Dyes and Pigments 83 (2009) 21–30

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

The reduction of dispersed indigo by cathodically formed 1,2,4-trihydroxynaphthalene

Sunsanee Komboonchoo [a,b,*], Aurora Turcanu [a], Thomas Bechtold [a]

[a] Research Institute for Textile Chemistry/Physics, University of Innsbruck, Hoechsterstr. 73, A-6850 Dornbirn, Austria
[b] Department of Industrial Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

ARTICLE INFO

Article history:
Received 23 December 2008
Received in revised form
6 March 2009
Accepted 10 March 2009
Available online 24 March 2009

Keywords:
Cyclic voltammetry
Spectroelectrochemistry
2-Hydroxy-1,4-naphthoquinone
Lawsone
Indigo
C.I. Vat Blue 1
C.I. Natural Blue 1
C.I. Natural Orange 6
C.I. Vat Yellow 1

ABSTRACT

The indirect cathodic reduction of the vat dye indigo (C.I. Vat Blue 1) by cathodically reduced Lawsone (2-hydroxy-1,4-naphthoquinone; C.I. Natural Orange 6) was studied in aq. solution at different pH values. Cyclic voltammetry and spectroelectrochemistry were used to investigate the electrochemical behavior of 2-hydroxy-1,4-naphthoquinone at a hanging mercury drop electrode. The cathodic peak potential $(E_p)_d$ measured at 0.1 mM lawsone solution at a scan rate of 50 mV s^{-1} changed from −425 mV at pH 7, to −730 mV at pH 11.5 and −750 mV at pH 13 (vs. Ag/AgCl, 3 M KCl). Particularly at pH values of 8–9 and 11.5–13 voltammograms indicated successful, indirect cathodic reduction of the dye in which the cathodically reduced 2-hydroxy-1,4-naphthoquinone acted as soluble mediator. The linear relationship obtained for $(I_p)_d$ vs. $v^{1/2}$ is indicative of a diffusion-controlled electrode reaction mechanism. In the presence of dispersed indigo, the overall cathode reaction is similar to the E_{cat} process with continuous regeneration of the electroactive species. Spectrochemical experiments were used to prove the indirect cathodic reaction of dispersed vat dyes by 2-hydroxy-1,4-naphthoquinone.

1. Introduction

Lawsone, 2-hydroxy-1,4-naphthoquinone (C.I. Natural Orange 6) is the major natural colorant in the leaves of henna (*Lawsonia inermis*), which have been used since ancient times for medical purposes, as a cosmetic colorant for dyeing hair and staining skin as well as for textile dyeing. The structural formula of lawsone is given in Fig. 1a [1,2].

A mixture of henna with indigo leaves constitutes the so-called "Reng" or "Rang" natural colorant which has been used for hair dyeing for many centuries [3,4]. The use of both henna and indigo is based on empirical knowledge and little information is available about the chemistry of the combination of indigo and henna [4,5]. Furthermore henna has been added to indigo vats in Morocco and southern Tunisia to stimulate and maintain the bacterial fermentation, and madder, containing alizarin, has long been used for this purpose in woad vats [6].

2-Hydroxy-1,4-napthoquinone present in henna leaves is formed by oxidation of 1,2,4-trihydroxynaphthalene which is presumed to be released as aglycone from the hydrolysis of corresponding glycosides (hennoside A, B and C). In dried leaves from the indigo plant, the dye being present as indigo (C.I. Natural Blue 1; C.I. Vat Blue 1, Fig. 1b) [7].

Indigo is insoluble in aqueous solution and thus for the dyeing of protein and cellulose fibres has to be converted into the corresponding water-soluble leuco-form by a reduction stage, that is normally carried out in alkaline, aqueous solution at a pH of ∼11–14 using strong reducing agents such as sodium hydrosulfite ($Na_2S_2O_4$) [8,9].

Many attempts have been made to minimize the production of polluting wastes from the reduction step for example, usage of sugar type reducing agents and the electrochemical and biological reduction of indigo [8–12]. 9,10-Anthraquinoides with formal redox potential more negative than −700 mV (vs. Ag/AgCl, 3 M KCl) were shown to be suitable to serve as a mediator for the indirect cathodic reduction of dispersed organic dyestuffs [9,13]. The use of reversible redox couples for indirect cathodic reduction of dispersed vat dyes and sulfur dyes is currently under research by several groups [8,9,12–20].

---

* Corresponding author. Research Institute for Textile Chemistry/Physics, University of Innsbruck, Hoechsterstr. 73, A-6850 Dornbirn, Austria. Tel.: +43 5572 28533; fax: +43 5572 28629.
E-mail address: textilchemie@uibk.ac.at (S. Komboonchoo).

0143-7208/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2009.03.008

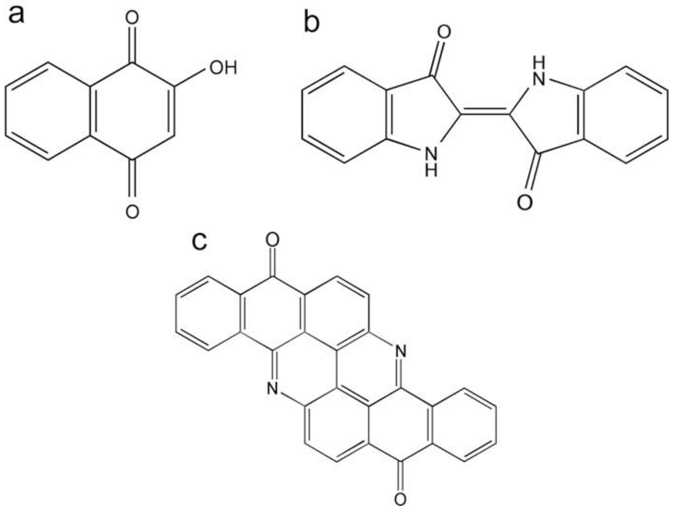


Fig. 1. The structural formulae of lawsone (a), indigo (b), C.I. Vat Yellow 1(c).

The application of henna and indigo in "Reng" is performed as a cataplasma (poultice) dyeing in which a soft moist and warm paste of powdered henna leaves is applied directly on the hair [4]. Assuming that 1,2,4-trihydroxynaphthalene is released from the hydrolysis of the hennoside during the preparation of the paste, this component could act as a reducing agent for indigo pigment. Both lawsone and reduced indigo then would be reaction products, which then form the active coloration system for hair dyeing.

In this study the system 2-hydroxy-1,4-naphthoquinone/indigo has been characterized as a model in order to establish possible reaction mechanisms occurring in Reng. 1,2,4-Trihydroxynaphthalene was formed by direct cathodic reduction from 2-hydroxy-1,4-naphthoquinone (lawsone). The electrochemistry of 2-hydroxy-1,4-naphthoquinone (lawsone) in the presence of indigo solution was studied by cyclic voltammetry (CV). Information about electron transfer from reduced lawsone to dispersed indigo particles thus will be of interest for both the improved understanding of the reaction occurring in Reng and the further development of the electrochemical vat dye reduction.

When 2-hydroxy-1,4-naphthoquinone is reduced in CV to 1,2,4-trihydroxynaphthalene, a catalytic current will be observed in the case of a redox reaction between 1,2,4-trihydroxynaphthalene and dispersed indigo.

Catalytic processes in electrochemistry occur when a substance O is reduced to R (equation (1)) and R regenerates O by chemical reaction with substance X (equation (2)). The reduction of X yields the product Y [21,22]. In the case of indigo, R reacts with dispersed indigo particles on their surface to generate O and dissolved leuco-indigo dianion (equation (3)).

$$\mathrm{O} + \mathrm{ne}^- \leftrightarrow \mathrm{R} \tag{1}$$

$$\mathrm{R} + \mathrm{X} \rightarrow \mathrm{O} + \mathrm{Y} \tag{2}$$

$$\mathrm{R} + \text{surface of dispersed indigo} \rightarrow \mathrm{O} + \text{dissolved leuco} - \text{indigo dianion} \tag{3}$$

Cyclic voltammetry thus permits the evaluation of the electrochemical behavior of 2-hydroxy-1,4-naphthoquinone in the presence of dispersed indigo. In addition spectroelectrochemical experiments were used to observe possible dyestuff reduction during a CV scan.

2. Experimental

2-Hydroxy-1,4-naphthoquinone (Sigma–Aldrich Chemie GmbH, Steinheim, Germany), KH_2PO_4, $Na_2HPO_4 \cdot 2H_2O$, HCl, Na-D-gluconate (Merck, Darmstadt, Germany), NaOH (Carl Roth GmbH, Karlsruhe, Germany) H_3BO_3, KCl (Zeller GmbH, Dornbirn, Austria) were analytical grade chemicals.

$FeCl_2$ (BASF, Ludwigshafen, Germany), Triethanolamine/TEA (K. Deuring GmbH, Hörbranz, Austria) were technical grade products.

Indigo solution 40% DyStar (hydrogenated C.I .Vat Blue 1), Dystar Indigo gran (C.I. Vat Blue 1) and indanthrene yellow G (C.I. VAT Yellow 1) were used as supplied by DyStar Textilfarben GmbH, Frankfurt a.M., Germany. For experiments employing dispersed indigo, this was prepared by adding a defined amount of reduced hydrogenated indigo solution to a buffer of desired pH followed by air oxidation of the reduced indigo by stirring overnight. The concentration of indigo in the dispersion was determined by photometry of the leuco-indigo dianion by reduction with an Fe(II)-complex salt solution [23]. Dispersed indigo solution (1 mL) was added to a solution of H_2O (14 mL), 0.1437 M $FeCl_2$, 0.825 M NaOH, 1.71 M triethanolamine and 0.0042 M citric acid $C_6H_8O_7 \cdot H_2O$. The absorbance of the added dyestuff then was measured at 405 nm (1 mm path length cuvette, single beam filter photometer Merck SQ 300). The concentration of indigo dye in the dispersion then can be obtained from a calibration curve established with indigo granulate.

The test solutions were prepared by dilution of stock solutions with buffers. Stock solutions were 0.001 M 2-hydroxy-1,4-naphthoquinone solution and a dispersion containing 0.003 M indigo. Solutions were prepared in the range from pH 7–13. The pH of the solution was checked with glass electrode and potentiometer (Hamilton-flush-trode, ORION 720 A, Orion Research Inc., Boston, USA). The composition of the solutions used in the cyclic voltammetry experiments is given Table 1.

Cyclic voltammetric experiments were carried out by employing a three electrode configuration. The apparatus used for the experiments was an EG&G 264 A potentiostat with a 303A HMDE (small drop size, drop area 0.96×10^{-2} cm^2). A platinum wire served as counter electrode. The cyclic voltammograms were recorded on a Rikadenki X–Y recorder. All potentials given were measured vs. a (Ag/AgCl, 3 M KCl) reference electrode. The test solutions were purged for 8 min with Ar to eliminate interfering oxygen. All experiments were performed at room temperature. The scan rates were varied from 5 to 100 mV s^{-1}.

The spectroelectrochemical experiments were performed using a plastic cuvette with path length $d = 1$ cm in which a three electrode arrangement was mounted. A stainless steel fabric (geometric area of fabric 209 mm^2, electrode area 626 mm^2, diameter of wire 0.025 mm, width of aperture 0.025 mm, Haver & Boecker, Oelde, Germany) was used as cathode, strips of Pt foil outside the optical path served as anodes. A (Ag/AgCl, 3 M KCl) reference electrode was used. The EG&G 264 A potentiostat was employed for the CV scan which was recorded on a Rikadenki X–Y recorder. With a diode

Table 1
Composition of solutions used for the cyclic voltammetry experiments.

Component	C. I. Vat Blue 1 (mM)	2-Hydroxy-1,4-naphthoquinone (mM)
1	1.50	
2		0.05
3		0.10
4		0.25
5	1.50	0.05
6	1.50	0.10
7	1.50	0.25

Table 2
Composition of solutions used for spectroelectrochemical experiments.

Component	2-Hydroxy-1,4-naphthoquinone (mM)	NaOH (M)	CI Vat Blue 1[a] (g L^{-1})	CI Vat Yellow 1[a] (g L^{-1})
8	0.287	0.1	0.12	
9	0.287	0.1		0.03

[a] Technical products.

array photometer (Zeiss CLH 500/MCS 521 UV–VIS, Carl Zeiss Jena, Jena, Germany) photometric spectra were recorded during the CV scan. The direction of the light beam of the photometer was perpendicular to the plane of the steel fabric cathode. Deoxygenation of the electrolyte was achieved by degassing the prepared electrolyte and starting the CV scan immediately after filling the cell.

The concentrations used in the spectroelectrochemical experiments are given in Table 2.

The species distribution of the 2-hydroxy-1,4-napthoquinone and 1,2,4-trihydroxy-napthoquinone as a function of pH was calculated with the program SPE using pKs values according to Petrova et al. [24,25].

3. Results and discussion

3.1. Cyclic voltammograms

Cyclic voltammograms of 2-hydroxy-1,4-naphthoquinone were recorded in the aqueous buffer solutions from pH 7–13. Then voltammograms with addition of dispersed indigo were measured under the same conditions.

The voltammograms were recorded as a function of the scan rate in the potential range of −200 mV to −900 mV (pH 7.0–7.1; and pH 8.1–8.2) and −300 mV to −900 mV (pH 9.1–9.3; pH 10.0–10.2; pH 11.5–11.7 and pH 12.9–13.0). Scan rates were varied from 5 to 100 mV s^{-1}. The voltammograms of 2-hydroxy-1,4-naphthoquinone at pH 7 and 13 are shown in Figs. 2a and 3a.

The voltammograms of 2-hydroxy-1,4-naphthoquinone in the aqueous buffer solutions show a single reduction peak and a corresponding anodic oxidation peak. However a small shoulder was observed in some voltammograms, which can be attributed to an impurity of either lawsone or indigo, the adsorption of lawsone on the electrode surface, or also to aggregation of the lawsone molecules.

The diffusion-controlled electrode reaction $(I_p)_d$ was analyzed as function of the square root of the scan rate. In Fig. 4 the cathodic peak current $(I_p)_d$ of 0.01 mM 2-hydroxy-1,4-naphthoquinone solutions is shown as function of the square root of the scan rate for different solution pH. For all conditions studied a linear relation between $(I_p)_d$ and $v^{1/2}$ is observed. This supports the assumption of a diffusion-controlled electrode reaction mechanism, which is in accordance with the literature data [21]. Small derivations from linearity are observed in experiments at pH 9 and 10.

The variation of peak potential separation between the anodic current peak and the cathodic current peak of 2-hydroxy-1,4-naphthoquinone in the aqueous buffer solutions, $(E_p)_a - (E_p)_c$ is shown in Table 3. The variation of peak potential separation can be used to study the electrochemical mechanism [26]. It was found

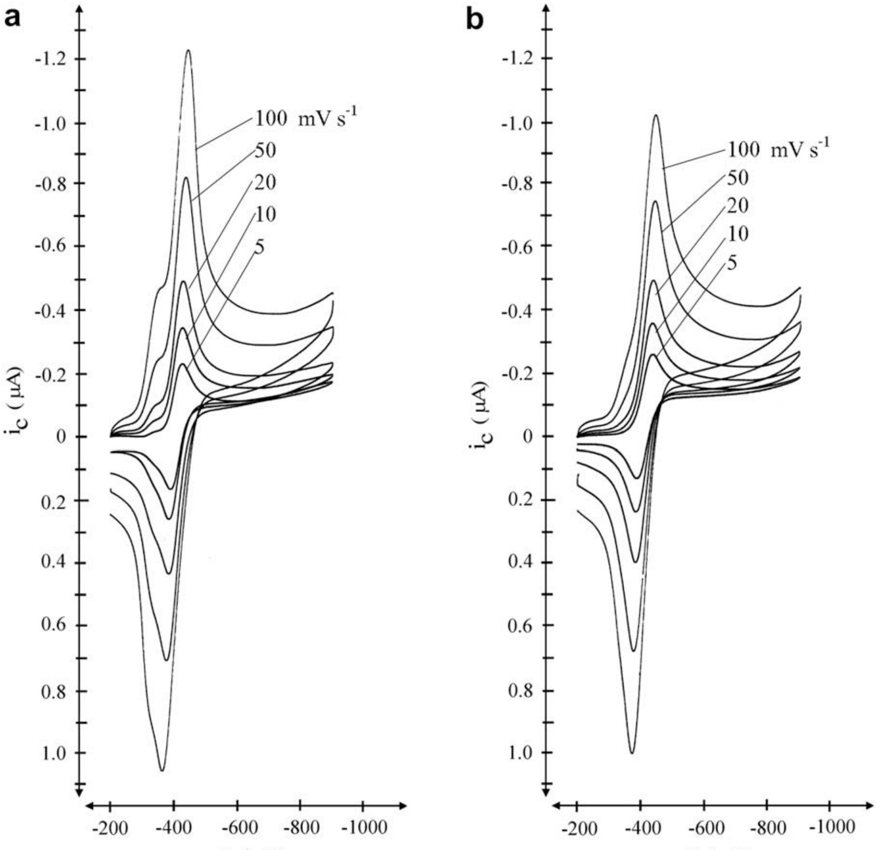


Fig. 2. CV of 0.1 mM 2-hydroxy-1,4-naphthoquinone at pH of 7.0–7.1 in the potential range between −200 and −900 mV at scan rate of 5, 10, 20, 50, 100 mV s^{-1} (solutions 3, 6) a) 0 mM indigo b) 1.5 mM indigo.

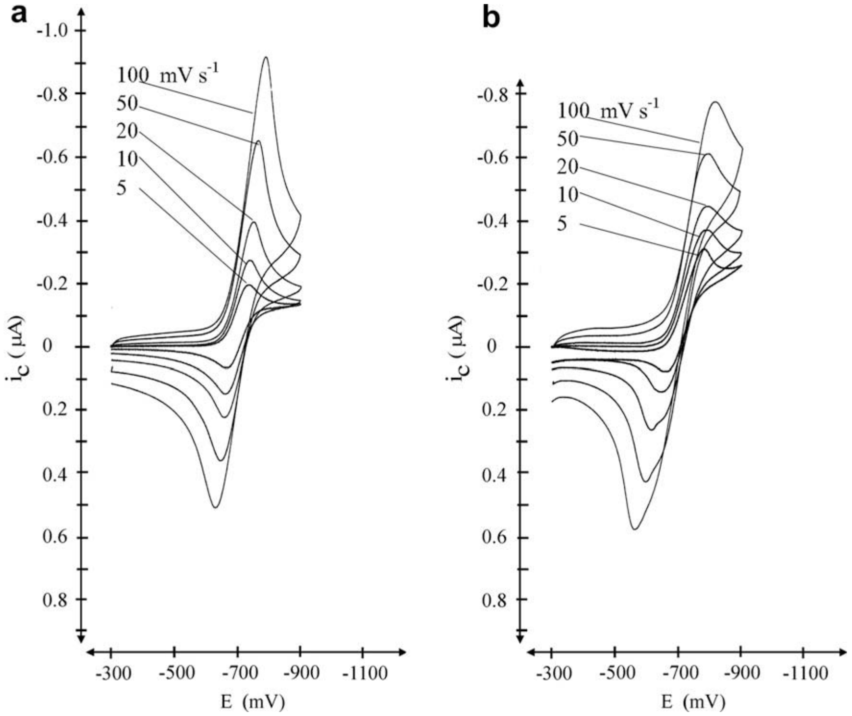


Fig. 3. CV of 0.1 mM 2-hydroxy-1,4-naphthoquinone at pH of 12.9–13.0 in the potential range between −300 and −900 mV at scan rate of 5, 10, 20, 50, 100 mV s^{-1} (solutions 3, 6) a) 0 mM indigo b) 1.5 mM indigo.

that the peak potential separation of lawsone in the buffer solutions tends to increase with increasing scan rate and pH of solutions. The increasing peak potential separation with increasing scan rate indicates that reversibility of the electron transfer cannot be assumed. The electron transfer reaction more probably follows a quasi-reversible mechanism. This assumption is supported by the diagnostic criteria described for quasi-reversible systems, such as: $|I_p|$ increases with $v^{1/2}$ but is not proportional to it, $|(I_p)_a/(I_p)_c| = 1$ provided $\alpha_C = \alpha_A = 0.5$, ΔE_p is greater than 59/n mV and increases with increasing scan rate and $(E_p)_c$ shifts negatively with increasing scan rate [21]. However the redox pathway is rather complicated due to coupled protonation steps and the different species of reduced lawsone formed [24,27–29].

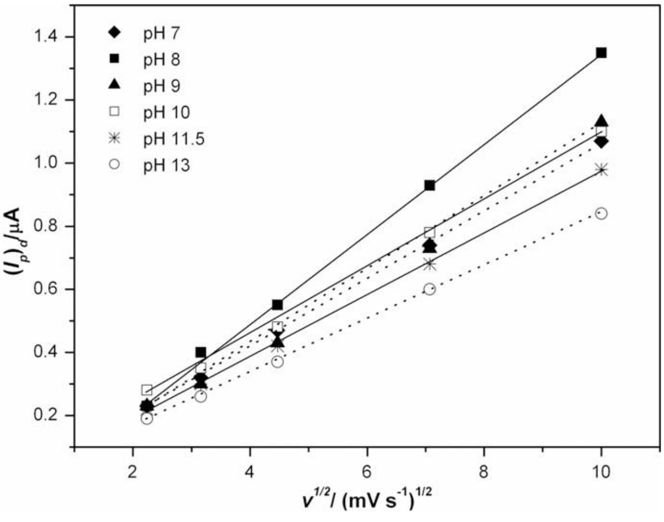


Fig. 4. Diffusion-controlled cathodic peak current, $(I_p)_c$ as function of the square root of scan rate, $v^{1/2}$ for 0.10 mM lawsone solution at different pH values.

In Table 4 cathodic peak currents $(I_p)_d$, anodic peak currents $(I_p)_a$ of 0.1 mM 2-hydroxy-1,4-naphthoquinone in the buffer solutions from pH 7 to 13 are summarized for various scan rates. The anodic-to-cathodic peak current ratio $(I_p)_a/(I_p)_d$ can be used as a measure for the overall reversibility of the electrode reaction. Up to pH 9 $(I_p)_a/(I_p)_d$ values are approximately 1 which indicates chemical stability of the reduction products, however particularly between pH 10 and pH 11.5 $(I_p)_a/(I_p)_d$ values decrease below unity indicating a more complex reaction pathway following the cathodic electron transfer reaction. For example consecutive reactions following the cathodic electron transfer reaction such as protonation of products and formation of more than one reduced species due to protonation equilibrium between different forms of 1,2,4-trihydroxynaphthalene could form an explanation for this finding [21,30].

Petrova et al. reported that depending on the pH in solution the redox reaction of 2-hydroxy-1,4-naphthoquinone involves up to five different species [24]. To determine the pH range in which a certain species is present in solution, the species distribution of lawsone and corresponding reduced forms was calculated with the program SPE [25]. The dissociation reactions and the corresponding pKs values are shown in equations (4)–(6) [24]. In Fig. 5 the species distribution of the different reduced and oxidized forms of lawsone is shown as a function of pH.

$$AH \leftrightarrow A^- + H^+ \qquad pK_{AH} = 4.16 \tag{4}$$

$$AH_3 \leftrightarrow AH_2^- + H^+ \qquad pK_{AH3} = 8.47 \tag{5}$$

$$AH_2^- \leftrightarrow AH^{2-} + H^+ \qquad pK_{AH2-} = 10.86 \tag{6}$$

In the oxidized state 2-hydroxy-1,4-naphthoquinone (AH) and the corresponding naptholate (A^-) are present, with A^- being the main species at pH values above pH 6. Thus in the investigated pH range of 7–13 the cathodic electron transfer at the cathode involves only the phenolate form of lawsone A^-.

Table 3
The variation of peak potential separation, ($(E_p)_a - (E_p)_c$) of 2-hydroxy-1,4-naphthoquinone in the aqueous buffer solutions at various scan rates and concentrations.

Lawsone/mM	Scan rate/mV s^{-1}	ΔE_p/mV					
		pH 7	pH 8	pH 9	pH 10	pH 11.5	pH 13
0.05	5	35	30	30	45	70	60
	10	30	40	40	60	80	70
	20	45	25	45	70	85	80
	50	50	45	40	100	135	90
	100	70	55	55	120	145	115
0.1	5	35	40	40	65	85	55
	10	45	40	40	80	95	70
	20	30	50	45	95	115	85
	50	40	50	40	130	150	110
	100	90	65	50	170	185	160
0.25	5	45	35	45	105	105	95
	10	45	40	50	125	135	110
	20	40	55	60	145	170	140
	50	70	75	70	190	200	160
	100	85	70	60	235	250	210

Depending on the pH different reduced forms are present in solution, which are 1,2,4-trihydroxynaphthalene (AH_3) or the deprotonated forms AH_2^- or AH^{2-}. Thus three different redox reactions are supposed to occur as result of the cathodic electron transfer (equations (7)–(9)).

$$A^- + 3H^+ + 2e^- \leftrightarrow AH_3 \tag{7}$$

$$A^- + 2H^+ + 2e^- \leftrightarrow AH_2^- \tag{8}$$

$$A^- + H^+ + 2e^- \leftrightarrow AH^{2-} \tag{9}$$

The electrochemical behavior of A^- involves the transfer of two electrons and protonation reactions with 1–3 protons to yield AH_3 (below pH 9; equation (7)), AH_2^- (pH 8–11.5; equation (8)), AH^{2-} (above pH 10.5; equation (9)).

The species distribution diagram indicates pH-regions in which the cathodic reduction of A^- yields one major product for example at pH 7 AH_3 will be the major product and AH_2^- will be present only in minor amounts, at pH 13 AH^{2-} will be the major species.

However also for a pH value where the species distribution indicates the presence of only one oxidized and reduced species e.g. A^- and AH^{2-} the reduction of A^- to the final product is expected to proceed through a complicated series of sequential electron transfer on protonation steps [27].

Table 4
Peak current of 0.1 mM 2-hydroxy-1,4-naphthoquinone in the aqueous buffer solutions at various scan rates and ratio of anodic peak current to cathodic peak current $(I_p)_a/(I_p)_d$.

	Scan rate/mV s^{-1}	pH 7	pH 8	pH 9	pH 10	pH 11.5	pH 13
$(I_p)_d$	5	0.23	0.23	0.23	0.28	0.22	0.19
	10	0.32	0.4	0.3	0.35	0.3	0.26
	20	0.47	0.55	0.43	0.48	0.42	0.37
	50	0.74	0.93	0.73	0.78	0.68	0.6
	100	1.07	1.35	1.13	1.1	0.98	0.84
$(I_p)_a$	5	0.24	0.23	0.23	0.23	0.2	0.17
	10	0.35	0.43	0.3	0.3	0.23	0.23
	20	0.48	0.6	0.43	0.4	0.3	0.32
	50	0.78	0.93	0.68	0.55	0.41	0.47
	100	1.14	1.35	0.98	0.73	0.51	0.65
$(I_p)_a/(I_p)_d$	5	1.04	1.00	1.00	0.82	0.91	0.89
	10	1.09	1.08	1.00	0.86	0.77	0.88
	20	1.02	1.09	1.00	0.83	0.71	0.86
	50	1.05	1.00	0.93	0.71	0.60	0.78
	100	1.07	1.00	0.87	0.66	0.52	0.77

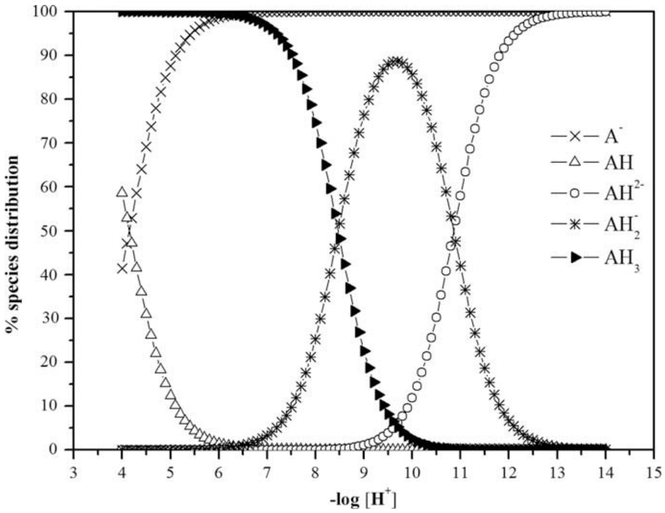


Fig. 5. Species distribution diagram for lawsone as a function of −log [H$^+$].

Table 5 shows the midpoint potential which is the half sum of cathodic and anodic peak potentials measured in the aqueous buffer solutions. The results indicate that the midpoint potential is independent of scan rate but it undergoes a pH dependent shift because of the various species of reduced lawsone present in solution.

During the anodic scan of the CV the reduced species formed will be reoxidized. At a pH of 10–11.5 AH_2^- and AH^{2-} will equilibrate in the diffusion layer of the cathode, thus two products will be reoxidized, which could explain the lowered $(I_p)_a/(I_p)_d$ ratio in this range. Particularly at higher scan rates of 50–100 mV $(I_p)_a$ are supposed to decrease because two species with different anodic current peak potential $(E_p)_a$ will be oxidized to form A^-.

Therefore the reduction process, which includes follow up reactions (protonation steps), will yield more than one reduced species. This complicated reaction pathway makes the analysis of the cathodic reduction of A^- difficult.

In a series of electrochemical experiments, the possibility of 2-hydroxy-1,4-naphthoquinone as a mediator for indirect cathodic reduction of indigo was studied by cyclic voltammetry. In the oxidized state indigo is present in a dispersed form as a blue pigment which is insoluble in aqueous solution. This dispersion has been shown to be electrochemically inactive under the conditions applied in this study [14].

The reduction of indigo into its alkali soluble leuco-form can be achieved at a potential around −700 mV (vs. Ag/AgCl/3 M KCl). For 2-hydroxy-1,4-naphthoquinone, the reduction potential of 2-hydroxy-1,4-naphthoquinone in alkaline solution was described to be more negative than −700 mV, thus lawsone could serve as a soluble mediator system for indirect cathodic reduction of dispersed indigo [14,15,31].

Bond et al. studied the electrochemical reduction of indigo in solid state and reported a reversible reduction of microcrystalline indigo mechanically attached to a basal plane pyrolytic graphite electrode. This process showed a pH dependent cathodic peak potential between −700 mV at pH 13 and −500 mV at pH 7 (vs. SCE reference electrode) [32].

The midpoint potential of lawsone in aqueous buffer solutions undergoes pH dependent shift in the negative direction with increasing pH. For example, at pH 7, the midpoint potential of lawsone is in the range of −400 to −410 mV while at pH 13 it is in the range of −700 to −715 mV. Thus the reduction potential of lawsone is sufficiently negative to reduce dispersed indigo to the leuco-indigo dianion.

Table 5
The midpoint potential, $((E_p)_c + (E_p)_a)/2$, of 2-hydroxy-1,4-naphthoquinone in the aqueous buffer solutions at different concentrations and various scan rates.

Lawsone/mM	Scan rate/mV s^{-1}	Midpoint potential/mV					
		pH 7	pH 8	pH 9	pH 10	pH 11.5	pH 13
0.05	5	−402.5	−445	−505	−587.5	655	−705
	10	−405	−440	−505	−590	655	−705
	20	−402.5	−442.5	−507.5	−595	662.5	−705
	50	−405	−437.5	−505	−590	667.5	−705
	100	−405	−432.5	−507.5	−600	677.5	−712.5
0.1	5	−407.5	−450	−510	−592.5	657.5	−702.5
	10	−402.5	−450	−510	−590	662.5	−705
	20	−405	−445	−502.5	−592.5	662.5	−702.5
	50	−405	−445	−505	−595	675	−705
	100	−410	−437.5	−505	−595	682.5	−710
0.25	5	−407.5	−452.5	−507.5	−592.5	667.5	−707.5
	10	−407.5	−450	−505	−592.5	667.5	−705
	20	−410	−447.5	−510	−587.5	665	−710
	50	−405	−447.5	−505	−595	670	−710
	100	−407.5	−445	−510	−597.5	685	−715

Table 6
Enhancement factors (*e.f.*), $(I_p)_c/(\ I_p)_d$ observed with solutions 5, 6, 7 containing 2-hydroxy-1,4-naphthoquinone in buffer solutions in the presence of dispersed indigo.

Molar ratio of lawsone(A) to indigo(B) A:B	Scan rate/mV s^{-1}	pH 7	pH 8	pH 9	pH 10	pH 11.5	pH 13
1:30	5	1.56	0.87	1.00	0.67	1.36	1.56
	10	1.62	0.76	0.75	0.65	1.05	1.62
	20	1.26	0.77	0.66	0.81	0.83	1.26
	50	0.91	0.67	0.65	0.63	0.67	0.91
	100	0.73	0.59	0.55	0.58	0.57	0.73
2:30	5	1.04	1.09	0.87	0.71	1.23	1.74
	10	1.06	0.95	1.00	0.80	1.07	1.38
	20	0.96	0.96	1.00	0.83	0.93	1.16
	50	1.05	0.86	0.89	0.77	0.81	0.90
	100	0.86	0.84	0.82	0.71	0.72	0.81
5:30	5	1.09	0.93	0.91	0.86	1.10	1.23
	10	1.04	1.02	1.00	0.83	1.03	1.12
	20	1.00	1.00	1.05	0.79	0.98	1.00
	50	0.99	0.98	0.99	0.86	0.84	0.95
	100	0.92	0.96	0.95	0.75	0.88	0.84
corrected *e.f.*							
1:30	5	2.14	1.47	1.82	1.16	2.39	2.14
	10	2.22	1.29	1.36	1.12	1.84	2.22
	20	1.73	1.31	1.20	1.40	1.46	1.73
	50	1.25	1.14	1.18	1.09	1.18	1.25
	100	1.00	1.00	1.00	1.00	1.00	1.00
2:30	5	1.21	1.30	1.06	1.00	1.71	2.15
	10	1.23	1.13	1.22	1.13	1.49	1.70
	20	1.12	1.14	1.22	1.17	1.29	1.43
	50	1.22	1.02	1.09	1.08	1.13	1.11
	100	1.00	1.00	1.00	1.00	1.00	1.00
5:30	5	1.18	0.97	0.96	1.15	1.25	1.46
	10	1.13	1.06	1.05	1.11	1.17	1.33
	20	1.09	1.04	1.11	1.05	1.11	1.19
	50	1.08	1.02	1.04	1.15	0.95	1.13
	100	1.00	1.00	1.00	1.00	1.00	1.00

Depending on the solution pH cathodically formed AH_3, AH_2^- or AH^{2-} diffuses to the electrochemically inactive organic pigment, where electron transfer to the surface of the dispersed indigo can take place.

In alkaline solution the reduced dyestuff dissolves as leuco-indigo dianion and diffuses into the bulk solution. At lower pH the leuco-indigo mono-anion or the insoluble leuco-indigo acids are formed.

The regeneration of the oxidized form A^- leads to an increase in the cathodic current peak of lawsone and the shape of the CV curves change. The overall electrode reaction thus is similar to the E_{cat} mechanism, which describes a cathodic electron transfer reaction with catalytic regeneration of the electroactive species (equations (1) and (2)) [21]. In case of dispersed indigo a heterogeneous system is present and equation (2) is replaced by equation (3). The surface of the dispersed indigo then substitutes for the concentration of reactant X. Furthermore the anodic current peak decreases due to lowered concentrations of AH_3, AH_2^-, AH^{2-} present in the diffusion layer of the electrode.

In CV-experiments the electrochemical activity of a redox couple to undergo E_{cat} reactions with dispersed reducible components can be described in a more quantitative way by a calculation of so-called enhancement factors *e.f.*. The enhancement factors *e.f.* are calculated as $(I_p)_c/(\ I_p)_d$, with $(I_p)_c$ being the catalytic cathodic current peak in the presence of dispersed reducible dyestuff (indigo) and $(I_p)_d$ being the cathodic current peak of A^- alone [16].

To study these reactions, the experimental conditions have to be chosen in such a way that the amount of dispersed indigo present in the diffusion layer of the electrode is in sufficient depth to result in a detectable impact on the cathodic electron transfer reaction to A^- according to Table 6

CV-experiments of lawsone in the presence of dispersed indigo were performed in the pH range 7–13 at three different molar ratios of lawsone to indigo. The respective CV graphs at pH 7 and 13 are given in Figs. 2b and 3b. In Table 6 the calculated enhancement factors $(I_p)_c/(\ I_p)_d$ are given.

The enhancement factors decrease with higher scan rate as the influence of the catalytic reaction regenerating A^- decreases. Similar behavior was found with other redox couples for the indirect reduction of dispersed dyes [16].

At low scan rates of 5 and 10 mV s^{-1} *e.f.* above 1 are observed in the pH ranges of pH 7–8 and pH 11.5–13. This demonstrates the presence of an E_{cat} reaction between cathodically reduced A^- and dispersed indigo.

However independent on molar ratio of lawsone to indigo at high scan rates enhancement factors were found to be below unity e.g. at 100 mV s^{-1} and a molar ratio lawsone:indigo 1:30 *e.f.* were only 0.5–0.7.

Values of *e.f.* below unity could be due to sorption of lawsone molecules on the surface of the dispersed indigo, thereby lowering the electrochemically active concentration of A^- present in the diffusion layer of the cathode. In experiments, not given in detail in this paper, the cathodic current peak of an alkaline lawsone solution was compared to a lawsone/dispersed indigo containing solution and the same solution after removal of the dispersed indigo by filtration through a 0.2 μm membrane filter. The filtered solution showed a decreased cathodic current peak current which proves sorption of lawsone on the dispersed indigo.

The partial removal of electroactive lawsone molecules by sorption on indigo particles also can be observed from the data presented in Table 4 at the *e.f.* measured at high scan rates. At a molar ratio of 1:30 and scan rate of 100 mV s^{-1} *e.f.* values range from 0.55 to 0.73. *e.f.* increase with higher relative concentration of lawsone as the influence of sorption decreases. Thus *e.f.* at 100 mV s^{-1} increase to values of 0.75–0.96 at molar ratio lawsone:indigo of 5:30.

Taking into account the removal of electroactive species A^- by sorption on dispersed indigo and that the catalytic reaction (equation (3)) is almost decoupled at rapid scan rates of 100 mV s^{-1} a correction of the *e.f.* values can be done $(I_p)_c$ at scan rate of 100 mV s^{-1} to replace the corresponding value for $(I_p)_d$.

The corrected *e.f.* clearly demonstrate the E_{cat} reaction of reduced forms of 2-hydroxy-1,4-naphthoquinone with dispersed indigo, particularly in the pH range of 7–8 and 11.5–13. In this range of pH reduced forms of 2-hydroxy-1,4-naphthoquinone are AH_3, and AH^{2-} which can reduce dispersed indigo are thereby are reoxidized to A^-. At pH 9–10 low catalytic reaction between reduced lawsone presented as AH_2^- and dispersed indigo is observed. From the species distribution curves given in Fig. 5 it can be seen that in this region mainly AH_2^- is present, thus under these experimental conditions AH_2^- does not exhibit significant reduction of dispersed indigo.

Another effect which has to be considered when discussing the mechanism of indirect cathodic reduction of dispersed indigo is the pH dependent deprotonation and solubility of leuco-indigo.

As investigated by Etters, depending on the pH value of the solution, leuco-indigo is present as fully dissociated dianion (IV), mono-anion (III) or insoluble leuco-acid form (II) [17]. The leuco-indigo dianion which is present at pH values above 12 and the mono-anion which exists in the pH range of 9–13 both are water-soluble molecules. The leuco-acid form (II) which begins to form at pH values below 10 tends to precipitate from the aqueous solution (Scheme 1).

The experimental results thus can be combined to the overall reaction scheme given in Scheme 1.

In alkaline solution of pH 11.5–13 cathodic reduction of lawsone yields AH^{2-} which reduces dispersed indigo to the soluble ionic leuco-indigo forms (III) and (IV). In solutions near to the neutral pH such as pH 7–8 cathodic reduction yields AH_3 which reduces indigo to the insoluble leuco-acid form. The formation of an insoluble reaction product at the surface of the dispersed indigo particles disturbs further reduction and lower *e.f.* is observed.

3.2. Spectroelectrochemical experiments

The spectroelectrochemical experiments were used to monitor the electrochemical activity of 2-hydroxy-1,4-naphthoquinone during a CV scan.

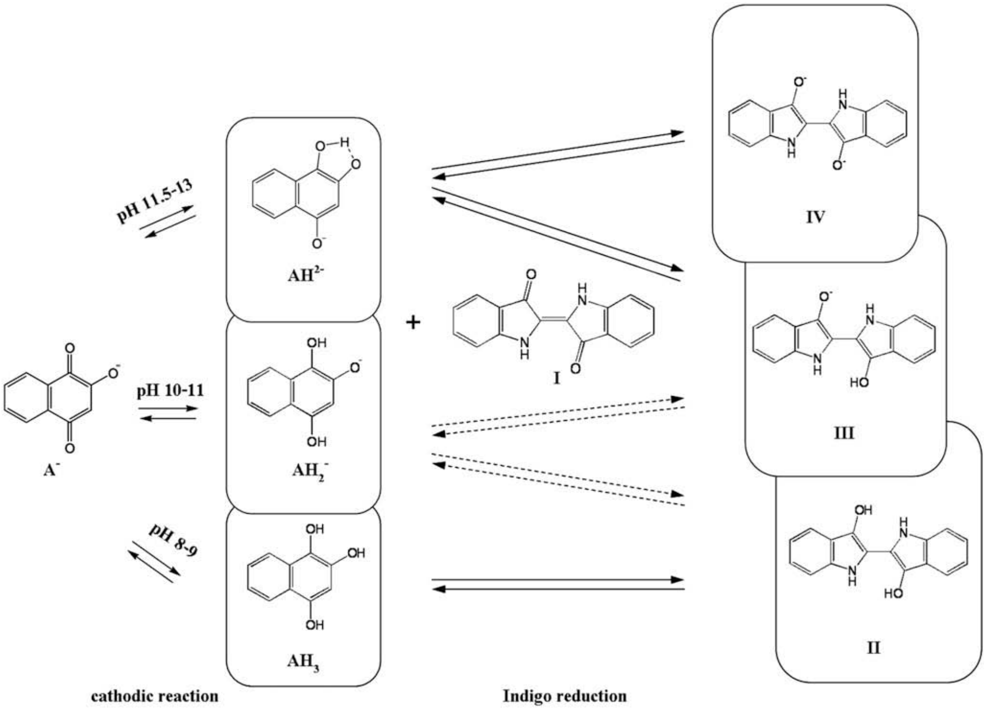
Besides indigo (C.I. Vat Blue 1), C.I. Vat Yellow 1 (Fig. 1c) was taken as probe because this dye shows a distinct change in color from yellow in its oxidized form to blue in reduced state. The indirect cathodic reduction of C.I. Vat Yellow 1 can be followed by two distinct absorption maxima near 600 nm and 640 nm [14]

The composition of solutions used for the experiments is given in Table 2.

The CV scans and simultaneously recorded absorbance spectra are given in Figs. 6–9. Figs. 6 and 7 show the reduction of 0.12 g L^{-1} C.I. Vat Blue 1 with 0.287 mM 2-hydroxy-1,4-naphthoquinone in 0.1 M NaOH. Figs. 8 and 9 show the reduction of 0.03 g L^{-1} C.I. Vat Yellow 1 with 0.287 mM 2-hydroxy-1,4-naphthoquinone in 0.1 M NaOH. The CV scans were recorded in the potential interval from −500 to −900 mV at a scan rate of 10 and 20 mV s^{-1}. While with C.I. Vat Yellow 1 successful indirect cathodic reduction of the dye was observed at scan rate of 20 mV s^{-1}, for indigo the scan rate was lowered to 10 mV s^{-1} to offer more time for possible indirect dyestuff reduction.

In presence of C.I. Vat Yellow 1, the spectra confirm the successful indirect reduction. The shape of the CV curves changes with the addition of the dyestuff and the cathodic current peak increases (Fig. 8). The reduction of C.I. Vat Yellow 1 also can be monitored by the increase in absorbance measured at 600 nm and 640 nm as shown in Fig. 9. Even during the reverse scan of the CV the reduction of the disperse dyestuff proceeds, as long as the concentrations of reduced 2-hydroxy-1,4-naphthoquinone remain sufficiently high to establish the negative redox potential required for dyestuff reduction. In the case of C.I. Vat Yellow 1 this potential is given in the literature with −520 to −550 mV (vs. Ag/AgCl, 3 M KCl) [18].

In the case of spectroelectrochemical experiments with addition of dispersed indigo the interpretation is more difficult. The cathodic peak current $(I_p)_c$ in the cell reaches the same magnitude as in absence of dispersed dye as can be seen in Fig. 6. However considering the already discussed decrease in $(I_p)_c$ due to lawsone adsorption on the dispersed dye the registered current already represents an increase in cathodic peak current. Interpretation of the photometric spectra is difficult because the yellow color of lawsone both oxidized and reduced form interferes with the yellow color of reduced indigo, which would be expected to be at 405 nm (Fig. 7). Thus in the case of indigo the spectroelectrochemical experiments do not yield further information with regard to the indirect cathodic dyestuff reduction.



Scheme 1. Reduction mechanism of lawsone and indigo [17,24].

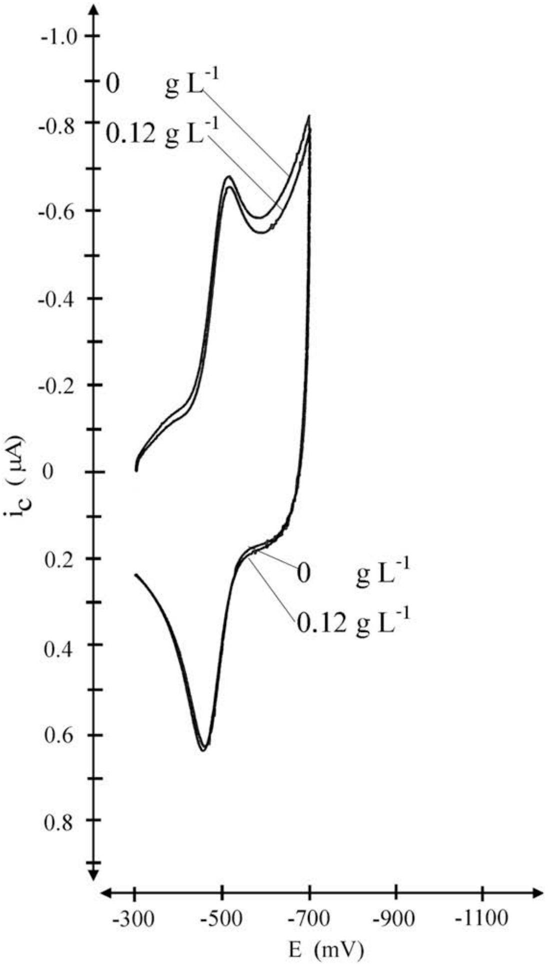


Fig. 6. CV Scan of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH and of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH in the presence of 0.12 g L^{-1} C.I. Vat Blue 1 in the potential interval from −500 mV to −900 mV (scan rate 10 mV s^{-1}). Absorption spectra see Fig. 7. (solution 8).

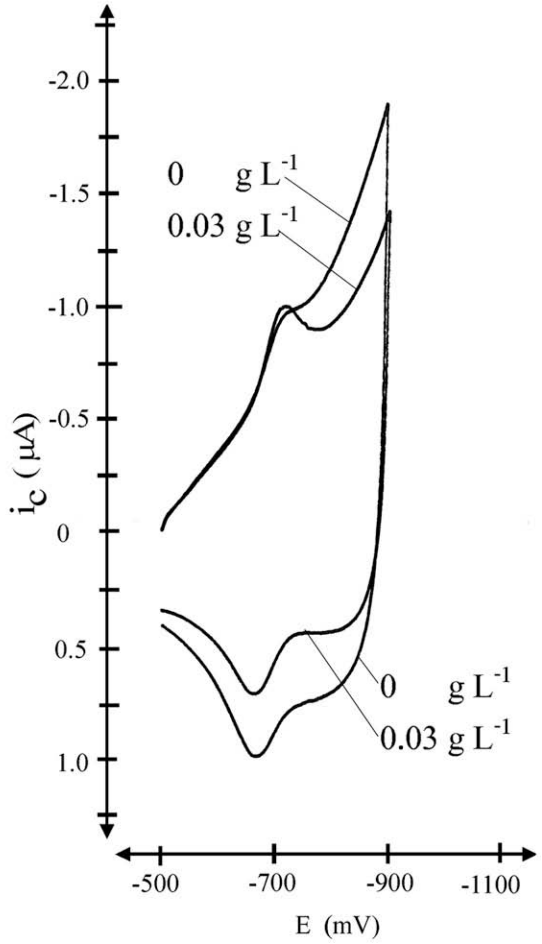


Fig. 8. CV Scan of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH and of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH in the presence of 0.03 g L^{-1} C.I. Vat Yellow 1 in the potential interval from −500 mV to −900 mV (scan rate 20 mV s^{-1}). Absorption spectra see Fig. 9. (solution 9).

4. Conclusions

In the dried leaves of the indigo plant part of the indigo is known to be present in oxidized form. Lawsone is assumed to be released from the glycoside as aglycone 1,2,4-trihydroxynaphthalene. This reduced form of lawsone is able to reduce indigo. Depending on the conditions applied redox reactions between reduced lawsone (1,2,4-trihydroxynaphthalene) and indigo are expected.

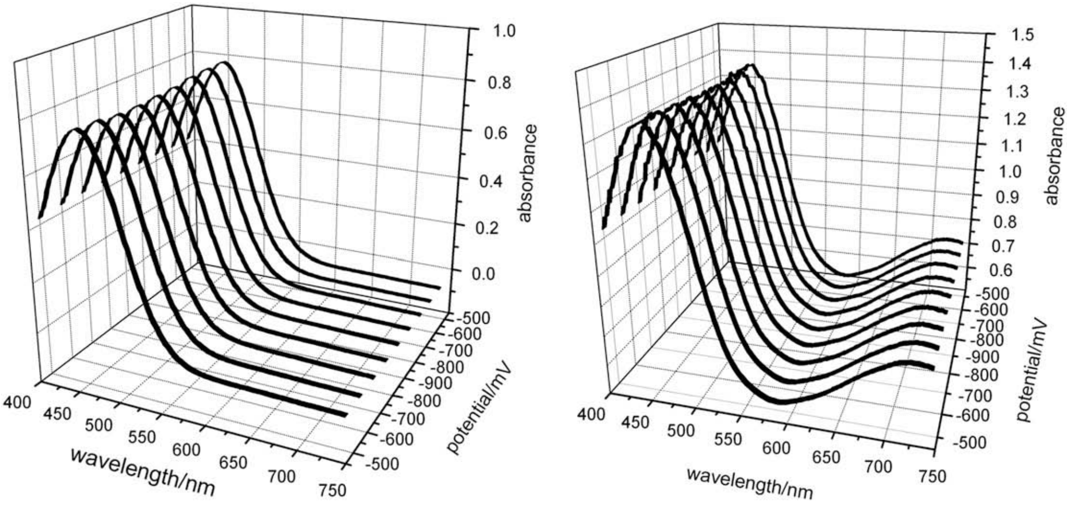


Fig. 7. Absorption spectra of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH and of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH in the presence of 0.12 g L^{-1} C.I. Vat Blue 1 in the potential interval from −500 mV to −900 mV(scan rate 10 mV s^{-1}). CV see Fig. 6. (solution 8).

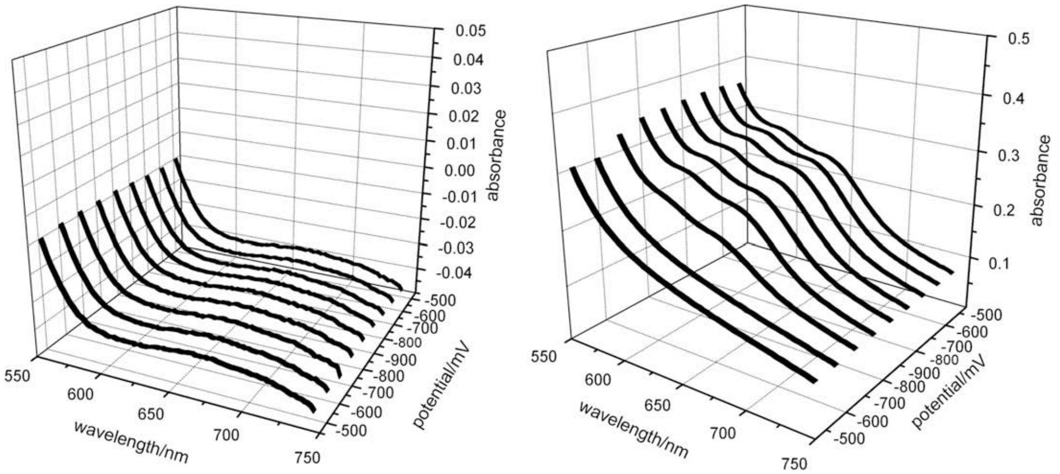


Fig. 9. Absorption spectra of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH and of 0.287 mM 2-hydroxy-1,4-naphthoquinone in aqueous 0.1 M NaOH in the presence of 0.03 g L^{-1} C.I. Vat Yellow 1 in the potential interval from −500 mV to −900 mV (scan rate 20 mV s^{-1}). CV see Fig. 8. (solution 9).

Cyclic Voltammetry can be applied to study the electrochemical behavior of 2-hydroxy-1,4-naphthoquinone in the presence of dispersed indigo. Under the investigated experimental conditions for 2-hydroxy-1,4-naphthoquinone a linear relation of $(I_p)_d$ vs. $v^{1/2}$ was observed which indicates a diffusion-controlled electrode reaction mechanism in the pH range of pH 7–13.

In the presence of dispersed indigo, the shape of CV curves changes as expected for a cathode reaction with catalytic regeneration of the electrochemically active species. The electrochemical activity was described by enhancement factors, *e.f.* which were calculated as $(I_p)_c/(I_p)_d$ with $(I_p)_c$ being the cathodic current peak in the presence of a dyestuff and $(I_p)_d$ the current peak of 2-hydroxy-1,4-naphthoquinone. Due to adsorption of lawsone on the dispersed indigo the cathodic current of lawsone was reduced in presence of dispersed indigo.

From the enhancement factors indirect cathodic dyestuff reduction was found to occur particularly at the pH range of 7–8 and 11.5–13. The calculated species distribution curves of the different deprotonated forms of 1,2,4-trihydroxynaphthalene showed that AH_3 and AH^{2-} are the main forms that act as mediator compounds. AH_3 is responsible for indirect dyestuff reduction in the pH range of 7–8 which then yields the insoluble leuco-acid form of indigo, while in more alkaline solution of pH 11.5–13 AH^{2-} reduces indigo to soluble ionic forms.

During application of Reng in the traditional hair dyeing 1,2,4-trihydroxynaphthalene is supposed to be released as aglycone from the corresponding glycoside. The results presented demonstrate that 1,2,4-trihydroxynaphthalene is able to reduce dispersed indigo at pH 7–8. The pathway presented for this pH range is shown in Scheme 1 and can be understood as a chemical model describing the reactions which occur during preparation of henna/indigo pastes. Thus a synergistic action between henna containing leaves and indigo containing plant material exists. At the end of the proposed redox reaction between AH_3 and oxidized indigo, henna (AH, A^-) and indigo in leuco-acid form will be formed in the paste as fibre attracting colorants.

Spectroelectrochemical experiments with C.I. Vat Yellow 1 proved the indirect cathodic dyestuff reduction, however in the case of dispersed indigo, the absorption spectra of lawsone hindered the successful monitoring of the dyestuff reduction.

Acknowledgements

Authors thank Dystar Textilfarben GmbH Frankfurt for supplying dyestuff. The authors wish to gratefully acknowledge the ÖAD (Österreichischer Austauschdienst) for the scholarship grant to Ms. Sunsanee Komboonchoo.

References

[1] Ali S, Hussain T, Nawaz R. Optimization of alkaline extraction of natural dye from Henna leaves and its dyeing on cotton by exhaust method. J Clean Prod 2009;17(1):61–6.
[2] Badri BM, Burkinshaw SM. Dyeing of wool and nylon 6.6 with henna and lawsone. Dyes Pigments 1993;22:15–25.
[3] Balfour-Paul J. Indigo. British Museum press, London. ISBN 0-71441-2550-4. p. 224–227.
[4] Cartwright-Jones C. Developing guidelines to Henna: a geographical approach. Essay for Masters degree. Kent, Ohio, USA: Kent State University, www.hennapage.com; August 2006.
[5] James KC, Spanoudi SP, Turner TD. The absorption of lawsone and henna by bleached wool felt. J Soc Cosmet Chem 1986;37(5):359–67.
[6] Cardon D. Natural dyes: sources, tradition, technology and science. Archetype Publications; 2007. p. 83–85.
[7] Schweppe H. Handbuch der Naturfarbstoffe: Vorkommen, Verwendung. Nachweis. Landsberg/Lech: ecomed; 1992.
[8] Božic M, Kokol V. Ecological alternatives to the reduction and oxidation processes in dyeing with vat and sulphur dyes. Dyes Pigments 2008;76: 299–309.
[9] Vuorema A, John P, Keskitalo M, Kulandainathan MA, Marken F. Electrochemical and sonoelectrochemical monitoring of indigo reduction by glucose. Dyes Pigments 2008;76:542–9.
[10] Blackburn RS, Harvey A. Green chemistry methods in sulphur dyeing: application of various reducing D-sugars and analysis of the importance of optimum redox potential. Environ Sci Technol 2004;38(14):4034–9.
[11] Nicholson SK, John P. The mechanism of bacterial indigo reduction. Appl Microbiol Biotechnol 2005;68(1):117–23.
[12] Bechtold T, Turcanu A, Schrott W. Dyeing behaviour of hydrogenated indigo in electrochemically reduced dyebaths. Color Technol 2008;124:324–30.
[13] Bechtold T, Gutmann R, Burtscher E, Bobleter O. Cyclic voltammetric study of anthraquinones-2-sulfonate in the presence of Acid Yellow 9. Electrochim Acta 1997;42:3483–7.
[14] Bechtold T, Turcanu A. Iron-complexes of bis(2-hydroxyethyl)-amino-compounds as mediators for the indirect reduction of dispersed vat dyes – cyclic voltammetry and spectroelectrochemistry experiments. J Electroanal Chem 2006;591:118–26.
[15] Bechtold T, Burtscher E, Turcanu A. Anthraquinones as mediators for the indirect cathodic reduction of dispersed organic dyestuffs. J Electroanal Chem 1999;465:80–7.
[16] Bechtold T, Burtscher E, Gmeiner D, Bobleter O. The redox-catalysed reduction of dispersed organic compounds. J Electroanal Chem 1991;306:169–83.
[17] Etters JN. Effect of dyebath pH on color yield in indigo dyeing of cotton denim yarn. Text Chem Color 1989;21(12):25–31.
[18] Bechtold T, Burtscher E, Amann A, Bobleter O. Alkali-stable iron complexes as mediators for the electrochemical reduction of dispersed organic dyestuffs. J Chem Soc Faraday Trans 1993;89(14):2451–6.
[19] Kulandainathan MA, Muthukumaran A, Patil K, Chavan RB. Potentiostatic studies on indirect electrochemical reduction of vat dyes. Dyes Pigments 2007;73:47–54.
[20] Vuorema A, John P, Jenkins ATA, Marken F. A rotating disc voltammetry study of the 1,8 dihydroxyanthraquinone mediated reduction of colloidal indigo. J Solid State Electrochem 2006;10:865–76.

[21] Pletcher D, Greef R, Peat R, Peter LM, Robinson J. Instrumental methods in electrochemistry. West Sussex: Horwood Publishing Limited; 2006. pp. 178–228.
[22] Saveant JM, Vianello E. Potential-sweep chronoamperometry: kinetic currents for first-order chemical reaction parallel to electron-transfer process (catalytic currents). Electrochim Acta 1965;10:905–20.
[23] Bechtold T, Burtscher E, Bobleter O. Konzentrationsbestimmung von Indigo in Ansatz- und Prozeßbädern. Textil Praxis Int 1992;47:44–9.
[24] Petrova SA, Kolodyazhny MV, Ksenzhek OS. Electrochemical properties of some naturally occurring quinones. J Electroanal Chem 1990;277:189–96.
[25] Martell AE, Motekaitis RJ. Determination and use of stability constants. 2nd ed. New York/Weinheim: VCH; 1992.
[26] Nicholson RS, Shain I. Theory of stationary electrode polarography, single scan and cyclic methods applied to reversible, irreversible and kinetic systems. Anal Chem 1964;36:706–23.
[27] Evans DH. Solution electron-transfer reactions in organic and organometallic electrochemistry. Chem Rev 1990;90:739–51.
[28] Grygar T, Hradil D, Kučková Š, Hradilová D. Electrochemical analysis of natural solid organic dyes pigments. J Solid State Electrochem 2003;7:706–13.
[29] Doménech-Carbó A, Doménech-Carbó MT, Saurí-Peris MC, Gimeno-Adelantado JV, Bosch-Reig F. Electrochemical identification of anthraquinones-based dyes in solid microsamples by square wave voltammetry using graphite/polyester composite electrode. Anal Bioanal Chem 2003;375:169–1175.
[30] Rieger PH. Electrochemistry. New Jersey: Prentice-Hall International, Inc.; 1987. pp. 216–219.
[31] Bailey SI, Ritchie IM. A cyclic voltammetric study of the aqueous electrochemistry of some quinones. Electrochim Acta 1985;30:3–12.
[32] Bond AM, Marken F, Hill E, Compton RG, Hügel H. The electrochemical reduction of indigo dissolved in organic solvents and as a solid mechanically attached to a basal plane pyrolytic graphite electrode immersed in aqueous electrolyte solution. J Chem Soc Perkin Trans 1997;2:1735–42.