

PYRIMIDINE IN AQUEOUS MEDIA

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(Received November 21st, 1968)

INTRODUCTION

Pyrimidine is a key compound in understanding the electrochemical behavior of pyrimidines and purines, including those of biological importance which are the essential bases of the nucleic acids, *e.g.*, the electrochemical reduction of purines occurs in the pyrimidine ring^{1,2}. The polarography of pyrimidine and its derivatives at the dropping mercury electrode (DME) has been studied by several investigators², the most detailed study being that of Smith and Elving³. The latter included controlled-potential electrolysis and coulometry as well as d.c. polarography and resulted in the following mechanism being postulated for the electrochemical reduction of pyrimidine at the DME, which results in five polarographic waves appearing over the pH-range, 0.5–13.

In highly acid media, pH-dependent one-electron ($1e$) wave I is seen. At about pH 3, pH-independent $1e$ wave II emerges from back-ground discharge. These two waves merge near pH 5 to form pH-dependent $2e$ wave III. Near pH 7.2, pH-independent $2e$ wave IV emerges from background and, at pH 9.2, merges with wave III to form pH-dependent $4e$ wave V. Wave I is postulated to be the $1e$ reduction of pyrimidine to the neutral radical; wave II is the $1e$ reduction of the latter to a dihydropyrimidine with wave III being the composite of these two steps. Wave IV is the $2e$ reduction of the dihydro species to a tetrahydropyrimidine with wave V being the composite of waves III and IV.

Recently, Timmer *et al.* presented a theoretical treatment⁴ and an experimental verification⁵ of a.c. polarographic waves for irreversible electrode reactions, which have done much to dispel the belief that only "reversible" polarographic waves exhibit a.c. polarographic peaks, and which should, therefore, promote the use of a.c. polarography in the elucidation of organic oxidation–reduction paths and the determination of kinetic parameters; the latter have not been as well studied as those of inorganic systems probably because of the generally irreversible nature of organic polarographic reactions. We have been prompted by these studies to investigate the a.c. polarographic behavior of pyrimidine in aqueous media in an effort to obtain further evidence for the proposed pyrimidine reduction mechanism (particularly in respect to the controlling step in each process) and to evaluate the various theoretical predictions for a rather complicated organic system.

EXPERIMENTAL

Chemicals

Pyrimidine (Mann Research Laboratories) was of chromatographic grade; its polarographic pattern afforded no evidence of any electroactive impurity. Buffer solutions (Table 1) were prepared from analytical reagent-grade chemicals; ionic strength was kept constant at 0.5 *M*. Nitrogen used for deoxygenating (Liquid Carbonic, O.P.) was purified and equilibrated by bubbling it successively through two acidic vanadous solutions, saturated calcium hydroxide, and distilled water. Triply-distilled mercury was used.

TABLE I

BUFFER AND BACKGROUND ELECTROLYTE SOLUTIONS^a

Buffer No.	pH range	Composition
1	0.5- 2.5	HCl + KCl
2	2.2- 8.0	Na ₂ HPO ₄ · 7 H ₂ O + citric acid monohydrate + KCl
3	8.0-10.2	H ₃ BO ₃ + KOH + KCl
4	10.5-13.0	KOH + KCl
5	11.0-13.0	(C ₂ H ₅) ₄ NOH + (C ₂ H ₅) ₄ NCIO ₄

^a All buffers used were 0.5 *M* ionic strength

Apparatus

The water-jacketted (thermostatted at 25.0 ± 0.1°), three-compartment polarographic cell employed was previously described⁶. The counter electrode was a coil of platinum wire dipped into a 0.5 *M* KCl solution; the reference was an aqueous saturated calomel electrode, to which all potentials are referred. The capillary (marine barometer tubing) had the following *m*-values (mg/sec) in 0.5 *M* KCl at 25° and open circuit: 1.326 at 52.7 cm of mercury, corrected for back pressure; 1.577 (62.7 cm); and 1.833 (72.7 cm). Drop-times, measured at potentials of interest, were generally between 3 and 10 sec; drop-times greater than 6 sec were encountered only at heights of 22 and 32 cm during height-variation experiments, where the response was normal.

Slow, single-scan d.c. polarography, used to check $E_{\frac{1}{2}}$ -values previously obtained³, was performed with a Sargeant Model XV polarograph.

A.c. polarograms were obtained with a potentiostatic control loop of conventional design using Philbrick K2-XA and K2-P operational amplifiers and a precision rectifier previously described by Smith⁷; they were recorded with a Moseley 7035A X-Y recorder. The d.c. ramp voltage was supplied by a utility integrator constructed using a Philbrick UPA-2 amplifier and a step-function module consisting of a mercury battery and a resistive voltage divider. Scan rate was constant at 1.0 mV/sec. The superimposed sine wave was supplied by a Hewlett-Packard 202A low frequency function generator, whose output was regulated to between 5 and 60 mV peak-to-peak by means of an external voltage divider.

The output of the precision rectifier was calibrated using a sine wave of known r.m.s. amplitude and a dummy cell of resistive components; the output was linear from about 100 mV to over 30 V. The performance of this unit was tested by examining

the ferrous/ferric couple in 0.25 M potassium oxalate–0.25 M oxalic acid buffer. The response was identical with that previously reported for this system and with that expected for a reversible system⁸: The alternating current was independent of mercury column height, linearly proportional to the amplitude of the superimposed alternating voltage, and proportional to the square root of frequency to about 65 Hz; the summit potential was identical with the $E_{\frac{1}{2}}$, within experimental error. The alternating current, Δi_s , for 0.969 mM Fe(III) in oxalate background was 8.29 μA ; the calculated, theoretically expected value⁸ is 11.37 μA ($A = 3.38 \cdot 10^{-2} \text{ cm}^2$, $V = 8.46 \text{ mV r.m.s.}$, $f = 50 \text{ Hz}$, $D_0 = 5.70 \cdot 10^{-6} \text{ cm}^2/\text{sec}$). The difference is due primarily to the attenuation by the damping capacitor in the precision rectifier.

A Beckman Model G pH-meter was used to measure the pH of buffer and test solutions.

Procedures

Stock solutions of background buffers and pyrimidine were prepared by dissolving weighed quantities and diluting to known volume. Test solutions were prepared by pipetting appropriate amounts of stock solutions into 10- or 50-ml volumetric flasks and diluting to volume with distilled water. Nitrogen was bubbled through the test solution in the polarographic cell for 5 min; the DME was then inserted and voltammograms were recorded with nitrogen passing over the test solution.

The summit potential, E_s , for a.c. voltammograms, was taken as that potential at which the total alternating current due to the electroactive species, Δi_s , reached a maximum; Δi_s was measured as the difference in current at E_s between solutions containing the background buffer plus electroactive species, and the buffer only.

The error in Δi_s is estimated to be $\pm 5\%$ for moderate to large currents, i.e., above 0.5 μA ; with smaller currents, the error is larger owing to the problem of subtracting a large base current.

A. C. POTENTIAL BEHAVIOR

Wave pattern

Over the pH-range 0.5–13, pyrimidine gives five recognizable, if distorted, a.c. polarographic waves, which correspond to the five observed d.c. polarographic waves (cf. Fig. 1 for examples). At low pH, a single pH-dependent wave is observed (wave I). At about pH 2.8, pH-independent wave II, whose current is much less than that for wave I, emerges from background. At about pH 4.8, waves I and II merge to form pH-dependent wave III, whose peak current is close to that observed for wave I. At about pH 7.0, wave IV emerges from background and merges with wave III at about pH 8.3 to form pH-dependent wave V. Above pH 9, wave V is observed only as an inflection on background discharge in KCl/KOH buffer; point-by-point subtraction of background is necessary to obtain even a distorted wave. In $\text{Et}_4\text{NClO}_4/\text{Et}_4\text{NOH}$ buffer, the background discharge is sufficiently removed from E_s that reproducible measurements can be made. E_s does not shift with change of background electrolyte from KCl to Et_4NClO_4 , as has been seen in some systems.

The linear E_s vs. pH relationships presented in Table 2 were obtained by a least-squares analysis of the experimental data.

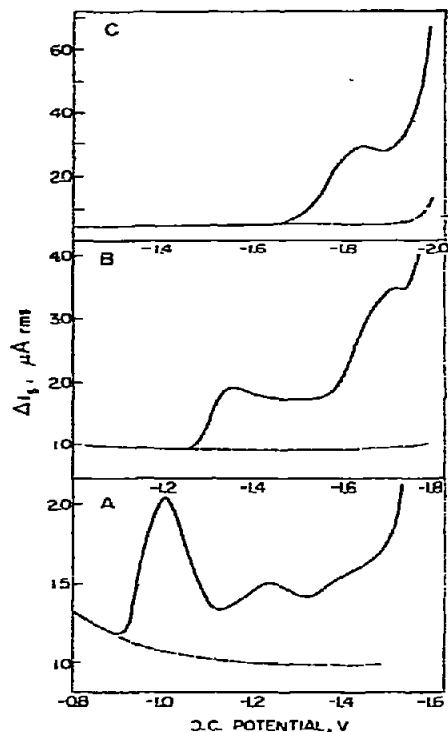


Fig. 1. Alternating current polarograms of 0.954 mM pyrimidine (—) and background alone (---) (A), Waves I and II, pH 3.96 (buffer 2); (B), waves III and IV, pH 7.48 (buffer 2); (C), wave V, pH 12.00 (buffer 5). Conditions: 62.7 cm mercury (corr.); 50 Hz applied frequency; 8.46 mV applied voltage. Only the peaks of drop oscillations are indicated.

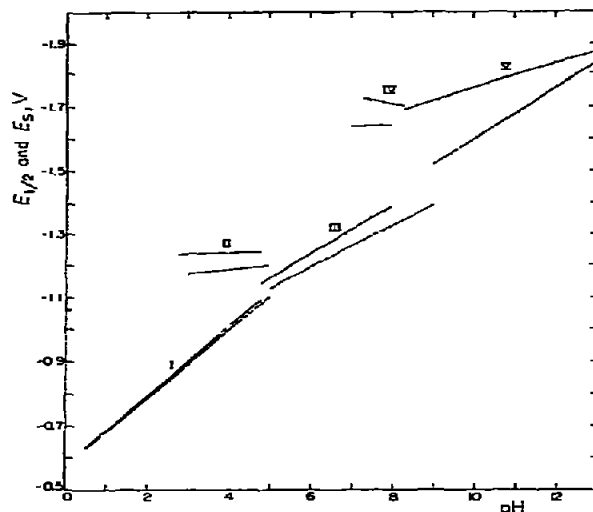


Fig. 2. Linear E_s (—) and E_3 (---) vs. pH relationships for pyrimidine. Each E_s -value is the average of two or more measurements (with an estimated uncertainty of ± 5 mV) for the following conditions: 0.954 mM pyrimidine concn.; 62.7 cm mercury (corr.); 50 Hz applied frequency; 8.46 mV r.m.s. applied voltage. E_3 -data taken from ref. 3.

TABLE 2

LINEAR E_s vs. pH RELATIONSHIPS FOR PYRIMIDINE^a

Wave	pH range	E_s^b (V)
I	0.5– 4.8	–0.575 – 0.108 pH
II	2.8– 4.8	–1.226 – 0.003 pH
III	4.8– 8.0	–0.766 – 0.078 pH
IV	7.0– 8.3	–1.982 + 0.023 pH
V	8.3–13.0	–1.365 – 0.039 pH

^a Conditions: 0.954 mM pyrimidine; 50 Hz; 62.7 cm mercury (corr.); 8.46 mV r.m.s. applied voltage.

^b The equations given indicate the E_s , when extrapolated to zero pH, and the variation of E_s with pH.

Relations of E_s and E_3

As can be seen from Fig. 2, the a.c. polarographic behavior of pyrimidine over the pH-range closely parallels the d.c. behavior at the DME. The deviation of E_s from E_3 can be considered as a measure of the reversibility of the originating electrode reaction⁸. E_s for waves II, IV and V, all corresponding to the direct formation of a

stable molecular species, are more negative than their corresponding $E_{\frac{1}{2}}$ -values by 60 to 200 mV. Wave I, on the other hand, which corresponds to a $1e$ addition to pyrimidine to form a radical species, shows little difference in E_s and $E_{\frac{1}{2}}$; this is, however, not to be taken in itself as an indication of the reversibility of the wave, as the observed alternating current is less than that theoretically expected for a $1e$ reversible system⁸. The derived linear E_s vs. pH relationships are only approximations to the true behavior of the system, e.g., a considerable departure from linearity is observable near the merging of two waves, which is particularly evident in the wave III data.

Effect of experimental conditions

E_s does not shift with amplitude of the applied alternating voltage (2–15 mV r.m.s.) or mercury column heights (drop-time), e.g., E_s for wave V is -1.836 ± 0.003 V (average deviation) for twelve different applied voltages and column heights. E_s for all five waves, however, shifts to more negative potential with increasing frequency of applied alternating voltage (Table 3), as expected for irreversible reactions. Plots of E_s vs. $\log f$ are approximately linear; however, the uncertainty of an individual datum (about ± 5 mV) is relatively large compared to the magnitude of the potential shift and precludes quantitative appraisal of the effect.

The width of the a.c. wave at half-height, $\Delta E_{s/2}$, for wave I at pH 0.92 and 3.96 does not vary, within experimental error, with changes in mercury column height or in magnitude of applied alternating voltage within the ranges studied; it is 124 ± 6 mV, as compared to the 90.5 mV predicted for a reversible, diffusion-controlled $1e$ reaction⁸. The a.c. wave is not symmetrical about E_s : $E_{(s/2)_1} - E_s = 52 \pm 3$ mV and $E_s - E_{(s/2)_2} = 72 \pm 3$ mV. In the frequency range, 5–100 Hz, $\Delta E_{s/2}$ appears to follow the same curvilinear trend as does the total current for change in frequency (*vide infra*), increasing from 106 mV at 5 Hz to 125 mV at 50 Hz and then decreasing to 118 mV at 100 Hz. The $\Delta E_{s/2}$ -values for the other waves cannot be determined because the current does not drop to one-half the peak value on the negative potential side of the a.c. wave.

TABLE 3
VARIATION OF E_s WITH FREQUENCY FOR PYRIMIDINE^a

Frequency (Hz)	Wave				
	I	II	III	IV	V
1	—	—	1.359	—	—
5	0.986	1.231	—	—	1.812
10	0.991	1.232	1.372	1.712	1.824
20	—	—	1.363	1.706	—
25	0.996	1.239	—	—	1.831
50	1.005	1.236	1.372	1.724	1.836
75	1.011	1.244	—	—	1.841
100	1.020	1.245	1.389	1.765	1.851

^a Conditions: Waves I and II, pH 3.96 (buffer 2); waves III and IV, pH 7.55 (buffer 2); wave V, pH 12.00 (buffer 5). Pyrimidine concn., 0.954 mM; 8.46 mV r.m.s. applied voltage; 62.7 cm (corr.). E_s -values are in negative volts vs. SCE. Dash indicates measurement not made.

A. C. CURRENT BEHAVIOR

Over the pH-range, the presence of pyrimidine shows no tendency to elevate or depress the a.c. base current before a reduction peak, regardless of the nature of the buffer; under all conditions, the base current was identical, within experimental error, with that observed in the absence of pyrimidine. This would seem to indicate that little or no adsorption of pyrimidine occurs, since a.c. polarography is a very sensitive technique for detecting adsorption. There does appear to be, however, some increase in base current after a reduction peak, *e.g.*, at low pH, the current after wave I does not return to the base-line even though there is sufficient potential span before background discharge (*ca.* 0.35 V) to permit this, which would seem to indicate some adsorption of the pyrimidine reduction product(s). The presence of pyrimidine does shift the background discharge to more positive potential; the magnitude is irregular but continues across the pH-range; the cause of this effect is not known but may be due to the pyrimidine reduction products.

Effect of alternating voltage

The alternating current observed for all five waves is a linear function of the applied alternating voltage from 2 to 15 mV r.m.s. (Fig. 3); none of the plots intersects at the origin. With waves II and IV, part of this effect may be explained by the proximity of these two waves to the previously occurring wave; thus, a residual current

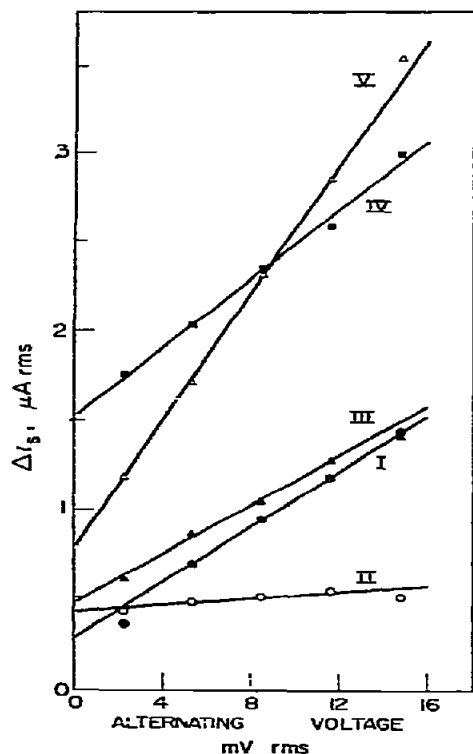


Fig. 3. Variation of alternating current, ΔI_5 , with amplitude of applied a.c. voltage: Waves I and II, pH 3.96 (buffer 2); waves III and IV, pH 7.48 (buffer 2); wave V, pH 12.00 (buffer 5). Conditions: 0.954 mM pyrimidine concn.; 50 Hz applied frequency; 62.7 cm mercury (corr.).

element may be incorporated. In addition, the apparent increased base current after a reduction wave, noted previously, may account for part of the increased current. The fact that plots for waves I, III and V also do not intersect the origin, however, indicates that other factors must be operative.

Effect of drop-time

Early theoretical treatments⁸ indicated that the faradaic alternating current for reversible and quasi-reversible electrode reactions should be independent of drop-time. Recently, several workers⁹⁻¹² have commented on the drop-time dependence of a.c. polarographic current with the general conclusion that such dependence will be exhibited whenever some rate process other than diffusion is kinetically influencing the faradaic process¹¹. Experimental evidence was presented on the drop-time dependence for (a) the quasi-reversible electron-transfer process and (b) reductions and oxidations involving coupled chemical reactions, rate limited surface coverage, and amalgam formation⁹. Timmer *et al.*⁴ supported their theoretical predictions that a.c. peak heights should be proportional to $t^{-\frac{1}{2}}$ for highly irreversible electrode reactions, with the experimental data of Aylward and Hayes⁹ on the reduction of the cadmium-EDTA complex.

In the case of pyrimidine, all five a.c. waves exhibit a considerable linear increase of current with $t^{-\frac{1}{2}}$. The relations for waves I, III, IV and V intersect near the origin (infinitely long drop-time) (Fig. 4); the small values of the intercepts can be attributed to experimental error. The relatively large positive intercept for wave II, as

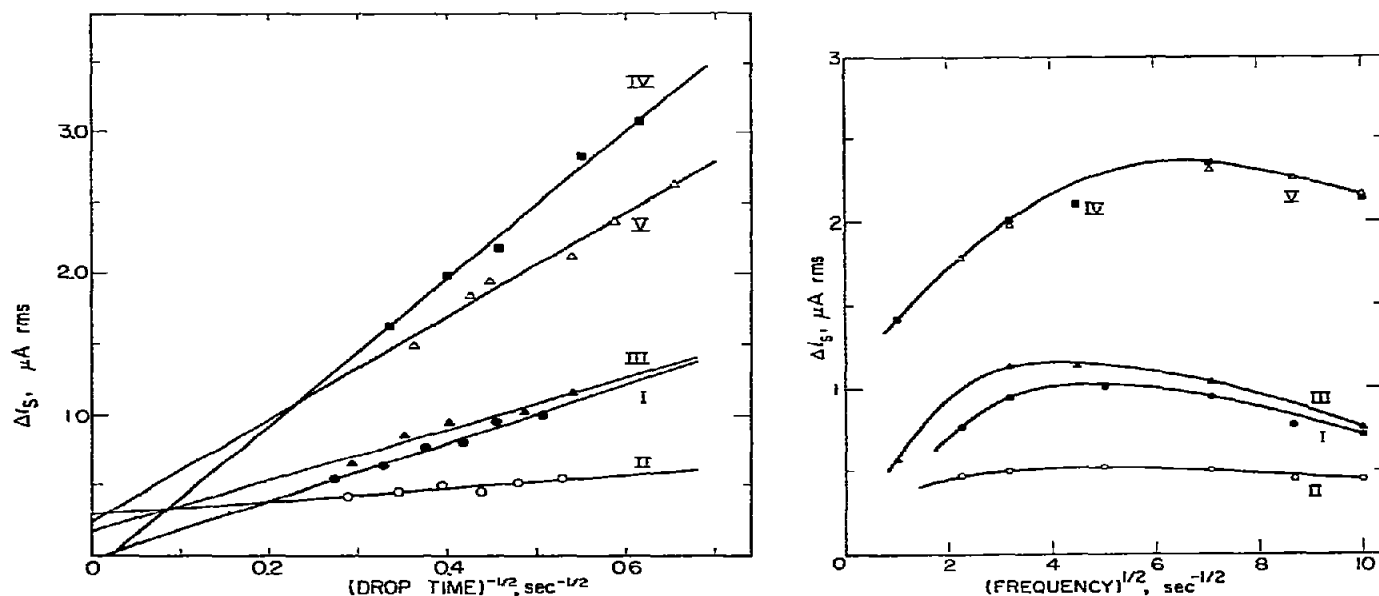


Fig. 4. Variation of alternating current, Δi_s , with drop-time, expressed as $t^{-\frac{1}{2}}$: Waves I and II, pH 3.96 (buffer 2); waves III and IV, pH 7.48 (buffer 2); wave V, pH 12.00 (buffer 5). Conditions: 0.954 mM pyrimidine concn.; 8.46 mV r.m.s. applied alternating voltage; 50 Hz applied frequency.

Fig. 5. Variation of alternating current, Δi_s , with the square root of applied frequency: Waves I and II, pH 3.96 (buffer 2); waves III and IV, pH 7.48 (buffer 2); wave V, pH 12.00 (buffer 5). Conditions: 0.954 mM pyrimidine concn.; 8.46 mV r.m.s. applied voltage; 62.7 cm mercury (corr.).

compared to the magnitude of the current observed, may be attributed to a residual current or capacitive current element from wave I, as mentioned previously. That waves IV and V exhibit such a relationship, despite the complexity of their electrode reactions, is significant.

Frequency-dependence

The frequency-dependence of the five waves (Fig. 5) shows the behavior generally expected for semi-reversible systems: increase in current to a maximum and then decrease. Wave II shows only a very small frequency-dependence, as would be expected with a highly irreversible wave. The almost identical response to frequency change shown by waves IV and V probably indicates that both waves involve a single rate-determining step, although a contributing factor may be their having almost identical currents at the applied voltage (8.46 mV) (Fig. 3).

Effect of concentration

The alternating current for wave I is linear with concentration in the range, 0.5–10 mM, with a small but definite change in slope at *ca.* 5 mM (Fig. 6). There seems to be a "toeing" below 0.5 mM.

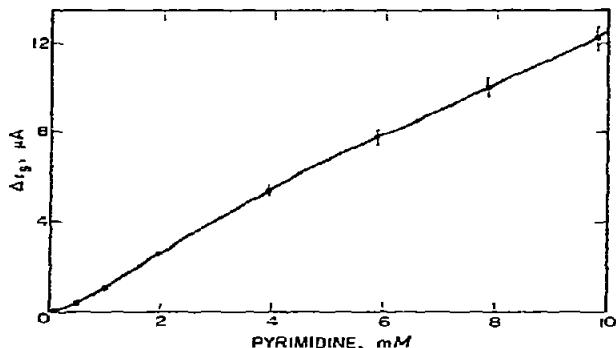


Fig. 6. Variation of alternating current, ΔI_a , with pyrimidine concn for wave I. Conditions: pH 0.92 (buffer 1); 50 Hz applied frequency; 8.46 mV r.m.s. applied voltage; 62.7 cm mercury (corr.).

REDUCTION MECHANISM FOR PYRIMIDINE

The a.c. polarographic behavior of pyrimidine generally substantiates the proposed reduction mechanism as outlined in Fig. 7. Wave I, associated with a $1e$, one-proton addition to pyrimidine to produce a neutral radical, is the most reversible of the pyrimidine waves on the basis of proximity of the $E_{1/2}$ - and E_s -values, and a general consideration of alternating current magnitudes. Furthermore, the increased proximity of $E_{1/2}$ - and E_s -values with increased hydrogen ion concentration (Fig. 2) supports the postulate that proton addition is part of the overall first step; as more of the pyrimidine is protonated, the coupled chemical reaction of protonation becomes less critical in determining the rate of the first step. (pK_a for protonation of pyrimidine is 1.30.) Owing to the relative simplicity of wave I, lack of preceding reactions and near-reversibility, it would be most amenable to analysis of kinetic parameters, particularly in highly acid media.

Wave II, associated with $1e$ addition to the neutral radical to form a dihydro-

pyrimidine, appears to be the most irreversible of the pyrimidine waves, as might be expected because of the stability of the dihydro species and the extreme difficulty of reoxidizing an aliphatic carbon site. The alternating current for this wave shows very little change with variation in applied voltage, drop-time or frequency; this behavior is expected since, once a reaction appears to be very irreversible under a given set of conditions, relatively small changes in variables would only slightly affect

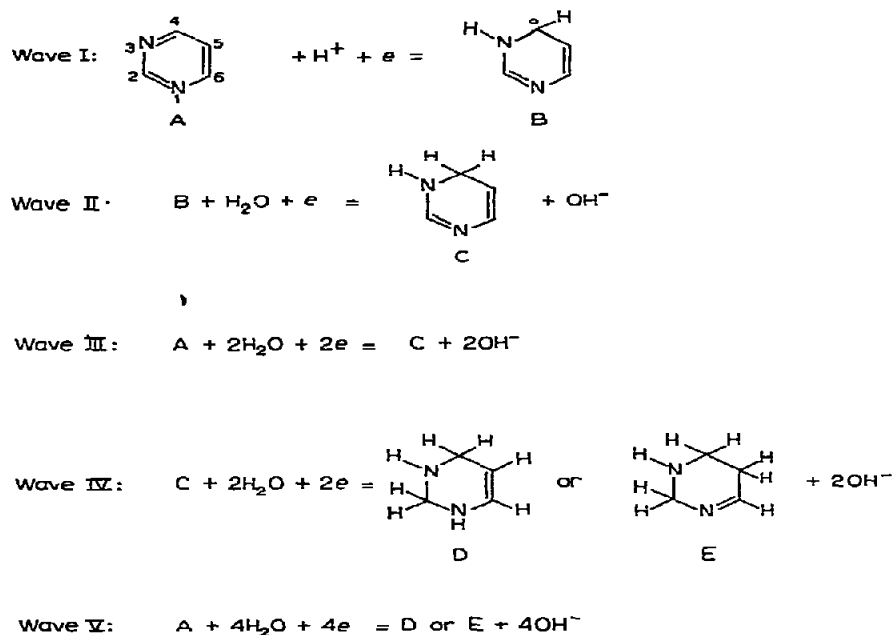


Fig. 7. Reactions producing the five polarographic waves observed in the electrochemical reduction of pyrimidine (compound A)

this irreversibility. Wave II differs most from the other waves of the reduction path in that its first step involves attack on a neutral radical, whereas the initial step for waves I, III and V presumably involves initial attack of the 3,4-carbon–nitrogen double bond, and that for wave IV on the 1,2-carbon–nitrogen double bond. This difference would account for the difference in electrochemical properties relative to the other waves.

The very similar characteristics of waves I and III are attributable to the fact that wave III involves the same initial step as wave I, *i.e.*, a $1e$ attack on the 3,4-N=C bond. Thus, although wave III should have four times the alternating current of wave I (for both reversible and irreversible reactions, Δi_s should vary as n^2), its current is actually only slightly larger. However, wave III is somewhat less reversible than wave I, as attested to by the larger deviation of E_s from $E_{\frac{1}{2}}$ and the fact that its maximum current is reached at a lower frequency (15 Hz; 25 Hz for wave I).

Waves IV and V, associated with the formation of a tetrahydropyrimidine species known to be unstable under the alkaline conditions of its formation, are highly irreversible. The similarity in their behavior with variation in drop-time, applied voltage and frequency, mentioned earlier, is probably due to the composite nature of wave V. Waves III and IV both involve a $2e$ attack on a double bond. The current for wave IV, as a function of applied alternating voltage, is about twice that for wave

III; since the shape of the a.c. wave (Fig. 1B) indicates that wave IV sits on top of wave III, the doubling of the current could indicate approximately equal contributions from the two waves.

CONCLUSIONS

The present state of the art does not permit quantitative evaluation of kinetic parameters or detailed specification of the pyrimidine reduction mechanism. For example, since there have been no theoretical nor—to our knowledge—experimental treatments of a system as complicated as pyrimidine, which has five discrete reduction waves and numerous intermediate chemical reactions, the magnitude of the “expected” currents for waves III—V are really not known and the reversibilities of the pyrimidine waves cannot be assessed by comparison of the magnitudes of alternating current. However, the qualitative application of a.c. polarography to the understanding of a complicated, quasi-reversible organic reduction series has been shown to be possible, *i.e.*, a.c. polarography has yielded interpretable results for the five-wave reduction pattern of pyrimidine. The qualitative correspondence of the five pyrimidine reduction waves with theoretical predictions for irreversible waves (effect of drop-time, frequency, applied voltage, and concentration) indicates the validity of contemporary theory and the possibility that further theoretical and experimental work on more complicated reaction mechanisms may be fruitful.

Use of phase-sensitive a.c. polarography, useful for suppression of the base current which becomes a problem with small faradaic currents, and a more detailed examination of wave I under highly acid conditions would probably be most useful in further clarifying the pyrimidine reduction mechanism. Phase angle measurements under a variety of conditions may yield more easily interpretable results for the more complicated waves, II–V.

ACKNOWLEDGEMENTS

The authors thank the National Science Foundation for helping to support the work described. One of the authors (JEO) thanks the Department of Health, Education and Welfare, and the Michigan Department of Chemistry for an NDEA Fellowship.

SUMMARY

The electrochemical reduction of pyrimidine in aqueous media was studied using alternating current (a.c.) polarography. Over the pH-range, 0.5–13, five moderately to highly irreversible a.c. waves were observed, which corresponded to the five waves observed on d.c. polarography. Although the electrochemical reduction path of pyrimidine is complex and only partially understood, good qualitative agreement between the features of the mechanism and various polarographic parameters was achieved, providing further support for the proposed mechanism, and indicating the validity of current theoretical and experimental results in the area of the a.c. polarography of quasi-reversible electrode reactions. Summit potentials for the five waves, which vary linearly with pH, are independent of applied alternating voltage, mercury

column height (drop-time) and concentration, but shift to more negative potential with increasing frequency. Total alternating current for all waves increases linearly with amplitude of applied alternating voltage and the negative square root of drop-time; plots of total alternating current *vs.* square root of applied frequency exhibit curvilinear trends with a maximum in the range, 15–50 Hz.

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