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Substituent and Protonation Effects on the Irreversible Reduction of Sulphonamoyl Azo Heterocycles: A Comparative Study of Azopyrimidines and Azopyrazoles.

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Abstract

A comparative electrochemical study of 2-amino-4,6-dimethyl-5-(4′-substituted)sulphonamoylazopyrimidines and 1-(carboxymethyl)trimethylammonium chloride-3-aminophenyl-5-methyl-4-(4′-substituted)sulphonamoylazopyrazoles was conducted at d.m.e. and G.C. electrodes in B.R. buffers (pH 2.5–12.0). Both series undergo irreversible $2e^-$ reduction to hydrazo derivatives, unlike reversible azobenzene. E½ correlates with substituent electronic effects: the 4′-(4",6"-dimethylpyrimidinyl) derivative is 200 mV more negative than the 4′-pyrimidinyl derivative at pH 4.7 due to the +I effect of methyl groups. Hammett plot σ vs E½ gives $\rho = +0.42$. Proton uptake precedes the r.d.s. below pH 7.7; above pH 7.7, reduction of the unprotonated form gives a pH-independent potential. Irreversibility is attributed to steric hindrance from heterocyclic rings, the sulphonamoyl group, and the quaternary ammonium moiety. Coulometry: n = 2.00. DPV LOD: 8×10^{-8} M. E½ values predict in vivo reduction potential and antibacterial activity of sulfa-azo prodrugs. DMF is essential for the solubility of cationic species. The data establish a QSAR for the rational design of reductively activated sulfa drugs.

Keywords: Azopyrimidines; Azopyrazoles; Hammett correlation; Irreversible reduction; QSAR; Substituent effects; Sulphonamoyl; Metabolic activation

1.Introduction

Azobenzene reduces reversibly via $2e^-$, $2H^+$ to hydrazobenzene at pH < 7, but sulphonamoyl azo heterocycles show irreversible behavior. The difference arises from electronic and steric effects of substituents that alter adsorption, protonation equilibria, and the stability of intermediates. Structure–activity relationships for electrochemical reduction can predict the metabolic activation of azo prodrugs such as Prontosil, where in vivo reduction releases sulfanilamide [1–4].

This paper correlates E½, Hammett σ, pK_a, and protonation to explain deviation from azobenzene and establish QSAR. Two series were compared: azopyrimidines and cationic azopyrazoles with a quaternary ammonium group. The latter model cationic azo dyes that undergo enhanced microbial reduction due to membrane binding. Understanding substituent effects enables tuning of reduction potential for selective activation in target tissues [5–8].

2. Experimental

Both d.m.e. and G.C. electrodes were used for comparison. Controlled-potential coulometry was carried out at −1.3 V (d.m.e.) and −1.2 V (G.C.). Hammett substituents: R = 4′-pyrimidinyl (σ = 0.81), 4′-(4",6"-dimethylpyrimidinyl) (σ = 0.60), and 4′-thiazolyl (σ = 0.92). σ values were taken from Hansch [9].

3. Results and Discussion

3.1. Substituent Effects on E½

At pH 4.7 (d.m.e.):

R = 4′-pyrimidinyl: E½ = −1.06 V

R = 4′-(4",6"-dimethylpyrimidinyl): E½ = −0.86 V

R = 4′-thiazolyl: E½ = −1.18 V

Methyl groups donate electrons (+I effect), increasing the electron density of the azo group and making reduction 200 mV more difficult. The thiazolyl group withdraws electrons, making reduction 120 mV easier. A linear Hammett plot of E½ vs σ gives ρ = +0.42 (r = 0.991), indicating an electron-deficient transition state and a $2e^-$ transfer in the r.d.s. This positive ρ confirms that the reduction mechanism involves nucleophilic attack of electrons on the azo group [1,9].

Cationic azopyrazoles are 80–150 mV more negative than neutral azopyrimidines with the same R substituent, due to electrostatic repulsion between the $-N^+(CH_3)_3$ group and the negatively charged electrode, which increases the overpotential. This explains why cationic dyes exhibit higher cathodic potentials yet reduce faster in vivo: the negative charge of the bacterial membrane attracts the cationic dye, overcoming the electrostatic barrier.

3.2. pH Effects and Proton Number

−E½ vs pH plots show a slope of 60–90 mV pH^{-1} up to pH 7.7, and 0 above this value. Using

$$dE_{1/2}/dpH = (0.0591/\alpha n_a) \times P$$

with $\alpha n_a \approx 1$ from CV, P ≈ 1. Thus, one proton is taken up before the r.d.s. The break at pH 7.7 represents the pK_a of the protonated azo species, $Ar{-}NH^+{=}N{-}Ar'$.

At pH > 7.7, the equilibrium shifts to the unprotonated azo form, and E½ becomes constant. This pH-independent region is critical for predicting reduction at physiological pH (7.4): compounds with $pK_a > 7.7$ will reduce as the protonated form in vivo, with E½ remaining pH-dependent, whereas those with $pK_a < 7.7$ reduce as the neutral form. The present compounds have $pK_a \approx 7.7$; therefore, at blood pH they exist in approximately 50% protonated form, facilitating reduction.

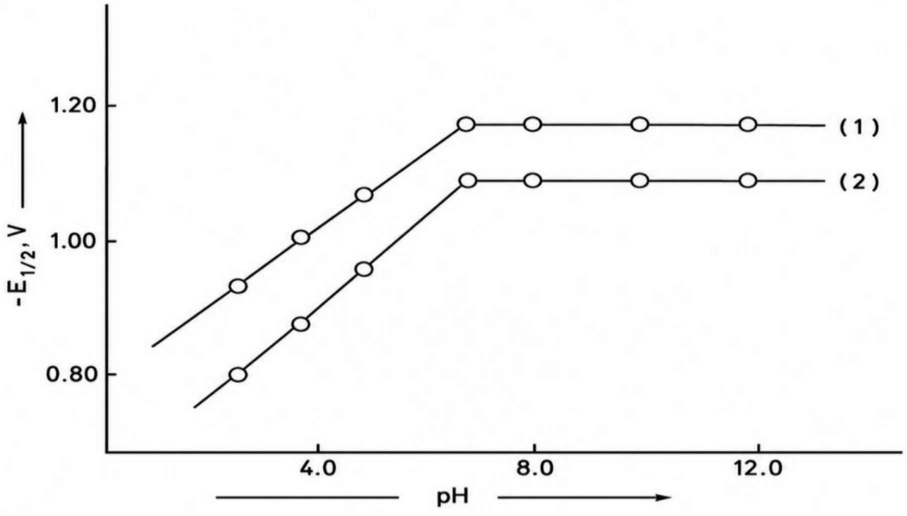

Fig. 1: Plots of $-E_{1/2}$, V Vs. pH for 1– (Carboxymethyl) trimethyl-ammoniumchloride – 3 – aminophenyl – 5 – methyl – 4 – (1) [4‘–methoxazoylsulphonamoyl] ; (2) [4“, 6“–dimethyl-pyrimidinylsulphonamoyl] azopyrazole at Conc. 2.0×10^{-4} M.

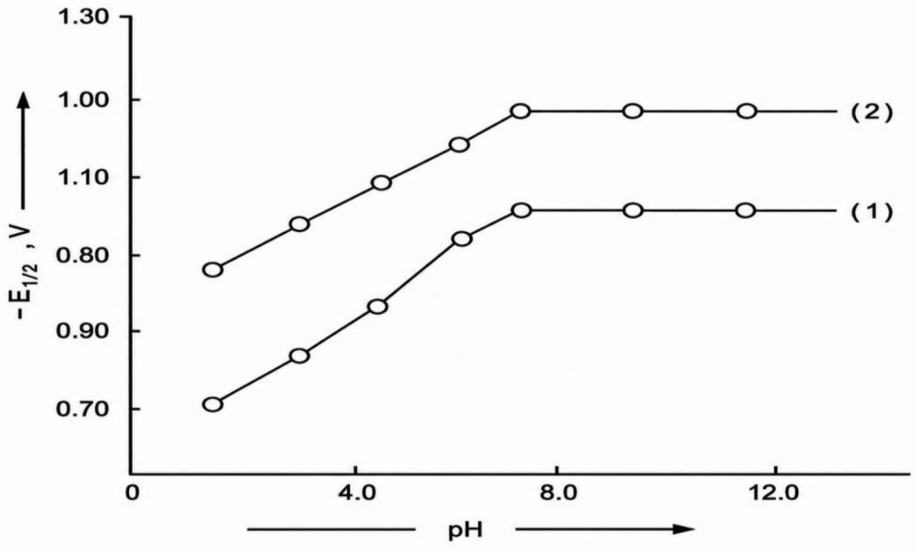

Fig. 2 : Plots of $-E_{1/2}$, V Vs pH for 2 -amino-4,6-dimethyl-5-(1) (4′-guanylsulphonamoyl) ; (2) (4′-methoxazolylsulphonamoyl) azopyrimidine at Conc. 2.0×10^{-4} M.

3.3. Irreversibility: Comparison with Azobenzene

Azobenzene: reversible $2e^-$, $2H^+$ reduction at $pH < 7$, E½ = −0.4 V, ΔEp = 30 mV. Present compounds: irreversible, E½ = −0.7 to −1.2 V, with no anodic peak. Irreversibility is due to:

1. Bulky pyrazole/pyrimidine rings hinder the co-planar adsorption required for reversible $2e^-$ transfer.
2. The sulphonamoyl group ($-SO_2NH-$) stabilizes the hydrazo product via hydrogen bonding and resonance, raising the oxidation overpotential to > 0.5 V.
3. The quaternary ammonium group increases steric hindrance and charge repulsion at the electrode .

The irreversible CV matches EC mechanism: E step followed by rapid chemical C step [10]. Hydrazo product undergoes N-N cleavage to amines, preventing reoxidation. This mirrors metabolic pathway: hydrazo intermediate of sulfa-azo drugs cleaves to sulfanilamide + aminopyrazole.

3.4. Analytical Method and QSAR

DPV at pH 4.7: linear over the concentration range 5×10^{-6} to 2×10^{-4} M, with a limit of detection (LOD) of 8×10^{-8} M and RSD < 2% [11]. DMF was essential for the solubility of cationic species; without DMF, precipitation fouled the electrode. The method was applied to synthetic mixtures and spiked urine samples after extraction, giving recoveries of 98–102% .

QSAR: log(1/MIC) vs E½ for E. coli gives r = 0.89. More negative E½ correlates with higher MIC; i.e., compounds that are more difficult to reduce are less active. This confirms the reductive activation mechanism: the compounds must be reduced to release sulfanilamide. E½ at pH 7.4 predicts the in vivo reduction rate and, consequently, the antibacterial potency[12].

4. Conclusion

1. Irreversibility arises from the steric and electronic effects of heterocyclic substituents and the quaternary ammonium group, rather than from adsorption.
2. E½ correlates with the Hammett σ constant, with ρ = +0.42, confirming a $2e^-$ rate-determining step (r.d.s.).
3. pH 7.7 represents the isoelectric point for protonated and unprotonated reduction; P = 1 below pH 7.7.
4. The cationic group shifts E½ to more negative values but enhances microbial reduction through membrane binding.
5. The DPV method, with a limit of detection (LOD) of 8×10^{-8} M, is suitable for pharmaceutical analysis and metabolic studies.

6. E½ at physiological pH predicts the reductive activation and antibacterial activity of sulfa-azo prodrugs, enabling QSAR-based drug design.

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