Polarographic Behavior of 5-Phenylazopyrimidinetrione and Its Substituted Derivatives

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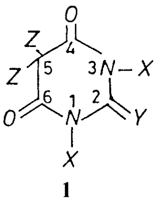
The polarographic behavior of 5-phenylazo-2-thiobarbituric acid and 5 of its substituted derivatives was investigated in a buffer solution of pH (2—12). A mechanism for the reduction pathway is suggested. Based on the results of the nitro derivative, the coupling product of 2-thiobarbituric acid with diazonium salt is most probably in the azo and not the hydrazone form. The most reliable $E_{1/2}$ values at different pH's were correlated to Hammett's σ constant which reflected a fair correlation.

Key words 5-phenylazopyrimidinetrione; polarographic behavior

The chemistry of pyrimidines has occupied unlimited attraction of researchers. Barbituric acid and barbiturates, as a class of these compounds, had received a great share of this attention. This is because of its wide medicinal applications, in particular its hypnotic action. The literature includes countless papers, reports and reviews. Representative of these articles are those dealing with its hypnotic action,[1,2] enzyme stimulation and inhibition[3,4] its anticonvulsant potency[5-7] and initiation of anesthesia.[8,9] Relevant reviews have also been published.[10-12] The use of these compounds as chelating agents for metal ions have been also reported.[13,14] However, the electroreduction of these compounds has received relatively little attention. Yet, several articles dealing with its polarographic reduction can be traced in the literature.[15,16]

Bearing such wide applicable importance, the polarographic reduction at a dropping mercury electrode (DME) of the coupling products of aryl diazonium salts with barbituric acid (**1a**) and its derivatives was the aim of a through investigation in our laboratory. Very recently,[17] the polarographic behavior of 1,3-dimethyl barbituric acid (**1c**) and its 5-phenylazobarbituric acid was published. Continuing in this direction we report here the polarographic and spectrophotometric behavior of 1,3-diethyl-2-thiobarbituric acid (**1b**) and its 5-phenylazo derivatives (**2a—f**) in buffer solution covering the pH range of *ca.* 2—12. The work aims to demonstrate the actual mechanistic reduction pathway taking place at the DME, and also to test the fidelity of linear free energy relationship (LFER) to the series under consideration through $E_{1/2}$–σ correlations.



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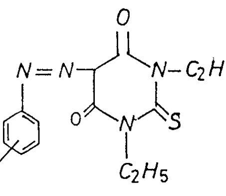

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1a: X=Z=H, Y=O
1b: X=–C$_2$H$_5$, Y=S, Z=H
1c: X=–CH$_3$, Y=O, Z=H
1d: X=H, Y=O, Z=–C$_2$H$_5$

R: a=H, b=*p*-CH$_3$, c=*p*-OCH$_3$
d=*p*-Cl. *e*=*m*-NO$_2$, f=*p*-NO$_2$

Experimental

Organic Syntheses 5-Phenylazo-2-thiobarbituric acid (**2a**) and the corresponding 5-phenylazo substituted derivatives (**2b—f**) were prepared by dissolving aniline or the relative substituted aniline (0.0068 mol) in concentrated hydrochloric acid (6 ml) and water (6 ml); these were then cooled to 0 °C and treated with a cold solution of sodium nitrite (0.6 g) in water (6 ml). The diazotized amine was added gradually to an ice-cooled solution of 1,3-diethyl-2-thiobarbituric acid (Aldrich product 1.5 g) dissolved in ethanol (50 ml) containing sodium acetate (2.6 g). The mixture was left overnight in a refrigerator. The separated product was filtered off, washed with water, and recrystallized from ethanol. The crystalline shape, mp, and elemental analyses for the 5-phenylazo-derivatives (**2a—f**) are listed in Table 1. The purity of the products was also checked by thin layer chromatography (TLC).

Polarography and Cyclic Voltammetry Apparatus: DC polarograms were recorded using a 693 VA processor supplemented with a multimode electrode 694 VA stand (Metrohm, Switzerland). The capillary possessed a drop time of 4 s d^{-1} and a flow rate of 1.46 mg s^{-1} under one atmosphere of nitrogen pressure. Cyclic voltammograms were carried out on a hanging mercury drop electrode (HMDE) using the same instrument.

Solution: 10^{-3} M-Stock solutions of (**2a—f**) were prepared by dissolving an accurately weighed quantity of material in the appropriate volume of absolute ethanol (n_D^{25} 1.359). Britton–Robinson modified universal buffer[18] (prepared from AnalaR grade chemicals) was used as the supporting electrolyte. The pH value of each buffer was measured using a digital pH meter (Hanna, Italy, ±0.01 pH unit) and a combined glass electrode.

Measurements: All experiments were carried out at 25±2 °C. The half-wave potentials were recorded graphically and expressed versus the Ag/AgCl (sat. KCl) electrode.

Procedure: Ethanol (7 ml) and the appropriate buffer solution (12 ml) were introduced into the polarographic cell. The solution was then deaerated by bubbling a stream of purified nitrogen for 20 min. The calculated amount of stock solution was then introduced into the cell so that the final concentration was 5×10^{-5} M in 20 ml of 40% (v/v) ethanolic buffer. The nitrogen gas was allowed to pass for another 2 min before the run was registered.

Controlled Potential Electrolysis (CPE) and Determination of the Number of Electrons Mercury pool electrolysis was carried out in 40% v/v ethanol 10^{-2} M HCl solution (200 ml), and substance **2a** (200 mg) taken as a typical example. The cell and detailed procedure were as previously published.[17] The progress of the electrolysis was followed by recording the decrease in current with time, and the number of electrons was computed from *i*–*t* curves following the procedure outlined by Lingane[19]; it was found to be 4*e*. After disconnecting the electrolysis cell, 1 ml of the resulting solution was withdrawn and the presence of aniline in this solution was revealed by a standard spot test.[20] The remaining reaction mixture was concentrated to one-fourth its original volume using a rotatory evaporator, then extracted with ether. The ether layer was in turn evaporated and the solid was then recrystallized from ethanol; pale yellow crystals were obtained (yield 55%) identified as 5-amnino-2-thio-1,3-diethylbarbituric acid mp 205 °C. IR (KBr): 3300

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Table 1. Analysis and Physical Data for the Synthesized 5-Phenylazo-2-thiobarbituric Acid **2a**—**f**

Compd.	Formula	Crystalline shape	mp ±1 °C	Yield[a] (%)	Analysis (%) C Calcd	C Found	H Calcd	H Found	N Calcd	N Found	S Calcd	S Found
2a	$C_{14}H_{16}N_4O_2S$ parent	Yellowish brown crystals	185	65	55.24	55.07	5.99	5.46	18.41	18.08	10.55	10.89
2b	$C_{15}H_{18}N_4O_2S$ p-CH_3	Brick-red needles	207	70	56.56	56.27	5.70	5.47	17.60	17.28	10.07	9.85
2c	$C_{15}H_{18}N_4O_3S$ p-OCH_3	Dark brick-red needles	182	74	53.88	53.95	5.42	5.09	16.76	16.37	9.59	9.83
2d	$C_{14}H_{15}N_4O_2ClS$[b] p-Cl	Orange needles	224	70	49.63	49.71	4.46	4.25	16.54	16.42	9.46	9.67
2e	$C_{14}H_{15}N_5O_4S$ m-NO_2	Yellow fluffy crystals	176	75	48.13	48.31	4.33	4.06	20.04	19.91	9.18	9.27
2f	$C_{14}H_{15}N_5O_4S$ p-NO_2	Bright brick-red long needles	240 (dec.)	73	48.13	48.35	4.33	4.45	20.04	19.88	9.18	9.35

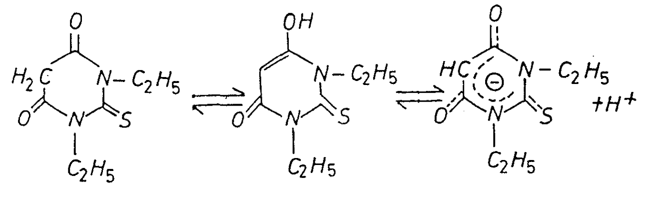
a) Calculated for pure recrystallized product. *b*) Cl: Calcd: 10.464. Found: 10.21.

(NH_2); 1770 (CO); 1600 (NH) cm^{-1}. *Anal.* Calcd for $C_8H_{13}N_3O_2S$: C, 44.63; H, 6.08; N, 19.52; S, 14.89. Found: C, 44.39; H, 5.96; N, 19.34; S, 14.60.

Spectrophotometric Measurement The apparent acid dissociation constant for compounds **1a**—**d** and **2a**—**f** were measured spectrophotometrically using a Unicam microprocessor version UV/Vis spectrometer UV2-ATI Unicam. Runs in the UV and visible range were carried out in 5×10^{-5}—10^{-5} M of **1a**—**d** and **2a**—**f**, 10% ethanolic buffers at increases of roughly 0.5 pH unit. The pK_a was obtained according to Issa and Zwail[21] by plotting pH *vs.* $\log[(A-A_{min})/(A_{max}-A)]$, where A_{min} is the absorbance of the undissociated acid, A the absorbance of the organic reagent at a given pH value, and A_{max} the absorbance of the completely dissociated acid at high pH medium. The intersection of the straight line with the pH axis gave the pK_a.

Results and Discussion

Model Compound 1b Similarly to barbituric acid **1a**, 1,3-diethyl-2-thiobarbituric acid (**1b**) didn't show any polarographic reduction wave throughout the whole range of study (pH *ca.* 2—12). The anions of these molecules are stabilized since the negative charge is well spread over the whole molecule, rendering it polarographically inactive:



Deactivation behavior through charge delocalization has been reported earlier for β-diketones.[22] On the other hand, the spectrophotometric pK_a of **1b** (*cf.* Table 2) showed marked acidity (pK_a *ca.* 4), a value which is near that for barbituric acid (**1a**) (pK_a *ca.* 4.1) and 1,3-dimethylbarbituric acid **1c** (pK_a=4.5),[17] but is quite different from that of 5,5-diethyl barbituric acid (**1d**) (barbital, pK_a *ca.* 8), where the 2H of the CH_2 group in position 5 is substituted by ethyl groups. The enhanced acidity of barbituric acid could, therefore, be unambiguously attributed to the CH_2 group in position 5.

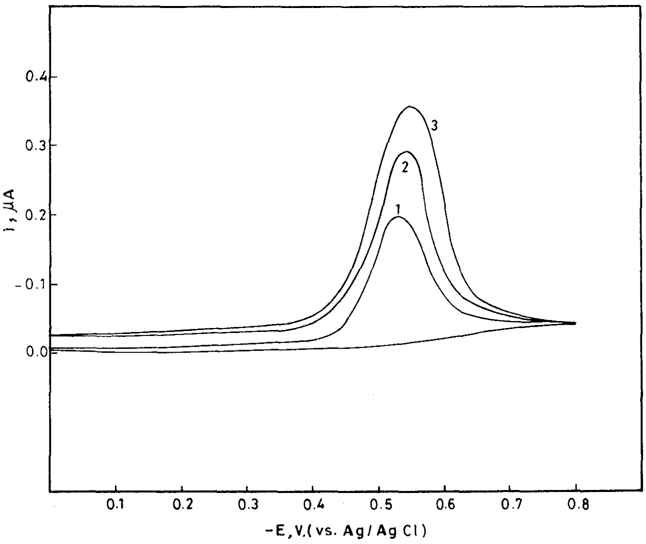


Fig. 1. Cyclic Voltammograms for 5×10^{-5} M of **2a** in 40% (v/v) Ethanolic Buffer (pH 4.03) at Scan Rates

1, 50 mV/s; 2, 100 mV/s; 3, 300 mV/s.

Compounds 2a—f The polarograms of 5×10^{-5} M solutions in 40% (v/v) ethanolic buffers of these compounds displayed a well-defined 4-electron diffusion controlled wave, except for the m-NO_2 and p-NO_2 derivatives (compounds **2e, f**) where additional waves for the nitro group have been registered. The diffusion nature of the wave was tested by the effect of time on i_l at a selected pH solution for a period of 3 h, which proved to be almost constant; also by the linear dependence of i_l with concentration (10^{-4}—10^{-5} M) at various pH values. The irreversible nature of the wave was proved from cyclic voltammetric measurement at the HMDE electrode at different pH and scan rates. As disclosed in Fig. 1, only a cathodic peak is displayed, confirming the irreversible nature of the wave. Further confirmation of the irreversibility of the wave could also be easily traced from the transfer coefficient obtained from logarithmic analysis (*cf.* Table 2). While the half-wave potential is pH-dependent, shifting to more negative values with an increase in pH ($dE_{1/2}$/dpH of **2a**= −0.073 mV/pH), the limiting current is practically pH-independent. The fact that this wave predominates over the whole range of study indicates that these molecules are highly basic in nature. The shifts in $E_{1/2}$ with pH, and also the i_l–pH relation of **2a** taken as

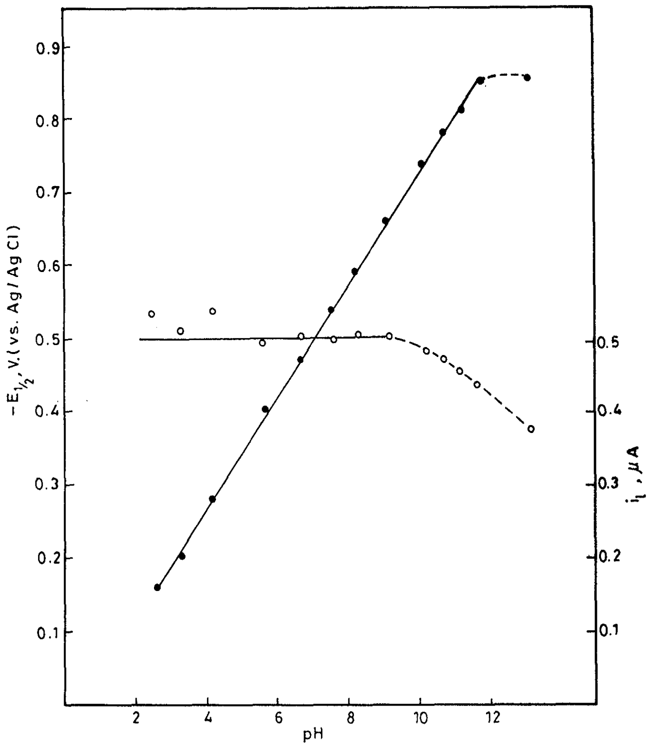


Fig. 2. $E_{1/2}$–pH and i_l–pH for 5×10^{-5} M of **2a** in 40% (v/v) Ethanolic Buffers

●, $E_{1/2}$–pH; o-i_l–pH.

Table 2. Polarographic Data and Spectrophotometric pK_a Values for Compounds **2a**—**g** and **1a**—**d**

Compd.	$E_{1/2}=f$(pH)	$\frac{2.303RT}{\alpha n}$[a]	αn[b]	pH	Spectro-photometric pK_a
2a parent	$E_{1/2}=-0.045-0.073$ pH	0.061	0.967	4.03	8.78
2b p-CH_3	$E_{1/2}=-0.065-0.066$ pH	0.057	1.035	4.03	8.53
2c p-OCH_3	$E_{1/2}=-0.085-0.074$ pH	0.055	1.072	4.87	8.81
2e p-Cl	$E_{1/2}=-0.024-0.061$ pH	0.062	0.952	5.61	8.66
2f	$E^{a}_{1/2}=+0.041-0.051$ pH	0.068	0.867	5.61	
m-NO_2					9.06
2g	$E^{b_1}_{1/2}=-0.181-0.083$ pH	0.086	0.686		
	$E^{a}_{1/2}=+0.048-0.046$ pH	0.064	0.922	5.61	8.97
p-NO_2	$E^{b_1}_{1/2}=-0.202-0.079$ pH	0.071	0.831		
1a					4.12
1b					4.75
1c					4.52
1d					8.23

a) Slopes of logarithmic analysis. *b*) Transfer coefficient. *c*) Individual pH at which logarithmic analysis was carried out.

a representative example of compounds (**2a**—**d**) are graphically shown in Fig. 2. The linear equation of $E_{1/2}=f$(pH) and other polarographic data are compiled in Table 2.

As the polarograms of (**2a**—**d**) displayed a single polarographic wave, the latter is assigned to a reduction and splitting of the CH–N=N-linkage. Proof of aniline in the sample after CPE (*cf.* Experimental) at the limiting current plateau of the wave supports this view. On the other hand, spectrophotometric measurements showed that these compounds behaved as weak organic acids according to their pK_a values (8.53—9.06). Chart 1 is thought to illustrate the acid-base equilibrium and the reduction mechanistic pathway. As the value of d$E_{1/2}$/dpH$=-0.073$ (*cf.* Table 2), it is not unreasonable to assume preprotonation of the molecule followed by its reduction in the seuqence described by Eqs. 4, 5. In the aforesaid scheme, an azo (–N=N–) and not hydrazono structure (–NH–N=) is proposed since if a hydrazone structure is thought to prevail, it will not allow tautomeric forms in step (1) and charge delocalization; hence, the deactivation of two carbonyl groups and the C=S linkage known to be polarographically active. Moreover, the shift of $E_{1/2}$ with an increase in pH, given in Table 2, is in good agreement with the reported values for the analogous azobenzene derivatives,[23,24] and deviates from those containing the hydrazone linkage[25,26] [at pH *ca.* 2, $E_{1/2}$ values of azo compounds ranging from 0.1—0.2 V (*vs.* Ag/AgCl), while for hydrazono compounds it ranges from 0.4—0.5 V (*vs.* Ag/AgCl)]. Clearly, the obtained $E_{1/2}$ values are more positive than those of the hydrazono compounds in the same pH solution. Further evidence for discrimination between a tautomeric azo/hydrazone structure could be ascertained in the light of the behavior of the nitro derivative. The importance of the nitro derivative lies in the fact that a literature survey of azo compounds containing a nitro group in any location of the molecule reveals that the azo group is always reduced before the nitro group,[27,28] while in hydrazones the opposite has been found.[25,26,29]At this point it seemed mandatory to run and inspect the polarograms of the nitro substituents.

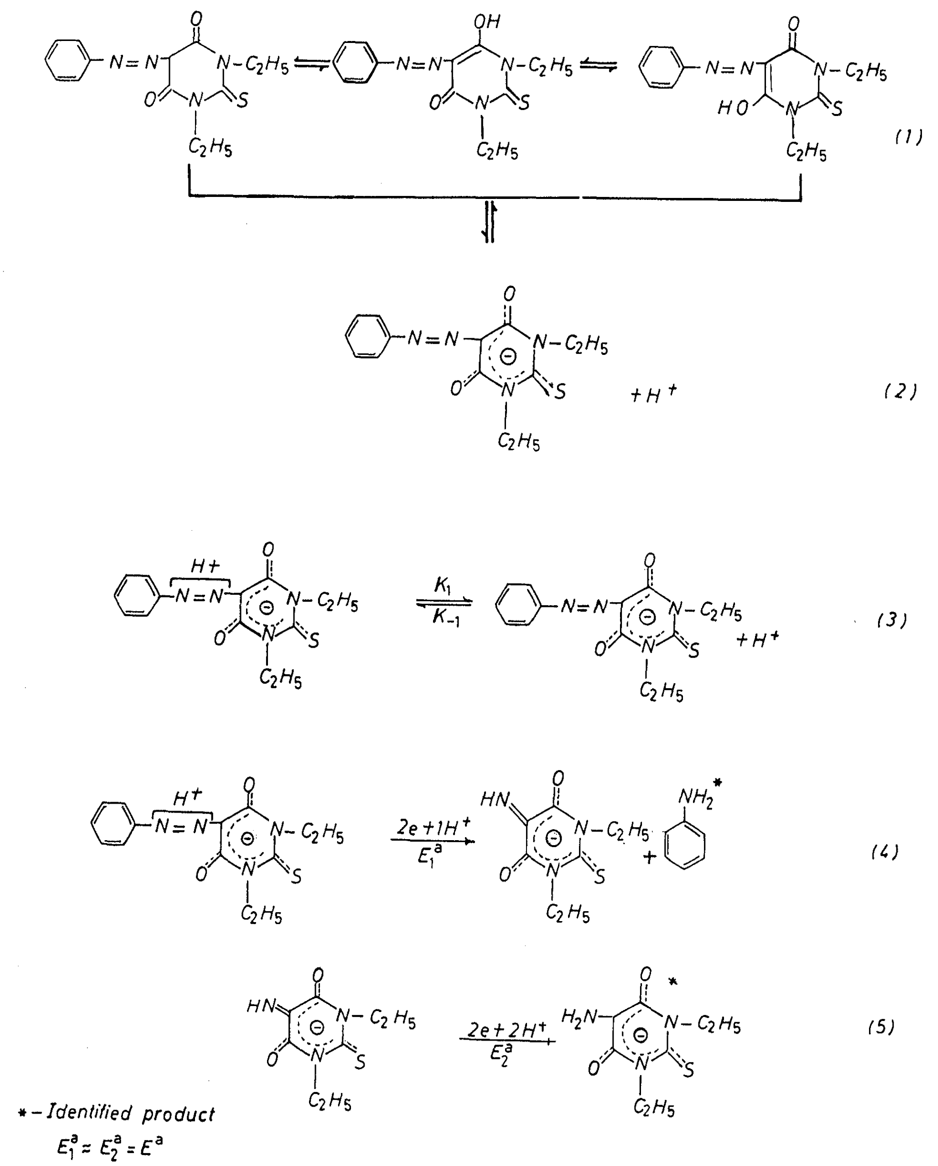
In addition to the polarographic wave (a) registered for compounds **2a**—**d**, the m-NO_2 (**2e**) and p-NO_2 (**2f**) derivatives displayed additional waves corresponding to a reduction of their nitro group. The behavior of the additional wave (b) can be described by the linear equation

$$E^{b}_{1/2(m\text{-}NO_2)}=-0.181-0.087\,\mathrm{pH}$$

Since the behavior of this wave is comparable to that known for the m-nitro group,[30,31] it is not unreasonable to attribute this extra wave to the reduction of the m-nitro group to the corresponding hydroxylamine

$$m\text{-}NO_2\xrightarrow[E^{b}]{4e,\,4H^+}\text{-}NHOH+H_2O$$

On the other hand, the p-NO_2 derivative (**2f**) afforded two extra waves (b_1 and b_2) for the reduction of the nitro group, as illustrated in Fig. 3. At this point it seemed worthy to investigate the polarographic behavior of p-nitroaniline. Figure 4 illustrates in detail the $E_{1/2}$ and i_l dependence of an authentic sample of p-nitroaniline under the same experimental conditions. Inspection and comparison of the NO_2 waves of **2e** (Fig. 3) with the waves of p-nitroaniline (Fig. 4) show that these waves are similar in all aspects. This could only be true if one assumed that in the reduced species there is a contribution of an azo form and not a hydrazone structure, since if the latter is assumed to be true, its reduction wave (a) should have followed and not preceded the six electron wave (b_1, b_2)



∗ - Identified product

$E_1^a = E_2^a = E^a$

Chart 1

which was definitely assigned to the reduction of the nitro group; obviously this is not the case. Further evidence sustaining an azo structure of the coupling product linkage of these compounds will be found through the σ–$E_{1/2}$ correlation discussed below.

Structure–Energy Relationship Constancy of the transition coefficient[32] (αn), a prerequisite for the quantitative study of the effect of substituents, was first ascertained by examination of the slopes obtained from logarithmic analysis. The obtained values were nearly constant (*cf.* Table 2). The most reliable values of $E_{1/2}$ at selected pH values have been correlated to Hammett's σ values as tabulated by Zuman.[32] In Fig. 5, σ–$E_{1/2}$ plots are illustrated at three different pH values. The plots have a reasonable linearity with ρ values varying between 0.23 and 0.36; these values are in good agreement with those reported for azo compounds.[33] That the points for the *p*- and *m*-nitro substituents lie on the $E_{1/2}$–σ plots further sustains the view that the coupling product linkage of these compounds are in the azo and not the hydrazone form. If this is not the case, one would expect reduction waves of the nitro group to precede that of the coupling product linkage, hence Hammett's σ-NHOH or σ-NH_2 should fit the correlation, which is not the case. Of note, the effect of the substituents is greater in an alkaline medium than in an acidic one (*cf.* Table 3). This could only be explained by the fact that in an acidic medium the reducible species is protonated while in an alkaline medium (due to lack of H^+) the substituents will exert more inductive effects on the unprotonated species. Consequently, the substituents will have a relatively greater influence on the electroreduction process of the unprotonated form than that displayed on the protonated species prevailing in an acidic medium.

Trials to correlate the spectrophotometric pK_a quoted in Table 1 to different σ sets ($\sigma, \sigma^\circ, \sigma^+$) showed weak dependence. This is to be anticipated since the negative charge is delocalized on the heterocyclic moiety of the molecule; also, the substituent in the benzene ring is located far from this center.

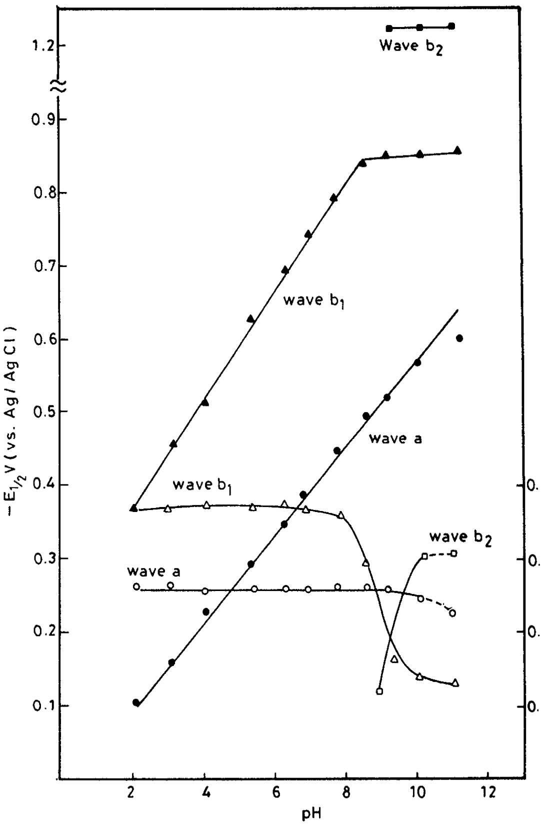


Fig. 3. $E_{1/2}$–pH and i_l–pH for 5×10^{-5} M of p-NO_2 Derivative (**2f**) in 40% v/v Ethanolic Buffers

$E_{1/2}$–pH (●, ▲, ■); i_l–pH (○, △, □).

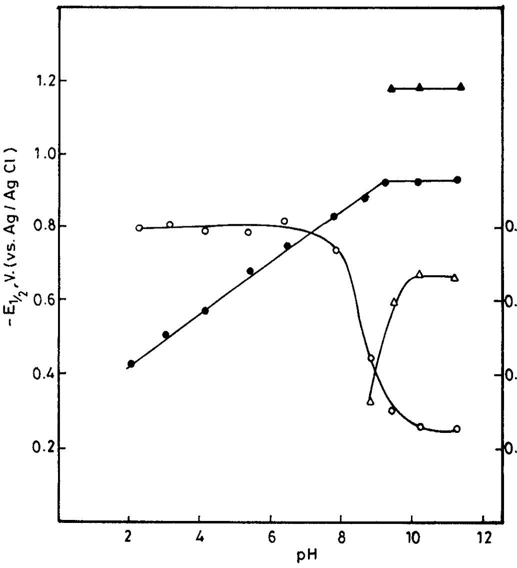


Fig. 4. $E_{1/2}$–pH and i_l–pH for 5×10^{-5} M of p-Nitro Aniline in 40% (v/v) Ethanolic Buffers

$E_{1/2}$–pH (●, ▲); i_l–pH (○, △).

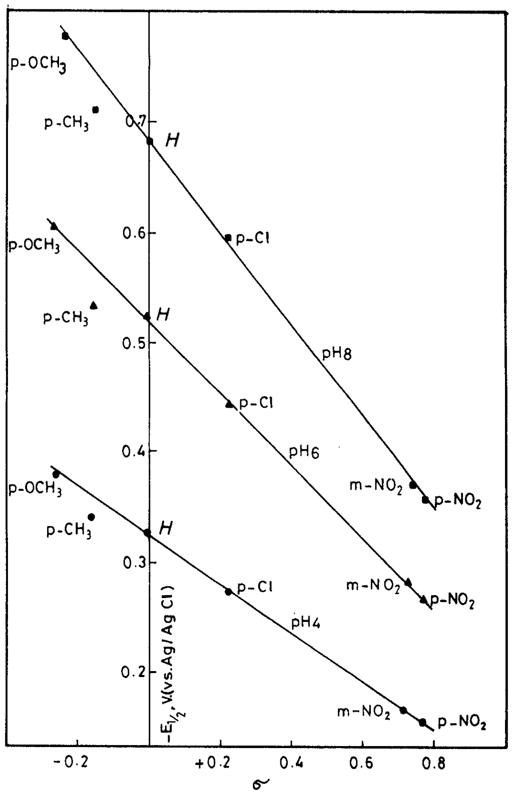


Fig. 5. $E_{1/2}$–σ Relation for 5-Phenylazo-2-thiobarbituric Acid at Different pH Values

●, pH 4; ▲, pH 6; ■, pH 8.

Table 3. Results of the Statistical Treatment of the $E_{1/2}$–σ Data for Compounds **2a**—**f** (Wave (a)) at Selected pH Values

pH	$\rho^{a)}$	$r^{b)}$	S.D.$^{c)}$	$n^{d)}$
4	0.231	0.986	0.0097	6
6	0.244	0.967	0.0196	6
8	0.367	0.958	0.0161	6

a) Reaction constant. *b*) Correlation coefficient. *c*) Standard deviation. *d*) Number of points.

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