Solubility and Properties of Two Crystalline Phases of Methylene Blue

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Microscopic and spectrophotometric techniques have been used to determine the solubility of methylene blue as function of temperature in aqueous solutions containing various concentrations of sodium chloride. The solubility data, combined with microscopic and x-ray diffraction studies, indicate that a phase change accompanied by a large enthalpy change occurs in the temperature range of 29° to 33° C. In addition, the optical crystallographic properties and the x-ray powder diffraction patterns of the two phases of crystalline methylene blue have been determined.

THE COMPOSITION and chemical properties of crystalline methylene blue have received considerable attention in the literature (*1*, *4*, *7-9*); however, they are not completely understood. The present investigation was undertaken to determine the solubility of methylene blue in aqueous solutions and to define the properties of two crystalline phases of methylene blue.

EQUIPMENT AND METHOD

Microscopic Examinations. The initial portion of the present investigation was concerned with a microscopic examination of methylene blue crystallized from a saturated aqueous solution. The Kofler micro hot stage and Thomas thermoelectric cold stage were employed in these studies. Samples were prepared which contained zinc-free methylene blue in amounts from 0.01 to 2.69 grams per 100 cc. and sodium chloride concentrations in the range of 0 to 7 weight %. These aqueous solutions were heated to 40° to 60° C. in a constant temperature bath to ensure complete solution of the dye.

Microscope slides were prepared to receive these samples by inscribing a square with a wax pencil. In this manner, an elevated "wall" of wax was created on the surface of the slide to form the boundaries of the liquid cell. One drop of methylene blue was quickly withdrawn by an eyedropper from a heated solution, placed in the cell and covered with a square cover glass 22 mm. on a side (slightly larger than the circumscribed area). The wax formed a barrier layer, inhibiting "creeping" of the liquid to the edge of the glass, and minimizing evaporation of the solvent during microscopic examination. The slide was immediately transferred to the hot stage, which had been preheated to the temperature of the sample, and adjusted to achieve a cooling rate of about 0.5° C. per minute.

Visual examination during cooling required a high-intensity zirconium arc light source to penetrate the highly opaque solution, particularly with methylene blue concentrations in excess of 1.5 grams per 100 cc. At a certain point (usually within a 1° to 2° C. range), micro crystals of methylene blue appeared suddenly in the solution, nucleating on particles of foreign matter. Temperatures at which birefringent needles first appeared were noted for each sample. Visual estimation of the nucleation temperatures was simplified by the fact that newly formed methylene blue crystal nuclei were the only anisotropic particles in the solution, appearing to twinkle or sparkle under crossed polars. Samples were observed at least twice to ensure reproducibility. A similar experimental procedure was used with the Thomas thermoelectric cold stage to obtain solubility data at temperatures below room temperature.

Spectrophotometric Examinations for Determination of Concentration. To ascertain the accuracy of the microscopic studies, the solubility curve for methylene blue in a 5 wt.% sodium chloride solution was established by a more conventional technique. An agitated vessel was filled with a methylene blue solution and an excess of methylene blue crystals. The vessel and its contents were then cooled very slowly from 50° C. to room temperature, and samples of the mother liquor were withdrawn periodically. Runs were made at different cooling rates to ascertain the reproducibility of the results.

The methylene blue concentration of the mother liquor was determined from a previously prepared calibration curve of log (per cent transmittance at the wavelength of maximum absorption) *vs.* concentration of methylene blue. This was necessary since methylene blue does not obey Beer's law even at concentrations as low as $10^{-6}M$ (*2*). A 1-ml. sample of the liquid phase was taken, weighed accurately, and then diluted to a liter. Further dilutions were made and recorded until the concentration was about $10^{-5}M$. At this concentration, transmittance measurements as a function of wavelength could most accurately be made using a double-beam spectrophotometer and a cell length of 1 cm. Comparison with a known standard could obtain the concentration of the unknown sample.

Photomicrographic and Optical-Crystallographic Studies. Photomicrographs of selected crystal samples were taken using a Polaroid MP-3 Camera in conjunction with an AO-Spencer binocular microscope (to achieve 7.5 × total magnification). Photomicrographs at higher magnifications were taken with a Reichert Zetopan Research Microscope under polarized light, utilizing a zirconium arc source. In addition to the foregoing, methylene blue crystals were also subjected to optical-crystallographic measurements.

X-Ray Diffraction Studies. X-ray powder diffraction patterns were recorded with a Norelco diffractometer equipped with scintillation detector, 0.006-inch receiving slit, and 1° divergence slits. Patterns of 1- × 2-cm. flat powder specimens were made with nickel-filtered copper K-alpha radiation and scanned at ⅛° and ¼° per minute over a range of 2° to 60° 2-θ diffraction angle. Patterns were also obtained on film with a Norelco 114.59-mm. diameter powder camera.

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Powders for x-ray specimens were prepared by grinding the methylene blue crystals to a particle size which passed through a No. 325 or No. 400 standard sieve. Crystal samples moist with mother liquor were first dried by exposing them to the air at room temperature (23° C.) for a few minutes.

RESULTS AND DISCUSSION

Solubility and Crystallization Studies. The solubility data obtained by the microscopic and spectrophtotmetric studies are shown in Figure 1. The data shown on these graphs were obtained using aqueous solutions, all of which had pH values of 2.0. However, in subsequent experiments, data were taken for solutions having pH values in the range 2.0 to 3.0; these data were substantially the same as those shown in Figure 1.

Figure 1,*A* indicates that the solubility data, as obtained by the two different experimental techniques, are in reasonable agreement, although some significant discrepancies occur in the lower temperature region. Since each set of experimental data is quite consistent within itself, the differences observed in Figure 1,*A* must arise from a constant experimental error or from different experimental techniques. The family of solubility curves shown in Figure 1,*B* is consistent with the curves of Figure 1,*A*; the anticipated shift in the solubility curves with a changing chloride ion concentration is evident in Figure 1,*B*.

The most significant aspect of the data shown in Figure 1 is the fact that an abrupt change is observed in the slope of each solubility curve. The temperature corresponding to the break point in each curve varies with the sodium chloride content of the aqueous phase. These temperatures increase from approximately 28° C. for experiments using pure water as a solvent to 36° C. for the data corresponding to 7 wt.% sodium chloride solutions.

If the aqueous solutions of methylene blue are assumed ideal, then the value of the slope of a line of Figure 1,*B* may be equated to $-\Delta H_f/R$. If the step change in the slope of each solubility curve on Figure 1,*B* is attributed to a solid state phase change, the difference in the slopes of the two portions of each line may be used to compute the enthalpy change accompanying the phase change. By this technique, an enthalpy change of about 30 kcal. per gram mole of methylene blue was calculated for the transition.

It is not prudent to be more precise about the magnitude of the enthalpy change, because the assumption of an ideal aqueous phase is obviously an invalid one. Indeed, as noted previously, the nonidealities of the liquid phase cause deviations from Beer's law at concentrations as low as $10^{-6}M$.

As a further stage of the investigation, a laboratory crystallization experiment was performed. In this experiment, an aqueous solution containing 2 wt.% methylene blue and 5 wt.% sodium chloride was cooled slowly from 50° C. to room temperature, and the temperature of the solution was recorded periodically. In plotting the slurry temperature *vs.* time, a curve with a continuous slope was obtained until the temperature reached 29° C. At this point, the temperature remained constant for several hours after which time the slurry temperature again decreased continuously until reaching room temperature. The discontinuity in the cooling curve can only be interpreted as a period during which an exothermic transformation takes place in the crystalline slurry. Both the temperature and approximate magnitude of the exotherm are quite consistent with the interpretation of the solubility data as previously presented.

Photomicrographic Studies. To elucidate the cause of the enthalpy change observed in the solubility and cooling-curve studies, a photomicrographic study was carried out. An aqueous solution containing 2.69 wt.% methylene blue

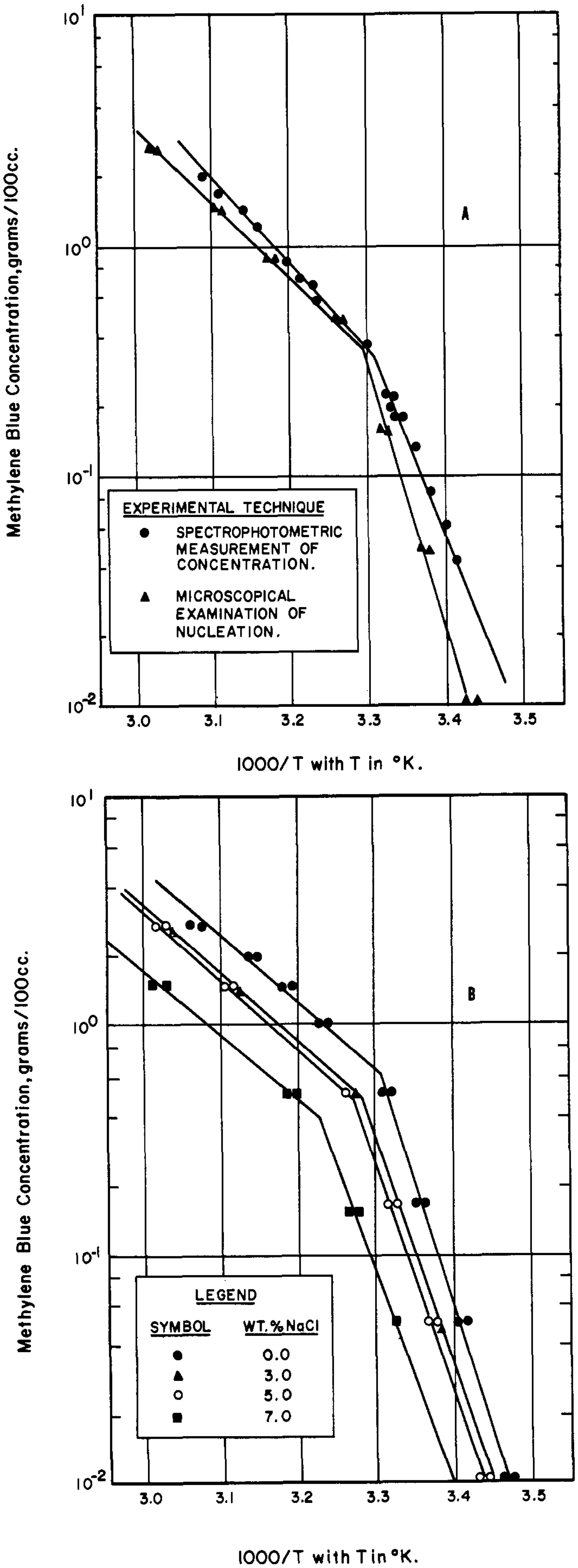

Figure 1. Solubility of methylene blue

A. Aqueous solutions containing 5% sodium chloride

B. Determined by microscopic examination of heterogeneous nucleation and 5 wt. % sodium chloride was cooled gradually from 50° to 23° C.; the temperature-time profile for this experiment was approximately the same as that in the cooling-curve experiment. Samples of the crystals formed were obtained at various temperatures during the crystallization. These crystal samples were carefully air dried at room temperature and were subjected to microscopic examination.

Figure 2 shows photomicrographs of typical crystals withdrawn from the mother liquor at various stages during the crystallization. These photographs are presented in consecutive order with Figure 2,*A* showing the crystals obtained at an early stage of the experiment and the remaining photographs showing the subsequent development of the crystals as the slurry was gradually cooled. Figure 2,*A* through 2,*C* depict the gradual growth of the individual crystals as the mother liquor–crystal slurry was cooled; the growth observed in these pictures appears to be ordinary. However, Figure 2,*D* shows a sudden decrease in the crystal size as well as a change in crystal habit that occurred between 40° and 32° C. This finding is consistent with the previous observation that a substantial enthalpy change takes place in an aqueous slurry of crystalline methylene blue as it is cooled. For a mother liquor containing 5 wt.% sodium chloride, the solubility data in Figure 1,*A* indicate that the phase change should occur in the temperature range of 29° to 33° C.

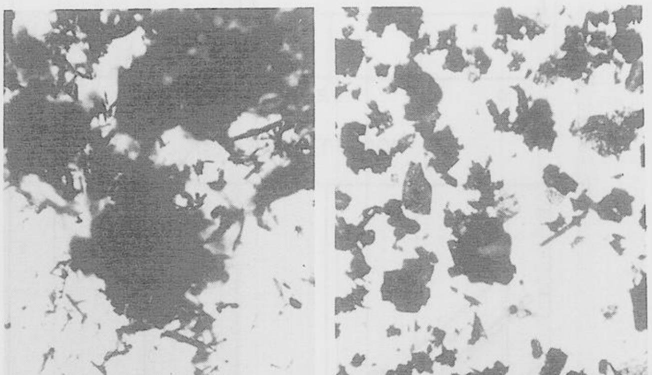

Figure 2. Methylene blue crystals

A. Obtained at 45° C. after 10 hours of crystallization (50X)
B. Obtained at 45° C. after 13 hours of crystallization (50X)
C. Obtained at 42° C. after 31 hours of crystallization (50X)
D. Obtained at 32° C. after 91 hours of crystallization (50X)

Optical-Crystallographic Studies. To facilitate the interpretation of the solubility data, crystals of methylene blue obtained from the crystallization study were subjected to a detailed optical examination. Two types of crystals were studied: Type I crystals, which were obtained at a temperature below the transition temperature (Figure 2,*D*); and Type II crystals which were obtained at a temperature above the transition temperature (Figure 2,*C*). These studies showed that methylene blue crystals less than 1 micron in thickness will transmit visible light. Under these conditions, the crystals display anisotropic absorption properties (dichroism) sufficient to characterize each phase.

The results of the microscopic examination are shown in Table I. The existence of two crystalline phases is clearly evidenced by the results shown in this table. Refractive indices were measured on the common orientation in every case. Crystals selected for optical examination varied in length from 1 to 10 microns with a 1-micron maximum thickness.

X-Ray Diffraction Studies. The results of the x-ray powder diffraction studies provide an additional quantitative means of characterizing and differentiating the different types of methylene blue crystals. The most clearly defined and reproducible x-ray diffraction pattern was that for the Type II crystalline phase. This pattern may be summarized by reference to the interplanar spacing values of the three most intense diffraction peaks—namely, 3.477, 9.60, and 9.17 A.

Table II presents the complete x-ray diffraction data for the forward reflection range and gives the *hkl* indices that have been assigned. Table II also presents the corresponding interplanar spacing values calculated from Warwicker's results (*9*) for comparison with the observed and calculated spacing values for Type II crystalline phase obtained in the present work. The calculated interplanar spacing values presented in Table II for Warwicker's unit cell data and for the unit cell data of the present work were done by IBM 1620 computer with a FORTRAN program (World List No. 279) of Haendler and Cooney (*3*) set up for the monoclinic crystal system. The agreement between observed and calculated interplanar spacing values shows that the Type II phase is identical to the crystalline methylene blue examined by Warwicker. Warwicker has established that the crystals he examined were the pentahydrate; thus, the Type II crystalline phase obtained in the present work is the pentahydrate.

Table III summarizes the lattice parameters and their estimated errors for the Type II crystalline phase derived from the x-ray examinations of the present investigation.

In a subsequent portion of the present investigation, crystals were prepared in a manner similar to that used by Warwicker—namely, by slow evaporation of a saturated aqueous solution of methylene blue containing no sodium chloride. The evaporation was carried out at a temperature of 23° C. over a period of several weeks. Crystals obtained

Table I. Optical-Crystallographic Data on Crystals of Methylene Blue

	Type I Crystals, Low Temperature	Type II Crystals, High Temperature
Crystal system	Monoclinic	Monoclinic
Habit	Bladed needles	Laths and plates
Refractive indices	$n_1 = 1.515 \pm 0.005$ $n_2 > 1.590$[a]	$n_1 = 1.480 \pm 0.005$ $n_2 = 1.590 \pm 0.005$
Extinction[b]	Parallel	Parallel
Pleochroism	/ / Blue violet ⊥ Olive green to black	/ / Pale violet ⊥ Rose-red

[a] Not precisely determined owing to opacity. [b] On common orientation.

Table II. X-Ray Powder Diffraction Data for Methylene Blue Type II Phase

			Calculated *d*, A.	
d, A.	I/I_1, %	*hkl*	Present results	Warwicker data (*9*)
15.63	38	020	15.65	15.55
9.60	97	100	9.66	9.56
9.17	55	110	9.23	9.14
8.18	26	120	8.22	8.14
7.80	5	040	7.83	7.78
7.05	2	130	7.09	7.03
6.79	3	011	6.79	6.74
6.36	6	021	6.36	6.31
6.06	10	140	6.08	6.03
5.79	4	031	5.79	5.75
5.57	1	$\bar{1}21$	5.61	5.56
5.26	2	111	5.27	5.23
5.19	7	041,$\bar{1}31$	5.21 5.20	5.16 5.16
5.06	3	121	5.06	5.02
4.746	18	131	4.758	4.720
4.643	5	$05\bar{1}$	4.655	4.622
4.586	18	160	4.590	4.557
4.405	2	141	4.415	4.380
4.354	8	230	4.382	4.340
4.313	3	$\bar{1}51$	4.334	4.301
4.160	8	061	4.175	4.146
4.084	8	240,$\bar{2}21$	4.109 4.070	4.071 4.033
4.044	8	170	4.058	4.029
3.921	5	$\bar{1}61$	3.938	3.909
3.852	2	250	3.826	3.790
3.803	2	071	3.762	3.737
3.639	5	221	3.655	3.621
3.616	5	180	3.627	3.601
3.570	6	$\bar{1}71$	3.587	3.561
3.477	100	002	3.480	3.454
3.416	39	081	3.411	3.388
3.398	54	022	3.397	3.372
3.377	38	241	3.388	3.358
3.318	8	$\bar{1}22$	3.331	3.306
3.262	19	190	3.272	3.250
3.229	6	$\bar{1}32$	3.241	3.216
3.206	8	310	3.202	3.170
3.176	21	042	3.180	3.157
3.219	16	0,10,0	3.130	3.110
3.122	11	$\bar{1}42$	3.126	3.102
3.054	6	$\bar{3}11$	3.053	3.023
3.043	5	052	3.042	3.020
3.023	4	132	3.019	2.995
3.000	4	$\bar{2}02$	3.002	2.974
2.973	2	340	2.977	2.948
2.922	8	142	2.925	2.903

Table III. Crystal Data for Methylene Blue Type II Phase

Monoclinic unit cell dimensions

a = 9.60 A. ± 0.01 A.
b = 31.3 A. ± 0.1 A.
c = 6.95 A. ± 0.01 A.
β = 97°

from the evaporation experiment were examined by x-ray diffraction and were the Type II crystalline phase identical to all other preparations of this phase obtained in the present study. Thus, the pentahydrate can be formed below the critical temperature range when the solvent is slowly evaporated.

A second type of x-ray pattern of methylene blue crystals was observed for the Type I crystals. Crystals of this nature had somewhat broader x-ray diffraction peaks and tended to show slight variations in interplanar spacing values from sample to sample. The x-ray pattern of these crystals exhibited interplanar spacing values for the three strongest peaks of 3.49, 7.6 to 7.8, and 16 A. and showed a characteristic peak of 11 A. spacing, which unequivocally distinguishes it from the pattern of Type II crystals.

Table IV. X-Ray Powder Diffraction Data for Methylene Blue Type I Phase

d, A.	I/I_1, %
16.	24
11.	13
7.7	52
7.4	12
6.9	10
4.85	6
4.59	5
4.31	5
3.90	7
3.49	100
3.41	46
3.01	8
2.93	6

Table IV presents the interplanar spacing and relative intensity values observed for the Type I crystals of methylene blue. Type I crystals could be produced by air drying (at 23° C.) the finely divided powder used for x-ray specimens of the Type II phase. Moreover, Type I crystals were also produced by vacuum drying of the Type II pentahydrate at room temperature to a water content of about 1 H_2O.

The reversion of Type I crystals back to Type II crystals also has been observed in the present study by subjecting the finely divided powder used for the x-ray specimens to high humidity conditions at room temperature. Conditions of 81% relative humidity at 23° C. were obtained in a closed vessel containing a saturated ammonium sulfate solution. Type I crystals which had been placed in this vessel converted completely to the Type II crystals in the course of a three-day exposure. These observations agree with those of Warwicker, who reported that the pentahydrate is the stable form of methylene blue at room temperature when the relative humidity is above 60%.

CONCLUSIONS

The present investigation has established the solubility of methylene blue in aqueous solutions and has demonstrated the existence of a phase transformation in crystalline methylene blue, which is accompanied by a large enthalpy change. The mechanism of the phase tranformation has not yet been ascertained. A change in the degree of hydration of the solid takes place at the transition temperature; however, a phenomenon more complex than a simple hydration is indicated. The possible existence of a liquid phase polymerization at the transition temperature, similar to that reported in references (*5*) and (*6*), needs to be investigated. Finally, the chemical composition of Type I crystals should be ascertained in order to determine the mechanism completely.

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NOMENCLATURE

a,b,c = lengths of unit cell edges on crystallographic axes a, b, and c, A.
d = interplanar spacing, the distance between adjacent planes of a given family of parallel, equally-spaced planes of a lattice, A.
hkl = indices of the x-ray reflection from a family of parallel planes, (hkl), of the lattice

I/I_1 = relative intensity, the intensity of a given x-ray diffraction line expressed as a percentage of the intensity of the most intense line of the x-ray powder diffraction pattern

n_1 = refractive index measured on the common orientation parallel to the length of a crystal

n_2 = refractive index measured on the common orientation parallel to the width of a crystal

R = universal gas constant, calories per gram mole -° K.

Greek

β = interaxial angle between the *a* and *c* crystallographic axes

ΔH_f = enthalpy change at constant temperature and pressure when 1 gram mole of methylene blue dissolves in an ideal solution; equal to the heat of fusion of methylene blue ($\Delta H_f > 0$ for heat being absorbed), calories per gram mole

Other

$//$ = refers to viewing a crystal when length of crystal is parallel to the plane of polarization

$\perp$ = refers to viewing a crystal when length of crystal is perpendicular to the plane of polarization

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Vapor-Liquid Equilibrium in the System Pyridine-Tetrachloroethylene

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Reliable vapor-liquid equilibrium data obtained with a modified Gillespie equilibrium still are presented for the binary system pyridine(1)-tetrachloroethylene(2) at 60°, 80°, and 100° C. A thermodynamic evaluation proved the data to be consistent. The thermodynamic correlation of the experimental data was made using a fourth-order Margules equation and the series expansion of relative volatility. A minimum boiling azeotrope is formed, whose composition changes very slightly with temperature.

THE literature has recently brought a whole series of theories (*4, 5, 8, 13, 16*) which deal with the thermodynamics of solutions. Most of these theories are, however, applicable only to systems whose components are practically nonpolar or only slightly polar. For proving the validity of those theories, therefore, reliable vapor-liquid equilibrium data are necessary for such systems. The literature dealing with vapor-liquid equilibrium data is very extensive (*5, 9*); nevertheless, there is a lack of good experimental data for systems with the aforementioned properties. Pyridine-tetrachloroethylene belongs to this group of systems, and what is more, the considerable difference in the densities of the components affords the exact analytical determination of both equilibrium phases.

EXPERIMENTAL

Chemicals. Fisher spectroanalyzed pyridine was twice distilled and purified as usual (*6*). The measured density at 30° C. (0.97285 gram per cc.) and the boiling point (115.31° C.) were in good agreement with the values given in the literature (*6, 7, 10, 17*). At atmospheric pressure, the boiling point and the condensation temperature measured with a Beckman thermometer in a modified Swietoslawski differential ebulliometer (*5*) differed by only 0.004° C. This result indicated that the sample did not contain sufficient impurities of different volatilities to have any significant effect on the boiling point measurement. The tetrachloroethylene, an Eastman-Kodak spectrograde product stabilized with thymol, was used without further purification. The experimental values of the density at 30° C. (1.60636 grams per cc.) and the normal boiling point (121.03° C.) agree with the literature values (*17*) very well. The boiling point and condensation temperature difference was 0.005° C. The apparent purity of the product stabilized with thymol, deduced from melting point studies by the time-temperature method, was 99.89 ± 0.04 mole %. Twice distilled deionized water was used as a standard for pressure measurements.

Apparatus and Procedure. The vapor-liquid equilibrium data were determined using a modified Gillespie still (*5*). The still was initially charged with approximately 200 cc. of a binary solution and operated for at least 2 to 3 hours. As has been proved (*2*), the time required to achieve equilibrium depends on the ratio of the condensate receiver volume to the boiling chamber volume. The theoretical calculation of Erdös and Pouchly (*2*) led to the conclusion that the contents of the condensate receiver should be exchanged six or seven times until equilibrium is established. The period can be shortened by decreasing the volume of the receiver and also by filling the receiver with a solution having approximately the equilibrium composition as estimated in some manner—e.g., using Raoult's law.

For the accurate measurement of vapor-liquid equilibrium data, it is necessary to have perfect control of both the temperature and pressure. To get the most accurate readings of the pressure, a Swietoslawski-type ebulliometer (*5*) filled with deionized and distilled water was connected in parallel into the system. From the boiling point of water, the corresponding pressure of the system was determined.