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ELECTROCHEMICAL AND NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY STUDIES OF PHENOL RED AND RELATED COMPOUNDS

by

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Ames, Iowa

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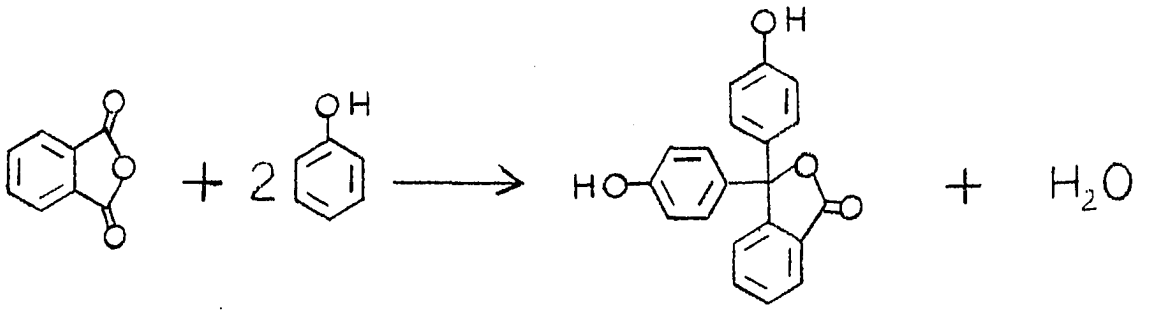
INTRODUCTION

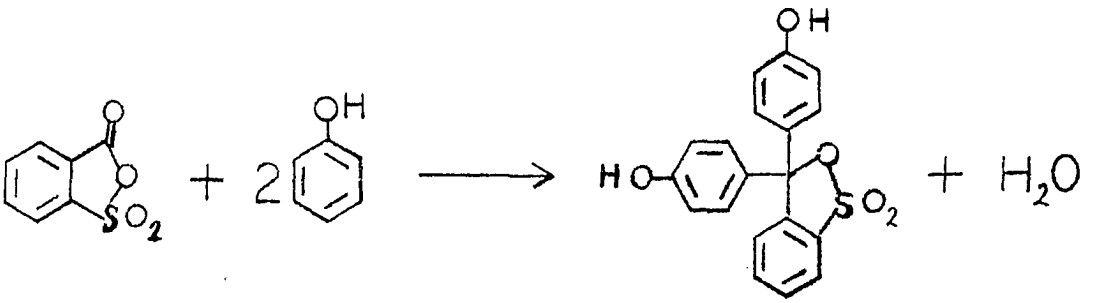
Properties of the Phthalein and Sulfonephthalein Indicators

The phthaleins and sulfonephthaleins have long been important compounds because they function as acid-base indicators. At least twenty-five of these compounds are available commercially, and many more have been synthesized, not so much because new indicators are needed (in the sulfonephthalein series alone, enough indicators are available to cover the entire pH region), but to gain general information about their behavior and functions. Some members of both series have purgative properties, phenolphthalein in particular having found wide use as a laxative. Recent work has shown that several sulfonephthaleins are excellent indicators in certain oxidation-reduction titrations, particularly those in which chlorite is the oxidizing agent (2).

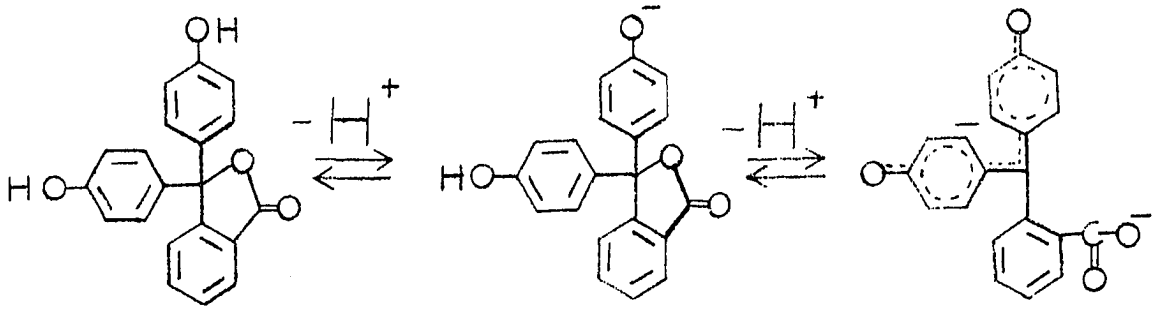
Phthaleins are usually prepared by a condensation of phenol or a substituted phenol and phthalic anhydride in the presence of zinc or tin salts, or of sulfuric acid.



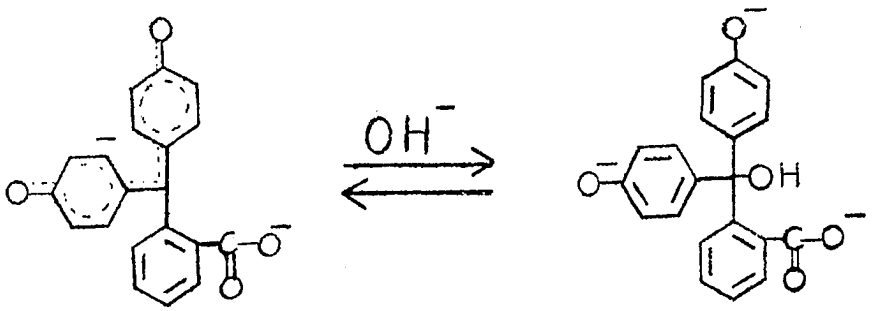
Similarly, the sulfonephthaleins are prepared by the reaction of either o-sulfobenzoic acid or o-sulfobenzoic anhydride with a phenol.



Extensive investigations into the mechanism of the color changes of these compounds with hydrogen ion concentration have been made, and the mechanism appears to be well understood. The reaction of phenolphthalein with base may be taken as representative of the phthaleins in general. In neutral to weakly acidic solutions the phenolphthalein molecule exists in a colorless form, generally considered to be the closed ring, or lactone structure. The two acid dissociation constants of phenolphthalein are sufficiently close that only a single break is obtained in the titration curve of the compound. The removal of one proton leads to the formation of the colorless phenolate anion. Removal of the second proton results in the formation of a dark red dianion, which is stabilized by resonance.

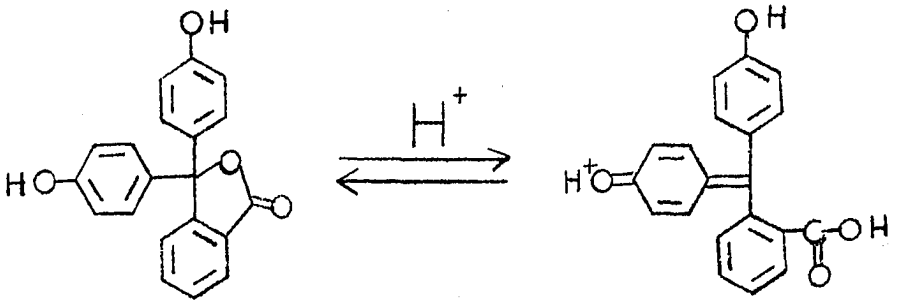


If a solution of phenolphthalein is made sufficiently basic, the dark red color fades owing to the formation of a colorless carbinol.



This fading does not take place instantaneously and a number of investigators have studied the rate of the reaction (3, 14).

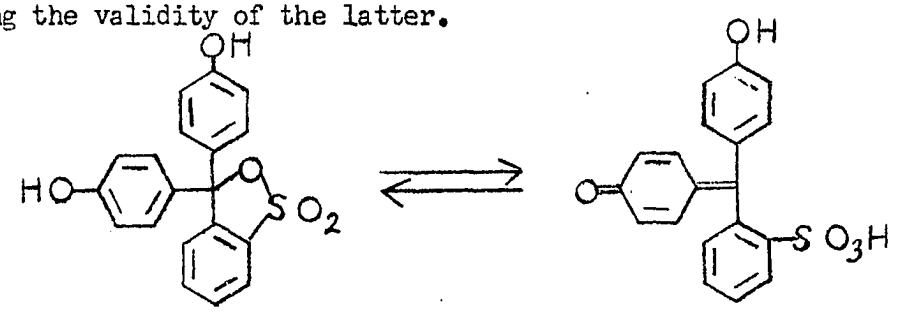
In addition to the reactions with alkali, most phthaleins are also colored in strongly acidic solutions. Thus, phenolphthalein is weakly rose-colored in 9 N and 12 N hydrochloric acid, and reddish-orange in concentrated sulfuric acid. This color is generally thought to be caused by the addition of a proton to the neutral molecule with the formation of a quinonoid structure (16).



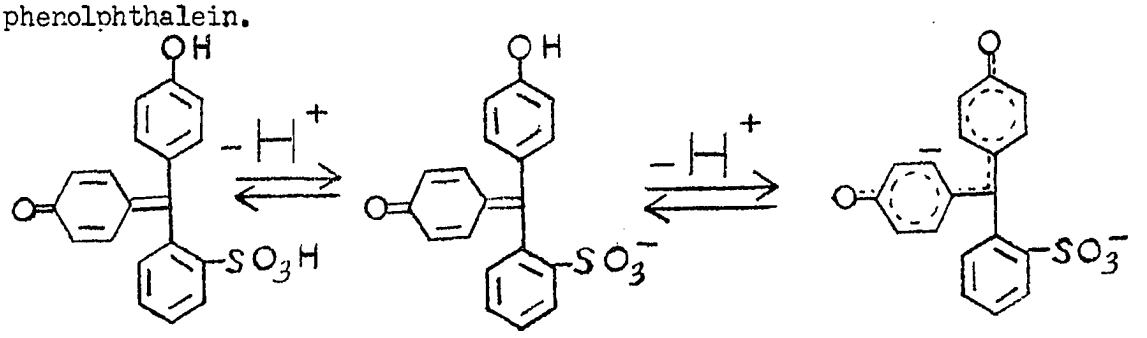
The behavior of the sulfonephthaleins is very similar to that of the phthaleins. However, the question of whether these compounds exist as the sultone (analogous to lactone) or quinonoid structure has not been completely settled. Early workers based their conclusions on the colors

of these compounds. They concluded that those sulfonephthalein indicators which were colored in the solid state and in solution exist in the quinonoid form, and that those which were colorless have the sultone structure. A great deal of evidence (spectrophotometric measurements, conductimetric measurements, and isolation of salts of the indicators) obtained on a number of compounds tends to confirm this postulate. In addition, the behavior (coloration in acid and base) of derivatives of the sulfonephthaleins is in agreement with the supposition that dyes having the closed sultone ring are colorless, and those in the open ring form are colored. It appears that much of the early confusion was due to the fact that both open and closed forms of a molecule may be coexistent.

All aqueous solutions of the sulfonephthaleins are yellow and, thus, the molecules exist either partially or wholly in the quinonoid form. Their colors in base are red, blue, or purple. The equilibria of the various forms in solution may be illustrated by considering phenol red, the behavior of which is typical of most sulfonephthaleins. On the basis of conductimetric measurements, White and Acree (34) have calculated that sixty to seventy-five per cent of the phenol red molecules in a saturated aqueous solution are present in the yellow, open-ring form, and the remainder in the colorless sultone form. They also cite spectrophotometric evidence to this effect, though Kolthoff (16) expresses doubt concerning the validity of the latter.



On addition of one mole of base, the open form is neutralized and the above equilibrium shifted until the monobasic salt of the open form is obtained. Addition of a second mole of base results in a dark red solution and the formation of a resonance-stabilized dianion, similar to the dianion of phenolphthalein.



Phenol red, like phenolphthalein, reacts with hydroxide ion in strongly basic solutions, forming a colorless carbinol (28). However, this reaction proceeds much more slowly with the sulfonephthaleins than with the phthaleins. For this reason, the sulfonephthaleins are often preferred in situations involving high concentrations of base.

Phenol red also reacts with strong acids in much the same way as phenolphthalein, producing reddish-orange solutions, the colors of which are again attributed to the protonated quinonoid structure (15).

Phthaleins and sulfonephthaleins in basic solutions are readily reduced by heating with zinc dust. Baeyer (1) first prepared phenolphthalin from phenolphthalein by this method. The white product obtained gave no color in basic solutions, but could be reoxidized to phenolphthalein by a number of common oxidizing agents. Baeyer also prepared ester and ether derivatives of phenolphthalin and concluded that the compound was dihydroxytriphenylmethanecarboxylic acid.

Sohon (29) reduced phenol red in the same manner, but found it necessary to work in an atmosphere of hydrogen to prevent oxidation. He assumed the reduction product to be dihydroxytriphenylmethanesulfonic acid. Orndorff and Sherwood (23) later found that a suspension of phenol red in water (in which it is practically insoluble) will slowly dissolve when boiled with zinc dust to give a colorless solution. Attempts to obtain a crystalline product failed but evaporation of these solutions to dryness yielded a zinc salt, which on analysis for zinc gave results consistent with the dihydroxytriphenylmethanesulfonic acid structure.

Fluorescein and sulfonefluorescein, which are similar in structure to the phthaleins and sulfonephthaleins, are reducible, and the reduction products have been assigned the triphenylmethane structure (21, 24).

The present work arose in connection with research on the acid-base properties of phthaleins in tert-butyl alcohol. A general survey of analytical methods for phenolphthalein was made and it was immediately evident that the electrochemical reduction of phthaleins and of sulfonephthaleins had been investigated in only a few cases and none of the work was definitive. An investigation of the electrochemical reduction of these compounds was therefore initiated in the hope that a clearer understanding of the electrochemical behavior would be obtained.

Electrochemical Techniques

Polarography.

Polarography was invented by Heyrovsky and Shikata in 1925 when they

devised an instrument for recording automatically the current-voltage curves obtained with a dropping mercury electrode (DME). Since that time polarography has proved immensely useful as an analytical tool for the determination of a large number of elements, and a great many organic compounds having reducible or oxidizable functional groups.

The basic components of a polarograph are simple; a potentiometer with a motor-driven sliding contact, a suitable cell, and a device for measuring the current through the cell. Into the cell are placed a reference electrode (a saturated calomel electrode is often used), the tip of a dropping mercury electrode, and an inlet tube for nitrogen or some other inactive gas. The inactive gas must first be bubbled through the test solution to remove dissolved oxygen which would otherwise interfere. By slowly moving the sliding contact to progressively more negative potentials, a potential will be reached at which reduction of the material in the test solution begins. As the potential becomes more negative, the reduction current will continue to increase until the rate of reduction equals the rate at which the reducible material diffuses to the electrode surface. At this point, the current reaches a limiting value (known as the limiting current) which is maintained until at some more negative potential the supporting electrolyte or the solvent is reduced. The portion of the curve recorded before the breakdown of electrolyte is generally S-shaped, and yields useful data. The limiting currents observed are small, usually from one to one hundred microamperes. Because the saturated calomel electrode has a relatively large surface area, it is not polarized to a measureable extent. Thus, the entire

potential drop (minus a negligible IR drop through the solution) will appear across the mercury-solution interface.

The wave height, half-wave potential, and shape of the wave provide useful data. The wave height is the difference between the residual current and the limiting current. The residual current is the current required to charge the electrical double layer around the mercury drop as it is formed. The wave height is important because it is directly proportional to the concentration of electroactive species when the reduction is diffusion controlled.

The half-wave potential is that potential on a polarogram at which the current reaches one-half of the wave height. When other factors are constant, this term is independent of the concentration of the electroactive species. It does, however, vary from species to species and, thus, may be used for identification. The half-wave potential is usually quite close to the standard reduction potential; it is important in the analysis of fundamental electrochemical problems.

The shape of the polarographic wave is important in that it provides clues to the number of electrons involved in the reduction, and to the degree of reversibility of the system.

A mathematical equation relating the variables of the system was derived by Ilkovic in 1934:

$$i_d = 706nFCD^{1/2}m^{2/3}t^{1/6}$$

in which

i_d is the current in microamperes,
n is the number of electrons involved in the reduction,
F is the Faraday constant,
C is the millimolar concentration of the electroactive species,
D is the diffusion coefficient in cm^2/ sec.,
m is the mass of mercury falling in mg./sec., and
t is the drop time in seconds.

The constant 706 is used when the instantaneous current is measured. If the mean current is computed, the constant used is 607. This equation makes it possible to calculate either n or D with reasonable accuracy (2%) when the other terms are known, and it is valid for both reversible and irreversible systems when the current is diffusion controlled.

An important equation relating the potential of the DME to the current on the steeply rising portion of the wave was derived by Heyrovsky and Ilkovic:

$$E = E_{\frac{1}{2}} - (0.0591/n)\log\left[i/(i_d-i)\right]$$

in which

E is the potential of the DME,
$E_{\frac{1}{2}}$ is the half-wave potential,
i is the current at any point on the wave, and
i_d is the diffusion current.

This equation was derived for reversible systems in which only the oxidized form is present in solution. It is apparent that a plot of E versus $\log\left[i/(i_d-i)\right]$ will be a straight line with a slope of 0.0591/n. This is an important test for reversibility and for the determination of n. For irreversible systems a straight line is often obtained but the slope does not yield an integral value for n.

The hydrogen ion is usually involved in the reduction of organic compounds. For reversible systems of the type:

$$O + xH^+ + ne^- = R^{x-n}$$

in which O and R represent the oxidized and reduced forms, the half-wave potential generally shifts to more negative values as the pH of the solution is raised. For reversible systems, this shift is quantitatively described by the equation:

$$E = E^o - (0.0591/n)\log(1/H^+)^x - (0.0591/n)\log [i/(i_d-i)].$$

The first two terms on the right hand side may be set equal to $E_{\frac{1}{2}}$ which gives:

$$E_{\frac{1}{2}} = E^o - (0.0591x/n)pH.$$

Thus, a plot of the half-wave potential as a function of pH will yield the number of hydrogen ions involved when n is known (19).

By application of these equations to polarographic data much can be learned about the reduction mechanisms of organic compounds. Unfortunately, kinetic complications accompanying the reduction of organic molecules are very common. A type commonly observed occurs when a chemical reaction precedes electron transfer. For example, compound A may be in tautomeric equilibrium with compound B. If both forms are reducible with A having a more positive half-wave potential than B, then two reduction waves will be observed. If the equilibrium is established very slowly, then the limiting currents of A and B will represent the ratio of the concentrations of A and B in solution. If, on the other hand, the rate of interchange between A

and B is very rapid, only a single wave produced by the reduction of A will be seen. In the intermediate case in which the equilibrium is established at a rate that is neither fast nor slow, the two waves will again be seen, but the height of the waves will be a function of both the equilibrium concentrations of A and B, and of the rate of conversion of B into A.

A similar situation is often encountered in the reduction of organic acids in which the free acid is reduced at a different potential than the anion of the acid. In acidic solutions, only one wave is observed, which is due to the reduction of the free acid. Solutions buffered at a pH near the pKa value of the acid yield two waves because both the acid and anion are present. Only a single wave due to the reduction of the anion is observed in basic solutions. The relative heights of the two waves is then a function of both the pH and the rate at which the equilibrium is established for the reaction:

$$HA = H^+ + A^-.$$

Another type of complication may occur when the product of the electron transfer is unstable and undergoes rearrangement, dimerization, or disproportionation. Such systems are best studied by electrochemical techniques other than polarography, such as cyclic voltammetry or chronopotentiometry with current reversal. However, polarography may provide clues to the mechanism, and in some cases, yield sufficient data for an approximation of the rate constants involved. An example is the second order disproportionation of U(V) in perchloric acid medium (8, 17, 22). At appropriate hydrogen ion concentrations, two reduction waves are observed for a solution of U(VI). The first wave constitutes the reduction

of U(VI) to U(V), and the second, U(V) to U(IV). The height of the first wave, however, is noticeably greater than that of the second owing to the disproportionation of U(V) to U(VI). The U(VI) formed by the disproportionation is very near the electrode surface and practically all of it is reduced again. This "recycling" of the U(VI) results in the inequality of the wave heights. Because the rate of disproportionation is second order in U(V), a plot of the limiting current of the first wave against the U(VI) concentration is nonlinear, bending upwards at high concentrations. Also, the height of the first wave is very temperature dependent. The rate of disproportionation (and, thus, the height of the first wave) increases rapidly with temperature. Orleman and Kern (22) devised a method for the approximation of the second order, homogeneous rate constant, and were able to estimate its value within one order of magnitude.

Reduction mechanisms can become quite complicated, requiring for their elucidation information as to the structure of the reduction product, the effect of surfactants, and the existence of tautomeric equilibria. Thus, polarographic data must be supplemented by data from other chemical and electrochemical studies.

Controlled potential coulometry.

This technique is useful in that it provides a determination of n which is independent of kinetic complications and the degree of reversibility of the system. It also permits a macro synthesis of the reduction product under conditions almost identical to those occurring at the DME. Such information aids in the interpretation of polarographic data when the

wave shapes and heights are dependent on kinetics, the presence of surfactants, and the degree of reversibility.

Controlled potential coulometry is usually carried out in a two compartment cell (Figure 1) containing a large mercury pool cathode which is separated by a glass frit or agar plug from a platinum or silver-silver chloride anode. In addition, a reference electrode is placed in the cathode compartment so that the potential of the mercury pool may be monitored. A known amount of electroactive species is placed in the cathode compartment and the electrolysis is carried out at the desired potential. An electromechanical or chemical coulometer is included in the circuit and the total charge passed is measured until the current has fallen to that of the supporting electrolyte only. By use of Faraday's law of electrolysis the number of electrons transferred per molecule is then calculated.

The potential controlling device is called a potentiostat. Early potentiostats were rather bulky and expensive to construct but the advent of transistors and the development of stable, high-gain, direct current, operational amplifiers has circumvented many of the difficulties.

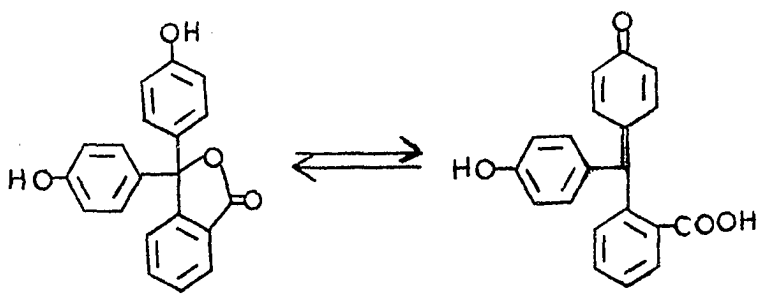
Several chemical coulometers have commonly been used. These include gas, colorimetric, gravimetric, and titration coulometers. Perhaps the most commonly used chemical coulometer is the hydrogen-oxygen gas coulometer. This coulometer has been shown to be inaccurate at low current densities, and is therefore unsuitable for micro or semi-micro coulometric determinations (25). The hydrogen-nitrogen gas coulometer, on the other hand, is accurate at low current densities and may be readily substituted in place of the former.

Some workers have felt that the reduction products produced at a large, stirred mercury pool cathode may not be identical to those formed at the DME, particularly when the reaction is rate controlled. To allay such fears, microcoulometry at the DME was developed (5). Using sensitive current integrators and micro cells containing one milliliter or less of solution, n may be determined to about three per cent. The technique suffers from the usual inconveniences of micro methods and the time for analysis is long (several hours). Thus, the method has not come into general use.

Review of the Electrochemistry of the Phthaleins and Sulfonephthaleins

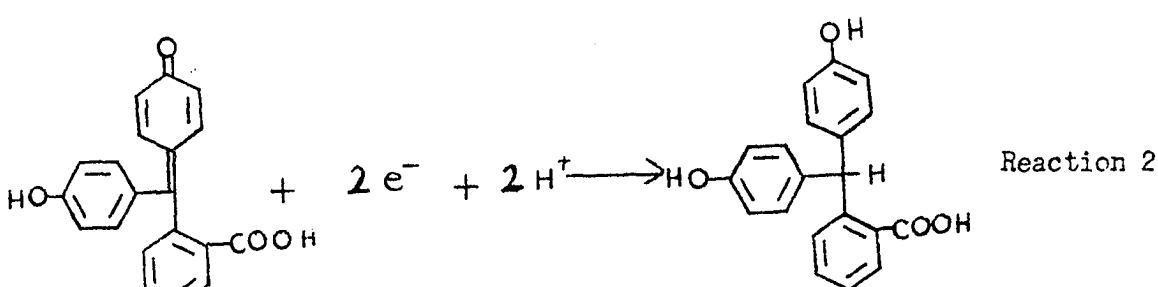
Previous work on the electrochemistry of the phthaleins, sulfonephthaleins, and related compounds is scant indeed. Of the phthaleins only phenolphthalein has been investigated in detail. Kolthoff and Lehmicke (18) studied the polarographic reduction of phenolphthalein in solutions of twenty-five per cent ethanol (certain conclusions in this article were later modified (20)). A shift in the half-wave potential of phenolphthalein in the negative direction with increasing pH was observed, indicating that the hydrogen ion was involved in the reduction. In the pH region between zero and 8.5, a single, two electron, reduction wave was observed; at pH 9, this wave split into two waves of unequal height, the first being larger than the second. Between pH 9 and 10.25 the height of the second wave increased and the first decreased. Above pH 10.25 the limiting currents of the two waves were equal. It was also observed that increasing the alcohol concentration of acidic solutions beyond forty per

cent resulted in large decreases in the limiting current, the latter becoming vanishingly small at ethanol concentrations of seventy per cent. Similar results were obtained when acetone or methanol were added. To account for the observed behavior, the authors proposed the following reactions:



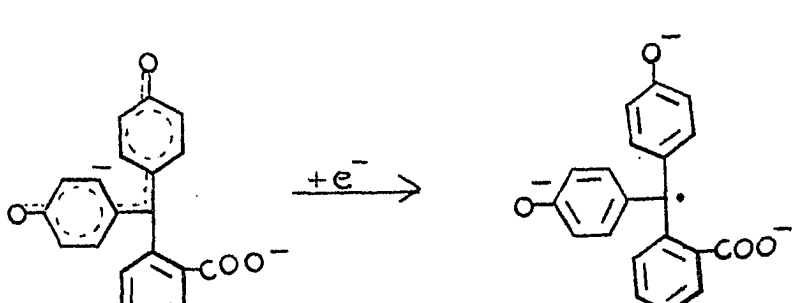
I Ia

Reaction 1



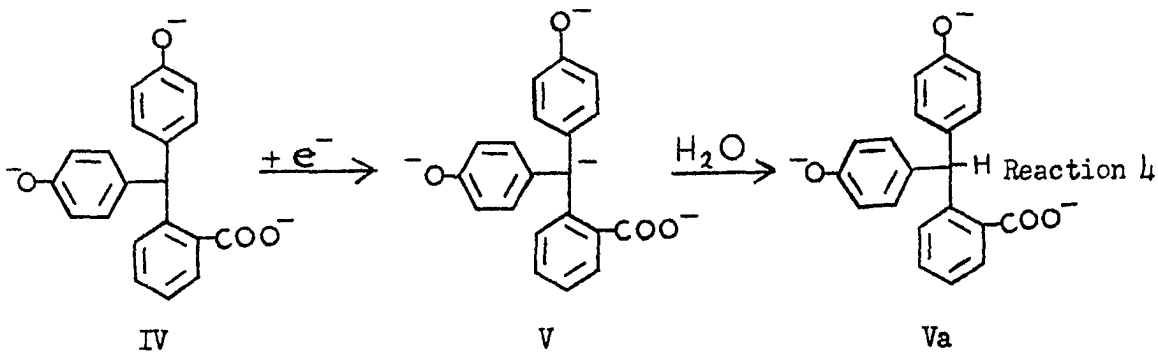
Ia II

Reaction 2



III IV

Reaction 3



IV V Va

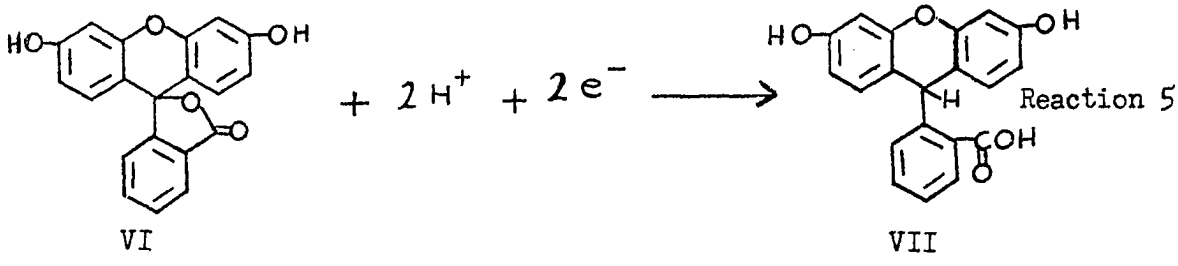
At pH 8.5 or less, reaction 1 yielded the electroactive species Ia which was reduced in a single step to phenolphthalin (reaction 2). At pH 10.25 or greater, only III existed in solution and was reduced in two, one electron steps (reactions 3 and 4) to give V, which reacted with water to form the trianion of phenolphthalin (Va). Between pH 8.5 and 10.25, both III and the carboxylate anion of Ia were present and both were reduced, but only two waves were observed. The authors consequently assumed the half-wave potentials of Ia and the first step of III to be identical. Reaction 1 was proposed to account for the effect of large amounts of nonaqueous solvents on the wave. Such a reaction would be favored in highly polar solvents, and the addition of alcohol or acetone to an aqueous solution would shift the equilibrium to the left. This would reduce the rate of production of Ia and result in the disappearance of the wave.

The authors assumed the final reduction product to be phenolphthalin. Although this was not established with certainty, the assumption seems valid since phenolphthalin is the only product obtained in the reduction of phenolphthalein by chemical means.

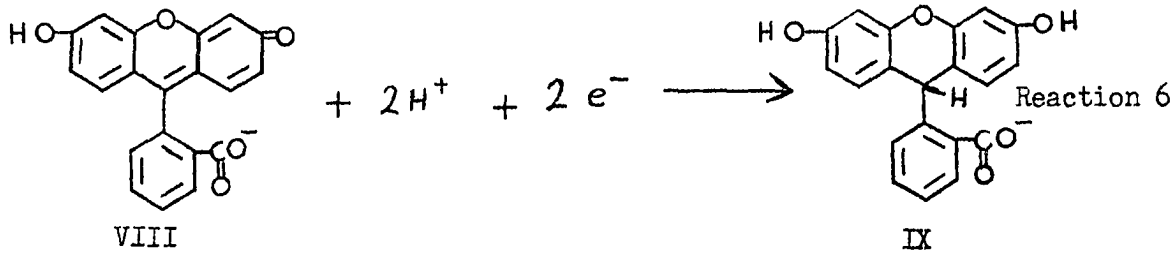
It was also observed that the carbinol form which is generated in strong base was not reducible.

Suzuki (31), using alternating current polarography, has shown reactions 3 and 4 to be reversible, and reaction 2 to be irreversible.

Further work on the phthaleins is lacking, but fluorescein which is structurally similar to phenolphthalein has been studied by Delahay (7). He observed a single, two electron, reduction wave below pH 6. Above pH 9.5, two waves were seen, each corresponding to a one electron transfer. In the region between 6.5 and 9.5 two waves appeared, the relative heights of which depended on the pH of the solution. This situation was similar to that of phenolphthalein, and Delahay proposed that below pH 6, reaction 5 occurred in one step while at pH 9.5 only the quinone form VIII existed and was reduced in two steps (reaction 6).



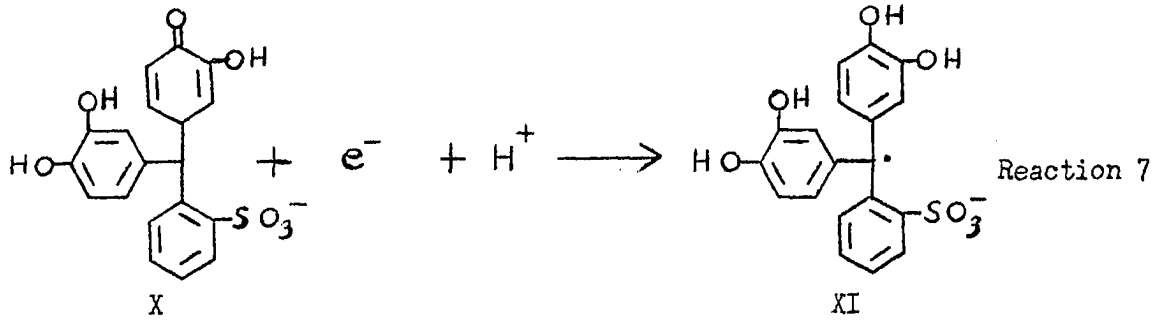
VI VII



VIII IX

Between pH 6.5 and 9.5 both VI and VIII were present giving three waves, only two of which were seen experimentally owing to overlap of the half-wave potentials. The final reduction product was assumed to be fluorescin (IX).

In the sulfonephthalein series, the reduction of pyrocatecholsulfonephthalein (X) has been reported. Pang and Lin studied the reduction of X and the stability of the pyrocatecholsulfonephthalein-germanium complex by the polarographic method (26). They found that between pH 3.2 and 7, X gave a well defined, one electron, reduction wave and a second poorly defined wave. The half-wave potential of the first wave of X was a linear function of the pH (0.07 volt/pH unit) within the range studied, and the plot of potential of the DME versus $\log [i/(i_d - i)]$ had a slope of 0.066. From this they concluded that the first wave corresponded to a one electron, reversible step (reaction 7).



X XI Reaction 7

Although the authors said nothing of the reduction product, the free radical XI would logically result from a one electron transfer. Further investigations into the nature of the second wave were not reported, though the final product of a two electron reduction would probably be the sulfonephthalin.

Polarographic studies of pyrocatecholsulfonephthalein were also

reported by Wang and Sung (33). They observed only one wave at pH values less than 5 (In this respect, their results disagree with those of Pang and Lin). Above pH 6, two waves were seen, and in very strong base a third wave appeared which they attributed to the reduction of the product of air oxidation of the indicator.

The present work is concerned mainly with the electrochemical reduction of phenol red. This compound was chosen because it is the simplest of the sulfonephthalein dyes and is structurally similar to phenolphthalein. It is soluble in buffered, aqueous solutions above pH 3 and is not easily converted to the carbinol in strong base. The reduction is studied using the polarographic method, controlled potential coulometry, and cyclic voltammetry. The product of electrochemical reduction was isolated and shown to be identical to that from the chemical reduction of phenol red by metallic zinc. A mechanism for the reduction of phenol red is proposed, postulating the formation and disproportionation of a free radical intermediate. Nuclear magnetic resonance studies of some phthaleins, sulfonephthaleins, and their reduction products were begun. It is shown that by the use of nuclear magnetic resonance spectroscopy it is possible to distinguish between open and closed ring systems in both the lactone and sultone series of indicators.

ELECTROCHEMICAL STUDIES OF PHENOL RED

Experimental Work

Reagents.

Phenol red. Phenol red and other sulfonephthalein dyes were obtained from the Hartman-Leddon Company, Philadelphia, Pennsylvania. Phenol red was also synthesized by the method of White and Acree (35). Material from both sources was chromatographed on Whatman No. 3 MM paper by the ascending technique using a three per cent sodium chloride solution as solvent (32). Spots were detected visually both by exposure of the chromatogram to ammonia fumes and ultraviolet radiation. In both preparations only one impurity was found. It was yellow, strongly fluorescent, and had an R_f value greater than that of phenol red. As a further check, chromatograms were developed by the descending technique using a four to one mixture of tert-amyl alcohol and concentrated ammonium hydroxide as the developer (10). The results were the same as in the previous case except that the R_f values for phenol red and the impurity were smaller and the time for development longer (30 hours versus 10 hours).

To obtain larger amounts of purified phenol red, a column chromatographic method of purification was devised. An alumina column about 5 cm. in diameter and 25 cm. long was prepared, washed with water until the effluent contained no suspended material, and then washed with 150 ml. of 95 per cent ethanol. A slurry of about 150 mg. of impure phenol red was slowly added to the column and washed on the alumina with more alcohol. A mixture of water, concentrated ammonium hydroxide, and alcohol (20:20:60 by

volume) was used to elute all materials. When a flow rate of about one drop per second was maintained, the first substance to be eluted was the yellow, fluorescent impurity, which could be removed with complete separation from other visible bands on the alumina. At this point, a light blue band could be clearly seen near the bottom, incompletely separated from a pink band above. The latter, in turn, was not fully resolved from the very dark red band of phenol red above it. At the very top of the column was a final, yellow-orange band which remained stationary during the elution of the other bands (It was later removed with tenth normal sodium hydroxide). Since the pink impurity could not be resolved from the phenol red band, the first 200 ml. of effluent containing phenol red was discarded, and the remaining product eluted with 500 to 800 ml. of eluant. The effluent was placed in a large suction flask and evaporated to about 150 ml. by gentle heating and vacuum. The solution was filtered on medium grade sintered glass, and then acidified by the addition of 35 ml. of concentrated hydrochloric acid and allowed to stand overnight. The solid was separated by filtration on coarse sintered glass, and then redissolved by slowly adding tenth normal sodium hydroxide with constant stirring until the solution was permanently red. This solution was filtered on medium grade sintered glass, and the phenol red was again precipitated as above. The final product was washed with two or three small portions of water and oven dried at 110 ^{o}C. for two hours. No attempt was made to identify the impurities.

Titration curves of phenol red show it to be a strong acid in water. Because it is only slightly soluble in water, about 50 mg. of purified material was first dissolved in 4.0 ml. of dimethylformamide, diluted to

100 ml. with water, and titrated under nitrogen with standard calcium hydroxide. The endpoint was determined potentiometrically with a glass indicator electrode and a saturated calomel reference electrode. Calcium hydroxide was used rather than sodium hydroxide because it is inherently free of carbonate due to the insolubility of calcium carbonate. A 0.04 N solution was prepared by saturating water with calcium hydroxide, permitting the mixture to settle overnight, and then filtering the supernatant liquid through a fine filter paper. The base was standardized potentiometrically against potassium hydrogen phthalate.

The titration curve of phenol red shows one distinct break with the endpoint occurring at about pH 5.3. A second break is also present but it is not sufficiently sharp to permit analysis. Results of equivalent weight determinations for several batches of phenol red purified by the above method are shown in Table 1. The theoretical equivalent weight is 354.

Table 1. Equivalent weight of phenol red as determined by titration with standard calcium hydroxide.

Batch number	Equivalent weight
1	369
2	362
3	362
4	359
5	364
6	363

Values for the equivalent weight obtained by titration were consistently high by about three per cent. The residue on ignition was

determined for two samples and found to be 0.44 and 0.55 per cent. The residue, which was white and insoluble in tenth normal acid and base, was probably alumina. The water present in two samples from batch number six was determined by Karl Fisher titration and found to be 0.80 and 0.92 per cent. Thus, alumina and water together constituted about 1.5 per cent of the purified phenol red by weight, and no further attempts were made at purification.

Phenolsulfonephthalin. This material was synthesized both by the chemical reduction of phenol red with zinc dust (23), and by the controlled potential reduction of phenol red at a mercury cathode. Preparation by the latter method involved the reduction of about 200 mg. of phenol red in a solution of 0.1 M ammonium sulfate and 0.1 M ammonium hydroxide. The ammonium sulfate and ammonium hydroxide served the dual purpose of buffering the solution, and acting as supporting electrolyte for the electrolysis. When reduction was complete, the inorganic salt was precipitated by the addition of barium hydroxide solution, centrifuged, and separated from the supernatant by decantation. The supernatant (containing the phenolsulfonephthalin) was evaporated to dryness in a vacuum oven at 35 degrees. Thirty milligram portions of the residue were dissolved in 1 or 2 ml. of water and applied in a strip to a sheet of Whatman No. 3 MM chromatographic paper (previously washed with dilute ammonium hydroxide and dried), about one inch above the base of the paper. The chromatogram was developed overnight in a mixed solvent of n-butanol, concentrated ammonium hydroxide, water, and 95 per cent ethanol (50:25:25:20 by volume). The ascending technique was used. The air dried chromatogram was sprayed lightly with a 0.05 per cent solution of pinacryptol yellow, which reacted

specifically with sulfonic acids to produce spots which became visible under ultraviolet radiation. The phenolsulfonephthalin appeared as a rather broad, dark band near the top. This band was cut out and the material eluted off the strip with about 5 ml. of water. This solution was evaporated to dryness in the drying oven as before, and yielded a light orange solid which was apparently the monoammonium salt of phenolsulfonephthalin contaminated with a trace of phenol red. This material was stored in a desiccator under nitrogen, because it slowly reverted to phenol red on exposure to air. This compound, as well as the free acid, could not be obtained in crystalline form or completely free of water.

Phenolphthalein. This compound (N. F. powder) was obtained from the Mallinckrodt Chemical Works, St. Louis, Missouri.

Thymol blue. This indicator was obtained from the Hartman-Leddon Company, Philadelphia, Pennsylvania.

Triton X-100. This material was obtained from the Rohm and Haas Chemical Company, Philadelphia, Pennsylvania.

Other chemicals. Commercial prepurified nitrogen was used to deaerate the solutions used in polarographic work. All other chemicals were of commercial reagent grade and were used without further purification.

Apparatus.

Polarograph. All polarograms were obtained with a Leeds and Northrup Electro-Chemograph Type E. The instrument was specially equipped with a means of expanding the potential-time scale by a factor of two. The recorder had a full-scale response of one second.

Polarographic cell. The polarographic cell was of the H-cell type

consisting of three compartments separated by Corning ultra-fine glass frits. The central compartment contained saturated potassium chloride to minimize electrical resistance between the saturated calomel reference electrode and the test solution. The internal cell resistance was about 1000 ohms so that correction for IR drop through the cell was not necessary.

Dropping mercury electrodes. A number of different dropping mercury electrodes were used. Where necessary, as in calculations involving the Ilkovic equation, the value of $m^{2/3}t^{1/6}$ was determined for the particular capillary in use.

Potentiostat. A potentiostat was constructed using a Heathkit Model EUW-19 Operational Amplifier System. The basic circuit diagram is shown in Figure 1. Amplifier number one was essentially a control amplifier, and number two was a booster or power amplifier. The total system had a capacity of 20 milliamperes at ±50 volts. The variable resistor R was inserted to limit the total current to 20 milliamperes or less during the early stages of electrolysis and, thus, prevent damage to the amplifiers. The coulometer was of the hydrogen-nitrogen type discussed below. The variable 1.5 volt source permitted biasing of the mercury cathode from zero to - 1.5 volts relative to the S.C.E.

Coulometer. A hydrogen-nitrogen gas coulometer of the type described by Page and Lingane was used (25). Two lengths of platinum wire (0.025 inch diameter and two inches long) were sealed into a 10 ml. buret just above the stopcock. The graduated portion of the buret was surrounded by a water jacket, and a typical leveling bulb arrangement was used. The electrolyte was 0.1 M hydrazine sulfate. This coulometer was suitable when not more than 0.5 milliequivalents of material were to be reduced.

Before use, the solution in the buret was presaturated with hydrogen and nitrogen by passing a current of several milliamperes for 10 minutes. The top of the buret was then sealed, the initial reading taken, and the electrolysis begun. During electrolysis, the leveling bulb was periodically lowered as the level of the solution in the buret dropped. At the end of the electrolysis a final reading of volume was taken, along with measurements of the temperature and barometric pressure. Calculations of the total charge passed were based on the assumption that one milliequivalent of charge produced 16.74 ml. of gas at standard temperature and pressure.

The accuracy of the coulometer was checked by passing a constant current of known value through the apparatus for a given length of time. A Sargent Model IV Coulometric Current Source was used to generate the constant current. The results obtained with the coulometer were slightly low, averaging 1.7 per cent error in three trials. However, the accuracy was sufficient for the use intended.

Cell for controlled potential coulometry. A diagram of this cell is shown in Figure 2. Connections to the potentiostat were made as indicated in Figure 1. The salt bridge between the test solution and the S.C.E. was constructed from a length of 4 mm. (o. d.) glass tubing which was drawn out to a fine tip and cut to about 10 cm. length. A portion of a hot solution containing 3 g. of agar, 30 g. of potassium chloride, and 100 ml. of water was drawn into the tubing from the narrow end and allowed to cool. A small rubber tube placed over the other end and filled with saturated potassium chloride was used to make contact with the S.C.E.

All analysis were made in the following manner: About 30 ml. of solution containing adequate amounts of supporting electrolyte and buffer

were placed in the cathode compartment, and the anode compartment was filled to an equal height with an identical solution. The desired potential was then applied while the solution above the mercury was stirred and deaerated. Within twenty to thirty minutes the current had decayed to a constant value of 100 to 400 microamperes. A weighed amount of compound (or known volume of standard, deaerated solution) was then introduced and the electrolysis continued until the same background current was again observed. The total time for complete reduction was usually about one hour.

The entire system of potentiostat, coulometer, and cell was tested by reduction of a known amount of cadmium chloride. The maximum error in the determination of n for three individual results was less than two per cent.

Spectrophotometer. Quantitative spectrophotometric measurements of phenol red solutions were made using a Beckman Model DU Spectrophotometer.

Diaphragm cell. During the course of this study it became necessary to determine the diffusion coefficient of phenol red by a method independent of polarography. For this, the diaphragm cell technique was chosen (6, 11, 30). Two diaphragm cells were fabricated from Corning fine glass frit sealing tubes (Figure 3). Both upper and lower compartments contained one inch, teflon covered, magnetic stirring bars, and the volumes of the compartments were determined by filling with water from a buret. File marks on both necks of the cell were located such that both liquid levels were the same when the upper and lower compartments were filled to these marks. Small glass stopcocks mounted in rubber stoppers were used to seal the compartments. The cell was mounted by means of a universal

clamp about one-half inch from the bottom of a shallow constant temperature water bath which, itself, was supported by a magnetic stirrer. The solutions in the upper and lower compartments were stirred between 80 and 130 revolutions per minute. Rates of stirring faster than about 180 revolutions per minute resulted in a pumping action and were avoided.

Cell constants and diffusion coefficients were calculated from the equation

$$\log\left[(C_f' - C_f'')/(C_o' - C_o'')\right] = -DKt(1/V' - 1/V'')$$

in which

C_o' is the initial concentration in the lower compartment,
V' is the volume of the lower compartment,
C_o'' is the initial concentration in the upper compartment,
V'' is the volume of the upper compartment,
C_f' is the final concentration in the lower compartment,
D is the diffusion coefficient,
C_f'' is the final concentration in the upper compartment,
K is the cell constant, and
t is the time for diffusion (11).

The measurement of diffusion coefficients by this method required that the cell constant K be determined with some reference compound. Tenth normal potassium chloride has been suggested, and its diffusion coefficient in water at 25 °C. was taken as 1.87×10^{-5} $cm^2/sec.$ (30). For the measurement of K, a 0.1000 N potassium chloride solution was deaerated in a suction flask using a water aspirator, transferred to another container, and stoppered tightly. It was then warmed to 25 °C. in a constant temperature water bath. These steps insured the removal of the dissolved air in the solution which might otherwise form small bubbles in the glass frit. The liquid was then transferred to a 50 ml. buret, and working as quickly as possible, the lower cell compartment was filled. Some of the

solution was forced through the glass frit to remove any air bubbles and then the compartment was filled to the file mark and stoppered. The upper compartment was rinsed twice with water and each rinsing removed by suction with a line connected to a water aspirator. After filling the upper compartment to the mark with water (which had also been deaerated), the cell was stoppered and placed in a constant temperature bath over a magnetic stirrer operating between 80 and 130 revolutions per minute. At least four hours were allowed for the formation of a concentration gradient within the frit, after which time the solutions were simultaneously discarded by inversion of the cell and replaced by fresh portions of the original solutions, being careful not to disturb the gradient. The cell was then returned to the water bath and stirred for a suitable length of time. In the case of potassium chloride, this was about forty hours after which the solutions in both compartments were analyzed by titration with 0.1 N silver nitrate (9).

Cell constants for both cells were about unity when the cells were new, but a gradual increase in the constants was observed during the first month of nearly use. A cleaning in hot alcoholic sodium hydroxide resulted in a permanent increase in the cell constants on the order of five to ten per cent and these new values were more nearly constant from week to week. It should be noted that extreme care was necessary when transferring the solutions so as not to disturb the concentration gradient within the frit. With practice, cell constants were obtained that varied by no more than about four per cent (Table 2).

Table 2. Diaphragm cell constants listed in chronological order for cells 1 and 2.

K_1	K_2
1.06	1.14
1.06	1.13
1.10	1.22
1.12	1.20
1.15	1.20
1.27[a]	1.31[a]
1.21	1.34
1.22	1.32
1.24	1.30
1.22	1.29

[a]At this point, the cell was cleaned in alcoholic sodium hydroxide.

To test the accuracy of the cells, the diffusion coefficient of tenth molar calcium chloride was measured by a single determination in each cell. The experimental results for cells 1 and 2 were 1.15×10^{-5} and 1.12×10^{-5} cm^2./sec. respectively, while the literature value was 1.11×10^{-5} cm^2./sec. (12). Thus, for the purpose intended, the cells have the required degree of accuracy.

The procedure for determining the diffusion coefficient of phenol red was the same as that for obtaining the cell constants except that the dye was contained in a solution identical to that used in polarographic work and was permitted to diffuse into a solution containing the appropriate amounts of potassium chloride, phosphate buffer, and Triton X-100. Solutions from the upper and lower compartments were analyzed colorimetrically using standards prepared from the purified phenol red and buffered with tenth molar sodium phosphate and pH 7.2. Absorbances were measured at 445 millimicrons. The cell constant was determined immediately

before and after each experiment and the average of these two constants was used in calculating the diffusion coefficient of phenol red.

The polarographic reduction of phenol red.

The polarography of phenol red, like that of many other materials, is beset with the problem of maxima. In the absence of a maxima suppressor, polarograms of phenol red exhibited very large maxima and other distortions which rendered the wave form unintelligible. These deleterious effects were almost completely eliminated by the addition of Triton X-100 at a concentration of 0.015 per cent. Excessive foaming of solutions during deaeration owing to the presence of Triton X-100 was eliminated by the addition of one or two drops of a solution of 1-octanol in ethanol (1:4 by volume).

A typical polarogram of phenol red at pH 7.0 is shown in Figure 4. Unless otherwise stated, all polarograms were obtained with millimolar phenol red at room temperature in a solution of 0.1 M potassium chloride, 0.1 M sodium phosphate buffer (about pH 7.0), and 0.015 per cent Triton X-100. Two reduction waves were observed under all circumstances. The height of the first wave was greater than that of the second by a factor of 1.1 to 2.5. The magnitude of the factor depended on the temperature, the height of the mercury in the standpipe, and the concentrations of phenol red and Triton X-100. Attempts to meaningfully correlate changes in this factor with the above mentioned variables were not successful. An explanation for the inequality of the wave heights was not readily apparent, prompting a further investigation.

The rate of reduction of most substances at the D.M.E. is governed by

the rate at which the material diffuses to the electrode surface. Some compounds, however, may undergo a chemical reaction before or after electron transfer and the rate of this chemical reaction, along with diffusion, can determine the wave height. This "kinetic" control may or may not result in a linear relationship between concentration and the limiting current. The variation in the limiting currents of the first wave, second wave, and the total wave height with changes in the phenol red concentration is shown in Figure 5. As can be seen, strict proportionality exists only between the concentration of phenol red and the total wave height. This suggests (but does not prove) that the overall reduction is diffusion controlled. The non-linearity of the individual wave heights, however, does point to some type of kinetic complication.

Actual proof of diffusion control for the overall reduction was obtained by determining the diffusion coefficient (D) of phenol red, both polarographically and by the diaphragm cell technique (Table 3). By comparing the results of these two independent methods, it was readily apparent that the overall reduction was diffusion controlled and involved two electrons.

Table 3. The diffusion coefficient of phenol red[a] determined by the diaphragm cell (D.C.) technique, and by the polarographic method (P).

Conc. phenol red, M X 10^3	Method	Diffusion coefficient, $cm^2/sec.$ X 10^6 n = 1[b]	n = 2[c]	by D.C.
1.14	P	7.4		
1.14	P	8.2		
1.14	P	6.7	5.0	
1.04	P	7.5	5.4	
2.08	P	8.7	5.6	
	P	avg. 7.7	5.3	
1.00	D.C.			5.3
1.00	D.C.			6.3
1.20	D.C.			5.4
1.20	D.C.			5.4
1.08	D.C.			4.9
1.08	D.C.			4.7
	D.C.			avg. 5.3

[a]The diffusion coefficients were determined in solutions of 0.1 M potassium chloride, 0.1 M phosphate buffer (pH 7.2), and 0.015 per cent Triton X-100.

[b]Values of D in this column were calculated using the limiting current of the first wave and assuming a one electron transfer.

[c]Values of D in this column were calculated using the limiting current of the second wave and assuming a two electron transfer.

The excellent agreement between the polarographic and diaphragm cell methods is undoubtedly fortuitous, considering the precision of the measurements. However, the value of 5.3 X 10^{-6} $cm^2/sec.$ is quite close to that for phenolphthalein (5.2 X 10^{-6} $cm^2/sec.$) as determined by Kolthoff and Lehmicke (18). This would be expected for molecules of similar structure and molecular weight.

The variation of the half-wave potential of phenol red with pH (Figure 6) was studied using phthalate, phosphate, and ammonium chloride-

ammonium hydroxide buffer systems between pH 2.5 and 11.4. The half-wave potential of the first wave was a linear function of the pH, up to pH 10.6 with a slope of 0.069 volt per pH unit. This strongly suggested a reversible, one electron transfer involving one hydrogen ion, because the theoretical slope for such a system is 0.059 volt per pH unit. The lack of diffusion control for the first wave does not invalidate the previously stated conclusion, because the theoretical relationship between half-wave potential and pH does not require diffusion control as a prerequisite for validity.

The half-wave potential of the second wave was - 1.20 volt versus the S.C.E. and was essentially independent of the pH.

A further test for reversibility was performed by analyzing polarograms according to the equation

$$E = E_{\frac{1}{2}} - (0.0591/n)\log[i/(i_d - i)].$$

Plots of E versus $\log[i/(i_d - i)]$ were made for the first wave at varying values of pH, and the slopes of the lines and n values were computed (Table 4). Again, in the derivation of this equation, no assumptions were made concerning diffusion control. Thus, on the basis of the data in Table 4, it can be said with certainty that the first reduction wave represents a reversible one electron reduction.

Table 4. Slope of the line of E versus $\log[i/(i_d - i)]$ for the first wave at various values of pH[a].

Buffer system	pH	Slope	n
Phthalate	4.7	0.0564	1.05
Phosphate	7.0	0.0572	1.03
Ammonium chloride-Ammonium hydroxide	9.0	0.0565	1.05
Sodium hydroxide	13.0	0.062	0.95

[a]All solutions contained 0.75 M potassium chloride, 0.1 M buffer, and 0.015 per cent Triton X-100.

Similar plots for the second wave gave values for n which were considerably less than unity. Thus, the second wave represents an irreversible, one electron reduction.

The limiting wave heights of simple metal ions and compounds, the reductions of which are not controlled by kinetic factors, have temperature coefficients on the order of 1.3 to 2.3 per cent per degree (19). Wave heights which are limited or controlled by kinetics ordinarily show a great dependence on temperature. The temperature coefficients are usually much higher and the plots of limiting current versus temperature are generally nonlinear. Since the inequality of the wave heights of phenol red pointed to some type of kinetic control, a temperature study of the reduction was made (Figure 7). The range involved was approximately zero to forty-five degrees. Above this temperature, stirring of the solution due to thermal gradients became excessive and resulted in distorted polarograms. It is apparent from Figure 7 that the limiting currents increased linearly with temperature. In addition, the temperature coefficients of the three lines were all within 1.62 to 1.79 per cent per degree. These results were

totally unexpected, and can only be explained by assuming that either (a) kinetic complications do not exist, (b) the temperature range over which the studies were made was not sufficient to reveal kinetic complications, or (c) some other unknown effect is operative.

The controlled potential electrolysis of phenol red.

Polarograms of phenol red indicated that, by controlled potential coulometry at the appropriate potentials, an accurate value of n could be obtained for each of the reduction waves. For example, it is apparent from Figure 4 that electrolysis at - 1.40 volt versus the S.C.E. should yield an n value of two (because it was previously established by measurement of the diffusion coefficient that the overall reduction involves two electrons), while reduction at - 0.80 volt would be expected to give a value somewhat less than two. The results from the coulometry of phenol red at a mercury pool cathode are shown in Table 5.

There can be no doubt that the electrolysis of phenol red involved two electrons whether one electrolyzed at a potential corresponding to the limiting current of the first or the second polarographic wave. In both cases, the reduction product was the same, as evidenced by identical infrared and nuclear magnetic resonance spectra. The electrochemical reduction product was phenolsulfonephthalin, the same compound formed by the chemical reduction of phenol red with zinc.

Because it seemed possible that behavior of this sort might be characteristic of the phthaleins and sulfonephthaleins in general, a brief investigation of phenolphthalein and thymol blue was made. Phenolphthalein was chosen because its reduction in base proceeds in two steps, and its

Table 5. The controlled potential coulometry of phenol red.

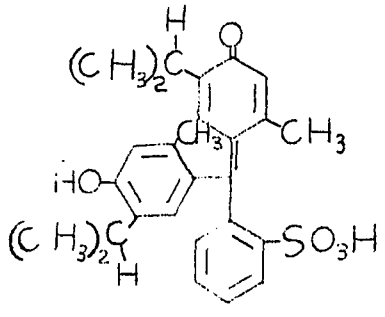
Trial	Applied potential, volts versus S.C.E.	pH	n
1	- 1.40 (Second wave)	7.2	2.21[a]
2	- 1.40 (Second wave)	7.2	2.17
3	- 0.90 (First wave)	7.2	2.20
4	- 0.90 (First wave)	7.2	2.16
5	- 0.90 (First wave)	9.0	2.02[b]
6	- 1.00 (First wave)	9.0	2.05
7	- 1.40 (Second wave)	8.9	2.02[c]
8	- 0.90 (First wave)	8.9	2.04

[a]Trials one through four were made in 0.1 M potassium chloride, 0.1 M sodium phosphate, and 0.015 per cent Triton X-100.

[b]Trials five and six were made in 0.1 M potassium chloride, 0.05 M ammonium sulfate-ammonium hydroxide buffer, and 0.015 per cent Triton X-100.

[c]Trials seven and eight were made in a solution similar to that used in five and six except that no Triton X-100 was present.

polarographic behavior is otherwise well known. Thymol blue was chosen because its polarographic reduction also proceeds in two steps of greatly different half-wave potentials at about pH 7. Thymol blue has the following structure:



The results of the controlled potential coulometry of these compounds are summarized in Table 6.

Table 6. The controlled potential coulometry of phenolphthalein and thymol blue.

Compound	Applied potential, volts versus S.C.E.	pH	n
Phenolphthalein	- 1.50 (Second wave)	10.85	2.03[a]
Phenolphthalein	- 1.10 (First wave)	10.85	2.16
Thymol blue	- 1.40 (Second wave)	7.10	1.89[b]
Thymol blue	- 1.40 (Second wave)	7.10	1.90
Thymol blue	- 0.80 (First wave)	7.10	1.74

[a]Phenolphthalein was reduced in a solution of 0.2 M potassium chloride and 0.4 M sodium carbonate adjusted to pH 10.85 with hydrochloric acid.

[b]Thymol blue was reduced in a solution of 0.2 M potassium chloride and 0.4 M potassium dihydrogen phosphate adjusted to pH 7.1 with potassium hydroxide.

Phenolphthalein and thymol blue behaved very much like phenol red. Reduction at a potential corresponding to the limiting current of the first wave yielded the same results as reduction at a potential corresponding to the limiting current of the second wave. This behavior is probably general for both the phthaleins and sulfonephthaleins, and indicates that the product from the one electron transfer is unstable with respect to some type of chemical reaction, probably leading to the fully reduced phthalin or sulfonephthalin. The reaction occurs both in the presence and absence of Triton X-100 (This has been confirmed for phenol red and is presumably true for the electrolysis of phenolphthalein and thymol blue, for which no maxima suppressor was used).

The cyclic voltammetry of phenol red.

After most of the electrochemical work had been completed, an apparatus became available for cyclic voltammetry studies. Because

reversible and irreversible systems may be readily distinguished with this technique, it was thought profitable to study the cyclic voltammograms of phenol red.

In cyclic voltammetry, as in polarography, the current at an electrode is recorded as a function of the applied potential. Unlike polarography, however, the potential is scanned very rapidly (ie., one volt per second) to some predetermined negative potential, at which point the direction of the scan is reversed. Substances which are reversibly reduced give rise to cathodic current peaks as the potential is scanned in the negative direction, and to anodic peaks on the reverse cycle. Species which are irreversibly reduced give rise to only cathodic peaks. This method is particularly useful in the study of transient species.

A cyclic voltammogram of phenol red is shown in Figure 8. The potential was scanned from - 0.40 to - 1.00 volt, and, thus, only one reduction wave was seen. The scan rate was 0.25 volt per second. The presence of both cathodic and anodic peaks immediately confirmed the existence of an intermediate which was reversibly reduced and oxidized. The total area under the cathodic portion of the curve was greater than that under the anodic portion, indicating that only a fraction of the reduced species was oxidized.

A cyclic voltammogram covering the region from - 0.40 to - 1.60 volt is shown in Figure 9. The cathodic and anodic peaks of the first wave were seen as before. In addition, a cathodic peak corresponding to the second reduction wave was seen, but no anodic peak appeared opposite. This confirms that the second wave constitutes an irreversible reduction, a conclusion drawn earlier from polarographic data.

Discussion

The first reduction wave of phenol red is reversible. This is indicated by plots of E versus $\log [i/(i_d - i)]$ which have slopes near the theoretical value of 0.0591 volt for reversible systems. In addition, the cyclic voltammogram of phenol red has peaks indicating reduction on the cathodic sweep and oxidation on the anodic sweep at potentials corresponding to the first reduction wave. Evidence for the involvement of one hydrogen ion in the reduction is derived from the plots of $E_{\frac{1}{2}}$ versus pH, where a slope of 0.069 volt per pH unit is observed (Reversible, one-electron reductions involving one hydrogen ion show $E_{\frac{1}{2}}$ dependencies of 0.0591 volt per pH unit).

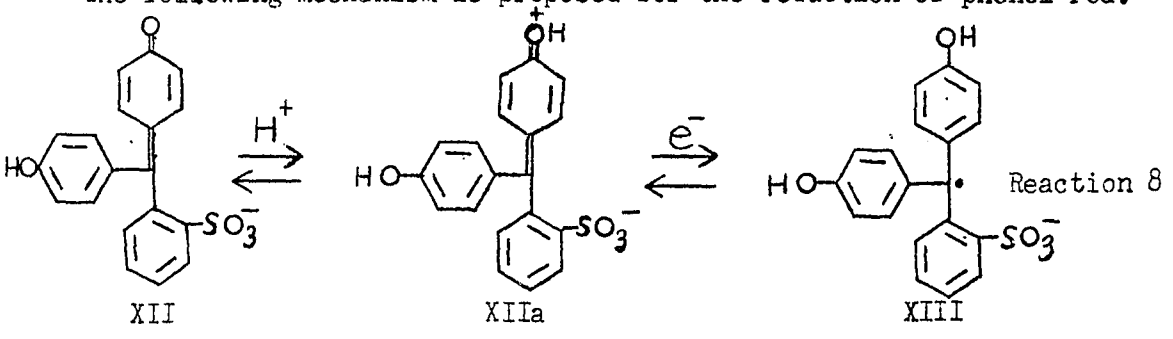
The second reduction wave is shown to be irreversible by the non-integral value of n calculated from the plot of E versus $\log [i/(i_d - i)]$. The irreversibility is confirmed by the cyclic voltammogram which lacks an oxidation peak, opposite the reduction peak, for the second wave. The half-wave potential for this wave was found to be - 1.20 volt and practically independent of the pH.

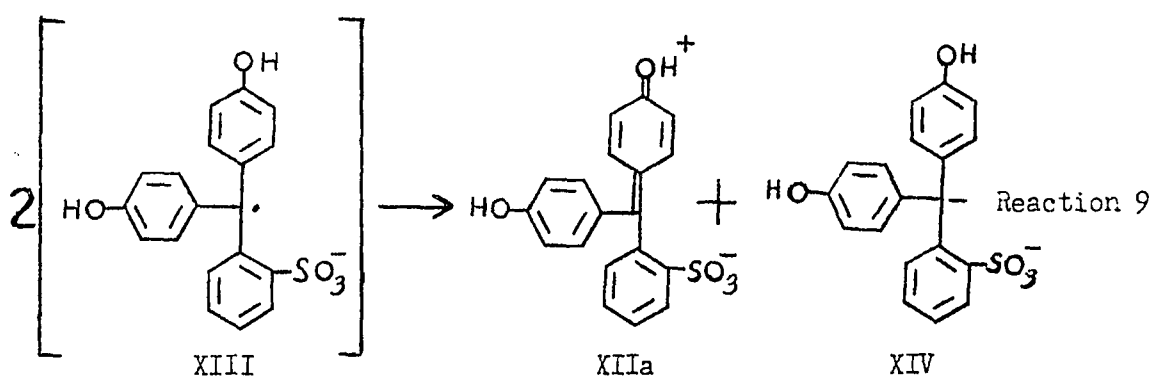
Phenolsulfonephthalin is the final product of electrochemical reduction. The product is obtained by electrolysis at potentials corresponding to the limiting currents of both the first and second waves. The product is identical to that obtained by the chemical reduction of phenol red with zinc dust.

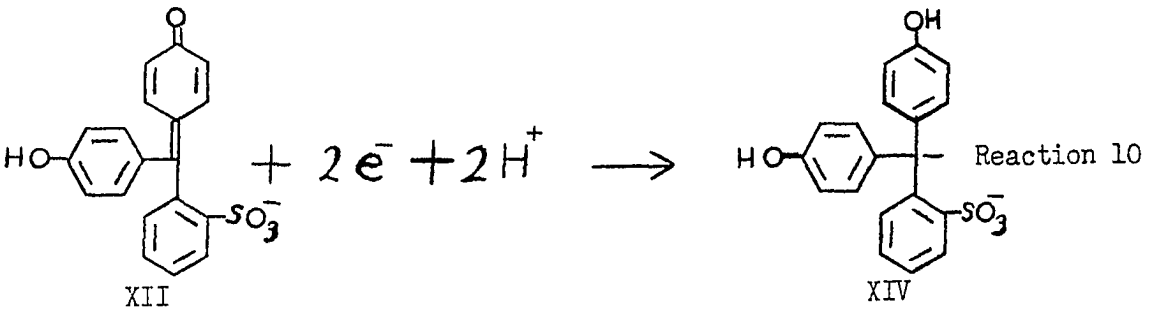
The height of the first wave is greater than can be accounted for by simple diffusion of phenol red from the bulk solution to the electrode surface. Furthermore, electrolysis at a potential corresponding to the

limiting current of the first wave invariably results in the transfer of two electrons per molecule, and the formation of phenolsulfonephthalin. These facts can only be explained by assuming that a chemical reaction follows the transfer of the first electron. Such a reaction is likely to be a second order, homogeneous disproportionation, similar to that observed for U(V). As discussed earlier, the reduction of U(VI) to U(V) is followed by the disproportionation of a considerable amount of U(V) to U(IV) and U(VI). The U(VI) regenerated by this reaction is reduced again, causing the reduction wave to be greater than expected for a normal, one-electron transfer. The height of the reduction wave has a greater-than-normal temperature dependence, and does not increase linearly with the U(VI) concentration. It is apparent that the electrochemical behavior of phenol red closely parallels that of U(VI).

The following mechanism is proposed for the reduction of phenol red:

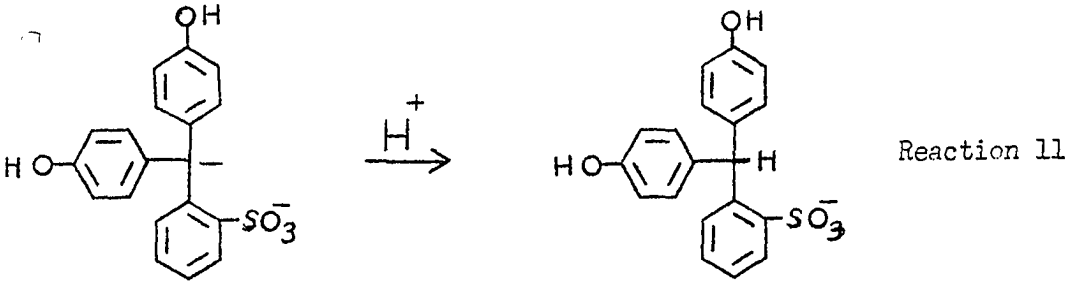






XII XIV

Reaction 10



XIV XIVa

Reaction 11

The species XII is protonated (XIIa) and reversibly reduced to the free radical XIII. The free radical disproportionates, giving the fully reduced product, XIV, and regenerating the protonated form XIIa. Practically all of the XIIa is reduced again, and results in the enhancement of the wave height. Thus, it is apparent why exhaustive electrolysis at a potential corresponding to the limiting current of the first wave results in the transfer of two electrons per molecule. This mechanism also accounts for the unequal areas of the cathodic and anodic peaks in the cyclic voltammogram of phenol red.

If, as indicated, the disproportionation is bimolecular, then the rate of the reaction should be proportional to the square of the concentration of XIII. Because the amount of XIII formed at the electrode

increases with the concentration of phenol red, a plot of wave height versus phenol red concentration for the first wave should be non-linear, curving upward at high concentrations. This was found to be the case (Figure 5).

At a potential of about - 1.20 volt, two electrons are transferred (Reaction 10). The overall process is irreversible and yields form XIV, which immediately gains a proton (Reaction 11).

Second order rate constants for reactions of this type have been calculated by the method of Orleman and Kern (22). Their theoretical calculations indicated that a plot of i/i_d versus i_d (where i is the observed limiting current of the first wave, and i_d is the theoretical diffusion controlled current) would be a straight line with a slope proportional to the rate constant. They verified this for the U(VI) system, but found that the results were correct only to within one order of magnitude. When similar plots were made for phenol red, the points were scattered rather badly. This probably indicates that the experimental conditions were not rigidly controlled, particularly with respect to the concentration of Triton X-100. The experiment was not repeated because, even under the best circumstances, the value of the constant would only be a rough estimate. A number of other electrochemical techniques are available which may be used for this determination, and are inherently more accurate.

The apparent lack of temperature dependence of the first wave is quite unusual. The species XIII is, of course, a free radical and can normally be expected to react quite rapidly. It is, therefore, somewhat surprising that two polarographic waves are seen, for if XIII reacted rapidly with

itself, only one wave would be seen. If it did not react at all, then two waves of equal height would appear. One explanation is that the free radical has little or no activation energy for disproportionation. However, it is probable that the electron may be transferred only when the radicals are correctly oriented with respect to each other at the moment of collision. This "steric effect" means that not all collisions between radicals result in electron transfers. Increasing the temperature merely increases the number of collisions (and the number of effective collisions in proportion), and no unusual temperature dependency is seen.

On the other hand, there may be a finite, but small, activation energy for disproportionation. As the temperature is increased, the rate of disproportionation increases. At the same time, however, the rate of diffusion of regenerated IIa away from the electrode increases. Thus, not all of the IIa produced by Reaction 9 is reduced. The overall result is that the two effects tend to cancel each other, and a normal temperature dependency is observed.

Thirdly, the temperature range over which the studies were made (zero to 45 °C.) may not have been sufficient to reveal any unusual behavior. However, judging from other reactions, this explanation appears less plausible than either of the previous two.

The behavior of phenol red, phenolphthalein, and thymol blue suggest that similar disproportionations would probably occur during the reduction of all of the phthalein and sulfonephthalein indicators. By application of such methods as chronopotentiometry with current reversal or electrolysis at a thin layer electrode, it should be possible to obtain accurate values of the second order disproportionation constants.

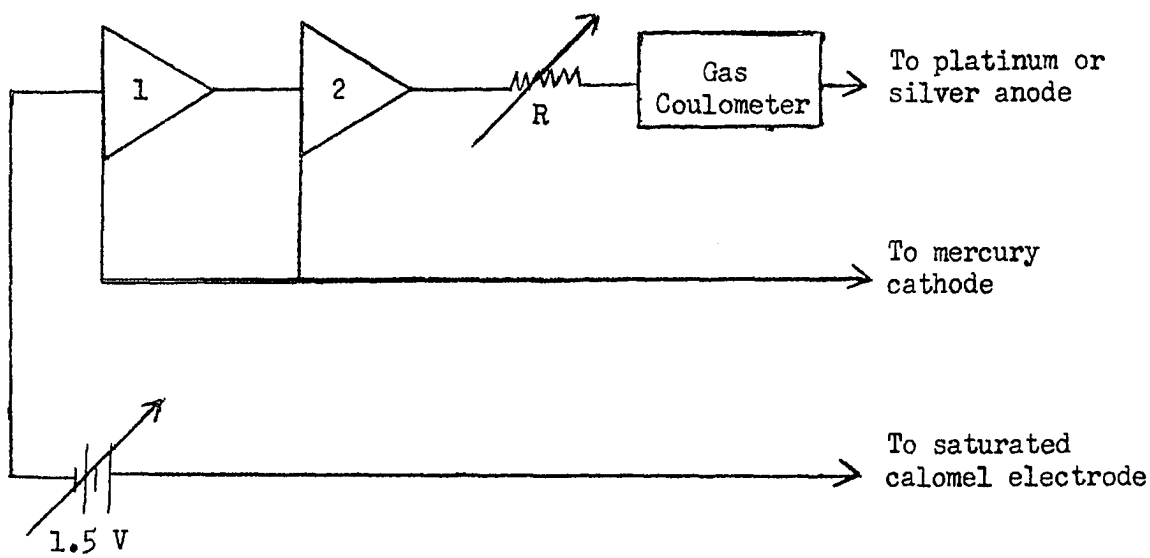


Figure 1. Potentiostat for controlled potential electrolysis.

1 Control amplifier

2 Booster amplifier

R Current limiting resistor

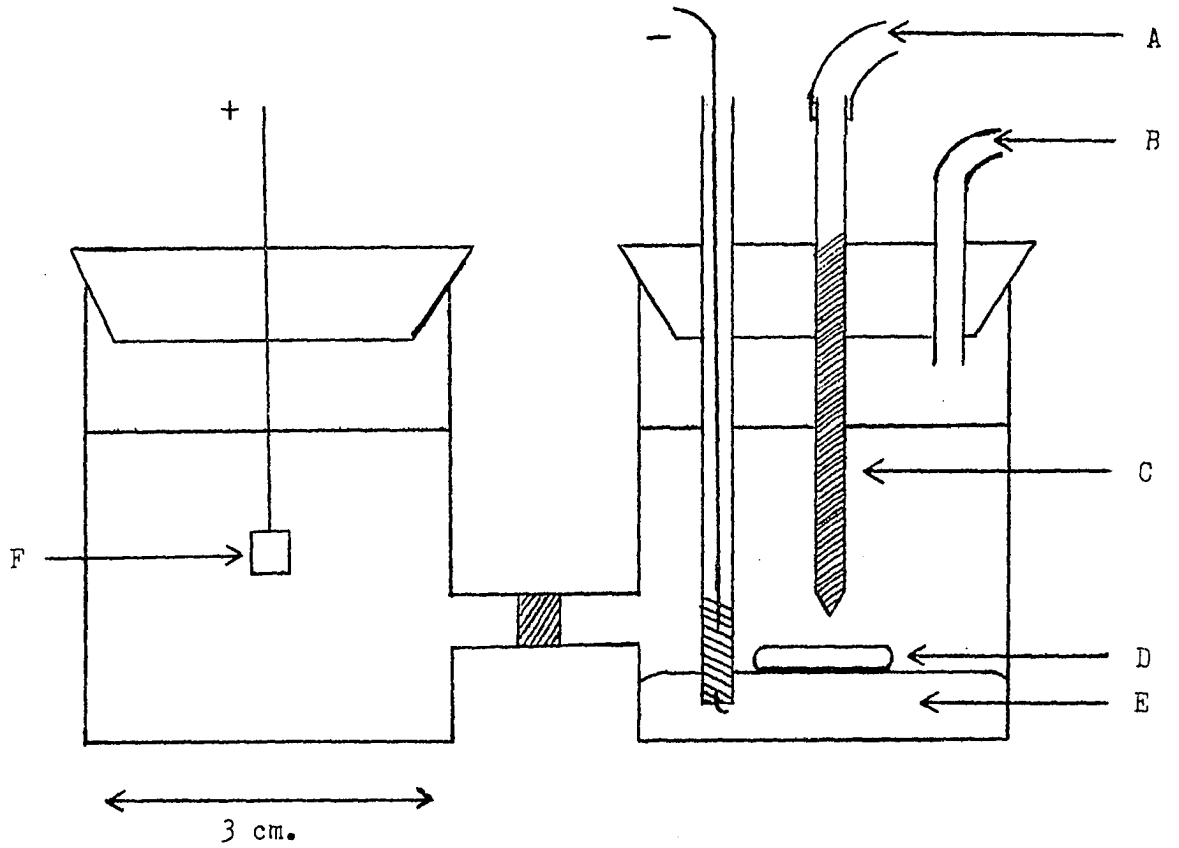


Figure 2. Cell for controlled potential electrolysis.

A. Saturated KCl salt bridge
B. Nitrogen inlet tube
C. Agar plug
D. Magnetic stirring bar
E. Mercury pool
F. Platinum anode

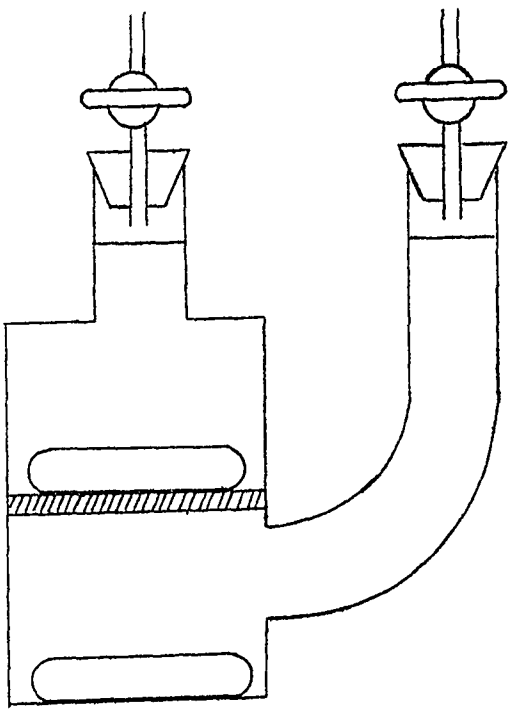


Figure 3. Diaphragm cell for the measurement of diffusion coefficients. Actual size.

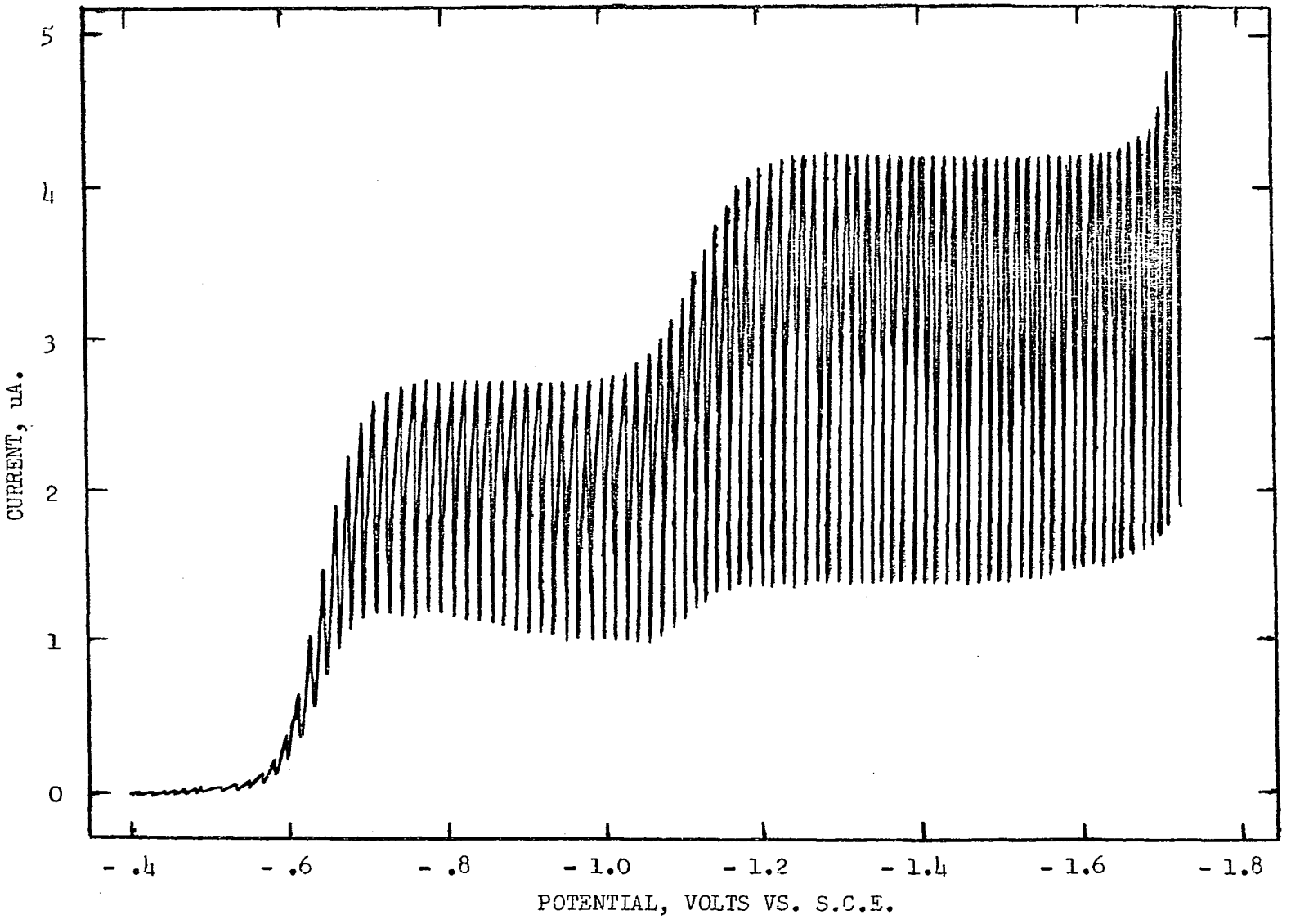


Figure 4. Polarogram of millimolar phenol red at pH 7.0.

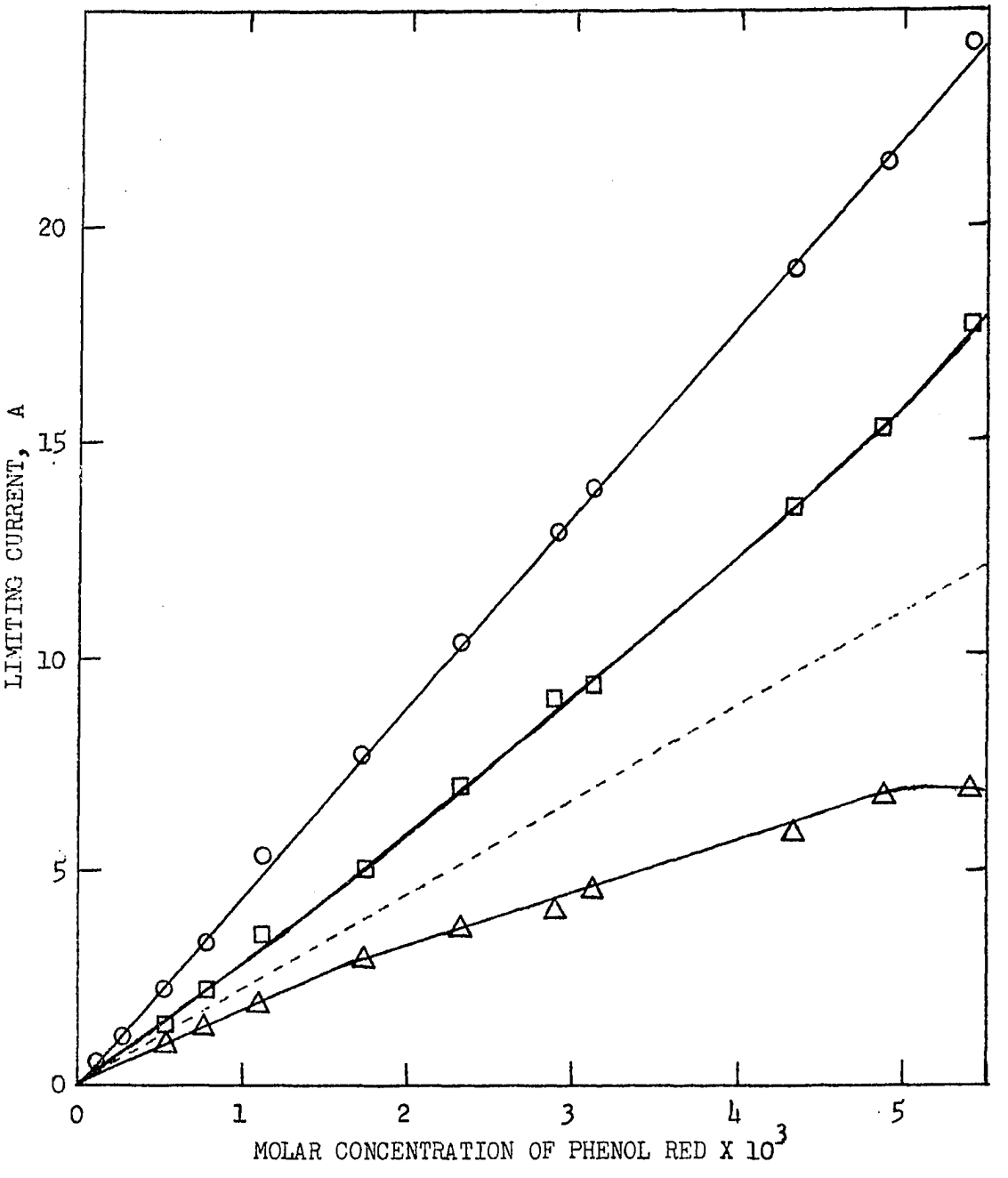


Figure 5. Concentration dependence of limiting currents.

□ First wave

△ Second wave

○ Total wave

---Theoretical wave height for a one-electron transfer

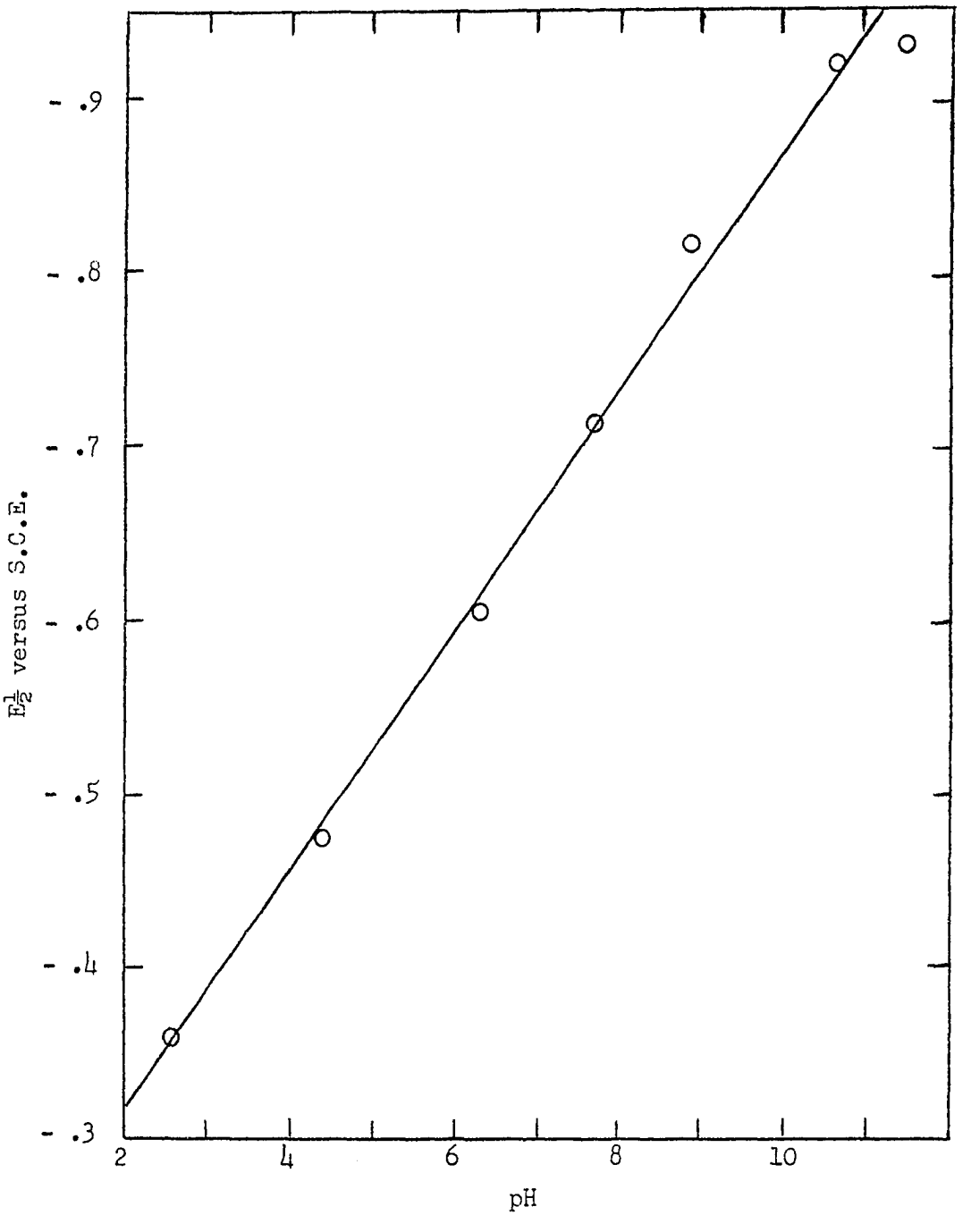


Figure 6. Half-wave potential of the first wave as a function of pH.

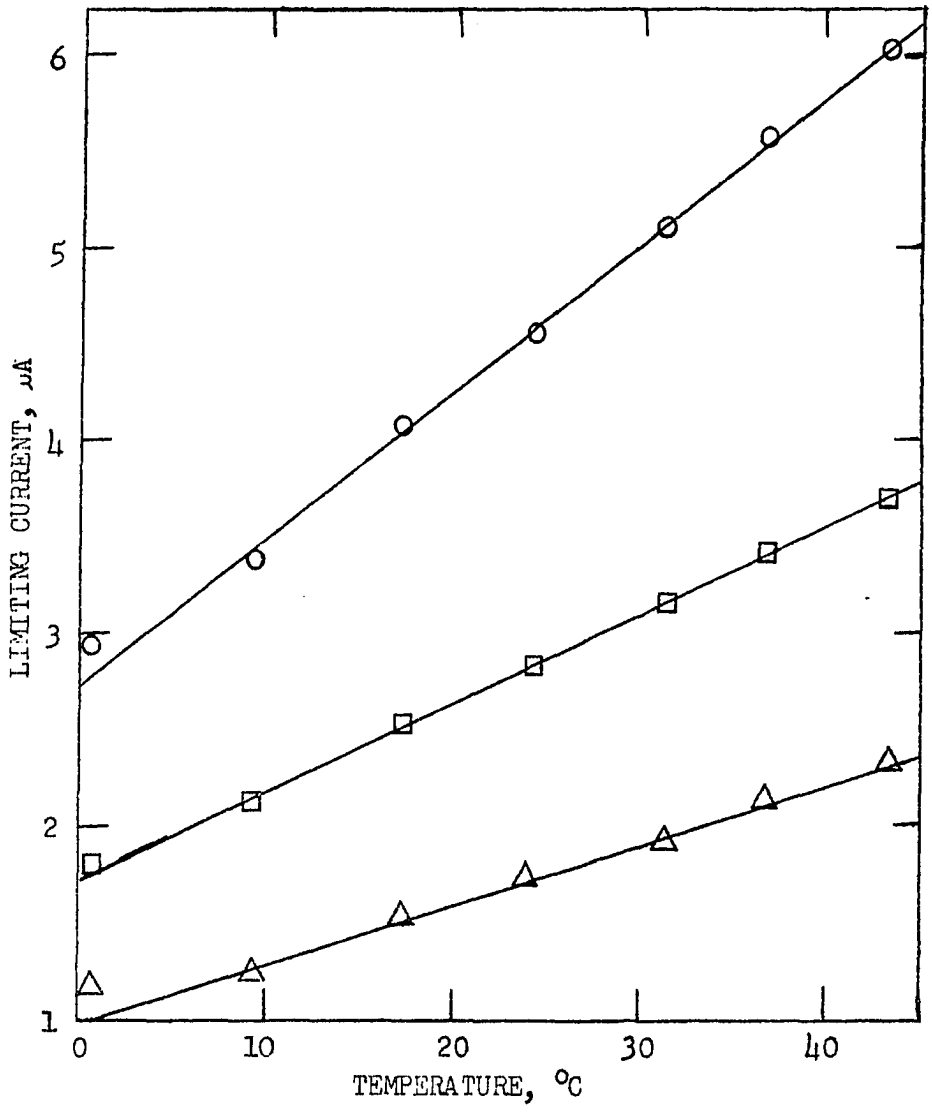


Figure 7. Temperature dependence of limiting currents.

□ First wave

△ Second wave

O Total wave

Figure 8. The cyclic voltammogram of millimolar phenol red. The scan rate was 0.25 volt/sec.

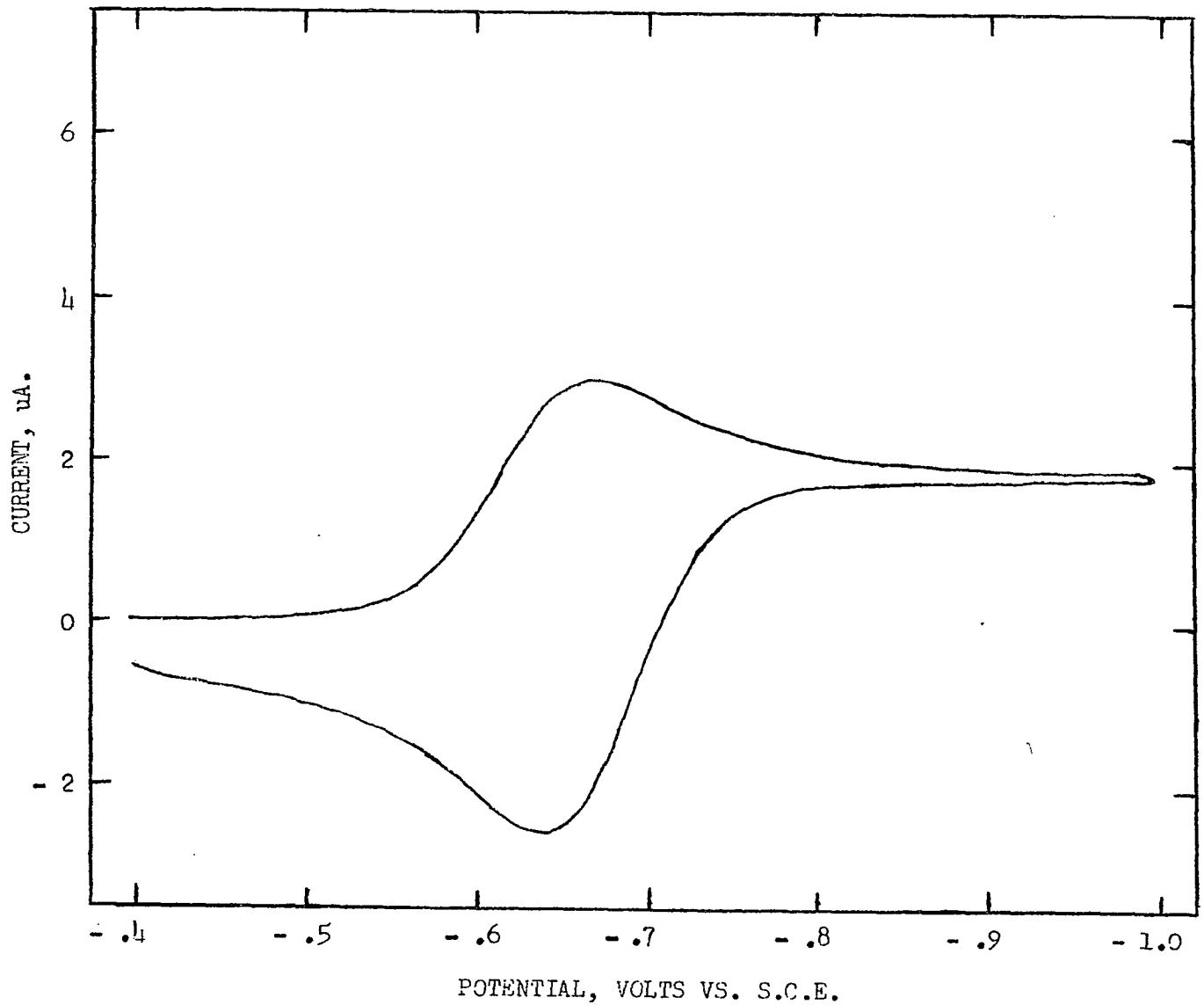
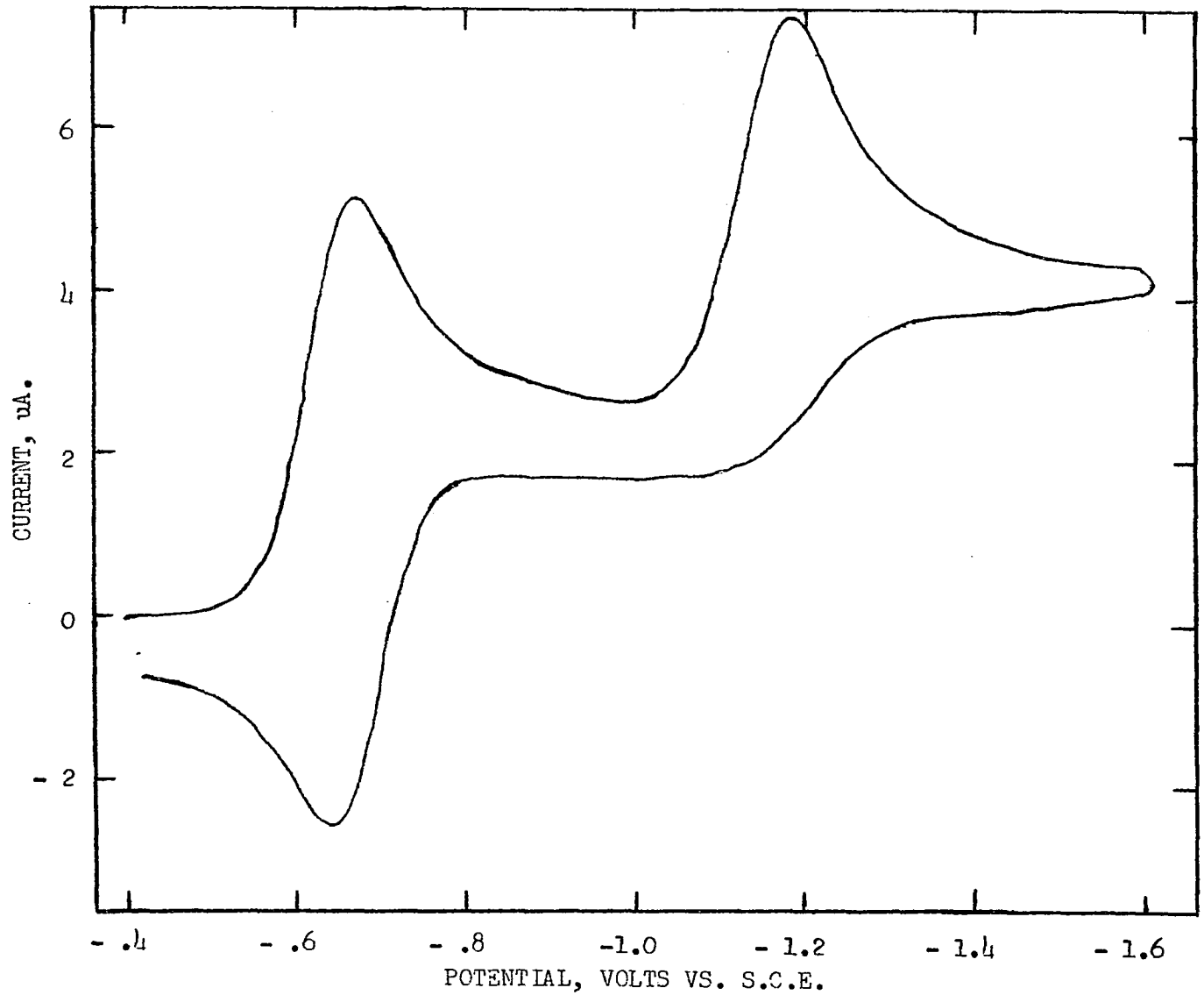

6
4
2
0
- 2
CURRENT, uA.
- .4
- .5
- .6
- .7
- .8
- .9
- 1.0
POTENTIAL, VOLTS VS. S.C.E.

Figure 9. The cyclic voltammogram of millimolar phenol red. The scan rate was 0.25 volt/sec.


6
4
2
0
- 2
CURRENT, uA.
- .4
- .6
- .8
-1.0
- 1.2
- 1.4
- 1.6
POTENTIAL, VOLTS VS. S.C.E.

NUCLEAR MAGNETIC RESONANCE STUDIES OF PHENOL RED, PHENOLSULFONEPHTHALIN, AND SOME RELATED COMPOUNDS

Introduction

As mentioned earlier, the reduction products of phenolphthalein, phenol red, and fluorescein have been shown to have the triphenylmethane structure, and it is very probable that similar structures would result from the reduction of other members of the phthalein, sulfonephthalein, and fluorescein series. During a routine study of phenolsulfonephthalin, a nuclear magnetic resonance (NMR) spectrum of the compound was obtained for comparison with the spectrum of phenol red. While the spectrum of phenol red was in agreement with the accepted structure, that of phenolsulfonephthalin was complex and difficult to interpret. In an attempt to clarify the situation, spectra of phenolphthalein and phenolphthalin were compared. Again, however, the spectrum of the reduced form was unexpectedly complex. As a result, a general study of related compounds was initiated.

The results of the study showed that the spectra of compounds known to have a closed lactone or sultone ring (for example, phenolphthalein in neutral solution) are characterized by an unresolved multiplet integrating to four protons in the region from 7.4 to 8.2 ppm. On the other hand, spectra of compounds known to exist in the open form (phenolphthalein in basic solution, for example) lack the multiplet. It has been replaced by three groups of smaller peaks in the region from 7.0 to 8.2 ppm with relative areas of 1:2:1. In most instances, color formation and the presence of the 1:2:1 pattern occur simultaneously, as expected. Thus, the appearance of the 1:2:1 pattern is shown to be strong evidence for the existence of the open form for both the phthalein and sulfonephthalein

series of indicators. In a few instances, both the open and closed forms are found to exist in tautomeric equilibrium.

The present work is concerned with illustrating the use of NMR spectroscopy as a means of distinguishing between open and closed forms of phthalein and sulfonephthalein indicators. To accomplish this, a number of closed ring compounds are presented, along with evidence (not from NMR studies) for the existence of the closed ring. In addition, some open ring compounds are presented. These two classes of compounds are then used as models from which generalizations about the spectra of each class can be made.

Experimental Work

Reagents.

The diacetate of phenol red. The method of Orndorff and Sherwood (23) was used to synthesize this compound from phenol red. The compound melted sharply at 132-133 °C., widely different from the reported value of 165 °C.

The monoammonium salt of phenol red. The salt was obtained by evaporating to dryness a solution of phenol red in excess ammonium hydroxide (23).

The disodium salt of phenol red. The salt was obtained by evaporating to dryness a solution of phenol red containing the theoretical volume of standard sodium hydroxide (23).

Phenolphthalin. This compound was synthesized by the method of Baeyer (1). It was pure white, gave no color on dissolution in dilute base,

and melted at 239-240 °C. (Baeyer reported 237.6-238.5 °C.).

Phthalide. Phthalide was synthesized by the reduction of phthalic anhydride with metallic zinc (27).

Tetrabromophenolsulfonephthalin. This material is the reduced form of bromphenol blue (tetrabromophenolsulfonephthalein). Two hundred to four hundred milligrams of bromphenol blue along with 1-2 g. of zinc dust were added to about 100 ml. of water. The mixture was boiled until the solution became colorless, and was then filtered through coarse filter paper to remove the zinc. The filtrate was placed in a small, thick-walled erlenmeyer flask and evacuated by aspiration while the flask was warmed over a steam cone. The solid zinc salt was obtained upon complete removal of the water. The freshly prepared compound was light gray in color but was rapidly oxidized back to the blue zinc salt of bromphenol blue.

The monosodium salts of bromthymol blue and bromphenol blue. These salts were obtained from the J. T. Baker Chemical Co., Phillipsburg, N. J.

Solvents for NMR studies. The dimethylsulfoxide (DMSO) was reagent grade and was not deuterated. No attempt was made to remove the last traces of water before use. The deuteroacetone and deuterium oxide were both 99.5 per cent pure.

Other chemicals. All other indicators were obtained from the Hartman-Leddon Company, Philadelphia, Penn. Other chemicals were reagent grade.

Apparatus.

Nuclear magnetic resonance spectrometer. Spectra were obtained using the Varian Associates A60 Nuclear Magnetic Resonance Spectrometer.

This is a 60 megacycle instrument operating at a magnetic field strength of about 14,092 gauss. Peak areas were measured using the built-in integrator.

Cells. Clear, precision ground glass NMR tubes, 0.5 cm. o.d., were used.

Reference standards. Tier's salt was used as the standard in D_2O solutions. In deuteroacetone and DMSO, tetramethylsilane (TMS) was the standard. Chemical shifts are reported in δ (parts per million) units downfield from the standard.

Closed ring compounds.

Whether a particular indicator exists in the closed ring form will depend upon the solvent, and, in aqueous solutions, upon the pH. Because indicators are primarily used in aqueous solutions, almost all previous experimental work (such as the determination of color change intervals and acid dissociation constants) was done in the medium. Caution must be excerised when applying this data to the nonaqueous solvents used in the NMR studies. However, one important rule seems to apply in both aqueous and nonaqueous solvents: When a solution of the indicator is colored, the quinonoid (open) form is present. This is because the fundamental process responsible for color is the establishment of a system of conjugated double bonds, and this occurs in both aqueous and nonaqueous solvents. The absence of coloration in these compounds, therefore, is a strong indication that the lactone or sultone form is present.

The phthaleins investigated were phenolphthalein, thymolphthalein, and o-cresolphthalein. They were chosed because of their ready availability, though it appears that any phthalein would have sufficed as a model of a

closed ring compound. The primary evidence for the closed ring structure of the phthaleins is the fact that neutral and weakly acidic solutions of these compounds are colorless, indicating the absence of an extensively conjugated system. In addition, they are very weak acids with dissociation constants on the order of 10^{-8} or less. This value is much too small for a free carboxylic acid which would have to be present in an open ring structure.

Of the sulfonephthaleins, very few have been obtained as colorless solids, indicative of a closed ring structure. However, a colorless form of bromphenol blue has been isolated (15). Solutions of this dye in deuteroacetone are faintly yellow, and, therefore, could not contain an appreciable amount of the open form. In this solvent, bromphenol blue is a suitable model of a closed ring compound.

The diacetate of phenol red was synthesized and found to be colorless both in solution and as a solid when freshly prepared. The infrared spectrum (KBr pellet technique) contains fairly strong bands at 1325 and 1359 cm^{-1} which are typical of a covalent sulfonate ($R-O-SO_2-R$). Further support for the closed form comes from the fact that it is difficult to draw a reasonable, open-ring structure for the compound.

Open ring compounds.

The monoammonium and disodium salts of phenol red (both of which are highly colored) exist in the open form. This is evident from a study of the properties of the parent acid. Aqueous solutions of phenol red are known to exist, at least partially, in the open form. The yellow color

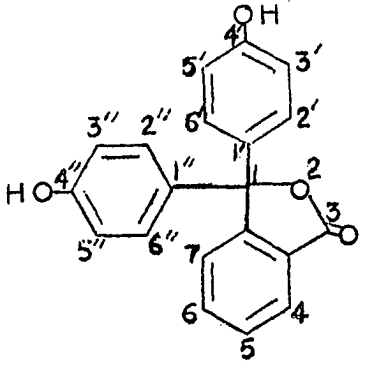
of the solutions, and, more significantly, the fact that it is a strong acid attest to this fact. Its acid strength can only be accounted for by assuming the presence of a free sulfonic acid group, which, in turn, requires the existence of an open ring. Neutralization of either one or both protons will lead to the formation of a salt with the open ring structure.

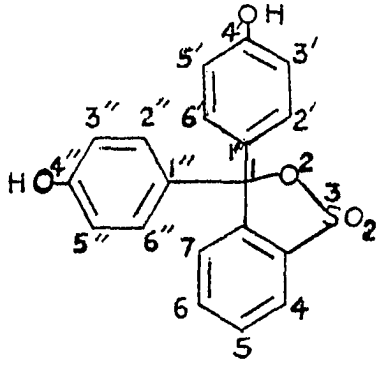
For reasons similar to those mentioned above, the monosodium salts of bromphenol blue and bromthymol blue are also suitable models of open ring compounds.

As the salts of phenolphthalein are rather difficult to isolate, and undergo considerable hydrolysis in solution, the open form was obtained by dissolving phenolphthalein in a solution of NaOD in D_2O. The dark red solution rapidly faded to a much lighter red, indicating that the colorless carbinol species was present as the major constituent. This species differs from the previously mentioned compounds in this class in that the valence of the central carbon atom is saturated.

Discussion of the spectra of individual compounds.

The following numbering system will be used in the discussion of the phthaleins and sulfonephthaleins:





The diacetate of phenol red. The NMR spectrum of this compound in deuteroacetone is shown in Figure 10, and the interpretation of the spectrum is straightforward. The sharp singlet at 2.23 ppm represents the six identical protons of the two acetate groups. The region from 7.05 to 7.5 ppm contains a quartet integrating to eight protons. The peaks are coupled with a coupling constant of 9 cycles per second (c/s), which is characteristic of ortho protons. This part of the spectrum represents an AB (para-substituted) pattern, A and B being protons of relatively close chemical shift which are spin-spin coupled. Protons 2', 2", 6', and 6" are of type A while 3', 3", 5', and 5" are of type B. The symmetry of the quartet and the sharpness of the 2.23 ppm peak confirm that the two phenolate rings are identical. The multiplet in the region from 7.6 to 8.1 ppm (four protons) can only be assigned to protons 4, 5, 6, and 7.

Phenolphthalein. The spectrum of phenolphthalein in DMSO (Figure 11) shows marked similarities to that of the diacetate of phenol red. The symmetrical AB quartet (eight protons centered at 7.02 ppm with a coupling constant of 9 c/s) is again present and may be readily assigned to the hydrogen atoms on the identical phenolic rings. The region from 7.4 to 8.1 ppm contains a series of peaks integrating to four protons. This multiplet must arise from protons 4, 5, 6, and 7. The two hydroxyl protons are relatively acidic and appear downfield at 9.68 ppm. Addition of one drop of D_2O to this sample resulted in the disappearance of this peak and the appearance of a corresponding water peak at 4.7 ppm.

The spectrum of phenolphthalein in deuteroacetone was identical to that in DMSO except for the position and shape of the 9.68 ppm peak. In deuteroacetone the peak had shifted to 8.88 ppm, was very broad, and

integrated to less than two protons. The peak broadening indicated that proton exchange was occurring more rapidly in deuteroacetone than in DMSO. This is usually the case because DMSO forms such strong hydrogen bonds that the exchange of acidic protons is slowed considerably, resulting in sharp peaks. The non-whole-number integral was evidence of the exchange of deuterium atoms for the acidic protons. This exchange occurred very slowly and the peak would probably have disappeared altogether in time.

Phenolphthalein in D_2O-NaOD solution. As mentioned earlier, the species in solution is mainly the carbinol form of phenolphthalein. This is an open ring compound, and a comparison of the spectrum (Figure 12) with that of phenolphthalein in DMSO reveals that a considerable alteration of structure has occurred. The AB quartet (9 c/s coupling constant) is still quite prominent, and these peaks must be assigned to the protons on the identical phenolic rings. The acidic protons are not seen because they have exchanged with the solvent. The remaining protons 4, 5, 6, and 7 no longer form a collapsed multiplet but have split into three groups of peaks: a doublet of doublet at 7.73 ppm (one proton), a possible triplet at 7.35 ppm, and one or more peaks at about 6.8 ppm. The latter two groups of peaks were not sufficiently resolved from the AB quartet so that separate integrals could be obtained. The assignment of these peaks to protons 4, 5, 6, and 7 for this and other open ring compounds is discussed at the end of this section.

Thymolphthalein. The spectrum of this compound in deuteroacetone is shown in Figure 13. The sharp singlet (two protons) at 8.18 ppm is assigned to the two hydroxyl protons. As in the case of other closed ring systems, the multiplet from 7.4 to 8.1 ppm (four protons) can be assigned

to protons 4, 5, 6, and 7. A pair of singlets (two protons each) appear at 6.90 and 6.72 ppm and are not spin-spin coupled. The identical protons 3' and 3" give rise to one of the peaks (probably the 6.90 ppm peak because of the inductive effect of the adjacent hydroxyl group), and protons 6' and 6" represent the other peak.

The region from 2.9 to 3.5 ppm (two protons) contains a nearly symmetrical heptet of peaks (only six of which are seen) and must be assigned to the hydrogen atom attached to the number two carbon of the isopropyl group. The two isopropyl substituents are not identical as evidenced by the doublet of doublets at 1.10 and 1.00 ppm (twelve protons). Apparently these bulky groups are not free to assume any position in space and, therefore, they experience slightly different magnetic field strengths. The protons of the 2' and 2" methyl groups form a sharp singlet at 2.08 ppm too near the solvent peak for integration.

The spectrum of thymolphthalein in DMSO is nearly identical to that in deuteroacetone, the only difference being the shift of the two phenolic protons from 8.18 to 9.33 ppm. This type of behavior has been explained for phenolphthalein, where a similar shift is observed.

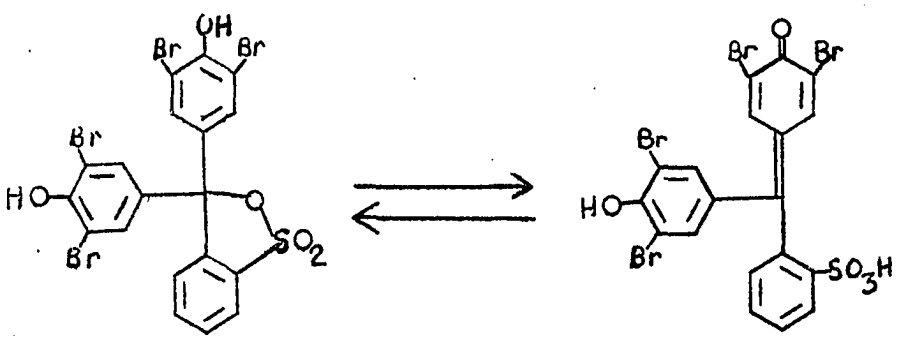
o-Cresolphthalein. The spectrum of this compound in deuteroacetone is shown in Figure 14. The broad singlet (less than two protons) at 8.33 ppm is due to the rapidly exchanging hydroxyl protons. As with phenolphthalein, these acidic protons are slowly being replaced with deuterons from the solvent. The multiplet from 7.3 to 8.0 ppm (four protons) arises from the phthalate ring protons. The second multiplet (6.5 to 7.2 ppm, six protons) arises from the unsymmetrically substituted phenolic ring protons. The two ortho methyl groups absorb near 2.1 ppm and are lost under the

solvent peak.

Bromphenol blue. The spectrum of bromphenol blue in deuteroacetone is shown in Figure 15. The sharp singlet (7.57 ppm, four protons) is readily assigned to the four identical protons 2', 6', 2", and 6". The multiplet from 7.8 to 8.3 ppm (four protons) represents the protons 4, 5, 6, and 7. The very broad singlet centered at 8.95 ppm (less than two protons) belongs to the hydroxyl protons which are undergoing rapid exchange.

The spectrum of bromphenol blue in DMSO (Figure 16) was significantly different from that obtained in deuteroacetone. The four identical protons of the two phenolic rings absorbed sharply at 7.55 ppm as expected. However, protons 4, 5, 6, and 7 no longer appeared as a compact multiplet but were spread over the region from 7.0 to 8.2 ppm, overlapping the 7.55 ppm singlet. The explanation for this change obviously involves the nature of the solvent. The solvating ability of DMSO compared to most organic liquids is known to be quite strong. It is possible that opening of the sultone ring occurs in this solvent, leading to the observed change in the NMR spectrum. Solutions of such an open ring compound would be expected to show color, and, indeed, solutions of bromphenol blue in DMSO are dark reddish-brown. This is in contrast to the deuteroacetone solution which is light yellow and to the colorless solutions of the phthaleins in both deuteroacetone and DMSO.

The lack of well-defined peaks for protons 4, 5, 6, and 7 suggests that a rapid equilibrium may exist between the open and closed ring forms.



If ring opening and closing are rapid with respect to the NMR time scale (about 0.1 sec.), then the chemical shift of the protons is a time average of the two situations. This can lead to a blurred or "smeared out" spectrum.

The two acidic protons of this molecule appeared as a sharp singlet at 8.70 ppm in DMSO, and this might be taken as evidence for the symmetrical closed ring form. However, protons which are acidic enough to undergo rapid exchange are often found to have the same chemical shift. Any differences in acidity are then erased as far as analysis by NMR is concerned.

The monosodium salt of bromphenol blue. Since opening of the sultone ring in DMSO was suspected of altering the pattern of the protons 4, 5, 6, and 7 of bromphenol blue, a form of the molecule in which the ring was known to be open was studied. The monosodium salt was investigated in both DMSO and D_2O (Figure 17). The latter solvent proved to be more useful because in DMSO, the water peak of the hydrated salt coincided almost exactly with the sharp singlet at 7.60 ppm, making integration impossible. In other respects, the spectra of the salt in DMSO and D_2O were identical. In D_2O, three groups of peaks were present: a doublet (one proton)

centered at 8.13 ppm, a sharp singlet overlapping a multiplet from 7.0 to 7.8 ppm (total integration, six protons), and a doublet (one proton) centered at 6.8 ppm. The sharp singlet at 7.60 ppm can only be due to the four protons of the two phenolic rings. All acidic protons having exchanged, it must be concluded that protons 4, 5, 6, and 7 have split into a 1:2:1 pattern.

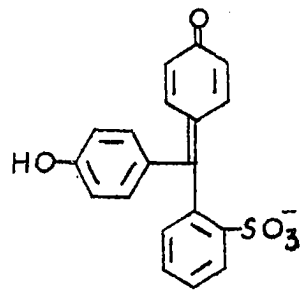
The fact that the spectrum of the acid form in DMSO did not show the well-defined 1:2:1 pattern observed with salt is additional evidence for the existence of a rapid tautomeric equilibrium between the open and closed forms of the acid.

Phenol red. The spectrum of phenol red (Figure 18) was obtained on a solution of the dye in DMSO. Deuteroacetone could not be used because of the low solubility of phenol red. The quartet (eight protons) centered at 7.03 ppm may be assigned to the hydrogen atoms of the phenolic rings. The coupling constant is 9 c/s and the symmetry of the pattern shows the rings to be identical. The series of peaks from 7.5 to 8.2 ppm represents the aromatic protons 4, 5, 6, and 7. The two acidic protons appeared as a singlet at 8.67 ppm. This latter peak disappeared with the addition of one drop of D_2O to the sample, indicating that the protons were readily exchangeable.

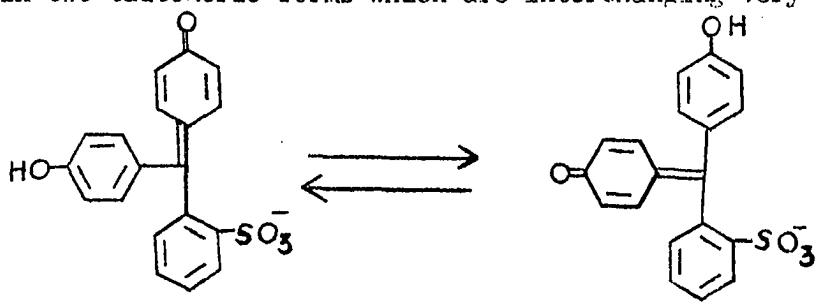
Conclusions drawn from the spectra of compounds known to exist in the closed form indicate that phenol red in DMSO is also a closed ring species. However, the DMSO solution has a moderately intense yellow color which is generally indicative of the open species. A possible explanation is that a small per centage of the molecules are in the open form, while the remainder have the sultone ring structure. The amount in the open form

could not exceed five to ten per cent or it would be detected by a non-integral number of protons for the multiplet from 7.5 to 8.2 ppm.

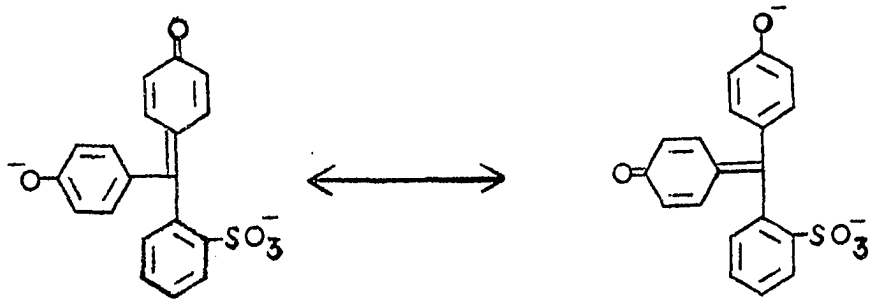
The monoammonium salt of phenol red. This compound exists in the open form in aqueous solutions. The NMR spectrum of a D_2O solution (Figure 19) is seen to contain both similarities and differences to that of phenol red. The AB quartet is present and centered at 6.97 ppm. Though the center of the quartet has shifted very little, the two sides have moved further apart, indicating that protons A and B are now less alike than before. The two rings, however, are still identical. The most striking difference between this spectrum and that of phenol red is the disappearance of the downfield multiplet. It has been replaced by peaks at 8.1, 7.4 to 7.7, and 6.85 ppm with relative areas of 1:2:1. The classical structure of the monoammonium salt of phenol red is



However, the NMR spectrum shows clearly that the two phenolic rings are identical. The most probable explanation for this is that the molecule exists in two tautomeric forms which are interchanging very rapidly.



The disodium salt of phenol red. The spectrum of this salt in D_2O (Figure 20) is only slightly different than that of the monoammonium salt. The AB quartet is centered at 6.90 ppm, although the separation of the A and B peaks is somewhat greater than in the case of the ammonium salt. The phenolic rings are identical, and the peaks of the 1:2:1 pattern appear at 8.05, 7.3 to 7.8, and 6.8 ppm. Again, the classical structure is inadequate to describe the molecule, which must be considered as a resonance hybrid of the two structures



Thymol blue. This compound is the sulfur analog of thymolphthalein. Solutions of thymol blue in DMSO are intensely colored, indicating that the molecule exists in the open form. Unfortunately, a spectrum of the closed form, if it exists, could not be obtained because of low solubility in less polar solvents. The upfield portion of the spectrum (Figure 21) is essentially identical to that of thymolphthalein, though some of the peaks are obscured by solvent bands. Downfield, the 1:2:1 pattern is clearly evident with the peaks centered at 8.0, 7.67, and 7.12 ppm. The doublet (four protons) at 6.67 ppm may be assigned to protons 3', 6', 3", and 6", while the sharp singlet at 6.23 ppm is due to one water of hydration and the two hydroxyl protons.

Bromthymol blue. A DMSO solution of this dye is a very dark red

color. The molecule, therefore, would be expected to be in the open form. The upfield portion of the spectrum (Figure 22), except for the four methyl groups of the isopropyl substituents, is obscured by solvent bands. In the region of interest, however, three peaks are seen at 8.05, 7.45, and 7.00 ppm with relative areas of 1:2:3. The explanation here is simple; the expected 1:2:1 pattern is present, but the peak near 7.0 ppm is overlapped by the protons 6' and 6". Further downfield at 10.18 ppm, the two acidic protons appear as a sharp singlet.

The spectrum of the monosodium salt of bromthymol blue in DMSO was identical to that of the free acid in DMSO, which confirms the existence of the open ring form of the free acid in DMSO.

Fortunately, bromthymol blue is also soluble in deuteroacetone, giving rise to a yellow solution. The upfield portion of the spectrum (Figure 23) is practically identical to that of previously discussed compounds containing the thymol grouping. Downfield, neither a clear cut 1:2:1 pattern nor a simple multiplet is present. Rather it appears that a mixture of both is present. Integration of the three groups of peaks (7.92, 7.33, and 6.78 ppm) gives proton ratios of $2\frac{1}{2}:1:2\frac{1}{2}$. This is readily explained by assuming that the two forms of the molecule are present in approximately a one to one ratio.

The monoammonium salt of cresol red. The salt of cresol red is an open ring compound. It is not a good model compound for this class, however, because the AB quartet has been altered by the unsymmetrical substitution of the phenolic rings, and overlaps the 1:2:1 pattern. The spectrum of the salt in D_2O (Figure 24) was obtained mainly for purposes of comparison with that of the free acid.

The sharp singlet at 2.05 ppm (six protons) represents the identical methyl protons. The three groups of downfield peaks (8.20, 7.00 to 7.77, and 6.50 to 7.00 ppm) have relative areas of 1:6:3. Subtraction of the expected 1:2:1 pattern from the observed pattern leaves a peak ratio of 4:2 for the two multiplets above 7.8 ppm. These are the six protons of the phenolic rings.

Cresol red. Cresol red is readily soluble in DMSO. The solution is very dark red, indicating that the molecule exists, at least partially, in the open ring form. The upfield portion of the spectrum (Figure 25) containing the two methyl groups is blanked out by solvent peaks. Downfield the sharp singlet at 6.43 ppm represents the hydroxyl protons plus one and a half waters of hydration. The six protons of the phenolic rings appear to be confined within the region from 6.6 to 7.1 ppm. The remainder of the spectrum has no definite pattern or recognizable portions. It can be said with certainty that the acid form in DMSO does not exist completely in either the open or closed form. Like bromthymol blue in deuteroacetone and bromphenol blue in DMSO, the two forms of cresol red are probably in tautomeric equilibrium.

Phenolsulfonephthalin. This compound, of necessity, has an open ring structure. However, it is significantly different from previously discussed open ring compounds in that the valence of the central carbon atom is saturated. The spectrum (Figure 26) is complex, but a step-by-step analysis based on previous experience reveals much. First, the AB quartet centered at 6.85 ppm is readily discernable owing to the height and symmetry of the peaks, and the 9 c/s coupling constant. The small multiplet centered at 7.97 ppm integrates to one proton and is undoubtedly

the downfield portion of what appears to be a 1:3 pattern rather than a 1:2:1. The remaining three protons overlap the AB quartet.

The sharp singlet at 6.53 ppm does not appear to be coupled, and is too far upfield to be a part of a 1:2:1 pattern. It was sufficiently resolved that its area was integrated and found to correspond to one proton. The proton attached to the central carbon atom probably gives rise to this peak.

The spectrum of this compound in DMSO and deuteroacetone is practically identical to that in D_2O. The classical structure adequately describes this molecule.

Phenolphthalin. The spectrum of this compound in DMSO (Figure 27) is similar to that of phenolsulfonephthalin. The groups of peaks centered at 7.80, 7.34, and 7.11 ppm were sufficiently separated that an integral was obtained. The ratio of the areas was 1:2:1. The AB quartet is still recognizable but has almost collapsed into a singlet. The singlet at 6.47 ppm integrated to one proton and was assigned to the hydrogen atom attached to the central carbon atom. The two hydroxyl protons appear downfield at 9.22 ppm but the carboxylate proton was not seen. It was apparently undergoing rapid exchange with the solvent, giving rise to a broad, low-profile peak below 9 ppm.

Tetrabromophenolsulfonephthalin. This compound is the reduced form of bromphenol blue, and was obtained as the water soluble zinc salt. It was chosen for study because the spectrum was inherently simpler than that of either phenolsulfonephthalin or phenolphthalin. The spectrum in D_2O (Figure 28) shows poor resolution and broad peaks. This effect was later observed with several other samples that had been reduced with a particular

batch of zinc powder. This powder apparently contained some paramagnetic material which adversely effected the relaxation time of the molecule and, thus, the spectrum. Nevertheless, three groups of peaks are clearly seen with relative areas of 1:7:1. The largest peak at 7.37 ppm must arise from the four identical protons of the phenolic rings. The 8.1 ppm peak must be the downfield portion of a 1:3 pattern for protons 4, 5, 6, and 7. The singlet at 6.63 ppm is well separated from other peaks, and its assignment to the proton on the central carbon atom can be made with certainty.

Phthalide. Phthalide is not an indicator and it differs greatly in structure from the phthaleins and sulfonephthaleins. However, a gamma lactone ring is present in the molecule. The appearance of its spectrum with respect to protons 4, 5, 6, and 7 is therefore of interest. The spectrum of a sample in DMSO is shown in Figure 29. The two benzylic protons appear sharply at 5.45 ppm, while the multiplet from 7.4 to 8.0 ppm contains the remaining protons. The appearance of the complicated multiplet is in agreement with previous spectra of closed ring compounds.

o-Toluic acid. The reduction product and open ring form of phthalide is o-toluic acid. The spectrum (Figure 30) of the acid in CCl_4 has four peaks. A sharp singlet at 2.67 ppm represents the three methyl protons and the other singlet at 12.73 ppm is the carboxylic acid proton. Of the two multiplets, the one centered at 8.1 ppm integrates to one proton and that at 7.3 ppm to three protons. While a 1:2:1 pattern was not observed, this should not be considered contradictory to the spectra of other open ring compounds because the influence of two benzene rings substituted for two of the methyl protons would result in changes in the spectrum. These rings would introduce a number of factors, such as

shielding and steric effects, which might easily cause further splitting of the 7.3 ppm multiplet, resulting in the expected pattern. Equally as important is the fact that the valence of the central carbon atom is saturated in o-toluic acid. This was not true for the unreduced indicators.

The positions of peaks other than the carboxylate proton were only slightly altered by dissolving the compound in DMSO.

The assignment of protons 4, 5, 6, and 7. Regarding the assignment of these protons to the various peaks of the 1:2:1 pattern, it is instructive to consider Hefley's work on the substituted fluoresceins (13). The NMR spectra of ten substituted fluoresceins in DMSO revealed the presence of a 1:2:1 pattern (doublet: triplet: doublet), which could only be attributed to protons 4, 5, 6, and 7. The pattern was much more evident than for the phthaleins and sulfonephthaleins because most of the hydrogen atoms attached to the phenolic rings, which generally overlap the 1:2:1 pattern, had been removed by substitution.

The downfield peak (8.0 to 8.3 ppm) appeared as a doublet. This splitting pattern is indicative of ortho coupling. Only protons 4 and 7 are adjacent to one other proton and eligible for assignment to this peak. The final choice was made by noting the downfield position of the doublet. Hefley assigned this peak to proton 4, arguing that the inductive (electron withdrawing) effect of the carboxylate group would result in a deshielding of proton 4, causing a downfield shift. Having assigned proton 4, the upfield doublet could only arise from proton 7. Protons 5 and 6 were then assigned to the triplet. These fluorescein compounds were felt to exist in the closed lactone ring form in DMSO.

Similar studies by Birze (4) on various isomers of dihydroxydimethyl-

fluorescein in NaOD-D_2O solutions revealed the presence of the 1:2:1 pattern. These compounds are either colorless or light yellow in most solvents, but in base they are highly colored, which is suggestive of the open ring form.

Some of the arguments used in making proton assignments for the fluoresceins apply directly to the phthaleins and sulfonephthaleins. In agreement with the fluoresceins, a doublet is seen for the downfield proton of the monosodium salt of bromphenol blue (Figure 17) and the monoammonium salts of phenol red and cresol red (Figures 19 and 24). The coupling constant is 7 to 8 c/s. Resolution is particularly good in the spectra of phenolphthalin (Figure 27) and the disodium salt of phenol red (Figure 20), and a doublet of doublets appears. The major splitting (7 to 8 c/s) arises from a proton ortho to the proton giving rise to the peak, and the minor splitting (2 c/s) is due to long range coupling with a meta proton.

In most other cases, the upfield portion of the 1:2:1 pattern has been partially obscured by the phenolic ring protons. However, an upfield doublet is clearly seen with the disodium salt of phenol red (Figure 20) and the monosodium salt of bromphenol blue (Figure 17). In addition, a triplet can be seen in the spectra of both salts of phenol red (Figures 19 and 20) and of phenolphthalein in NaOD-D_2O (Figure 12). In general, the overall pattern appears to be doublet: triplet: doublet, and very similar to that of the fluoresceins.

It is apparent that Hefley's deductions based on splitting patterns must apply here as well. One doublet must arise from proton 4, the other from proton 7, and the triplet from 5 and 6. However, the reasoning by which the downfield doublet was assigned to proton 4 cannot apply to the

phthaleins and sulfonephthaleins. The open forms of these compounds, aside from their reduction products, are salts. The carboxylate and sulfonate groups carry negative charges, the inductive effect of which would be to increase the electron density of the ring, particularly in the vicinity of ortho protons. As a result, the ortho protons would be shifted upfield instead of downfield.

Other factors, however, must also be considered. Mobile pi electrons of the carboxylate anion are caused to circulate by the imposed magnetic field. This circulation generates an opposing magnetic field which will enhance or diminish the actual field strength experienced by other atoms in close proximity. This is known as diamagnetic anisotropy. When the carboxylate anion is in the plane of the ring, the effect is to deshield (shift downfield) ortho protons, while the meta and para positions are unaffected. Little or no effect would be expected when the anion is out of the plane of the ring.

A third effect, resonance delocalization, may also affect the chemical shift of aromatic protons. Resonance delocalization occurs when the pi electrons of substituents on an aromatic ring are delocalized into the aromatic pi system. An example is sodium benzoate, in which the negative charge normally associated with the carboxylate anion is partially delocalized over the entire molecule. The charge, however, appears to be concentrated at positions ortho and para to the substituent. The effect, as relates to NMR spectroscopy, is to shield (shift upfield) the protons at the ortho and para positions. Resonance delocalization is most effective when the aromatic ring and substituent are coplanar.

It is difficult to predict the preferred orientation (coplanar or not)

of the carboxylate group with respect to the aromatic ring, even with the aid of molecular models. Because the effects of diamagnetic anisotropy and resonance delocalization are both a function of the degree of coplanarity, it is impossible to determine what the overall effect will be on chemical shifts. Thus, it is equally difficult to assign with certainty either proton 4 or 7 to the downfield doublet.

A way out of this predicament may come from the example of o-toluic acid. The spectrum of this compound in both CCl_4 and DMSO shows a 1:3 ratio of peaks in the aromatic region. The downfield peak (one proton) is a doublet of doublets centered at 8.05 ppm and is undoubtedly analogous to the downfield doublet of the 1:2:1 pattern observed with indicators. With this compound it is difficult to see how the doublet could arise from any proton other than the one ortho to the carboxylic acid group. By analogy, it may be said that the assignment of proton 4 to the downfield doublet of the 1:2:1 pattern is probably correct, not because of inductive effects, but by the interplay of diamagnetic anisotropy and resonance delocalization.

The assignment of the downfield doublet to proton 4 necessitates the assignment of the upfield doublet to proton 7, and the triplet to protons 5 and 6.

Having established that protons 4, 5, 6, and 7 appear as a multiplet in the closed ring indicators, and in a 1:2:1 pattern for open ring forms, it is natural to assume that the fluorescein compounds of Hefley were in the open form. While this is probably true, there is some question as to the validity of the assumption. The phenolic rings of the closed forms of the phthaleins and sulfonephthaleins are, in most cases, able to undergo

free rotation. Molecular models of the monoanions and dianions, however, appear to be considerably more restricted, with a tendency toward coplanarity of the two phenolic rings. The phenolic rings of fluorescein, on the other hand, must be coplanar in both acid and base. While the examples of phthalide and o-toluic acid suggest that ring opening is of major importance in the transformation of a collapsed multiplet into a 1:2:1 pattern, it is quite possible that the positions of the phenolic rings in space also play a part in this transformation. Thus, one cannot state with absolute certainty that the compounds of Hefley, by virtue of the 1:2:1 pattern, were in the open form.

Summary

In summary it can be said that:

a) The colorless lactone or sultone forms are characterized by a multiplet in the region of about 7.4 to 8.2 ppm. This multiplet integrates to four protons and can only be assigned to protons 4, 5, 6, and 7.

b) Spectra of indicators known to be in the open ring form (monobasic and dibasic salts) are characterized by a 1:2:1 pattern (doublet: triplet: doublet) in the region from 6.8 to 8.2 ppm. The downfield doublet is assigned to proton 4, the upfield doublet to proton 7, and the triplet to 5 and 6.

c) Certain dyes which exist in the closed form in deuteroacetone are converted into the highly colored open form, or mixtures of both forms when dissolved in DMSO. The spectra of mixtures of both forms contain both a multiplet (as in part (a) above) and the 1:2:1 pattern.

d) Spectra of the reduced forms of three indicators were obtained.

Table 7. Structures of some indicators and their salts in various solvents.

Compound	Deuteroacetone	DMSO	D_2O	Color
Phenol red		closed		mc[a]
Diacetate of phenol red	closed			c[b]
Monoammonium salt of phenol red		open	open	mc, mc
Disodium salt of phenol red			open	ic[c]
Bromphenol blue	closed	mixture		lc[d], ic
Monosodium salt of bromphenol blue		open	open	ic, ic
Cresol red		mixture		ic
Monoammonium salt of cresol red			open	mc
Bromthymol blue	mixture	open		mc, ic
Monosodium salt of bromthymol blue			open	ic
Thymol blue		open		ic
Phenolphthalein	closed	closed		c, c
Phenolphthalein in NaOD-D_2O			open	mc
Thymolphthalein	closed	closed		c, c
o-Cresolphthalein	closed			c

[a]Moderately colored.

[b]Colorless.

[c]Intensely colored.

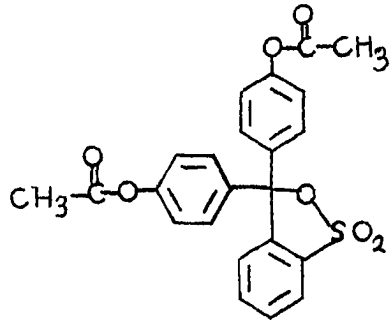
[d]Lightly colored.

The proton attached to the central carbon atom appeared as a singlet between 6.47 and 6.63 ppm. The remaining portions of the spectra were easily interpreted because of the obvious presence of the AB quartet and the 1:2:1 or 1:3 pattern.

The results on the indicators studied are summarized in Table 7.

Figure 10. The NMR spectrum of the diacetate of phenol red in deuteroacetone.

A sharp singlet at 2.23 ppm (six protons) is not shown.



Form which is present in solution

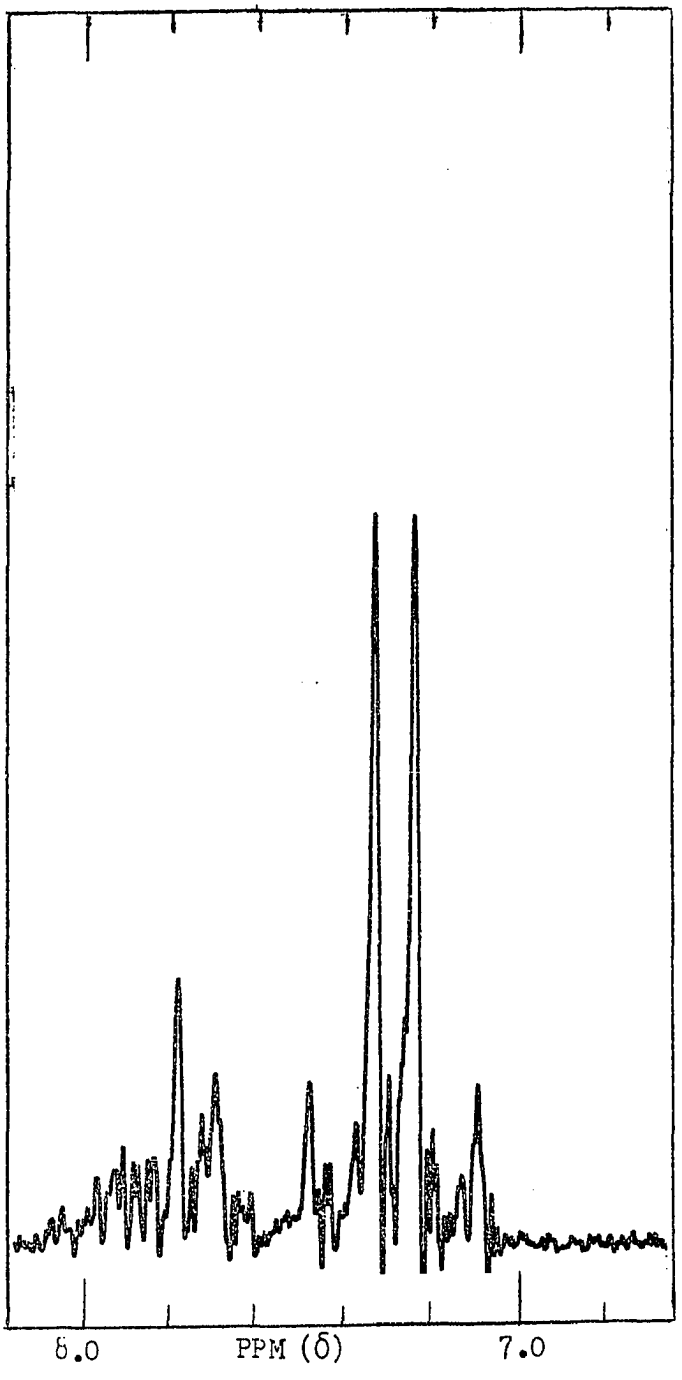
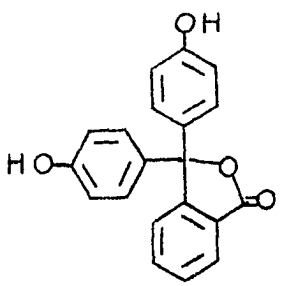

8.0
PPM (δ)
7.0

Figure 11. The NMR spectrum of phenolphthalein in DMSO.

The chemical shift of the offset peak (two protons) is 9.68 ppm.



Form which is present in solution

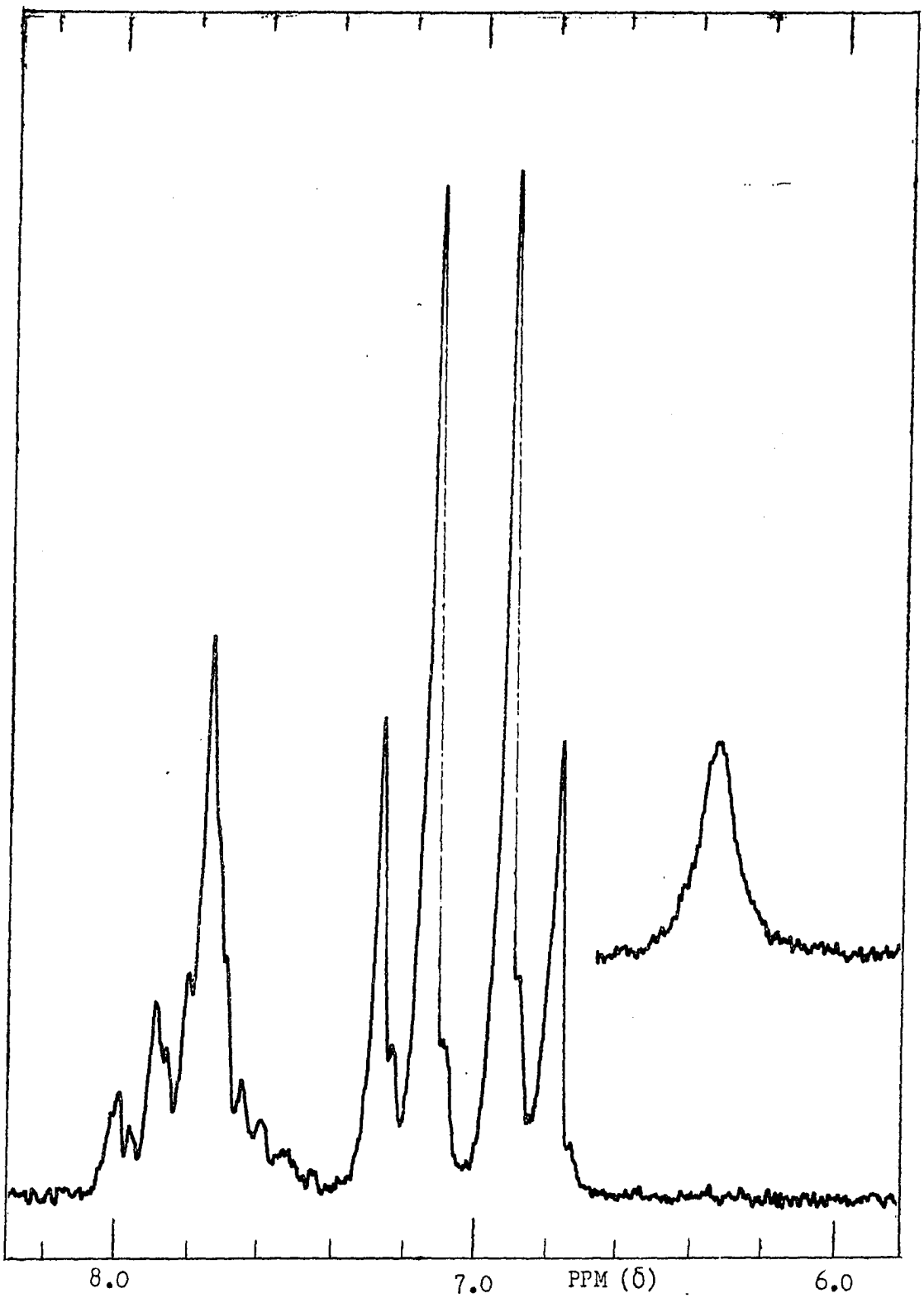
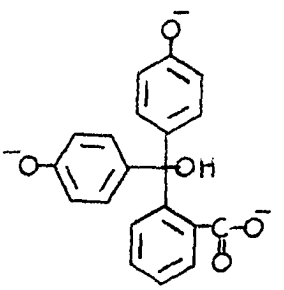

8.0
7.0
PPM (δ)
6.0

Figure 12. The NMR spectrum of phenolphthalein in NaOD-D_2O solution.



Form which is present in solution

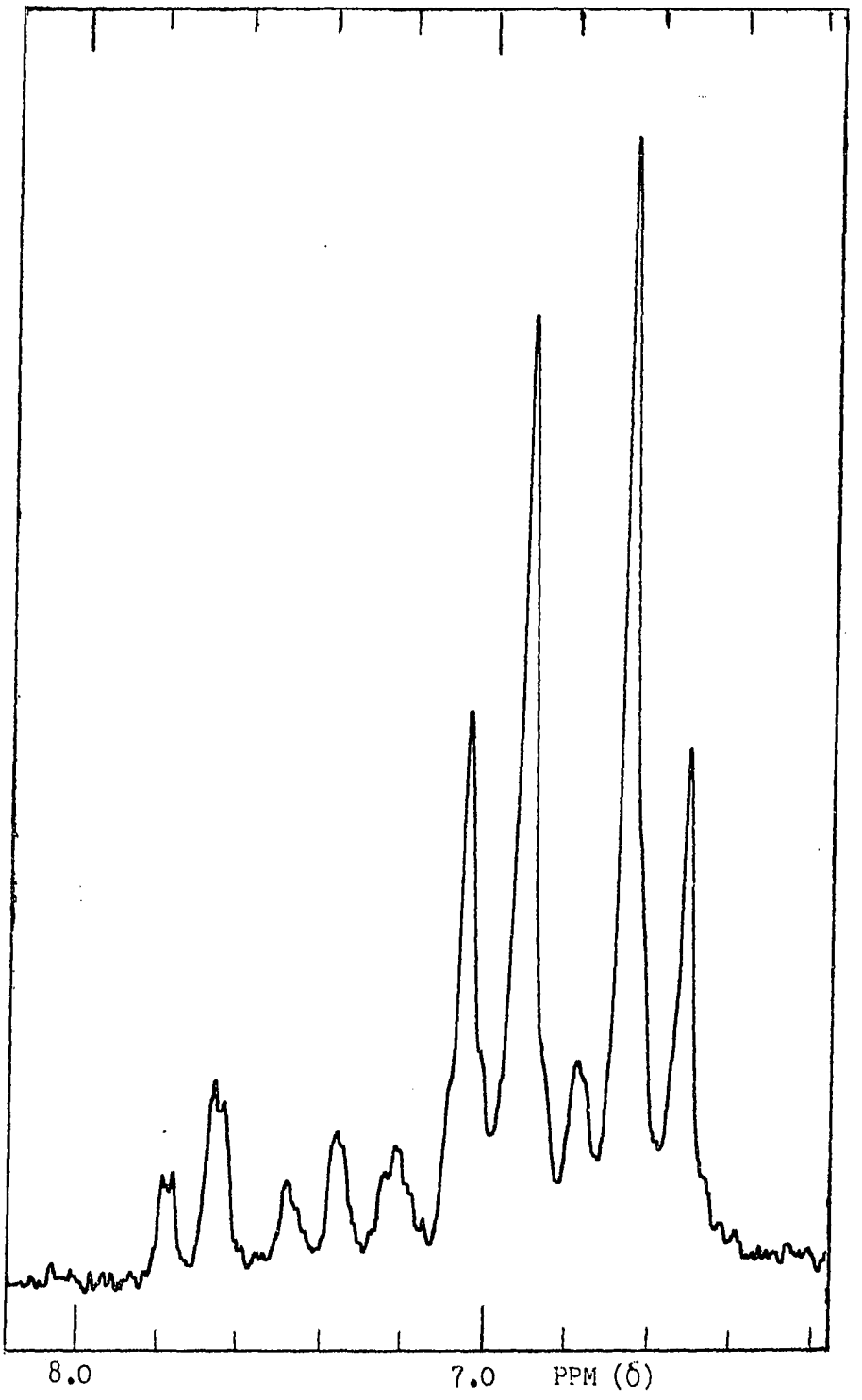
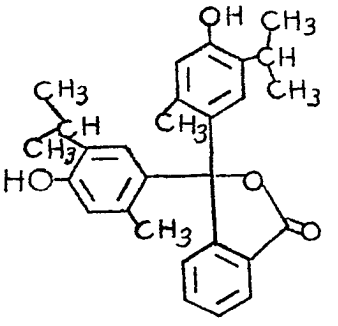

8.0
7.0
PPM (δ)

Figure 13. The NMR spectrum of thymolphthalein in deuteroacetone.



Form which is present in Solution

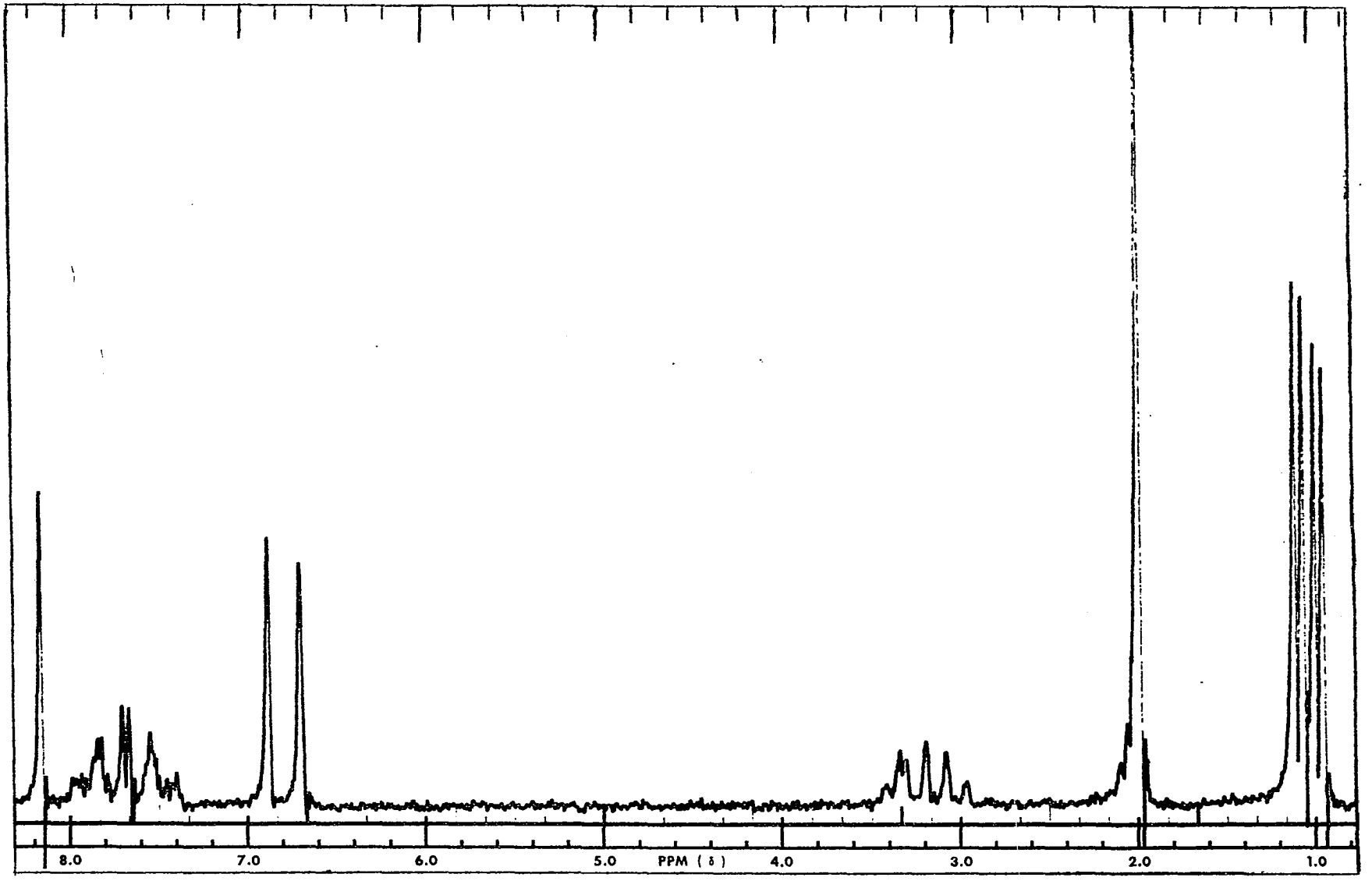
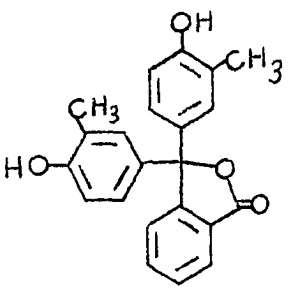

8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0

Figure 14. The NMR spectrum of o-cresolphthalein in deuteroacetone.

The peak of the 3', 3" methyl groups occurred near 2.10 ppm and was lost under solvent bands.



Form which is present in solution

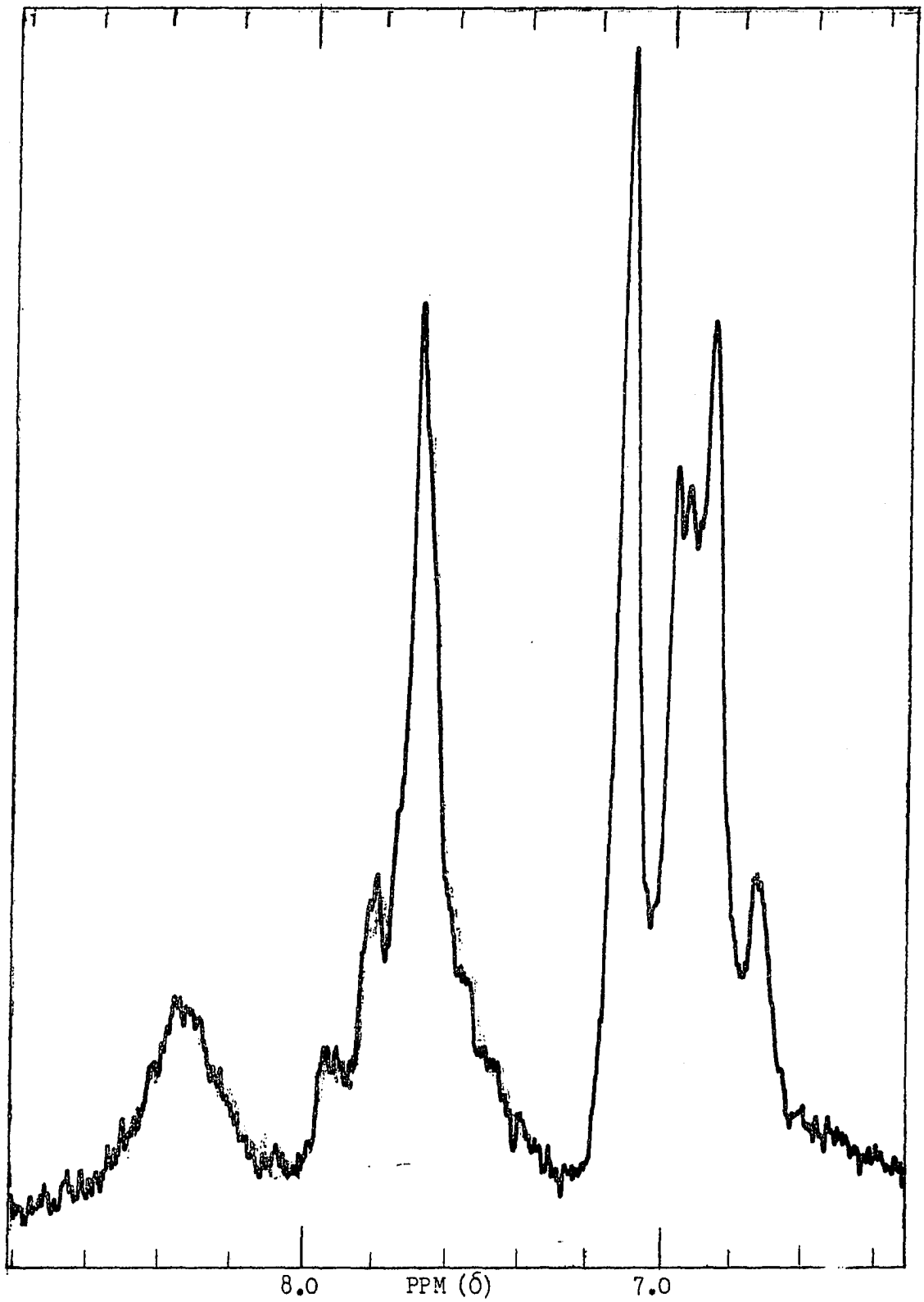
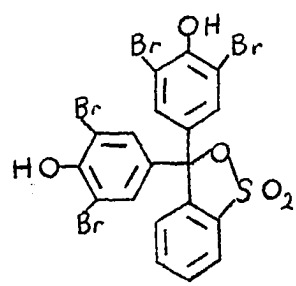

8.0
PPM (δ)
7.0

Figure 15. The NMR spectrum of bromphenol blue in deuteroacetone.



Form which is present is solution

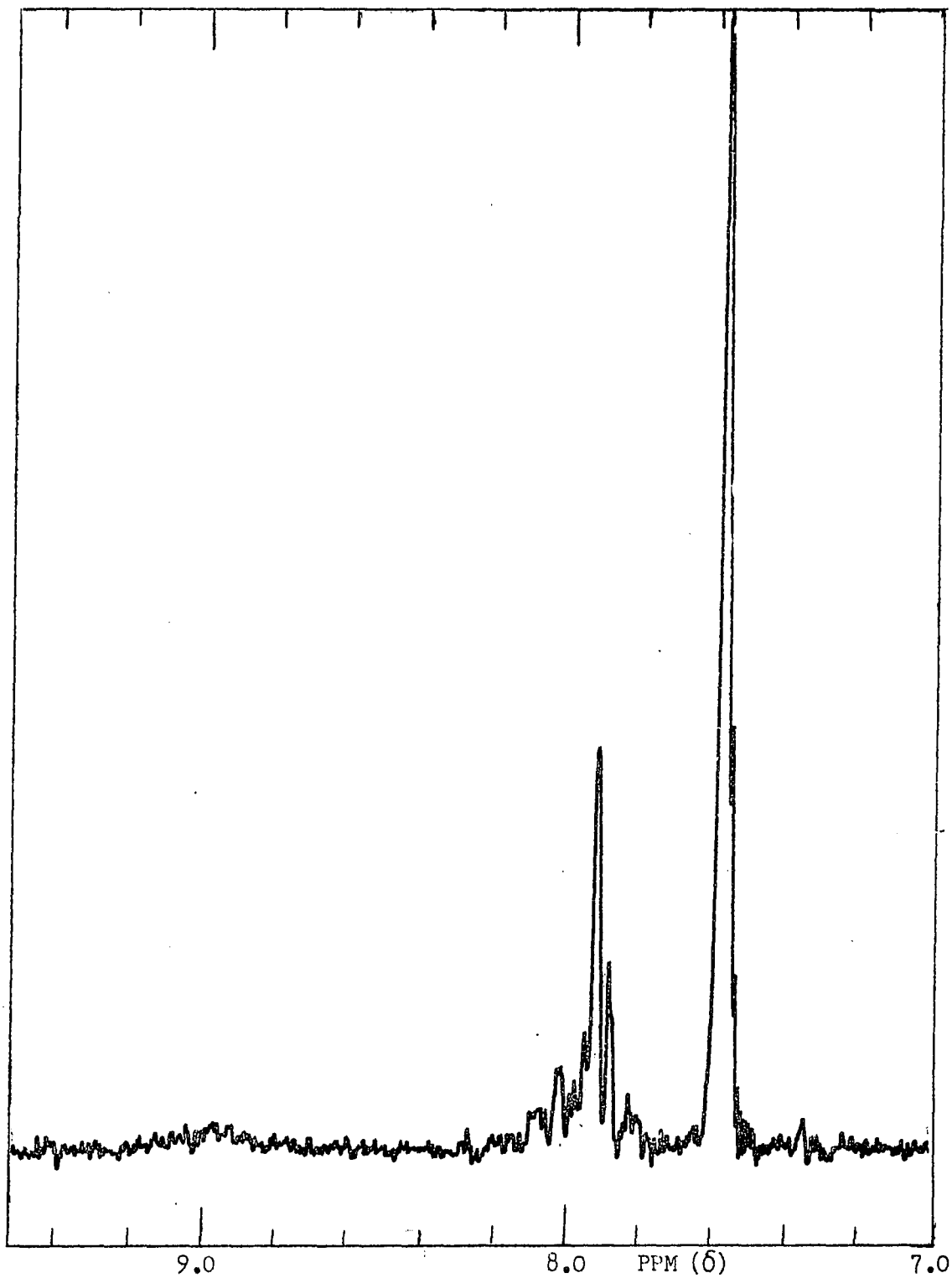
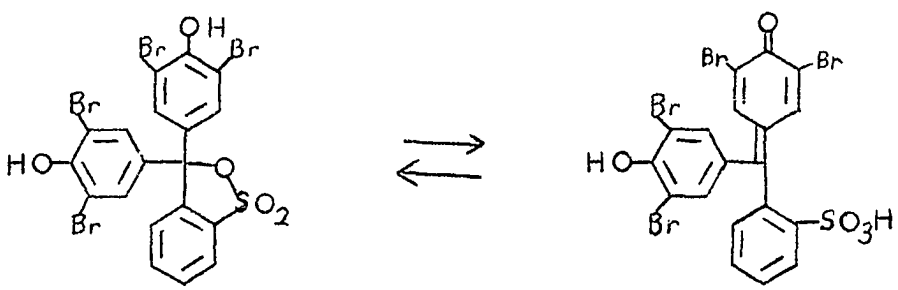

9.0
8.0
PPM (δ)
7.0

Figure 16. The NMR spectrum of bromphenol blue in DMSO.



Forms which are present in solution

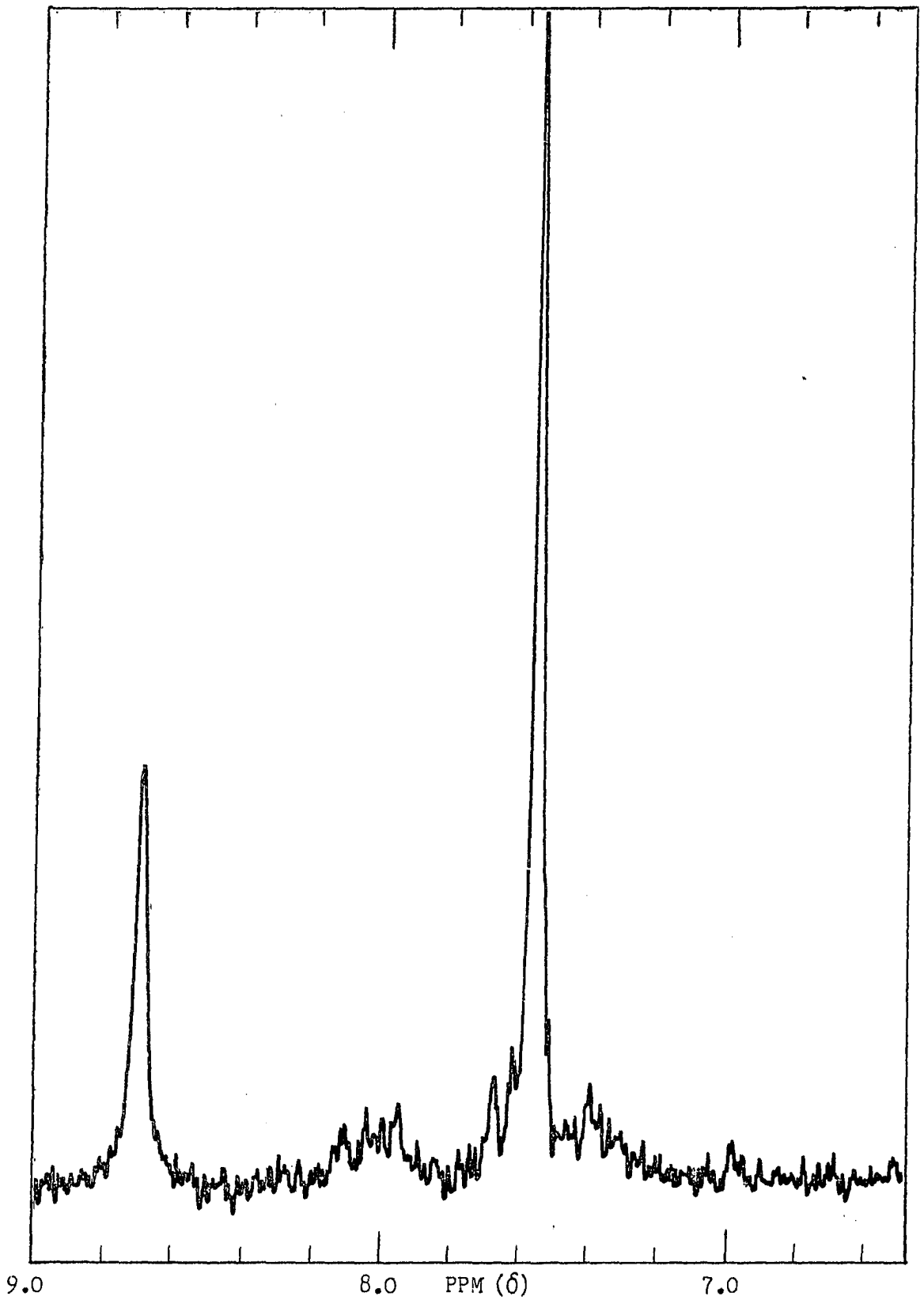
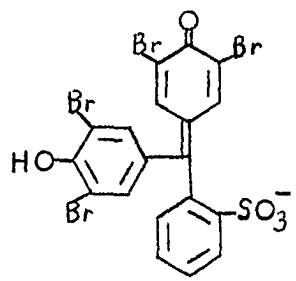

9.0
8.0
PPM (δ)
7.0

Figure 17. The NMR spectrum of the monosodium salt of bromphenol blue in D_2O.



Form which is present in solution

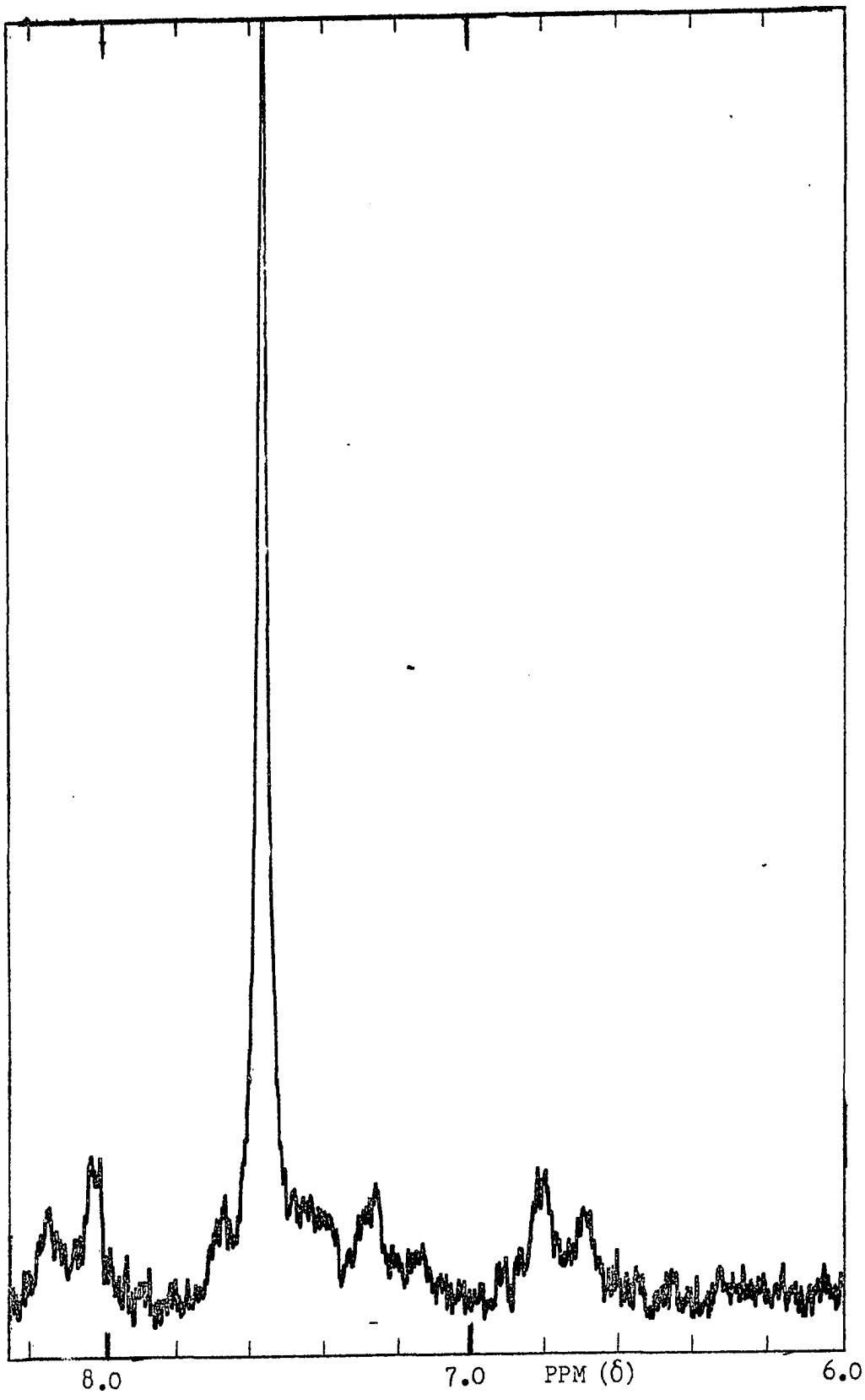
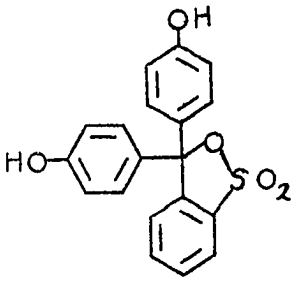

8.0
7.0
PPM (δ)
6.0

Figure 18. The NMR spectrum of phenol red in DMSO.



Form which is present in solution

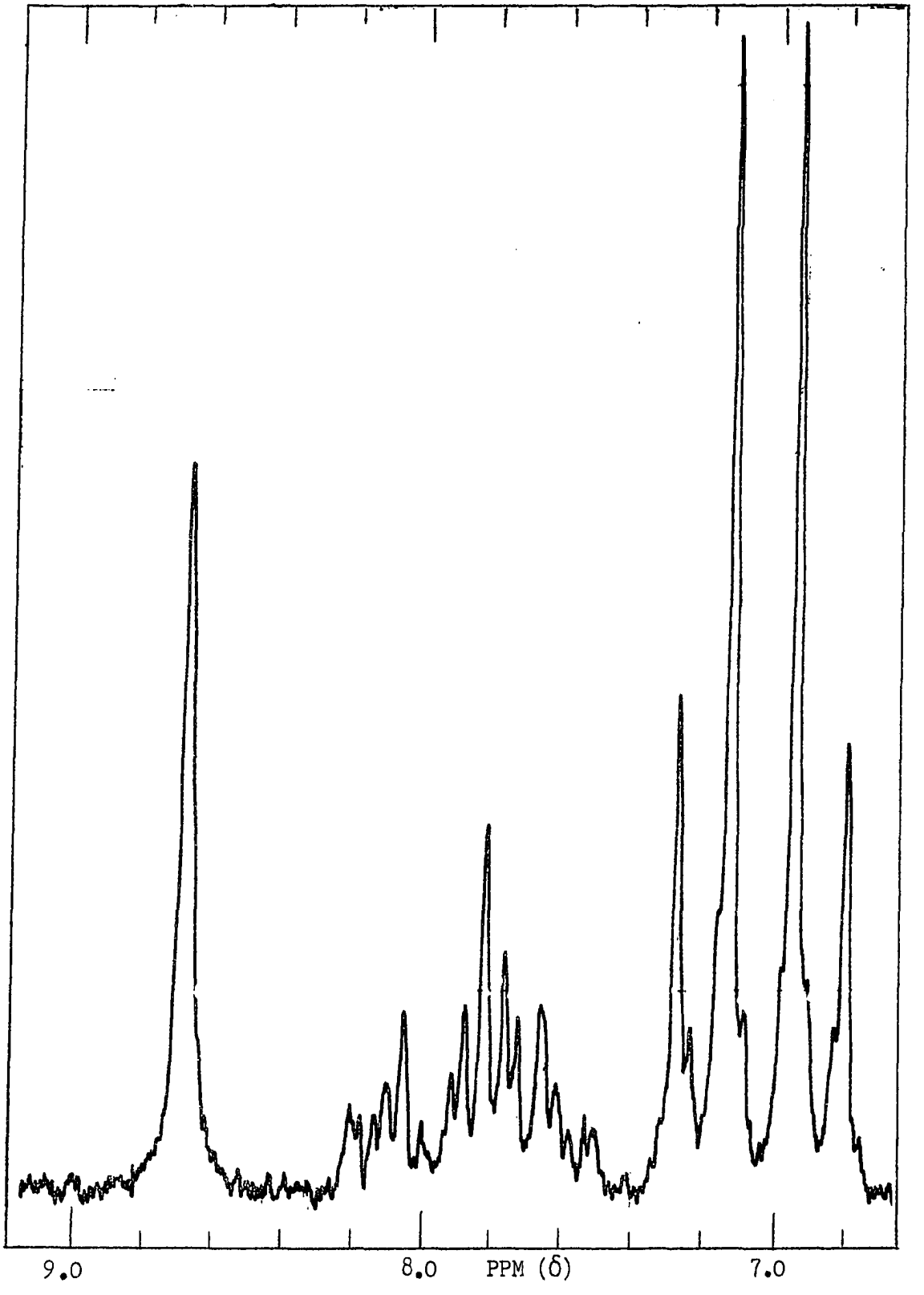
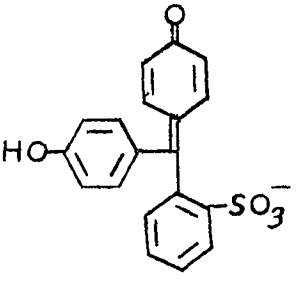

9.0
8.0
PPM (δ)
7.0

Figure 19. The NMR spectrum of the monoammonium salt of phenol red in D_2O.



Form which is present in solution

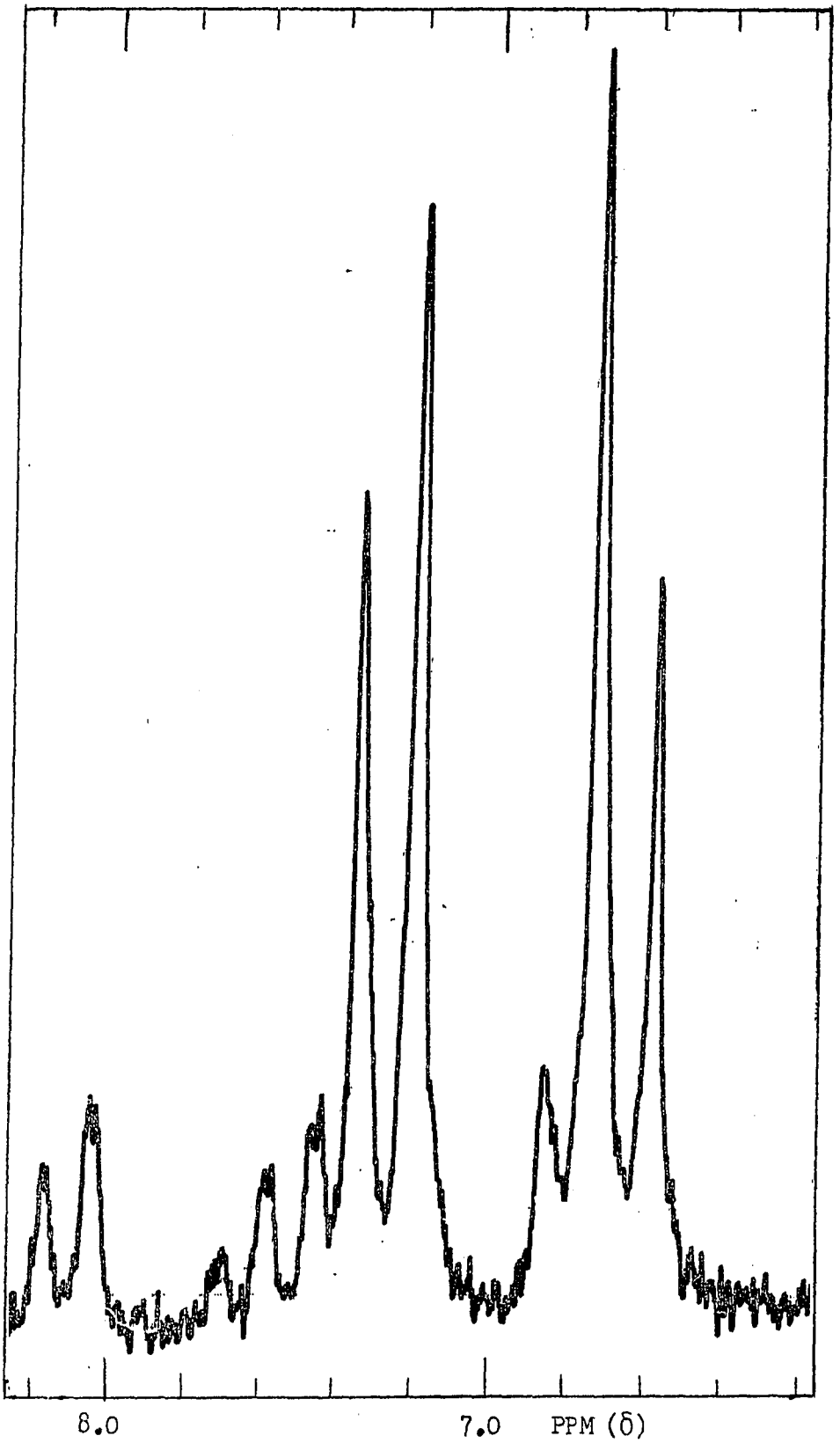
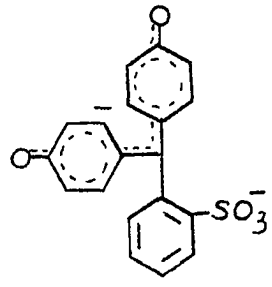

8.0
7.0
PPM (δ)

Figure 20. The NMR spectrum of the disodium salt of phenol red in D_2O.



Form which is present in solution

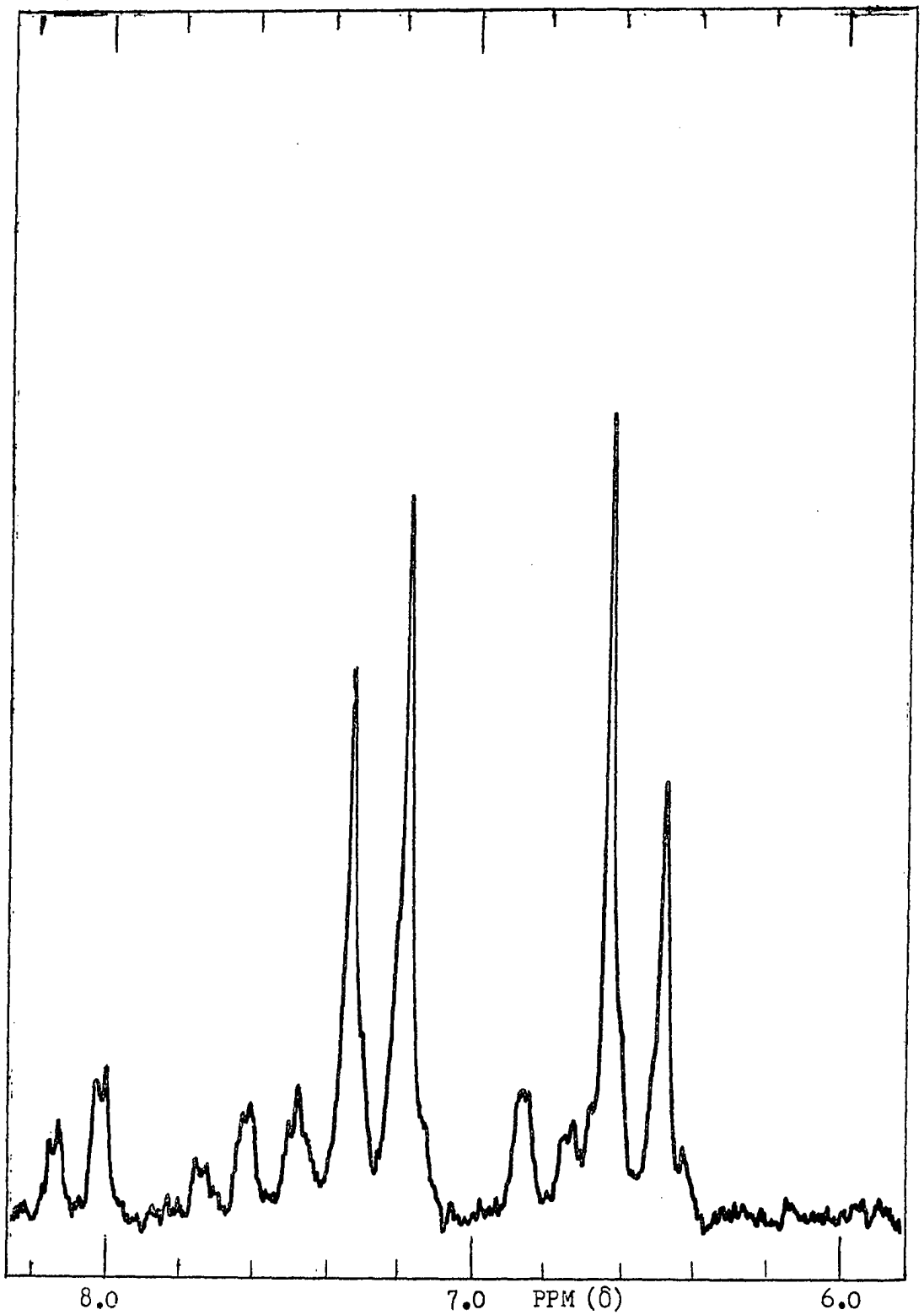
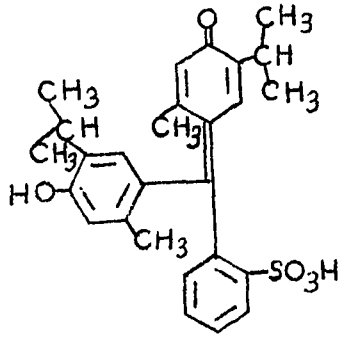

8.0
7.0
PPM (δ)
6.0

Figure 21. The NMR spectrum of thymol blue in DMSO.

The methyl groups of the 5' and 5" isopropyl substituents appeared as a doublet of doublets at about 1.0 ppm (see Figures 13 and 23).

Other upfield peaks were obscured by solvent bands.



Form which is present in solution

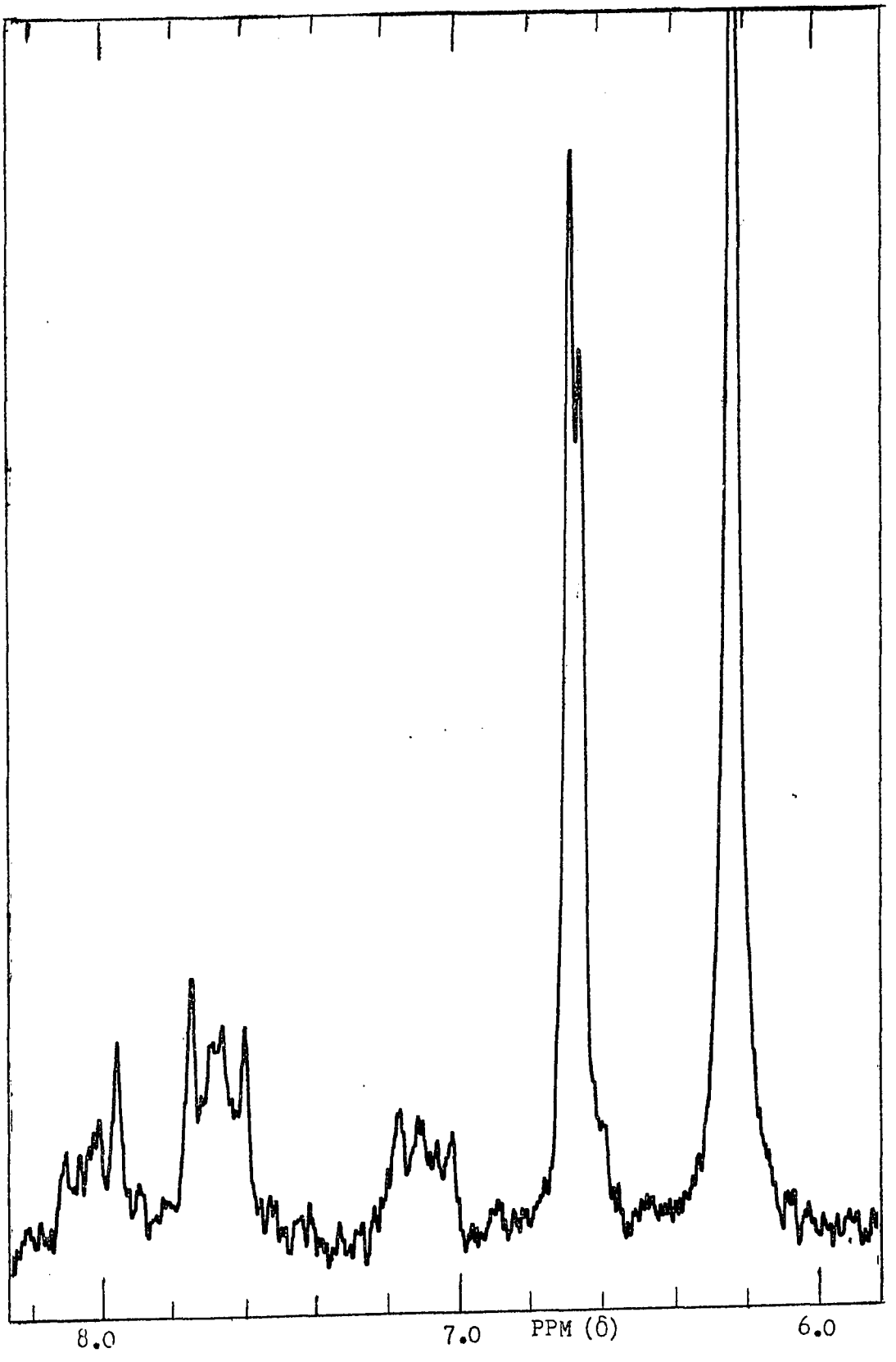
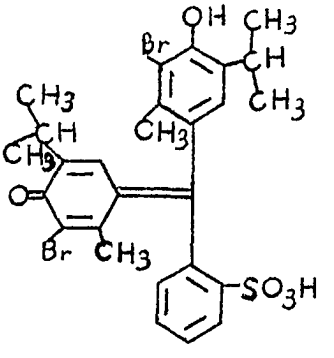

8.0
7.0
PPM (δ)
6.0

Figure 22. The NMR spectrum of bromthymol blue in DMSO.

The chemical shift of the offset peak (two protons) is 10.18 ppm.

The methyl groups of the 5' and 5" isopropyl substituents appeared as a doublet of doublets at about 1.0 ppm (see Figure 23).

Other upfield peaks were obscured by solvent bands.



Form which is present in solution

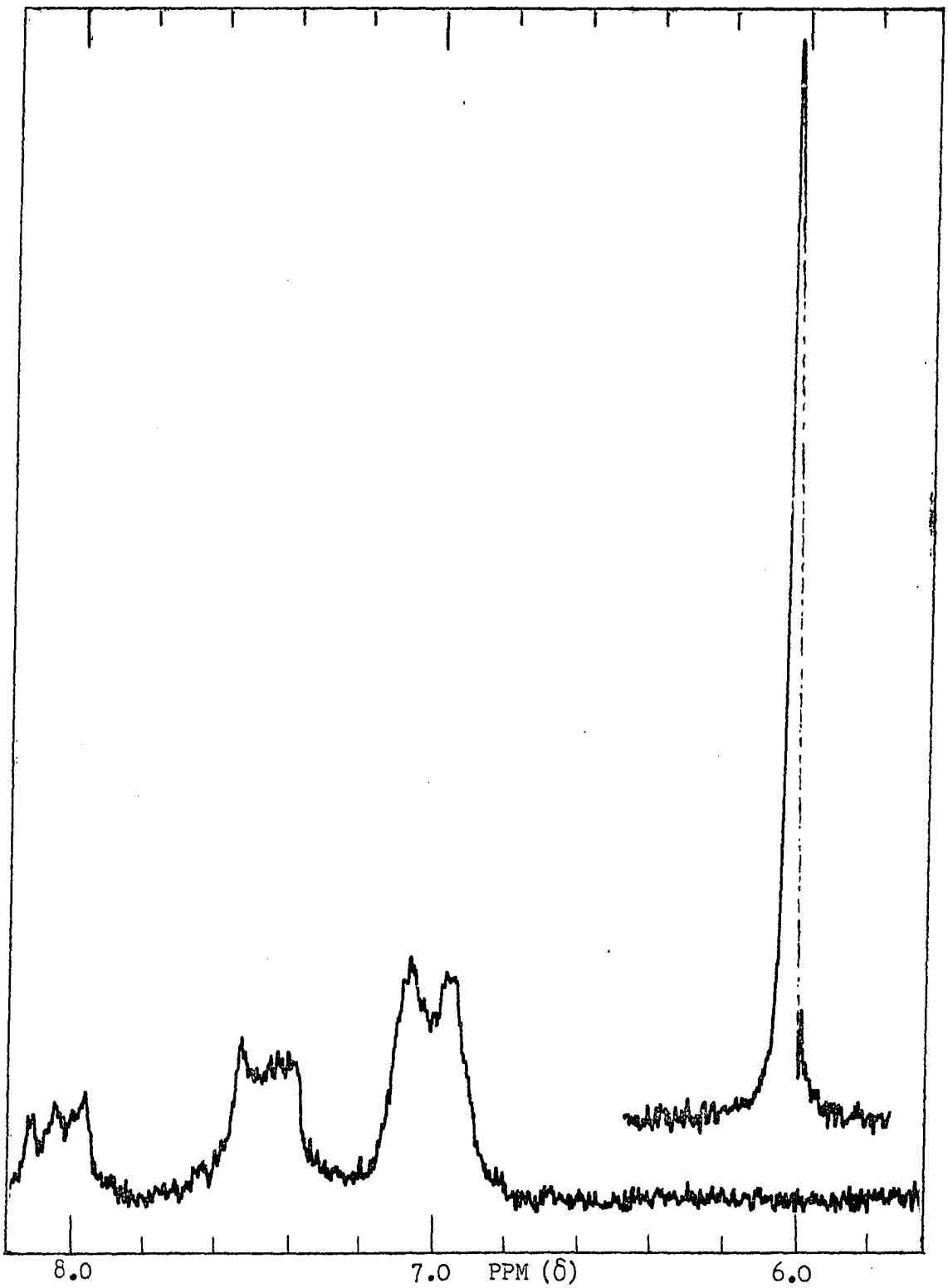
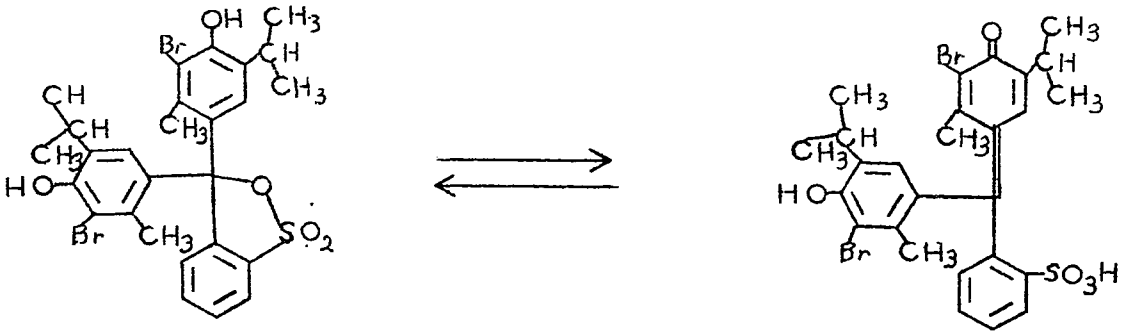

8.0
7.0
PPM (δ)
6.0

Figure 23. The NMR spectrum of bromthymol blue in deuteroacetone.



Forms which are present in solution

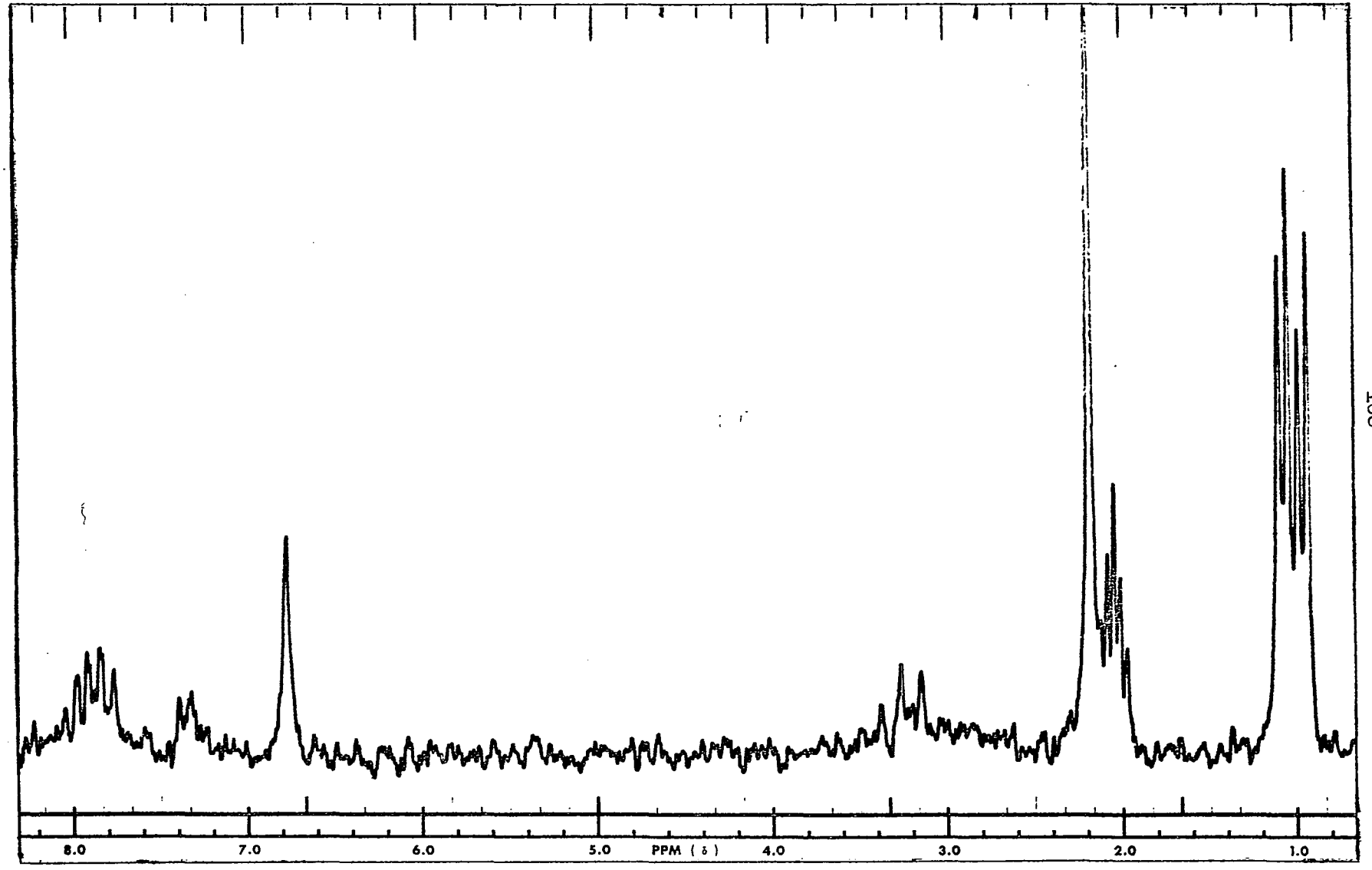
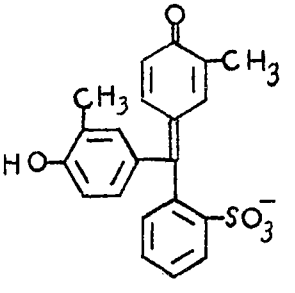

8.0
7.0
6.0
5.0
PPM (δ)
4.0
3.0
2.0
1.0

Figure 24. The NMR spectrum of the monoammonium salt of cresol red *in* D_2O.

A sharp singlet (six protons) at 2.17 ppm is not shown.



Form which is present in solution

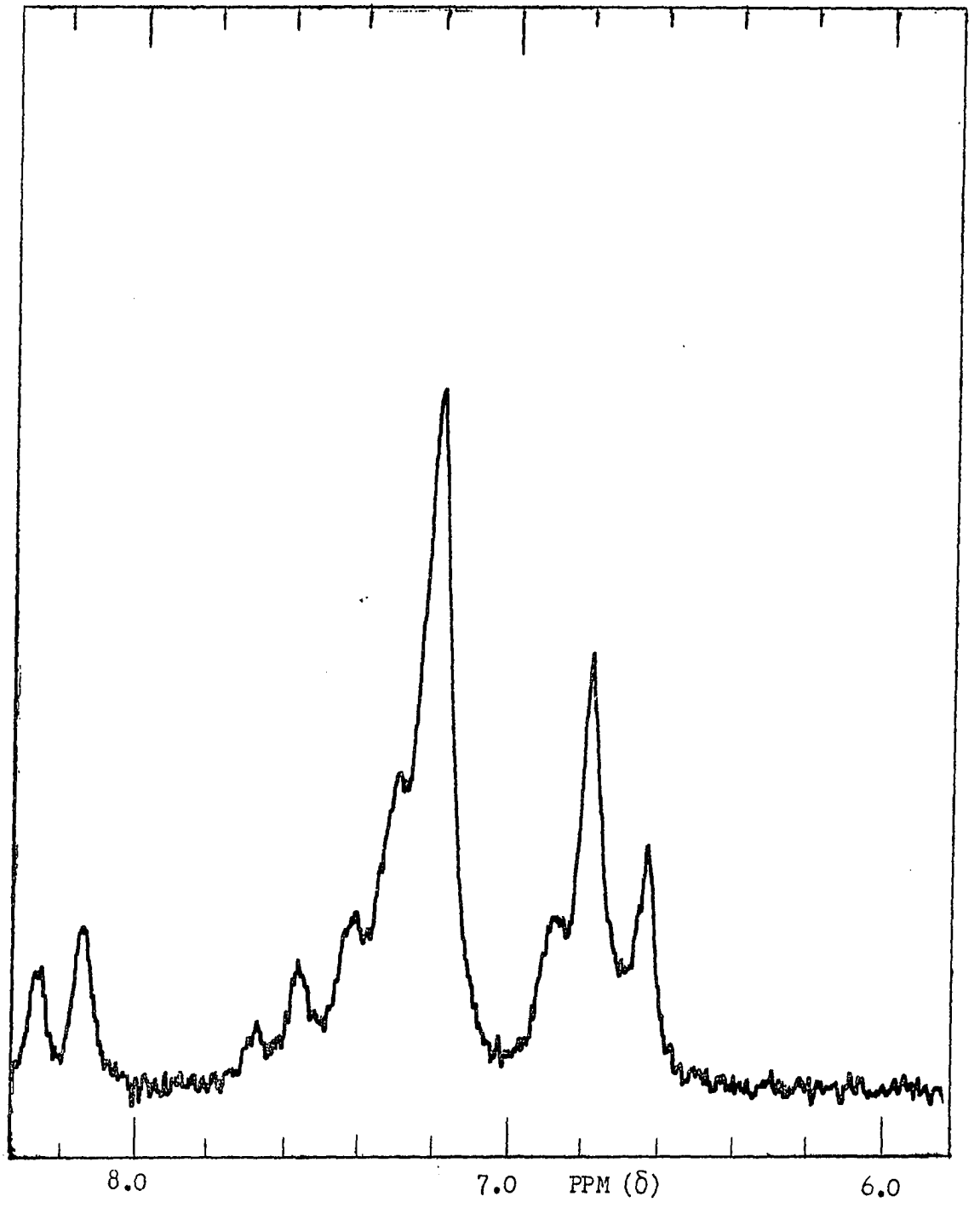
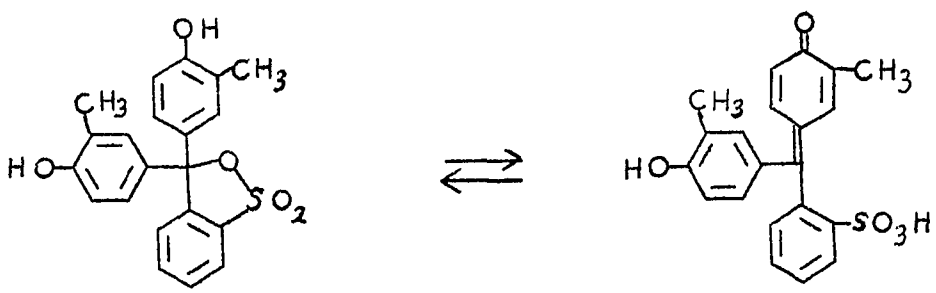

8.0
7.0
PPM (δ)
6.0

Figure 25. The NMR spectrum of cresol red in DMSO.

An upfield peak arising from the methyl substituents was obscured by solvent bands.



Forms which are present in solution

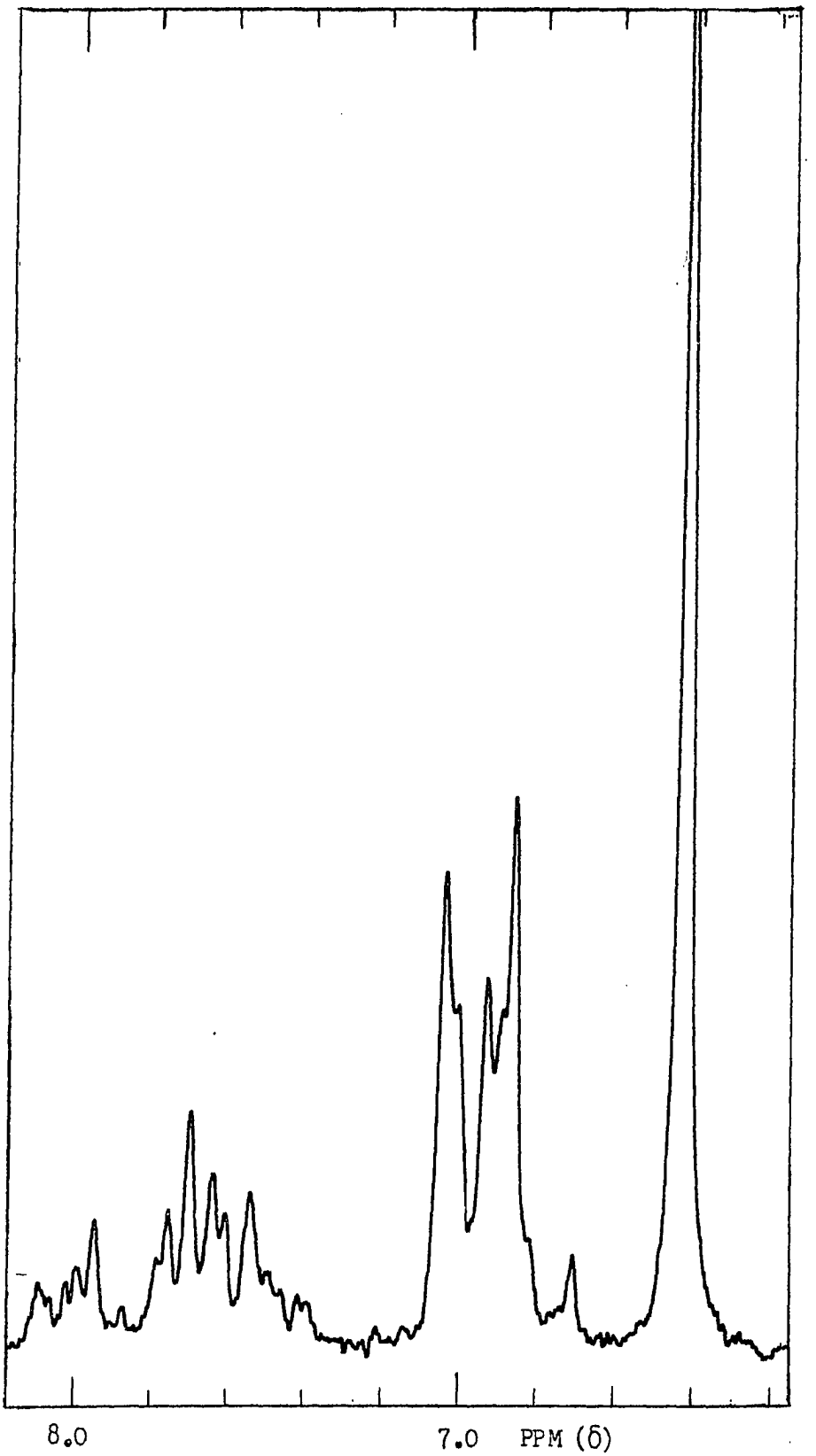
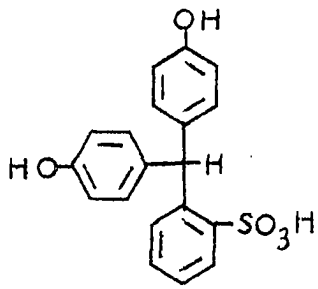

8.0
7.0
PPM (δ)

Figure 26. The NMR spectrum of phenolsulfonephthalin in D_2O.



Form which is present in solution

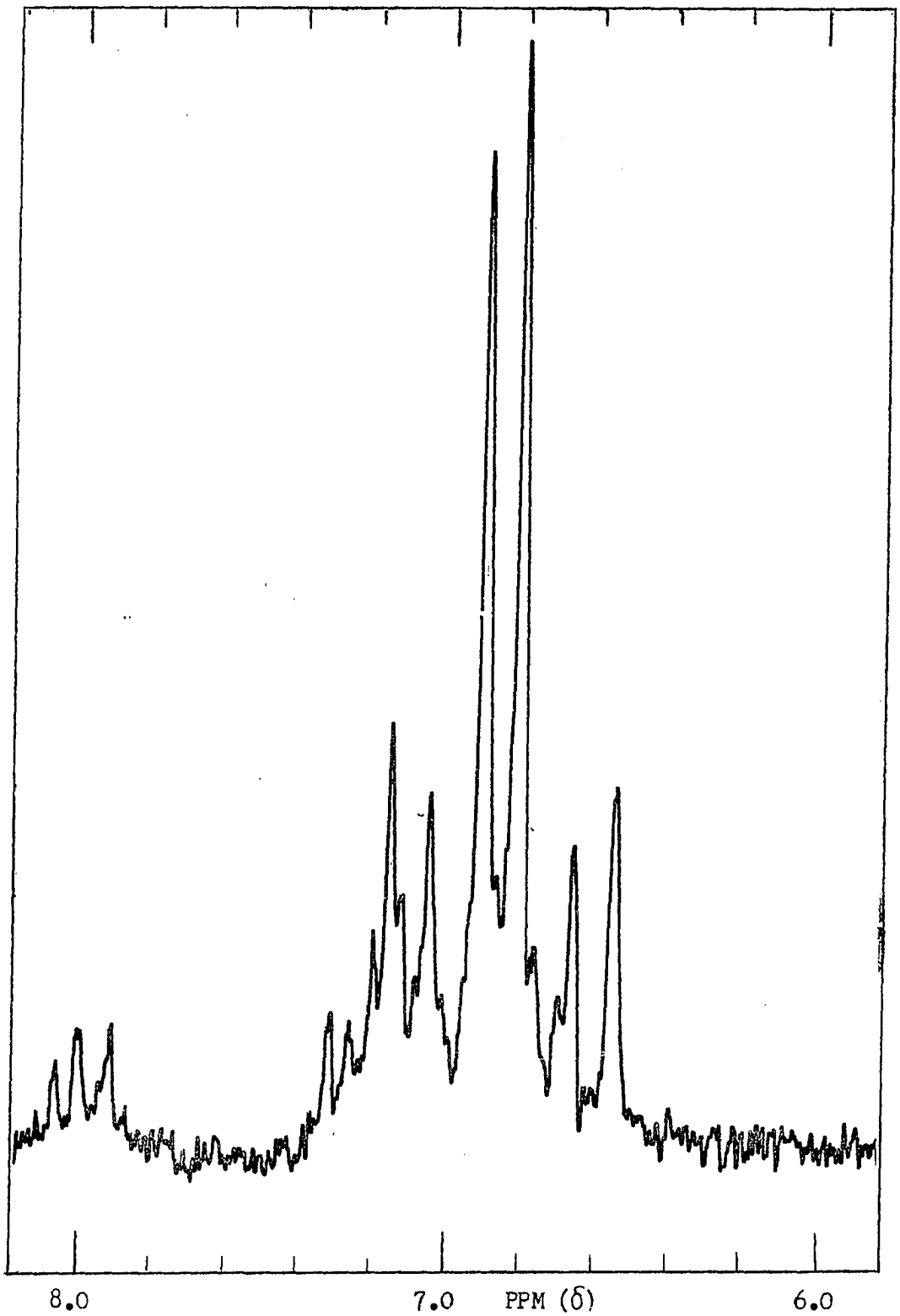
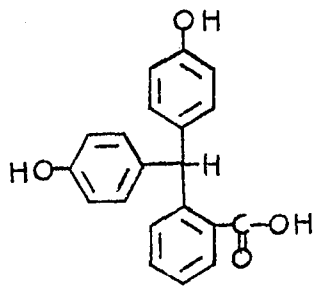

8.0
7.0
PPM (δ)
6.0

Figure 27. The NMR spectrum of phenolphthalin in DMDO.

The chemical shift of the offset peak (two protons) is 9.22 ppm.



Form which is present in solution

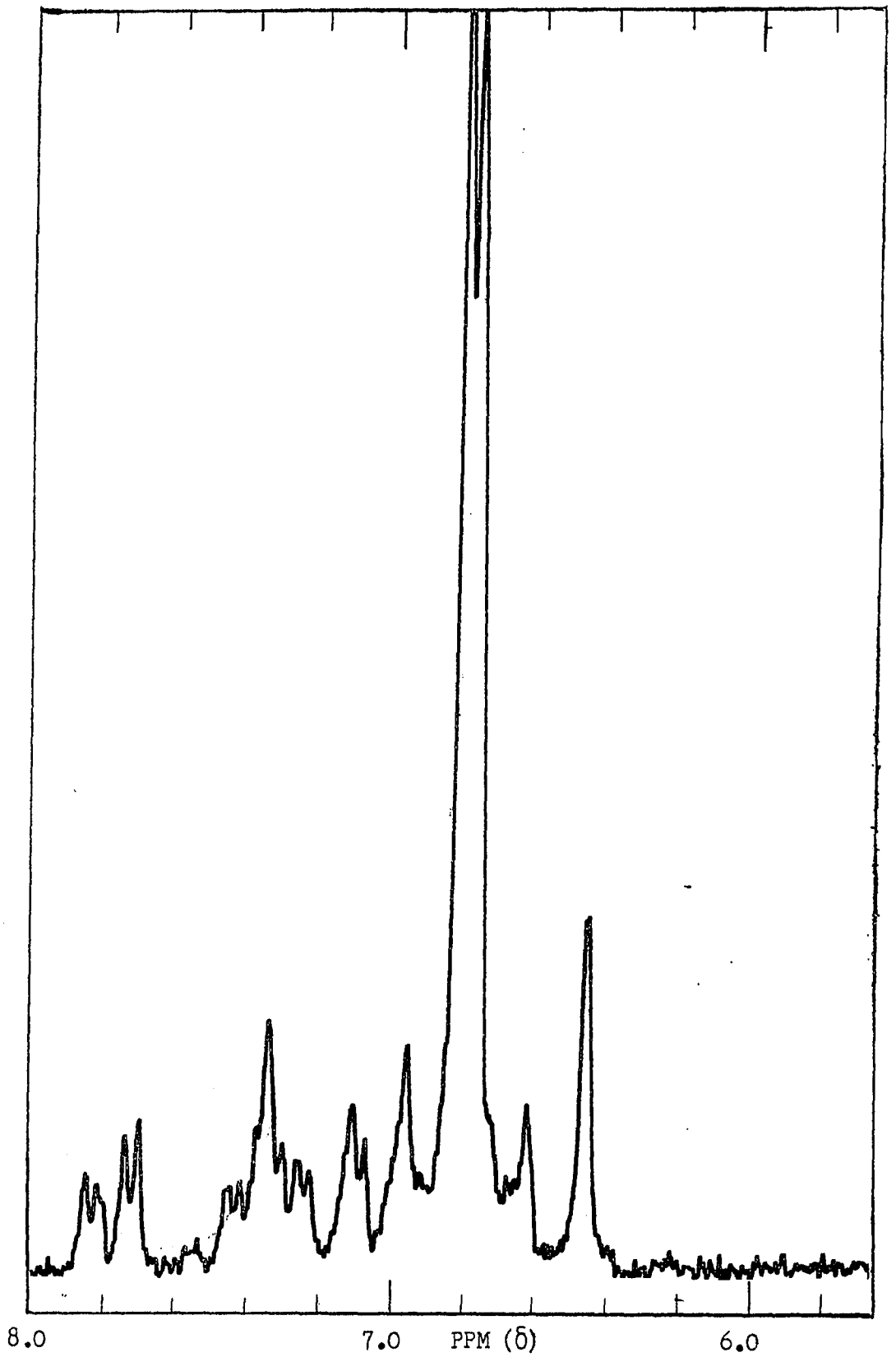
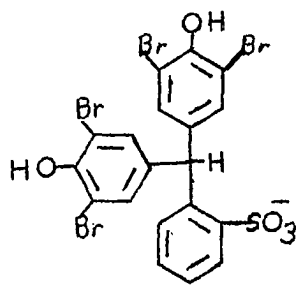

8.0
7.0
PPM (δ)
6.0

Figure 28. The NMR spectrum of tetrabromophenolsulfonephthalin in D_2O.



Form which is present in solution

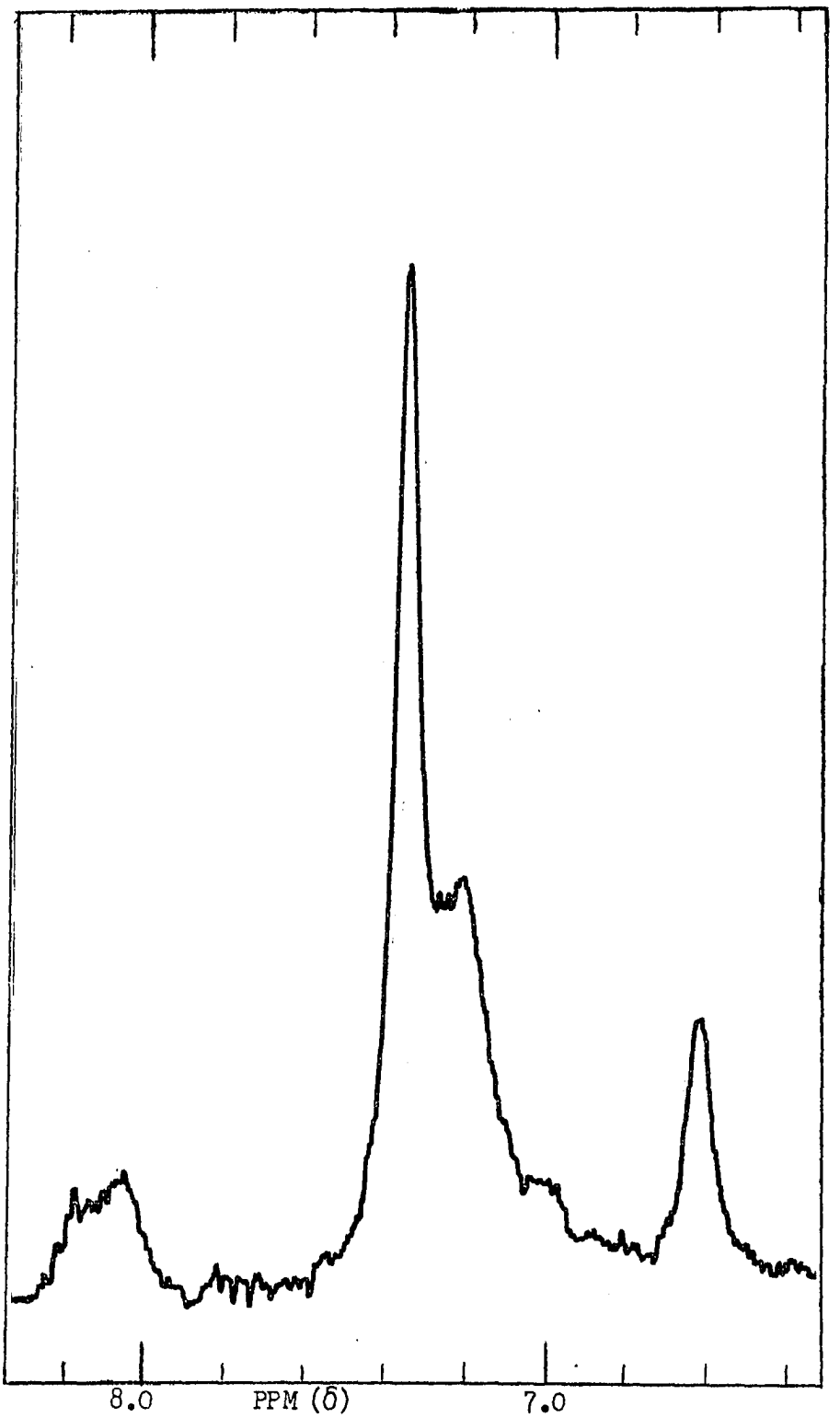
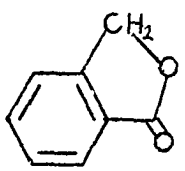

8.0
PPM (δ)
7.0

Figure 29. The NMR spectrum of phthalide in DMSO.



Form which is present in solution

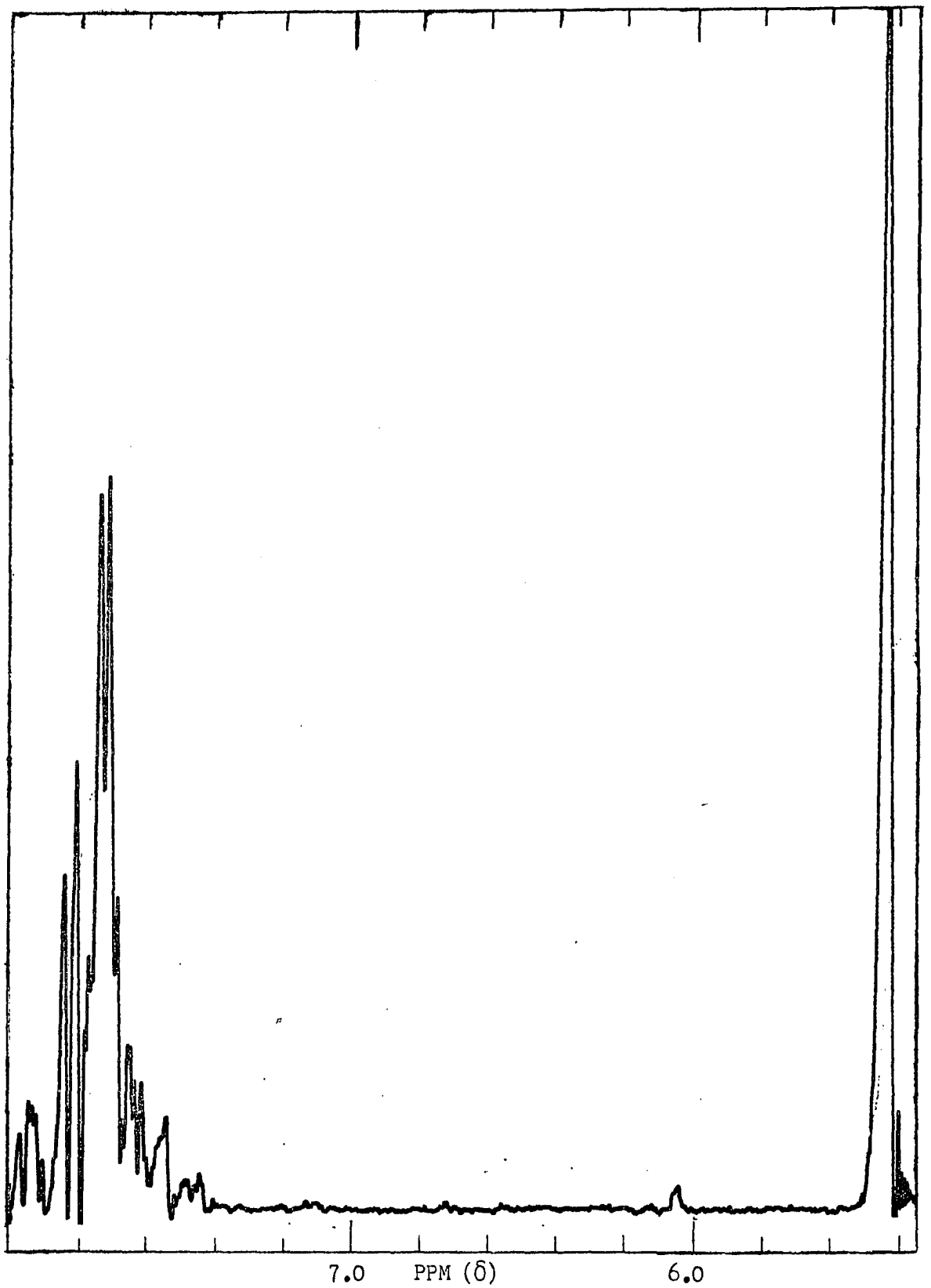
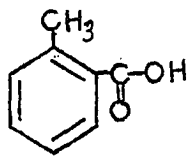

7.0
PPM (δ)
6.0

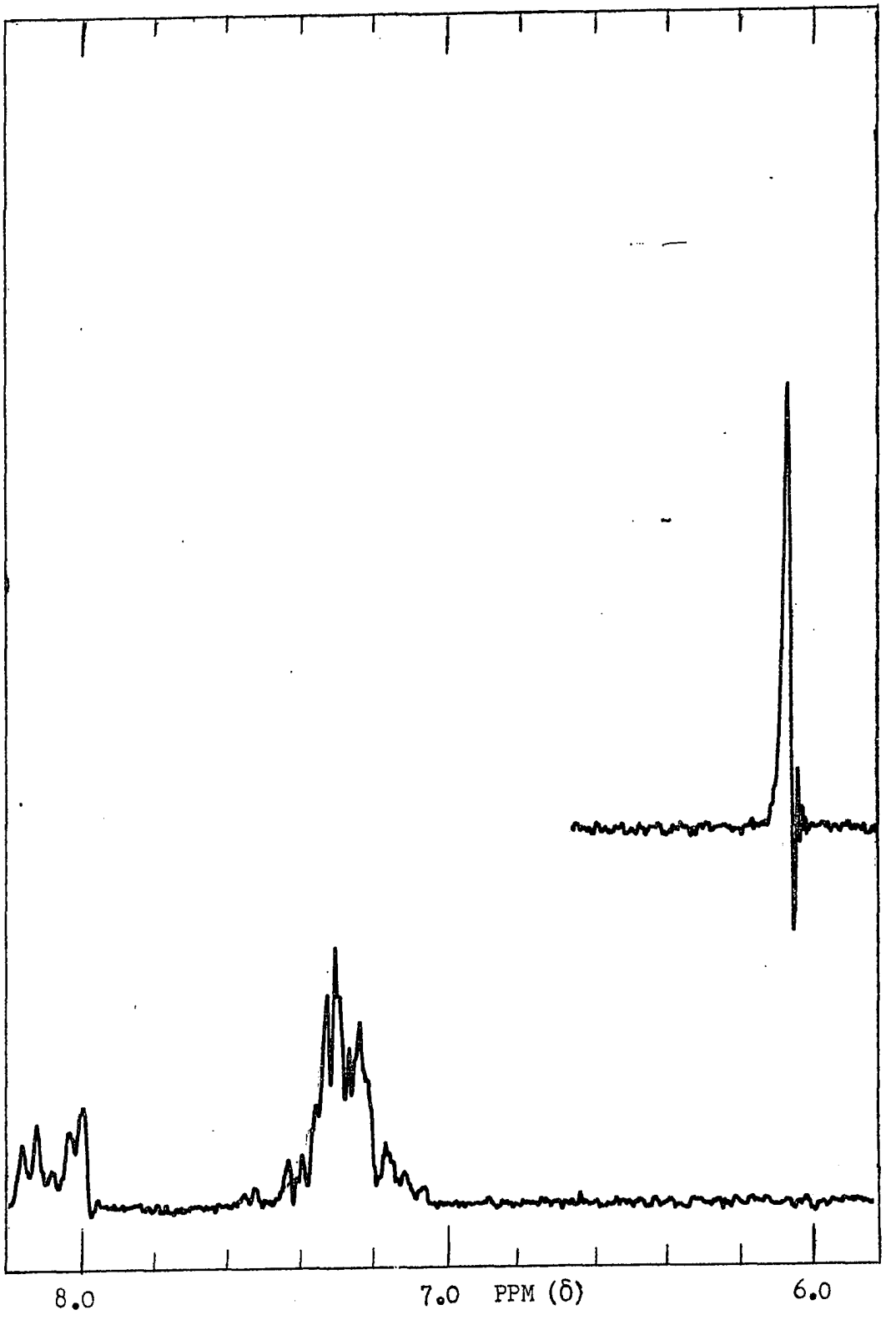
Figure 30. The NMR spectrum of o-toluic acid in CCl_4.

A sharp singlet (three protons) at 2.67 ppm is not shown.

The chemical shift of the offset peak is 12.73 ppm.



Form which is present in solution


8.0
7.0
PPM (δ)
6.0

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