

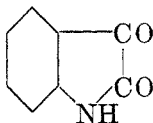
## THE POLAROGRAPHIC BEHAVIOR OF ISATIN

WARD C. SUMPTER, JOSEPHINE L. WILLIAMS,<sup>1</sup> PHIL H. WILKEN, AND BETTY LOU WILLOUGHBY<sup>2</sup>

Received December 11, 1947

Although polarographic studies of many organic compounds have been reported (1) including studies of keto-enol tautomerism (2) no such studies have been made of lactim-lactam types. It was felt that studies of the behavior of compounds of this type in the polarographic cell might serve to establish the existence of such tautomers in solutions of the compounds.

Isatin seemed ideally suited to this purpose since it contains the essential —CO—NH— grouping and since the *beta*-carbonyl group is readily reducible. To this end polarographic studies were conducted on carefully buffered solutions of isatin.



### EXPERIMENTAL

All polarographic data were taken with a Fisher Electropode which was operated manually. A piece of marine barometer tubing about 12 cm. in length was used as a capillary. A stock solution of 0.002 *M* isatin was used in the polarographic work and this solution was diluted to 0.001 *M* by mixing with an equal volume of the desired buffer solution. McIlvaine's standard buffer solutions were used in the *pH* range 2.2 to 8.0 and mixtures of sodium borate and sodium hydroxide solutions were used as buffers in the range 8.0 to 12.0.

The *pH* values of all solutions were determined with a Beckman *pH* meter using a glass electrode. The values reported for alcoholic solutions are "apparent values" not corrected for the nonaqueous solvent errors.

Removal of oxygen from the cell solutions was accomplished by passing natural gas through the solutions for a period of ten minutes before taking the polarographic data. The polarographic cell was maintained in an atmosphere of natural gas during the measurements to prevent absorption of oxygen. The natural gas was passed through an absorption train consisting of a solution of lead acetate, an alkaline solution of pyrogallol, a soda-lime tube and finally through a sample of the cell solution before entering the polarographic cell. All measurements were made with the polarographic cell connected to the saturated calomel electrode by means of an agar bridge saturated with potassium chloride. All measurements were made at a constant temperature of  $25 \pm 0.25^\circ$ . The resistance of the polarographic cell and bridge was of the order of 2000 ohms. The applied voltage was accurate to  $\pm 0.005$  volts.

*Results.* The results obtained with a solution of isatin 0.001 *M* and buffered at *pH* 7.10 are shown in Fig. 1. Curve 1, obtained with a freshly mixed solution,

<sup>1</sup> Present address: Department of Chemistry, Northwestern University, Evanston Illinois.

<sup>2</sup> Present address: Joseph E. Seagram and Sons, Inc., Louisville, Kentucky

exhibits four distinct waves. A second polarogram (curve 2) run on this solution after standing twenty-four hours exhibits only the two waves obtained at higher negative potentials. The solution which was originally yellow in color has now become nearly colorless. In the sequel the four waves shown in Fig. 1 are referred to as waves A, A', C, and D respectively, wave A being the wave observed at lowest potential and wave D that observed at highest potential. The initial and equilibrium polarograms given by a solution of isatin buffered at  $pH$  8.00 are shown in Fig. 2. These polarograms exhibit waves due to the same molecular (or ionic) varieties as did the solutions buffered at  $pH$  7.10.

The time required for equilibrium to be established varies with the  $pH$  value at which the solution is buffered and also with the buffer employed. In general

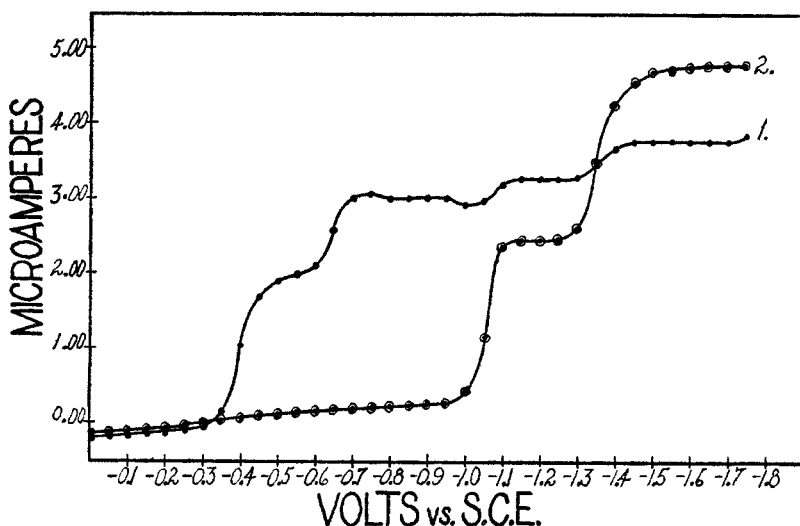


FIG. 1. 0.001 *M* SOLUTION OF ISATIN BUFFERED AT  $pH$  7.10  
Curve 1, initial polarogram. Curve 2, equilibrium polarogram.

equilibrium is established more rapidly as  $pH$  is increased and more rapidly when phosphate buffers are employed than when borate buffers are used. Seemingly the opening of the lactam ring is catalysed by phosphate ion. In all cases equilibrium can be established more quickly by heating the solution on the steam-bath for several hours.

A solution buffered at  $pH$  4.00 gave a polarogram (Curve 1, Fig. 3) exhibiting three waves (A, A', and B). The polarographic behavior of this solution did not change on standing.

The polarographic behavior of a solution of isatin buffered at  $pH$  2.88 is shown in Fig. 4. Curve 1 shows the behavior (waves A, A', and B) of a freshly mixed solution and curve 2 that of a solution after equilibrium has been established (waves A and A' only).

In Fig. 3 the equilibrium curves of solutions buffered at  $pH$  values of 4.00,

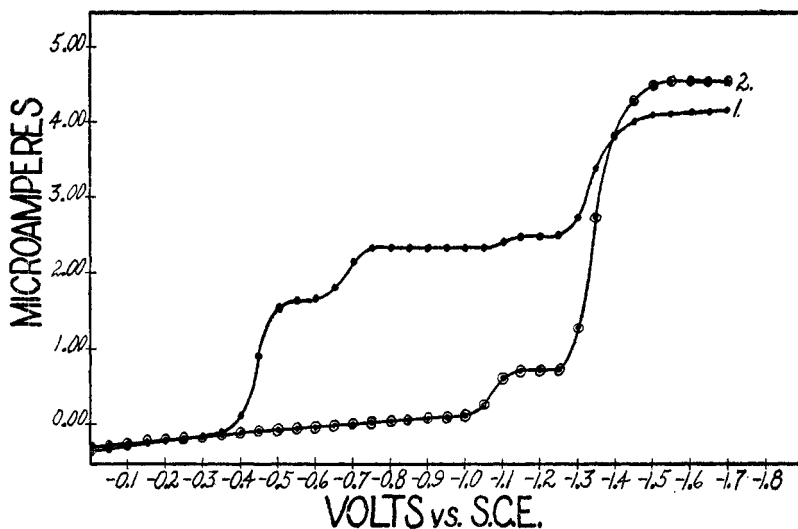


FIG. 2. 0.001 *M* SOLUTION OF ISATIN BUFFERED AT *pH* 8.00  
Curve 1, initial polarogram. Curve 2, polarogram at equilibrium.

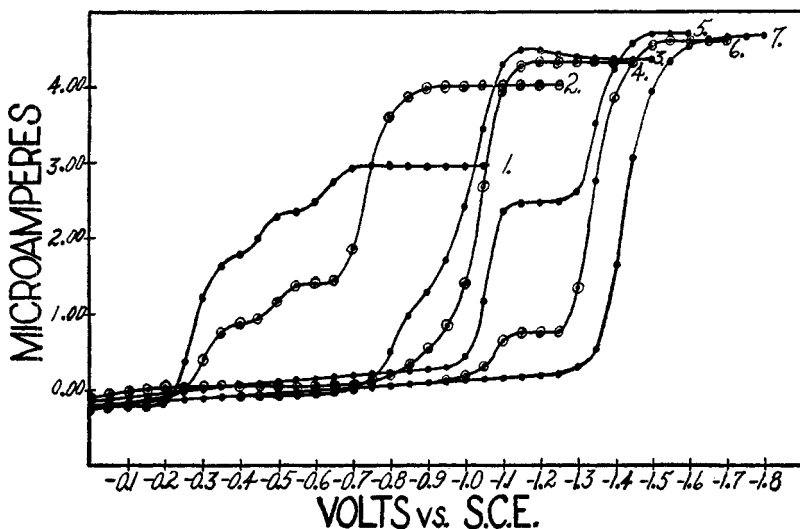


FIG. 3. 0.001 *M* SOLUTION OF ISATIN. EQUILIBRIUM POLAROGRAMS

Curve 1, buffered at *pH* 4.00. Curve 2, buffered at *pH* 4.35. Curve 3, buffered at *pH* 5.43. Curve 4, buffered at *pH* 5.83. Curve 5, buffered at *pH* 7.10. Curve 6, buffered at *pH* 8.00. Curve 7, buffered at *pH* 9.75.

4.35, 5.43, 5.83, 7.10, 8.00, and 9.75 are plotted. Curves 1 and 2 exhibit waves A, A', and B. Curve 3 exhibits waves B and C, while curve 4 shows only wave C. Curves 5 and 6 exhibit waves C and D while curve 7 exhibits only wave D (The explanation for the varying wave heights will be considered in the sequel).

A solution buffered at  $pH$  11.60 gives a curve quite like that given by the solution buffered at  $pH$  9.75 except that the single wave (D) appears at a potential slightly more negative.

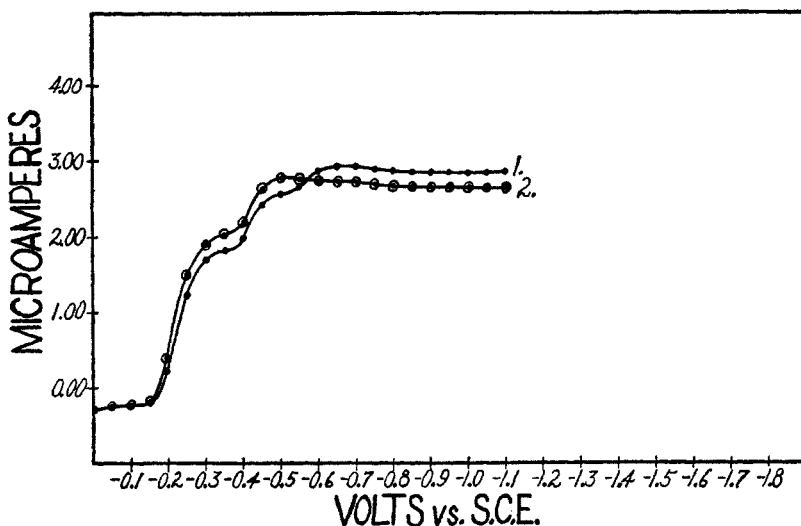


FIG. 4. 0.001  $M$  SOLUTION OF ISATIN BUFFERED AT  $pH$  2.88  
Curve 1, initial polarogram. Curve 2, polarogram at equilibrium.

TABLE I

$E_{1/2}$  VALUES (VOLTS vs. S.C.E.) FOR MOLECULAR VARIETIES A, A', B, C, AND D

$pH$	MOLECULAR VARIETY				
	A	A'	B	C	D
2.88	-0.22	-0.42			
3.01	-0.23	-0.43	-0.54		
3.40 <sup>a</sup>	-0.32				
4.00	-0.28	-0.46	-0.63		
4.35	-0.30	-0.49	-0.74		
5.43			-0.80	-1.02	
5.83				-1.04	
7.10	-0.39	-0.65		-1.06	-1.35
8.00	-0.44	-0.68		-1.08	-1.36
9.75					-1.44
11.10 <sup>a</sup>					-1.47

<sup>a</sup> "Apparent"  $pH$  values uncorrected for nonaqueous solvent errors.

Solutions exhibiting predominantly waves A and A' are yellow in color. This yellow color disappears as waves A and A' give way to waves B, C, and D. This disappearance of yellow color has long been supposed to be associated with opening of the lactam ring (3).

The solution of isatin of  $pH$  11.60 becomes a deep reddish-purple color when the solution is first made alkaline but rapidly loses color and is practically colorless by the time the solution has been degassed and is ready to be run polarographically. This solution then exhibits only wave D. The purple color is so transient that polarographic curves could not be obtained on the purple solutions. When the isatin solution is one in 50% ethanol the purple color is much more permanent. The polarographic behavior of these alcoholic solutions is somewhat complex and is now being studied further. The results of these studies will be reported subsequently. The purple color and the polarographic behavior of these solutions provide sufficient evidence to justify the assumption that another molecular variety (variety E) is present in these solutions.

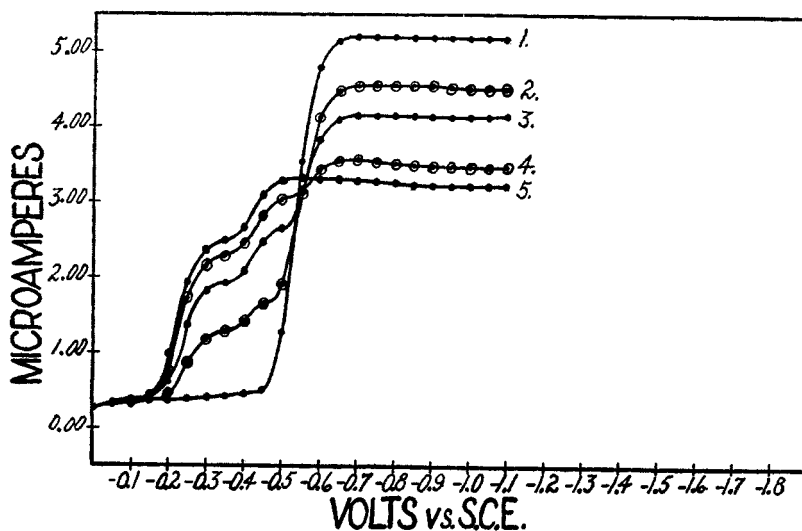


FIG. 5. 0.001  $M$  SOLUTION OF ISATIN BUFFERED AT  $pH$  3.01

Prepared as described in the text. Curve 1 is the initial curve, curve 5 the equilibrium curve, curves 2, 3, and 4 are intermediate curves.

Polarograms exhibiting only wave B can be obtained by preparing the polarographic solution in the following manner. To the isatin stock solution (0.002  $M$ ) is added first the alkaline constituent of the McIlvaine standard buffer. The solution is allowed to stand until the yellow color disappears. The citric acid portion of the standard buffer is then added to provide a solution 0.001  $M$  with respect to isatin and buffered at  $pH$  3.01. After degassing, curve 1 of Fig. 5 was obtained in the polarographic cell. As the solution aged curves 2, 3, 4, and 5 were obtained in succession, curve 5 being the equilibrium curve. It will be noticed that wave B alone is exhibited by curve 1 and that this wave gradually gives way to waves A and A'. The equilibrium curve is quite similar to that shown in Fig. 4 for a solution buffered at  $pH$  2.88. In Fig. 4 it will be observed that wave B also disappears as the solution approaches equilibrium.

*Discussion.* A possible rationalization of this behavior is shown in Chart I. In this rationalization it is postulated that the molecular (or ionic) varieties responsible for the several polarographic waves are: A, the lactam form of isatin; A', a hydrate of A; B, the cation; C, the dipolar ion; D, the anion; and E, the lactim (or the lactim ion) form of isatin each related to the other as sug-

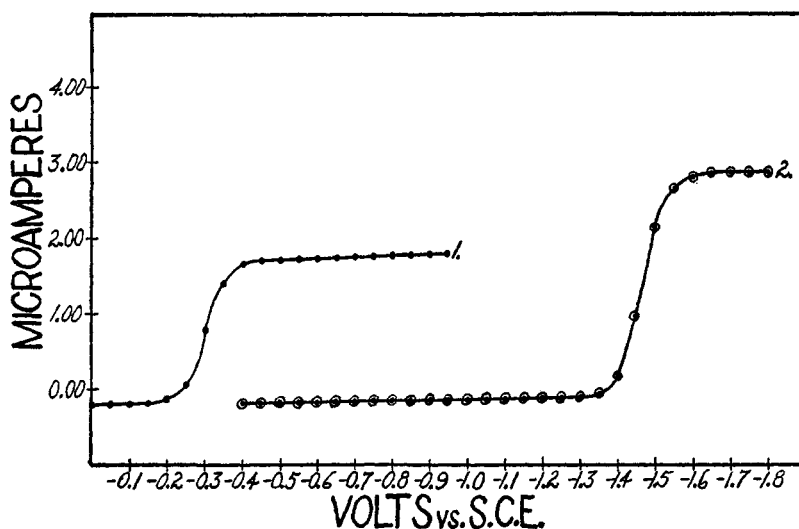
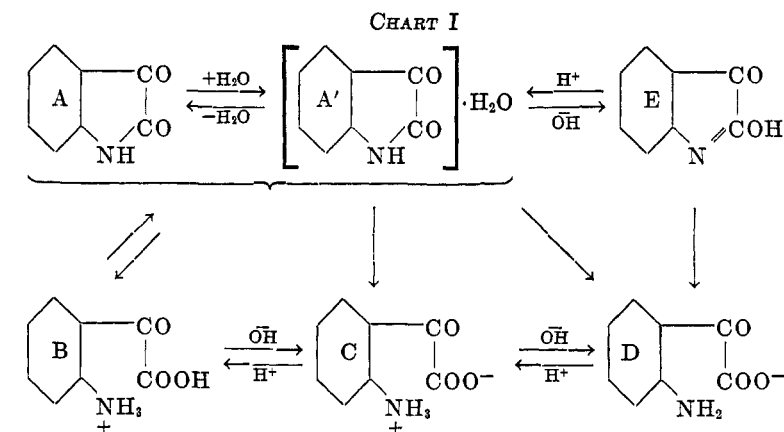


FIG. 6. 0.001 *M* Solution of isatin in 50% ethanol. Both curves are equilibrium curves. Curve 1, buffered at pH 3.40. Curve 2, buffered at pH 11.10.

gested in Chart I. In support of the postulate that variety A' is a hydrate of A it was found that wave A' became successively smaller when solutions containing 10%, 20%, 30%, 40%, and 50% ethanol were employed. In 40% and 50% ethanol solutions no A' wave was observed. In Fig. 6 curve 1, is shown the polarogram of a solution of isatin (0.001 *M*) in 50% ethanol at an apparent pH of 3.40.

Calculation of the number of electrons involved in the reduction by means of the Ilkovic equation. The number of electrons involved in the reduction of a substance at the dropping mercury electrode can be calculated by means of the Ilkovic equation.

$$n = I_d / 605 D^{1/2} C m^{2/3} t^{1/6}$$

Since experimental values for the diffusion coefficient  $D$  were not available a value for  $D$  was calculated by assuming that Stoke's law holds under the experimental conditions [ref. (1a), p. 48]. The value (1.51) used for the density of isatin was that given by Cox, Goodwin, and Wagstaff (4).

$$\begin{aligned} V_m &= \text{Mol. Wt./density} = 147/1.51 = 97.3 \\ D &= 3.32 \times 10^{-5} / V_m^{1/3} = 3.32 \times 10^{-5} / (97.3)^{1/3} \\ D &= 7.22 \times 10^{-6} \text{ cm.}^2 \text{ sec.}^{-1} \end{aligned}$$

Calculation of  $n$  for the  $D$  wave in aqueous solution (Curve 7, Fig. 3)

$$\begin{aligned} m &= 0.9824 \text{ mg./sec.} & t &= 5.76 \text{ sec. (at } -1.44 \text{ v.)} \\ m^{2/3} t^{1/6} &= 1.32 \text{ mg.}^{2/3} \text{ sec.}^{-1/2} & I_d &= 4.40 \text{ microamp.} \\ n &= 4.40/605 (7.22 \times 10^{-6})^{1/2} (1) (1.32) = 2.05 \end{aligned}$$

Calculation of  $n$  for the  $B$  wave (Curve 1, Fig. 5)

$$\begin{aligned} m &= 0.9824 \text{ mg./sec.} & t &= 7.28 \text{ sec. (at } -0.54 \text{ v.)} \\ m^{2/3} t^{1/6} &= 1.38 \text{ mg.}^{2/3} \text{ sec.}^{-1/2} & I_d &= 4.60 \text{ microamps.} \\ n &= 4.60/605 (7.22 \times 10^{-6})^{1/2} (1) (1.38) = 2.10 \end{aligned}$$

Calculation of  $n$  for the  $C$  wave (Curve 4, Fig. 3)

$$\begin{aligned} m &= 0.9824 \text{ mg./sec.} & t &= 6.92 \text{ sec. (at } -1.04 \text{ v.)} \\ m^{2/3} t^{1/6} &= 1.36 \text{ mg.}^{2/3} \text{ sec.}^{-1/2} & I_d &= 4.22 \text{ microamps.} \\ n &= 4.22/605 (7.22 \times 10^{-6})^{1/2} (1) (1.36) = 1.90 \end{aligned}$$

No calculation of  $n$  for the  $A$  wave can be made from diffusion currents measured in aqueous solution because in such solutions wave  $A$  is always accompanied by wave  $A'$ . When solutions in 50% ethanol are employed no evidence of the presence of variety  $A'$  is found and diffusion currents for variety  $A$  can be measured.

In such solutions no values are available for the diffusion coefficient  $D$ , the values calculated through the use of the Stokes-Einstein equation being valid only for aqueous solutions at 25°. However assuming that wave  $D$  in 50% ethanol (curve 2, Fig. 6) represents a two-electron reduction we can calculate a diffusion coefficient for isatin in 50% ethanol.

$$\begin{aligned} m &= 0.9824 \text{ mg./sec.} & t &= 5.56 \text{ sec. (at } -1.47 \text{ v.)} \\ m^{2/3} t^{1/6} &= 1.32 \text{ mg.}^{2/3} \text{ sec.}^{-1/2} & I_d &= 2.95 \text{ microamps.} \\ I_d &= 605nCD^{1/2} m^{2/3} t^{1/6} \\ 2.95 &= 605 (2) (1) D^{1/2} (1.32) \\ D &= 3.43 \times 10^{-6} \text{ cm.}^2 \text{ sec.}^{-1} \end{aligned}$$

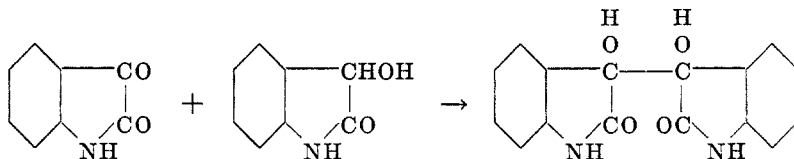
Then from the diffusion current for wave A (curve 1, Fig. 6) we can calculate a value for  $n$ .

$$\begin{aligned} m &= 0.9824 \text{ mg./sec.}^{-1} & t &= 6.84 \text{ sec. (at } -0.32 \text{ v).} \\ m^{2/3} t^{1/6} &= 1.36 \text{ mg.}^{2/3} \text{ sec.}^{-1/2} & I_d &= 1.80 \text{ microamps.} \\ n &= 1.80/605 (3.43 \times 10^{-6})^{1/2} (1) (1.36) = 1.18 \end{aligned}$$

It is thus rather definitely established that molecular variety A undergoes a one-electron reduction while varieties B, C, and D undergo two-electron reductions. This accounts satisfactorily for the increase in wave height as freshly prepared solutions exhibiting wave A age with wave A giving way to waves B, C, and D.

No calculation of a value of  $n$  is possible for variety A' since this wave cannot be isolated and since the relative concentrations of A and A' in these solutions are unknown. However, consideration of the relative wave heights in a large number of polarograms exhibiting waves A and A' leads one to the conclusion that the reduction of A' (wave A') is probably a two-electron reduction altho it must be recognized that a rigorous determination of this point has not been possible.

It is by no means surprising that variety A should exhibit a reduction wave corresponding to a one-electron reduction in view of the well known fact that the product of the two-electron reduction of isatin, dioxindole, condenses readily with isatin to give isatin pinacol (isatide).



Thus if variety A undergoes a two-electron reduction to dioxindole which in turn condenses with a molecule of isatin to form isatide the reduction wave exhibited by A will correspond to a one-electron reduction.

It is likewise not at all surprising to find that varieties A', B, C, and D undergo two-electron reductions and being incapable of condensing with their reduction products exhibit waves corresponding to two-electron reductions.

As previously stated the polarographic behavior of solutions containing variety E (the purple variety) is complex. These solutions are being studied further and it is hoped that quantitative treatment of the polarograms obtained can be given in a later paper. It can only be said now that from the appearance (color) and polarographic behavior of such solutions it is evident that another molecular variety (E) is present. This is thought to be the lactim (or the lactim ion) form of isatin since the N-alkylisatins (in which lactim formation is not possible) do not yield the transient purple color while isatin derivatives like 5-methylisatin, 5-bromoisatin, etc., which contain the  $-\text{CO}-\text{NH}-$  grouping do give this color behavior.

*Absorption spectra.* Absorption spectra measurements were made on solu-



tions known (from their polarographic behavior) to contain only one molecular (or ionic) variety. The results of these measurements are shown in Fig. 7. The similarity between the curves for varieties B, C, and D is in agreement with the rationalization postulated. Absorption spectra measurements made on isatin

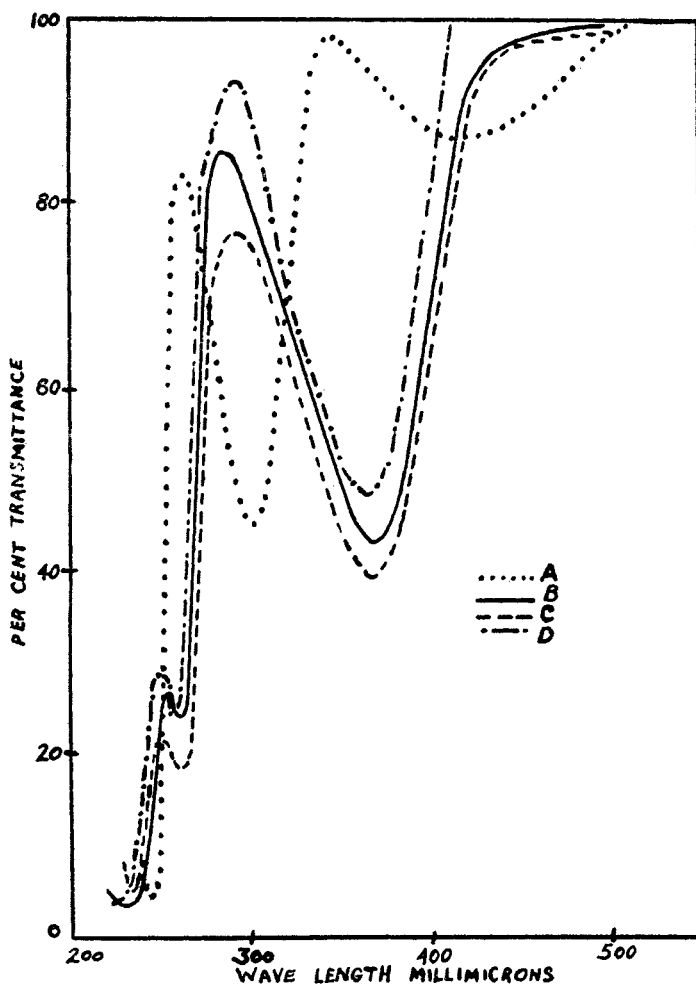


Fig. 7. Curve A. 0.0001 *M* isatin in 50% ethanol-H<sub>2</sub>O buffered at pH 3.00. Curve B. 0.0001 *M* isatin solution. Variety B isolated temporarily as in polarographic curve 1 of Fig. 5. Curve C. 0.0001 *M* equilibrated solution of isatin buffered at pH 5.83. Curve D. 0.0001 *M* equilibrated solution of isatin buffered at pH 11.60.

solutions have been reported previously by several workers (3, 5) but since the data reported by these workers are conflicting and their measurements were made for the most part on solutions which our work shows would certainly contain more than one molecular (or ionic) variety it appears that little value can be attached to the previous measurements. The absorption spectra measure-

ments shown in Fig. 7 were made with a Beckman DU spectrophotometer on solutions 0.0001 *M* with respect to isatin. Cells of 1.0 cm. depth were used.

*Acknowledgment.* The senior author (W. C. S.) is grateful to the Research Corporation for a grant which has made available the services of his co-workers and which made possible the purchase of necessary equipment.

#### SUMMARY

Polarographic evidence indicates that solutions of isatin contain (under different conditions) six different molecular or ionic varieties. A rationalization of this behavior has been presented which is in accord with the previously known facts of isatin chemistry.

BOWLING GREEN, KENTUCKY

#### REFERENCES

- (1) For extensive bibliographies see: (a) KOLTHOFF AND LINGANE, *Polarography*, Interscience Publishers, Inc., New York, 1941. (b) MÜLLER, *Chem. Revs.*, **24**, 95 (1939). (c) MÜLLER, Chapter on Polarography in Weissberger "Physical Methods of Organic Chemistry", Vol. II, Interscience Publishers, Inc., New York, 1946.
- (2) MÜLLER AND BAUMBERGER, *J. Am. Chem. Soc.*, **61**, 590 (1939).
- (3) AULT, HIRST, AND MORTON, *J. Chem. Soc.*, 1653 (1935).
- (4) COX, GOODWIN, AND WAGSTAFF, *Proc. Roy. Soc. (London)*, **A157**, 399 (1936).
- (5) ARBUZOV, *Bull. acad. sci. U.R.S.S., Classe sci. chim.*, 89 (1940); *Chem. Abstr.*, **35**, 2508 (1941); DABROWSKI AND MARCHLEWSKI, *Bull. soc. chim.*, **53**, 946 (1933); HARTLEY AND DOBBIE, *Trans. Chem. Soc.*, **27**, 640 (1899); KORCZYNSKI AND MARCHLEWSKI, *Ber.*, **35**, 4337 (1902); MENCZEL, *Z. physik. Chem.*, **125**, 161 (1927); MORTON AND ROGERS, *J. Chem. Soc.*, **127**, 2698 (1925).