CROATICA CHEMICA ACTA CCACAA **71** (3) 715–726 (1998)

ISSN-0011-1643
CCA-2523

Original Scientific Paper

Voltammetric Investigations of the Reduction of Direct Orange-31 a Bisazo Dye

Rajendra N. Goyal, Madhu S. Verma,** and Naveen K. Singhal*

Department of Chemistry, University of Roorkee, Roorkee – 247 667, India

Received December 2, 1997; revised March 26, 1998; accepted March 26, 1998

The electrochemical reduction of bisazo dye Direct Orange–31 has been studied at Pyrolytic graphite electrode (PGE) in the pH range 2.0–10.3. Two well-defined $4e^-$, $4H^+$ pH dependent reduction peaks (Ic & IIc) have been observed in linear and cyclic sweep voltammetry. It has been observed that reduction of azo groups in the dye does not stop at hydrazo stage but further reduction leads to the cleavage of –NH–NH– linkage to give amino compounds as the final products. At pH 3.0 and 7.0, 4-aminosalicylic acid and 3,3'-dimethylbenzidine have been characterized as the major products of reduction. A tentative mechanism for the reduction has also been suggested.

INTRODUCTION

Azo compounds are among the most profoundly explored classes of organic compounds both from theoritical and practical viewpoints. The presence of an azo linkage in aromatic compounds makes them highly important in dye-stuff industry, pharmacy and dosimetry.[1–5] Azo dyes which form complexes with a large number of metal ions have been used for determination of aluminium[6] and numerous other metals in trace quantity, including lanthanides.[7]

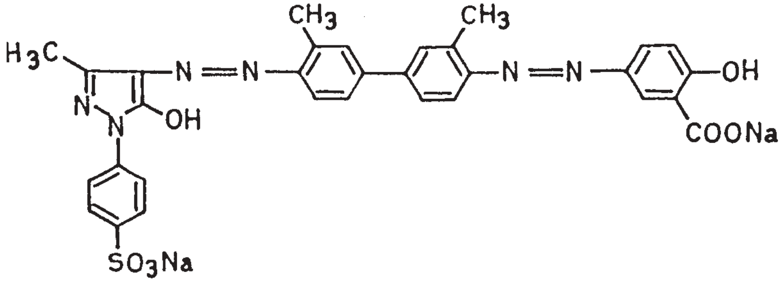
Application of moderately reactive azo compounds activated by various attached groups to biological studies has been reviewed.[8] Various synthetic azo dyes have been shown to induce a variety of tumors in mice and rats and to exhibit inhibitory effects on the biosynthesis of RNA, DNA and pro-

---

* Author to whom correspondence should be addressed.
** Present address: Department of Biochemistry, Medical College, Ihansi – 248 001.

teins.[9] When orally administered, azo compounds are found to reduce, mainly in liver and intestine, to the corresponding amines.[10]

Polarographic reduction of azo dyes has attracted considerable attention.[11] Electrochemical reduction of azo compounds has been reported to occur in $2e^-$, $2H^+$ proccess to give hydrazo products. However in the presence of strong electron donating groups, reduction has been found to occur in $4e^-$, $4H^+$ reaction to give amino compounds as the final products. An excellent review on the electroreduction of azo compounds has also appeared in the literature.[12] However, most of the studies are confined to the reduction at d.m.e. and very little is known about their reduction behaviour at solid electrodes. As the orientation of the molecule can be significantly altered at the electrode surface, it was considered interesting to study the electrochemical behaviour of an azo dye at solid electrode. As bisazo dyes have attracted less attention, despite their use as stains to demonstrate collagenous sepla,[13] Direct Orange-31 (I) has been selected for the present investigation.



Direct Orange-31 (I)

EXPERIMENTAL

Direct orange was obtained from Sigma Chemical Co. USA and was used as received. The stock solution of the dye (1.0 mM) was prepared in double distilled water. Voltammograms were recorded in phosphate buffers of ionic strength 0.1 M and were prepared[14] by mixing different volumes of $NaH_2PO_4 \cdot 2H_2O$ (0.5 M); $Na_2HPO_4 \cdot 2H_2O$ (0.5 M) and $Na_3PO_4 \cdot 12H_2O$ (0.05 M). The pH of buffer solution was measured using the ECIL expanded scale pH meter (model pH 821), after due standardization with potassium hydrogen phthalate and borax buffers. For recording voltammograms, 2.0 mL dye solution was mixed with 2.0 mL buffer of appropriate pH. Nitrogen gas was bubbled for 8–10 min. before recording the voltammograms. Details of the equipment used for recording voltammograms, controlled potential electrolysis and spectral studies were essentially the same as described earlier.[15–17] Pyrolytic Graphite Electrode (PGE) was prepared by the method reported earlier[18] and had an area ~ 1.8 mm^2. All potentials refer to SCE at an ambient temperature of 20±2 °C.

Controlled potential electrolysis of ~ 0.5 mM solution of the dye was carried out in a conventional H-type cell at potential 50 mV more negative than the peak potential observed for peak Ic or IIc in the buffer of desired pH. The working electrode was Pyrolytic graphite plate (area 6×1 cm^2), counter electrode was a cylindrical platinum gauze and the reference electrode was SCE. The value of '*n*' was determined by attaching a coulometer in series.

For product characterization about 8–10 mg of the dye was electroreduced at peak Ic or IIc potentials. When the peak current reduced to about 5%, the electrolysis was stopped and the electrolyzed solution was removed from the cell. The pH was adjusted to 3.0 to convert sodium salts into the corresponding acid and lyophilized. The freeze-dried material obtained was dissolved in 2–3 mL water and passed through a glass column (60×1.5 cm) packed with Sephadex G-10 (Sigma, bead size 4–120 µ). Doubly distilled water was used as an eluting solvent. Different fractions (5 mL each) were collected in test tubes. The absorbance of each tube was monitored at 210 nm and was plotted against volume. The volume collected between 160 to 200 mL (peak P_1) was found to contain phosphate and hence discarded. The other three peaks between volumes 220–250 (P_2), 250–270 (P_3) and 290–325 (P_4) were collected separately, freeze-dried and analyzed by m.p., IR and ^{1}H NMR spectra. IR spectra were recorded as KBr pellets using Perkin Elmer 1600 FTIR spectrometer and ^{1}H NMR were obtained with a FX-100 Jeol NMR spectrometer. TLC of the electrolyzed solution was carried out using silica Gel-G as adsorbent and methanol-benzene (25:75) as developer.

RESULTS AND DISCUSSION

Voltammetric Behaviour

Linear sweep voltammetry of Direct Orange-31 at a sweep rate of 20 mV s^{-1} at pyrolytic graphite electrode exhibited two well-defined reduction peaks (Ic and IIc) in the pH range 2.0–10.3 when the sweep was initiated in negative direction. The peak potential of the peaks was dependent on pH and shifted towards more negative potential with pH increase. The plots of *E*p *vs.* pH can be approximated as two segments of straight lines which intersect at pH 4.6. (Figure 1). The dependence of *E*p on pH can be represented by the following relations:

Peak Ic

$$-E\text{p (pH 2.0 – 4.6)} = [250 + 100\ \text{pH}]\ \text{mV}\ vs.\ \text{SCE}$$

$$-E\text{p (pH 4.6 – 10.3)} = [450 + 60\ \text{pH}]\ \text{mV}\ vs.\ \text{SCE}$$

Peak IIc

$$-E\text{p (pH 2.0 – 4.6)} = [-110 + 100\ \text{pH}\ ]\ \text{mV}\ vs.\ \text{SCE}$$

$$-E\text{p (pH 4.6 – 10.3)} = [100 + 65\ \text{pH}\ ]\ \text{mV}\ vs.\ \text{SCE}$$

The break in *E*p–pH curve at around pH 4.6 represents the pK_a of the dye. In cyclic sweep voltammetry at a sweep rate of 100 mV s^{-1}, two well-

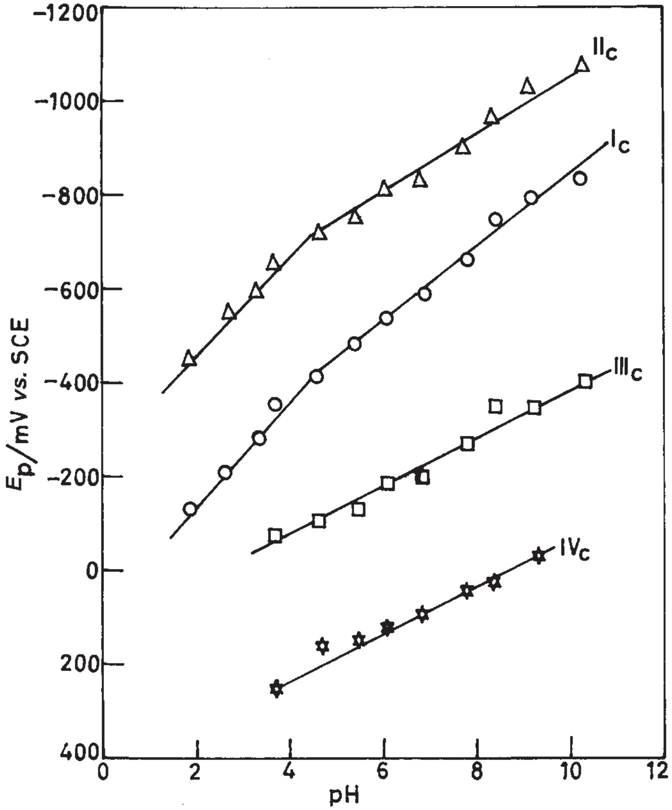


Figure 1. Observed dependence of peak potential (*E*p) of reduction peaks on pH for 0.5 mM Direct Orange-31, sweep rate 20 mV s^{-1}.

defined cathodic peaks (Ic and IIc) were observed. When the direction of sweep was reversed, two anodic peaks (IIIa and IVa) were observed in the entire pH range (Figure 2). At pH > 4.0, one more anodic peak (Ia) was occassionaly observed. When the sweep direction was again changed, two new reduction peaks IIIc and IVc were noticed at pH > 4.0 which formed a quasi-reversible couple with peaks IIIa and IVa. The peak currents of peaks Ic and IIc were more or less constantly below pH 7.0 and showed a decrease at higher pH. To check whether the anodic peaks were related to reduction peaks Ic and IIc, cyclic voltammograms were also recorded by initiating the sweep in the anodic direction. As no anodic peak was observed, it was concluded that peaks IIIa and IVa were due to oxidation of the products generated in peak Ic/IIc reaction. The peak potential of peaks IIIc and IVc was also pH dependent and shifted to more negative potential with pH increase. The dependence of *E*p on pH for these peaks can be represented by the following equations:

Peak IIIc

$$-E\text{p(pH 4.0–10.3)} = [120+55\ \text{pH}]\ \text{mV}\ vs.\ \text{SCE}$$

Peak IVc

$$-E\text{p(pH 4.0–10.3)} = [450+50\ \text{pH}]\ \text{mV}\ vs.\ \text{SCE}$$

To further distinguish whether the species responsible for peaks IIIa/IIIc and IVa/IVc were related to peak Ic or IIc, separate experiments were car-

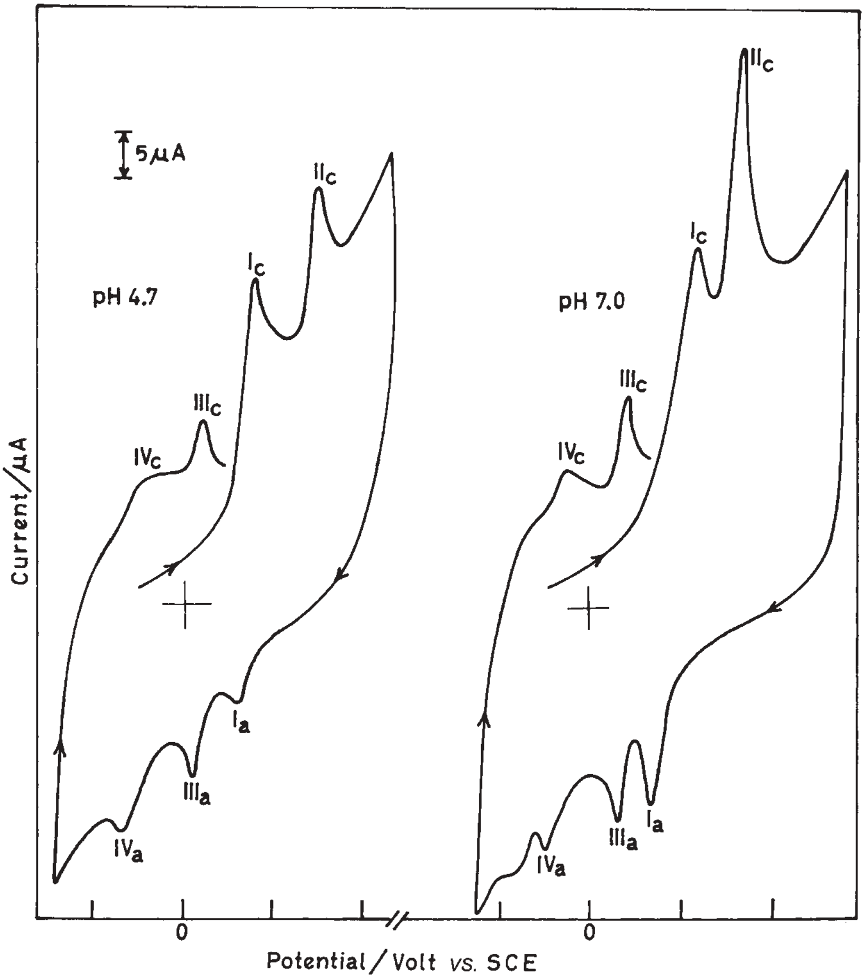


Figure 2. Cyclic voltammograms of 0.5 mM Direct Orange-31 in phosphate buffers, sweep rate 100 mV s^{-1}.

ried out in which direction of sweep was reversed after recording peak Ic. It was interesting to observe that redox couple IVa/IVc was clearly visible in such cases. Hence, it was concluded that the species responsible for peaks IVa/IVc are related to peak Ic reduction product whereas couple IIIa/IIIc is related to peak IIc. Some of the typical cyclic voltammograms of Direct Orange-31 are presented in Figure 2.

The peak currents of peaks Ic and IIc were found to increase with an increase in concentration of Direct Orange. The plots of i_p *vs.* concentration for both peaks were linear up to concentration of approximately 1.0 mM and had a tendency to limit at higher concentrations. The i_p (*I*c) *versus* concentration plot for Direct Orange-31 is presented in Figure 3. This behaviour indicated adsorption of the dye at the surface of PGE.[19] Adsorption was further confirmed by the linear increase of peak current with increase in sweep rate (Figure 4).

The values of '*n*' the number of electrons involved in the reduction, were determined at PGE in the entire pH range. The values of '*n*' obtained for peaks Ic and IIc at different pH and potentials are presented in Table I. It is clear from these values that reduction of the dye occurs in two steps, each involving 4.0 ± 0.1 electrons.

Spectral Studies

The progress of electroreduction of Direct Orange-31 was monitored by recording UV / VIS-spectra in the region 250–800 nm. Compound **I** exhib-

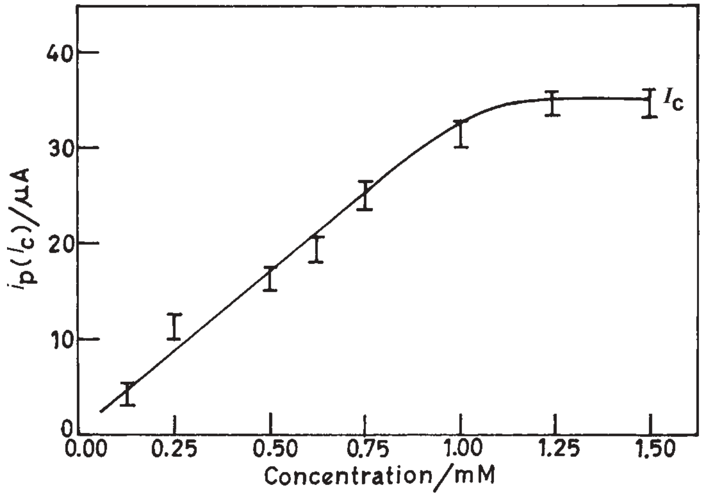


Figure 3. Peak current versus concentration behaviour for the reduction peak Ic of Direct Orange-31 at pH 7.0.

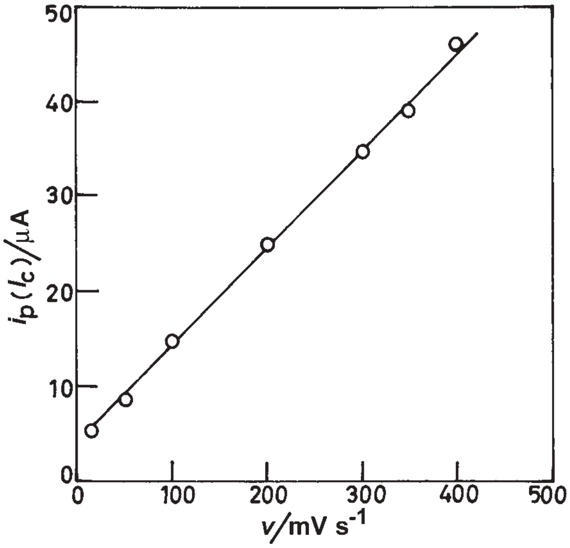


Figure 4. Variation of the peak current (i_p) observed for the reduction peak Ic with the variation in sweep rate for 0.5 mM Direct Orange-31 at pH 7.0.

ited two well-defined absorption bands at 257 and 356 nm in the pH range 2.0–4.6. At pH > 4.6, the band at 356 shifted to 407 nm. The shift in λ_{max} at pH > 4.6 again indicates the change in structure of the dye and suggests the pK_a of 4.6. A typical UV/VIS spectrum observed for 0.1 mM Direct Orange-

TABLE I

Coulometric *n*-values observed for the reduction of 0.5 mM Direct Orange-31 at PGE

pH	Potential (mV *vs*. SCE)	Experimental* *n* value
2.0	–200	4.08
	–600	3.96
3.8	–400	4.12
	–800	4.10
6.9	–700	4.11
	–900	3.86
8.4	–800	3.91
	–1050	4.14

* Average of at least three replicate determinations

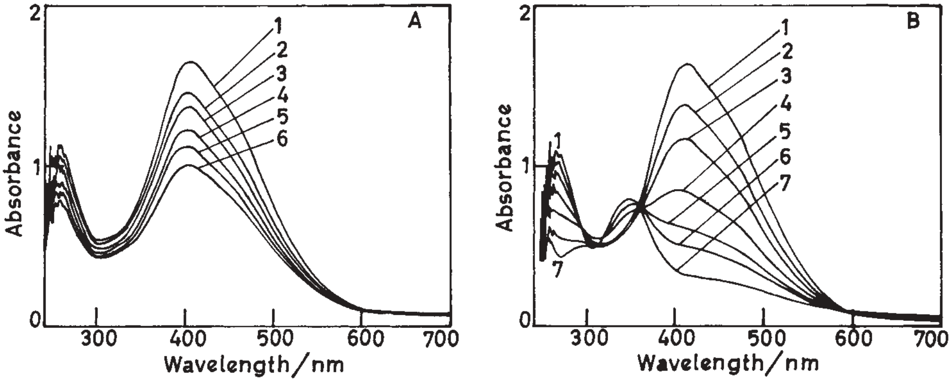


Figure 5. Observed spectral changes during reduction of 0.1 mM Direct orange –31(A) for peak Ic and (B) for peak IIc at pH 7.0, potential 0.7 and 0.9 V *vs.* SCE respectively. Curves (1–6) were recorded at 0; 10; 20; 40; 60 and 75 min of electrolysis. Curve 7 was rcorded after 120 min.

31 at pH 7.0 is presented by curve 1 in Figure 5. Upon application of the potential more negative than peak Ic, the absorbance at λ_{max} decreased systematically (Figure 5A). If potential is turned off at any stage during electrolysis, no change in absorbance was noticed. Thus, no intermediate capable of absorbing at longer or shorter wavelength was generated. In contrast, if 0.9 V potential was applied (Figure 5B), the absorbance in the regions 250–300 and 350–600 nm decreased and absorbance in the region 300–350 nm increased. After 2 h of electrolysis, a new band at 350 nm was observed. However, the change in absorbance ceased when the potential was turned off at any stage of electrolysis.

The progress of electrolysis was also monitored at pH 7.0 by recording cyclic voltammograms at different time intervals. The cyclic voltammograms of compound **I** exhibited two reduction peaks (Ic and IIc) and oxidation peaks (IIIa and IVa), as shown in Figure 2. When potential was applied 50 mV more to peak IIc, a systematic decrease in the peak current of peaks Ic and IIc was noticed. Redox couples IIIa/IIIc and IVa/IVc were clearly observed at the end of electrolysis.

Product Characterization

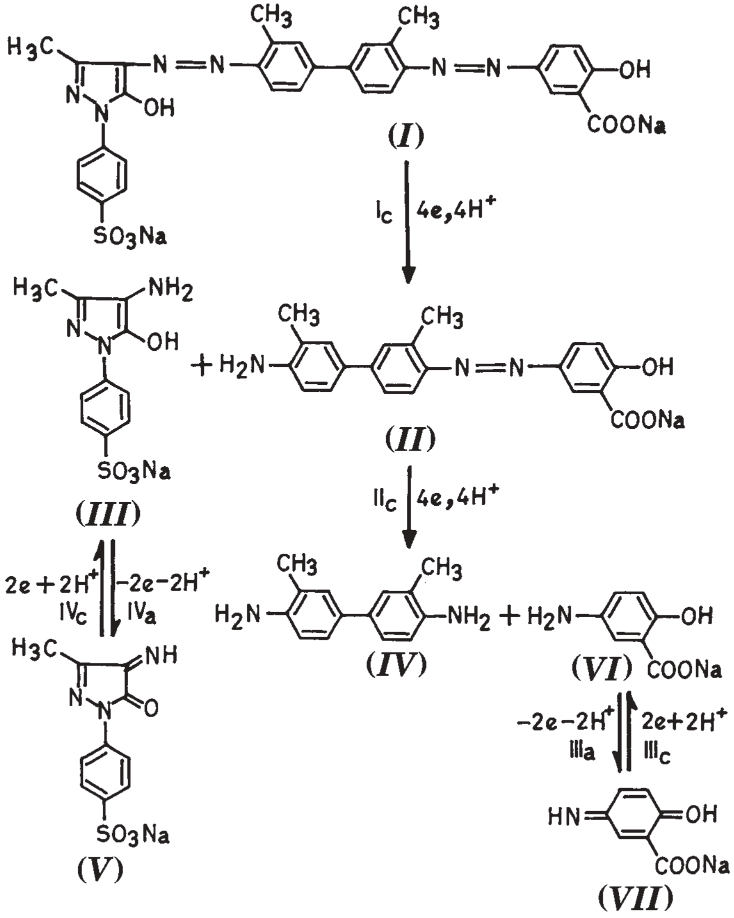
The products of electroreduction were characterized at pH 3.0 and 7.0. The exhaustively electrolyzed solution of the dye corresponding to peak Ic exhibited two clear spots in TLC at both pH, with R_f values 0.30 and 0.45. The presence of two spots indicated the presence of two compounds. Hence, it appears reasonable to conclude that one of the azo linkage breaks in peak Ic reaction. When electrolysis was carried out at peak IIc potentials and the

TLC of the resulting solution was carried out, three spots with R_f 0.30, 0.50 and 0.60 were observed. The product with $R_f \sim 0.30$ seems to be common to both reduction peaks. The electrolyzed solution was freeze-dried (see experimental) and the solutions under various chromatographic peaks were collected separately and lyophilized.

The colourless material under chromatographic peak P_2 exhibited a single spot in TLC ($R_f \sim 0.60$). It had m.p. 145–146 °C and exhibited the following prominent bands in the FTIR spectrum: 3429, 3163, 2598, 2085, 1666, 1609, 1441, 1297, 1153, 1029, 892 and 760 cm^{-1}. This compound was characterized as 4-aminosalicylic acid by comparing its IR with the authentic sample. Formation of 4-aminosalicylic acid as one of the products and the number of electrons involved in the reduction in peaks Ic and IIc clearly indicates that cleavage of –N=N– occurs. Hence, the other products should be 3,3'-dimethylbenzidine and substituted pyrazole. The volume collected under peak P_3 on lyophilization gave light brown coloured material having m.p. > 300 °C. The IR spectrum of the product exhibited the following significant bands: 3602, 3520, 3316, 3228, 1607, 1491, 1260, 817, 700 and 507 cm^{-1}. The ^1H NMR spectrum of the material exhibited the signals at δ/ppm 2.5 (s, 3H), 2.6 (s 3H) and 7.5 (m, 10H). Thus, the compound is characterized as 3,3'-dimethylbenzidine. The product corresponding to chromatographic peak P_4 ($R_f \sim 0.30$) had m.p. 220 °C and could not be completely characterized at this stage due to the very small amount obtained. The involvement of four electron reduction in each voltammetric peak suggests that it should be pyrazole derivative.

The experimental information presented above indicates that the electroreduction of Direct Orange-31 dye occurs in two well-defined reduction peaks at a PGE. As controlled potential electrolysis indicated that peak Ic is $4e^-$, $4H^+$ transfer process, either the two azo group can be reduced to a bishydrazo derivative, as reported for bisazobenzene,[20,21] or cleavage of one of –N=N– linkage may take place, as observed in the case of several azo dyes.[22,23] As TLC of the exhaustively electrolyzed solution corresponding to peak Ic exhibited two clear spots, it is concluded that reduction in peak Ic process leads to cleavage of one of the two azo groups present in the dye. As cleavage of –N=N– group attached to the bulkier moiety has been often observed due to steric hindrance to coplanarity,[24] it seems reasonable to conclude that, in peak Ic process, reduction of azo group attached to substituted pyrazole moiety takes place and cleavage of –N=N– would give substituted pyrazole **III** and species **II** as shown in Scheme 1.

The pyrazole species **III** possesses an amino and hydroxyl group at 4 and a 5 positions. As oxidation of aminophenols is well documented in the literature,[25] it seems reasonable to conclude that redox couple IVa/IVc observed on changing the sweep direction after recording peak Ic is due to oxidation of this compound. Further transfer of $4e^-$, $4H^+$ in peak IIc reaction



Scheme 1. A tentative reaction mechanism proposed for the reduction of Direct Orange-31.

causes cleavage of the second azo group *via* a hydrazo intermediate to give 4-aminosalicylic acid (**VI**) and 3,3'-dimethylbenzidine (**IV**). Compounds **IV** and **V** have been characterized on the basis of m.p., IR and NMR spectra. Redox couple IIIa/IIIc has been found to be due to the oxidation of 4-aminosalicylic acid as it has been confirmed by recording a CV of the authentic compound. When cyclic voltammograms of authentic 4-aminosalicylic acid were recorded by initiating the sweep in positive direction, peaks IIIa/IIIc were observed at the same potential as observed in the case of Direct Orange-31.

Thus, it is concluded that the electroreduction of Direct Orange-31 dye does not stop at the hydrazo stage but further reduction of hydrazo molecule yields amino compounds as the final products. The observed reduction behaviour of Direct Orange-31 was also compared with the reduction of *p*-bisazobenzene reported in literature.[20] It was interesting to observe that in acidic medium reduction of *p*-bisazobenzene occurred in two waves at d.m.e. involving close to 8.0 electrons, whereas in alkaline solution reduction stopped at hydrazo stage (n = 4.0). In the case of several other bisazo dyes having napthol moiety[26] or ethoxy groups,[23] reduction has been observed in 8.0 electrons to cause clevage of azo linkages in acidic and alkaline media. The present studies clearly indicate that the electroreduction of compound **I** at solid electrode occurs *via* hydrazo intermediate to give amino compounds as the ultimate product. The difference in behaviour from *p*-bisazobenzene can be attributed to the electrode material as well as to bulkier pyrazole moiety attached to one side of an azo group. The steric hindrance due to bulky groups has been reported to affect the reduction behaviour in a large number of organic compounds.[27]

Acknowledgement. – One of the authour (MSV) is thankful to the CSIR, New Delhi, for the award of Research Associateship.

REFERENECES

1. Y. Castriellezo and R. Pardo, *Electroanalysis* **2** (1990) 553.
2. H. Terayama, *Methods in Cancer Research*, Academic Press, New York, vol.1, 1967, p. 399.
3. Z. F. Liu, K. Hashimoto, and A. Fujishima, *J. Electroanal. Chem.* **324** (1992) 259.
4. A. A. A. Hafez, I. M. A. Awad, and K. M. Hanssan, *Collect. Czech. Chem. Commun.* **52** (1987) 2753.
5. G. L. Clark and P. E. Bierstedt, *Radiat. Res.* **2** (1955) 199.
6. H. H. Willard and J. A. Jean, *Anal. Chem.* **22** (1950) 1764.
7. M. L. Thakur, *Talanta* **21**(1974) 771.
8. T. Miyadera, *Biological Formation and Reactions of Hydrazo, Azo and Azoxy Groups*, S. Patai (Ed.), John Wiley and Sons, Inc., London, 1975, p. 495.
9. J. Barek, A. Berka, and J. Zima, *Collect. Czech. Chem. Commun.* **50** (1985) 1819.
10. M. Matsumoto, H. Terayama, *Gann.* **56** (1965) 169.
11. M. Matsumoto, *Seikagaku* **37** (1965) 37.
12. U. Eisner and E. K. Eisner, *Encyclopedia of Electrochemistry of the Elements,* A. J. Bard (Ed.), Marcel Dekker, N. Y., 1979.
13. P. C. Dobler and M. S. Spach, *Stain Technol.* **62** (1987) 23.
14. G. D. Christian and W. C. Purdy, *J. Electroanal. Chem.* **3** (1962) 363.
15. R. N. Goyal, A. K. Jain, and N. Jain, *Bull. Chem. Soc. Japan* **69** (1996) 1987.
16. R. N. Goyal, A. K. Jain, and N. Jain, *J. Chem. Soc., Perkin Trans. 2* (1995) 1055.
17. R. N. Goyal, N. Jain, and D. K. Garg, *Bioelectrochem. and Bioenerg.* **43** (1997) 105.
18. R. N. Goyal, S. K. Srivastava, and R. Agarwal, *Bull. Soc. Chim. Fr.* **4** (1985) 656.
19. R. H. Wopschall and I. Shain, *Anal. Chem.* **39** (1967) 1514.
20. T. M. Florence, *J. Electroanal. Chem.* **52** (1974)115.

21. A. M. Shams-El-Din, T. M. H. Saber, and N. M. Abed, *J. Electroanal. Chem.* **21** (1969) 377.
22. W. U. Malik, R. N. Goyal, and N. C. Mathur, *J. Electroanal.Chem.* **235** (1987) 225.
23. R. N. Goyal and Anoop Kumar, *Bull. Soc. Chim. Fr.* (1987) 577.
24. T. M. Florence, D. A. Johnson, and G. M. Batley, *J. Electroanal. Chem.* **50** (1974) 113.
25. W. M. Fox and W. A. Waters, *J. Chem. Soc.* (1964) 6010.
26. R. N. Goyal and A. Kumar, *Indian J. Chem.* **27A** (1988) 482.
27. P. Zuman, *Substituents Effect in Organic Polarography*, Plenum Press, N. Y., 1967.

SAŽETAK

Voltammetrijsko istraživanje biazo-boje *Neposredno narančasto 31* na pirolitičkoj grafitnoj elektrodi

Rajendra N. Goyal, Madhu S. Verma i Naveen K. Singhal

Istražena je elektrokemijska redukcija biazo-boje *Neposredno narančasto 31* na pirolitičkoj grafitnoj elektrodi u rasponu 2,0 < pH < 10,3. U linearnoj i cikličkoj voltammetriji uočena su dva dobro definirana odziva uzrokovana redukcijom koja uključuje prijenos 4 elektrona i 4 protona. Produkti redukcije azo-skupina te boje jesu amini jer se hidrazo-skupina (koja nastaje kao međuprodukt reakcije) cijepa u daljnjem toku redukcije. Pri pH = 3,0 glavni su produkti redukcije 4-aminosalicilna kiselina i 3,3'-dimetilbenzidin. Predložen je mehanizam redoks-reakcije.