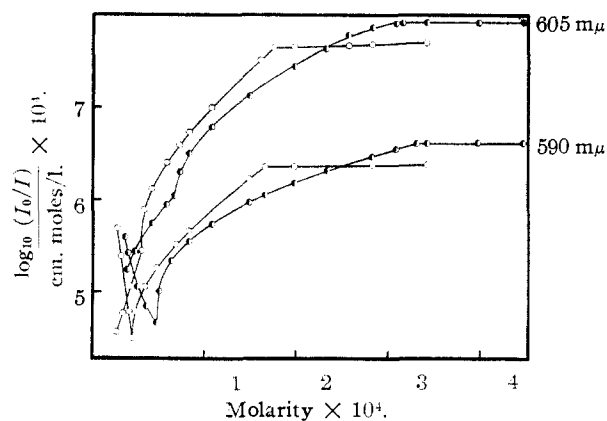
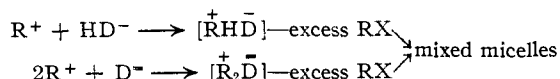


Fig. 1.—Extinction coefficient of B.P.B. at pH 6.50 and at band maxima vs. cetyltrimethylammonium bromide concentration: O, B.P.B. at $3.75 \times 10^{-6} M$; ●, B.P.B. at $7.50 \times 10^{-6} M$.

The C.M.C. and S.M.P. for a given quaternary ammonium salt (see Table I) vary directly in accordance with the change in concentration of brom phenol blue. It is seen, also, that $1.5 \times 10^{-5} M D^-$ has the same C.M.C. and S.M.P. values as $3.0 \times 10^{-5} M HD^-$. The requisite of charge and quantity neutralization, preliminary to micelle formation, is thereby demonstrated. The mixed micelle phenomenon can be represented as



where R^+ is the quaternary ammonium ion.

The spectral absorption curves of both HD^- and D^- in the presence of some of the quaternary ammonium salts are illustrated by the sample curves shown in Figs. 2-5. These curves were chosen to show the effects of quaternary ammonium salts on the absorption spectra of HD^- and D^- . The various quaternary ammonium salts in general have the same effect in displacing the different bands to the same wave lengths, but the extents of absorption differ even in the completely displaced curves (the S.M.P. values and above). HD^- in the ab-

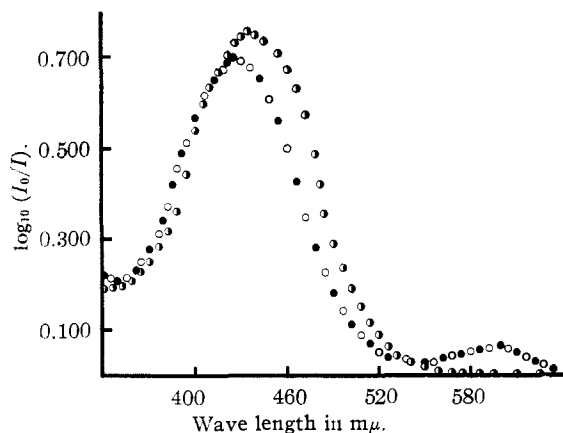


Fig. 2.—Effect of cetyltrimethylammonium bromide on spectral absorption of $3.00 \times 10^{-5} M$ B.P.B. at pH 1.00: O, dye alone; ●, at C.M.C. value; ●, at S.M.P. value and above.

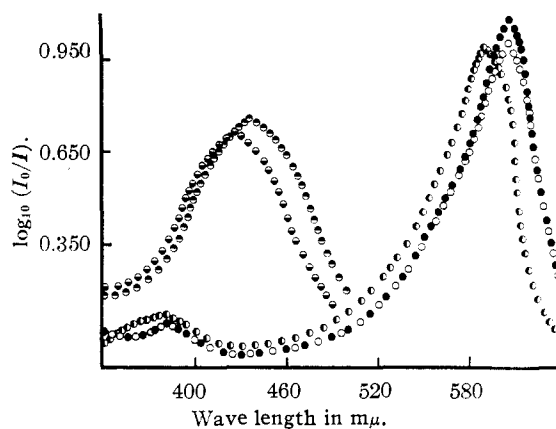


Fig. 3.—Effect of Hyamine 1622 and octadecyldimethylbenzylammonium chloride on spectral absorption of B.P.B.: ●, $1.5 \times 10^{-5} M$ B.P.B. alone at pH 6.50; O, same at $6.3 \times 10^{-4} M$ Hyamine 1622; ●, same at $11.0 \times 10^{-4} M$ Hyamine 1622 (the S.M.P. value) and above; ●, $3.0 \times 10^{-5} M$ B.P.B. alone at pH 1.00; ●, same at $6.0 \times 10^{-4} M$ octadecyldimethylbenzylammonium chloride (the S.M.P. value) and above.

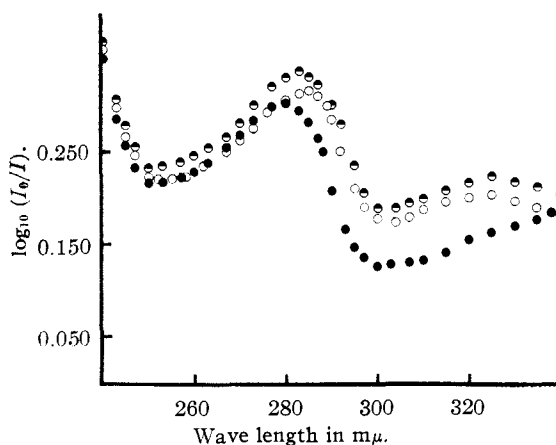


Fig. 4.—Effect of cetyltrimethylammonium bromide on spectral absorption of $3.00 \times 10^{-5} M$ B.P.B. at pH 1.00: ●, dye alone; ●, at C.M.C. value; O, at S.M.P. value and above.

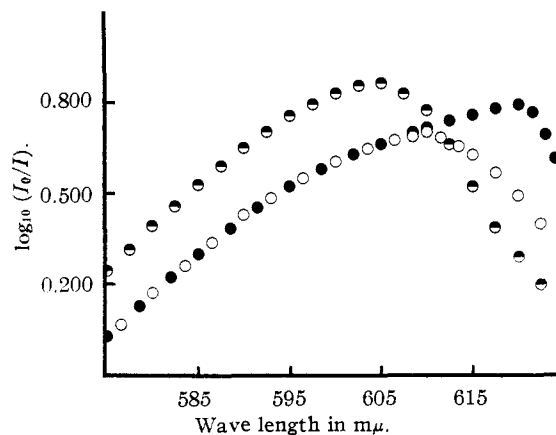


Fig. 5.—Effect of cetyltrimethylammonium bromide on spectral absorption of $1.5 \times 10^{-5} M$ B.P.B. at pH 6.50: ●, dye alone; O, at $3.00 \times 10^{-5} M$ cetyltrimethylammonium bromide; ●, at S.M.P. value and above.

sence of quaternary ammonium salts has two bands, one in the visible at 435 $m\mu$, and the other in the ultraviolet region at 280 $m\mu$. These two bands are displaced in the presence of quaternary ammonium salts to 425 and 285 $m\mu$ (see Figs. 2, 3 and 4). D^- in the absence of quaternary ammonium salts has three bands, two in the visible at 590 and 380 $m\mu$, and one in the ultraviolet region at 310 $m\mu$. Two of these bands are displaced in the presence of quaternary ammonium salts to 605 and 325 $m\mu$ (see Figs. 3 and 5). The 380 $m\mu$ band is not affected. This band cannot be attributed to any action by the phosphate buffer since 3.0 M sodium hydroxide solutions of D^- showed the same behavior (data not cited). It cannot be attributed to impurities since it is not found when brom phenol blue is in the form of HD^- . Figures 2 and 4 show that HD^- in the presence of quaternary ammonium salts exhibits two small additional bands, one at 605 $m\mu$ and the other at 325 $m\mu$. These are recognized as the two main bands belonging to D^- in the micelle form. Thus HD^- is partially converted into D^- as follows: $HD^- + R^+ \rightarrow D^- + R^+ + H^+$. Apparently, this conversion is appreciable even at pH 1.00 due to the stronger micelle forming ability of D^- . HD^- in the presence of quaternary ammonium salts changes color from yellow to a pale green. This is probably due to a blending of the yellow and blue forms (*i.e.*, the color of HD^- and D^- in their micelles). Evidence for the strong micelle forming ability of D^- is shown by the tremendous resistance to alkaline decomposition¹⁶ that D^- solutions show toward even 3.0 M sodium hydroxide solutions when quaternary ammonium salts are present at concentrations corresponding to their S.M.P. values.

Previously,⁶ it was shown that $2R^+ + 1D^-$ formed non-conducting ion pairs in aqueous solution. The compound R_2D is at least partially in the form of micelles even below the C.M.C. (*i.e.*, before the excess RX is added). Under the conditions where only $2R^+$ are present to $1D^-$, the spectral absorption and band maximum of D^- are observed to have been altered considerably, and can only be accounted for by micelle formation. By working in solvents of low dielectric constant, Lewis, *et al.*,¹⁷ show that the ion-pair phenomenon in itself does not alter the spectral absorption of associated ions; higher ordered ion clusters are necessary before significant shifts are observed.

The aqueous and displaced or micellar bands of brom phenol blue are summarized in Table II. All the bands are recognized as fundamental A bands due to electronic oscillations within the molecules. B bands^{17b} of partial oscillation are not found here since all bands are well separated and defined. The widely separated bands in the case of HD^- are easily identified as fundamental A bands of the first order, that is, x (435 and 425 $m\mu$) and y (280 and 285 $m\mu$) bands according to the concepts developed by Lewis, *et al.* The x band in the case

of D^- is seen to be at 590 $m\mu$ and when in its micellar form at 605 $m\mu$. No sudden enhancement with change in concentration characteristic of aggregation state differences,¹⁸ is found for any of the three D^- bands. Therefore, none of these bands is a z band. It has been found^{18b} that when x bands appear in the red or infrared, as is the case here, fundamental bands of the second order are usually observed. On these bases, either the 380 $m\mu$ band or the 310 $m\mu$ band is an x' band, and the other one is then a y band. The intensities of these two bands are not far different, and thus the effect of the quaternary ammonium salts on their absorption spectra should be seen with approximately equal precision. It is, therefore, significant that the D^- does not have its 380 $m\mu$ band altered on forming micelles while the 310 $m\mu$ band is shifted to 325 $m\mu$ when in the micelle. Furthermore, the 605 and 325 $m\mu$ bands appear in the spectrum of HD^- in the micellar form (see discussion above). A consideration of the above facts might lead to the conclusion that the 310-325 $m\mu$ band is a y band, and the unaltered 380 $m\mu$ band is a second order or x' band. However, this designation is contrary to the order usually found by Branch, *et al.*¹⁹ The band of the shortest wave length has always been termed the x' band when three absorption peaks are involved. It thus seems unwise to designate the 380 $m\mu$ band

TABLE I

TABULATED RESULTS OF CRITICAL MICELLE CONCENTRATIONS (C.M.C.) AND STABLE MICELLE POINTS (S.M.P.) OF THE VARIOUS QUATERNARY SALTS IN BROM PHENOL BLUE (B.P.B.) AS DETERMINED FROM ABSORPTION SPECTRA

Quaternary salt	Total concn. of B.P.B., $M \times 10^5$	pH	Wave length observed, $m\mu$	C.M.C., $M \times 10^4$	S.M.P., $M \times 10^4$
Cetyltri-methylammonium bromide	3.00	1.00	280	1.2	6.8
	3.00	1.00	310	1.2	6.8
	3.00	1.00	325	1.2	6.8
	3.00	1.00	425	1.1	7.0
	3.00	1.00	435	1.1	7.0
	1.50	1.00	425	0.6	4.0
	1.50	1.00	435	0.6	4.0
	1.50	6.50	310	1.1	6.0
	1.50	6.50	325	1.1	6.0
	0.375	6.50	590	0.25	1.7
Octadecyldimethylbenzylammonium chloride	0.375	6.50	605	.35	1.8
	0.750	6.50	590	.50	3.3
	0.750	6.50	605	.68	3.2
	1.50	6.50	590	1.2	6.5
	1.50	6.50	605	1.2	6.5
	3.00	1.00	425	..	6.0
	3.00	1.00	435	..	6.0
	1.50	6.50	590	..	5.2
	1.50	6.50	605	..	5.2
	Laurylpyridinium chloride	3.00	1.00	425	..
3.00		1.00	435	..	20.0
1.50		6.50	590	..	21.0
1.50		6.50	605	..	21.0
Hyamine 1622	3.00	1.00	425	..	10.0
	3.00	1.00	435	..	10.0
	1.50	6.50	590	..	11.0
	1.50	6.50	605	..	11.0

(16) Sager, Maryott and Schooley, *THIS JOURNAL*, **70**, 732 (1948), studied the effect of substituents on the ordinarily pronounced rate of fading of sulfonphthalein indicators in alkaline solutions. Reference is given here to the kinetic studies of such reactions by LaMer and Kilpatrick.

(17) (a) Lewis, Goldschmid, Magel and Bigeleisen, *ibid.*, **65**, 1151 (1943); (b) Lewis, Magel and Lipkin, *ibid.*, **64**, 1774 (1942).

(18) (a) Lewis and Calvin, *Chem. Revs.*, **25**, 273 (1939); (b) Lewis and Bigeleisen, *THIS JOURNAL*, **65**, 2107 (1943).

(19) Tolbert, Branch and Berlenbach, *ibid.*, **67**, 887 (1945).

here as an α' band without independent proof such as might be obtained by extremely difficult polarization experiments.^{18b} Therefore, these two bands are identified in Table II in general terms as merely λ_2 and λ_3 .

When both cationic and anionic species in an ionizable compound are interfacial modifying,⁷ lower²⁰ C.M.C.'s, as found here, are to be expected. Gegenion concentrations in the pH 1.00 and 6.50 buffer solutions are not far different, and thus the C.M.C.'s in these two buffers should be nearly comparable¹¹ as seems to be the case here.

(20) Scott, Tartar and Lingafelter, *THIS JOURNAL*, **65**, 698 (1943).

TABLE II
INFLUENCE OF LONG CHAIN QUATERNARY AMMONIUM SALTS ON COLOR AND ABSORPTION MAXIMA OF B.P.B.

Species	Band	Aqueous maxima, m μ	Micellar maxima, m μ
HD ⁻	λ_x	435 (yellow) ^a	425 (yellow-green)
HD ⁻	λ_y	280	285
D ⁼	λ_x	590 ^a & 592 ^b (purple)	605 (blue)
D ⁼	λ_2	380	380
D ⁼	λ_3	310	325

^a Results here check with value reported by Haring and Heller, reference 15. ^b Value reported previously by Brode, reference 14.

LOS ANGELES, CALIF.

RECEIVED AUGUST 24, 1950

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, INSTITUTE OF TECHNOLOGY, UNIVERSITY OF MINNESOTA]

A Spectrophotometric Study of the Molisch Phase Test of Chlorophyll*

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The visible absorption spectra of the intermediate compounds, responsible for the "brown" phase of the Molisch phase test of chlorophylls a and b, have been determined. The compound derived from chlorophyll a has a broad region of absorption starting at about 5500 Å. and extending at least to 3800 Å. In addition there is a minor maximum centered at 6750 Å. The compound corresponding to chlorophyll b has its major maximum at 5300 Å. This maximum has a distinct shoulder at about 5000 Å. There appears to be a region of general absorption starting about 4600 and extending at least to 3800 Å. The formation of the intermediate occurs within a second or less after the chlorophyll and base are mixed. It is postulated that the formation of the brown color is due to the neutralization of the proton on carbon 10 of ring V of the chlorophyll molecule.

It has been known for many years that an ethereal solution of freshly prepared chlorophyll exhibits a transitory brownish color when it is treated with a strong base (such as concentrated alcoholic potash). Conant and his co-workers¹ have demonstrated that the green product of this reaction is a chlorin formed by the oxidative hydrolysis of ring V. Measurements of the absorption spectrum of the intermediate were made in the hope that this might help in the identification of the reaction intermediate.

Experimental Materials and Methods

Materials.—The chlorophylls a and b were prepared and purified by a modification of the method of Zscheile and Comar.^{2a,b} The samples used were free from spectrophotometrically detectable quantities of pheophytin³ and allomierized chlorophyll.⁴

The solvents were purified anhydrous samples. The trimethylbenzyl ammonium hydroxide was prepared by prolonged drying of a commercial sample (Triton B) over phosphorus pentoxide in a vacuum desiccator.

(*) This work was made possible by the joint support of the Office of Naval Research (Contract N6ori-212, T. O. I. NR 059 028) and the Graduate School of the University of Minnesota, to whom the authors are indebted.

(1) J. B. Conant, S. E. Kamerling and C. C. Steele, *THIS JOURNAL*, **53**, 1615 (1931); J. B. Conant, J. F. Hyde, W. W. Moyer and E. M. Dietz, *ibid.*, **53**, 359 (1931); Steele, *ibid.*, **53**, 1371 (1931).

(2) (a) R. Livingston, D. Sickle and A. Uchiyama, *J. Phys. Colloid Chem.*, **51**, 775 (1947); (b) F. Zscheile and C. Comar, *Botan. Gaz.*, **102**, 463 (1941).

(3) C. Comer and F. Zscheile, *Plant. Physiol.*, **17**, 198 (1942).

(4) Unpublished work, W. F. Watson.

Apparatus.—The absorption measurements were made with a Beckmann spectrophotometer. An ethereal chlorophyll solution was mixed continuously with a relatively concentrated solution of a base, and the resulting solution flowed through the absorption cell (see Fig. 1). The cell proper (a) was made from 10 mm. precision square Pyrex tubing. The flow was controlled by applying gas pressure to the reservoirs containing the stock solutions. It was limited by the capillaries (b,b'). The small bulbs (c,c') were introduced to ensure complete mixing of the solutions before they reached the cell (a). The efficiency of mixing was checked by direct observation using a colored and a colorless solution under the conditions of the experiments. Connections between the solution reservoirs and the inlet tubes (d,d') were of glass, with the exception of short lengths of flexible tygon tubing. The flow rate and the composition of the mixture were determined directly during each measurement.

The stock solutions were prepared on a vacuum line. Known quantities of chlorophyll in ether and of base in methanol were added to separate reservoirs. The solutions were frozen and the reservoirs evacuated. The solvents were degassed by boiling at low temperature, and the appropriate volumes were distilled under vacuum into the reservoirs. Purified nitrogen was then admitted to the reservoirs, their stopcocks were closed, and they were transferred to the flow system. This system was flushed out with commercial "oxygen-free" nitrogen.

Experimental Results

Typical measured absorption spectra of the flowing mixtures are represented by the dotted curves of Figs. 2 and 3 for chlorophyll b and chlorophyll a, respectively.⁵ The ordinates are the optical densities of solutions when the total chlorophyll or chlorophyll derivative concentration is approximately 8.3×10^{-6} m. In addition to the results for the flowing mixtures, absorption curves are plotted for the chlorophylls and for the relatively stable products. Inspection of the curves for the flowing mixtures, and of other similar data

(5) These curves have been corrected for a slight absorption in the violet due to Triton B.

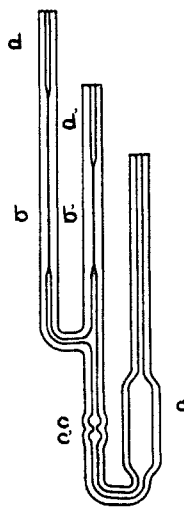


Fig. 1.—Absorption cell.