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# Electrochemical determination of plant-derived leuco-indigo after chemical reduction by glucose

Anne Vuorema · Philip John · Marjo Keskitalo · Frank Marken

**Abstract** Electrochemical determination of redox active dye species is demonstrated in indigo samples contaminated with high levels of organic and inorganic impurities. The use of a hydrodynamic electrode system based on a vibrating probe (250 Hz, 200 μm lateral amplitude) allows time-independent diffusion controlled signals to be enhanced and reliable concentration data to be obtained under steady state conditions at relatively fast scan rates up to 4 V s$^{-1}$. In this work the indigo content of a complex plant-derived indigo sample (dye content typically 30%) is determined after indigo is reduced by addition of glucose in aqueous 0.2 M NaOH. The soluble leuco-indigo is measured by its oxidation response at a vibrating electrode. The vibrating electrode, which consisted of a laterally vibrating 500 μm diameter gold disc, is calibrated with $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl and employed for indigo determination at 55, 65, and 75 °C in 0.2 M NaOH. Determinations of the indigo content of 25 different samples of plant-derived indigo are compared with those obtained by conventional spectrophotometry. This comparison suggests a significant improvement by the electrochemical method, which appears to be less sensitive to impurities.

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## 1 Introduction

Indigo is one of the oldest dyes used by mankind. Until the commercialisation of chemically synthesised indigo at the end of the 19th century, indigo was produced from plants such as *Indigofera tinctoria* in the tropics, and *Isatis tinctoria* in temperate zones [1, 2]. In response to consumer demand for more environmental sustainability in all aspects of the textile industry, new methods are being developed to produce plant-derived indigo [3, 4]. Indigo yielding plants do not contain indigo, but instead accumulate indoxyl derivatives, indican and isatans [5], which are transformed into indigo during the extraction process. These precursors are released from indigogenic leaves when they are steeped in water forming free indoxyl on hydrolysis by plant or microbial enzymes. Subsequent alkali addition and oxygenation of the steep water converts the indoxyl to indigo and indigoid side-products [6].

The extraction process results in the inclusion of variable amounts of impurities in the final plant-derived indigo product [7]. For example the indigo produced from *Isatis tinctoria* has been reported to have a typical purity of 20–40% [8]. The purity of the indigo product directly affects the quality of dyeing as a less pure product will dye to a lighter shade and dye less evenly. Therefore indigo purity needs to be determined accurately.

Indigo is a vat dye, which in the presence of a suitable reducing agent becomes water soluble. Sodium dithionite has been for long the main industrial reducing agent but its consumption in the vat contributes to environmentally

harmful wastes from the dyeworks. There is interest in alternative chemical and electrochemical means of reduction [9–12]. Glucose has been investigated previously for sulphur dye reduction [13] and it is considered to be a possible alternative reducing agent for indigo [14].

In this report, the focus is on the analytical determination of indigo in impure samples. Indigo is routinely determined by spectrophotometry of solutions using a variety of organic solvents (see for example [2, 8, 15]). Recently it has been pointed out [6] that these methods are subject to anomalies due to the long recognized [16] association of indigo molecules in the solvents routinely employed. N-Methyl-2-pyrrolidone (or NMP) was introduced as a solvent [6] that avoided some of these anomalies, and this solvent is employed in the present paper.

Glucose is employed as a convenient "electrochemically irreversible" reagent with no voltammetric response interfering in the potential range of leuco-indigo oxidation. Excess glucose can be added to give complete conversion of indigo to leuco-indigo and then the voltammetric response for the oxidation to indigo is a direct measure of dye content. This indirect methodology is related to previous work on the voltammetric leuco-indigo determination [17–19] and similar to the direct voltammetric determination, it is relatively insensitive to colloidal and other impurities.

The use of a hydrodynamic electrode system allows diffusion controlled signals to be enhanced, and in particular vibrating electrodes [20–22] provide a high rate of mass transport and simple operation. In this report, we measure indigo purity of raw natural plant-derived indigo in aqueous media and without the need for any of the organic solvents that are required in conventional spectrophotometric methods. The true indigo content is shown to be systematically over-estimated by conventional spectrophotometry.

# 2 Experimental

## 2.1 Reagents

Chemical reagents such as indigo (Fluka), NaOH (Aldrich), $K_4Fe(CN)_6$, and D-(+)-glucose (Sigma), N-methylpyrrolidone (Sigma), butylated hydroxyl toluene (Sigma) were obtained commercially and used without further purification. Demineralised water was taken from an Elga purification system with at least 18 MΩ cm resistivity. Argon (BOC) gas was employed for de-aeration. Plant-derived indigo samples were obtained from extractions made in MTT Agrifood Research Finland in summer 2004. Indigo was extracted from leaves of Dyer's woad (Isatis tinctoria L.) by extraction in water, using principles described in [3]. Before extraction, the leaves were washed

three times with cold water. $Ca(OH)_2$ (50 g L$^{-1}$) was used to raise the alkalinity of the steep water to pH 11 to accelerate indigo formation [6]. Five batches of leaf samples were harvested from different fields and at different times, and five separate samples of this leaf material were extracted independently to obtain 25 different samples of indigo. These samples contain variable amounts of indigo and inorganic and organic impurities [6].

## 2.2 Instrumentation

For voltammetric studies a micro-Autolab II potentiostat system (EcoChemie, Netherlands) was employed with a Pt wire counter electrode and a saturated calomel (SCE) reference electrode (Radiometer, Copenhagen). A vibrating 500 μm diameter gold disc electrode acted as the working electrode. The electrode was embedded in a home-build PEEK probe and connected to a sonic probe (Braun, Oral-B Sonic Complete) to produce a sonic vibration with approximately 1000 μm amplitude at high power or ∼200 μm amplitude at low power (see Fig. 1). The vibration frequency of 250 Hz was determined optically with a photodiode connected to an oscilloscope. Experiments were conducted in a thermostated electrochemical cell (with a Haake B3 circulator) under constant de-aeration with high purity argon and at constant temperature.

## 2.3 Calibration procedure for the vibrating electrode with aqueous $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl

A solution of 2.4 mM $K_4Fe(CN)_6$ in 0.1 M KCl was thermostated to 55, 65, or 75 °C in a jacketed three electrode electrochemical cell. Cyclic voltammograms were recorded with a scan rate ranging from 0.1 to 5.0 V s$^{-1}$. In the absence of agitation, conventional voltammetric responses were obtained which allowed the diffusion coefficient to be assessed as a function of temperature. Next, the vibrating electrode was activated and voltammograms obtained at high/low power setting to assess the mass transport effect under these conditions as a function of temperature.
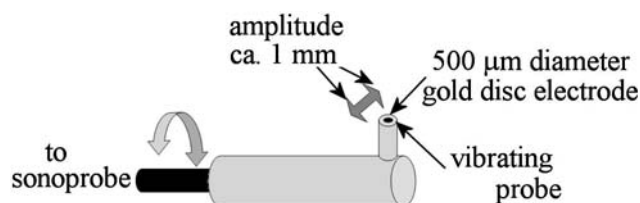


**Fig. 1** Schematic drawing of the sonoprobe design with a 500 μm diameter gold electrode embedded in a PEEK housing undergoing a 250 Hz lateral movement with ∼1,000 μm amplitude (high power) or ∼200 μm amplitude (low power)

### 2.4 Procedure for the sonovoltammetric determination of leuco-indigo in 0.2 M NaOH

An aqueous 0.2 M NaOH solution (100 mL) was thermostated to 55, 65, or 75 °C, and 10–50 mg (impure) indigo added after dispersion in a small volume of solution in a sonic bath. Next, glucose (600 mg to give a 33 mM solution in 100 mL of 0.2 M NaOH) was added under an atmosphere of argon, and voltammograms were recorded at regular time intervals. From the limiting currents observed for the oxidation of leuco-indigo the concentration of indigo was determined and at the endpoint of the reduction process the total amount of indigo in the sample was calculated.

### 2.5 Spectrophotometric indigo determination [6, 23]

Dried indigo samples were dissolved into 90% N-methyl-2-pyrrolidone (NMP, wet, with 0.1% butylated hydroxyl toluene and 3 mM citric acid). The colour of the resulting solution was blue. The absorbance of the solution was measured at a peak at 614 nm with a UV-160A UV-Visible spectrophotometer (Shimadzu). A calibration curve was obtained using synthetic indigo.

## 3 Results and discussion

### 3.1 Vibrating gold electrode calibration with the $Fe(CN)_6^{3-/4-}$ redox system

Hydrodynamic voltammetry provides time-independent steady-state responses, which, under conditions of mass transport control, are directly proportional to the bulk concentration. Hydrodynamic concentration monitoring techniques have been based on, for example, rotating disc voltammetry [12], jet voltammetry [24], microwave enhanced voltammetry [25], sonovoltammetry [26], and vibrating electrode systems [27]. Vibrating probe systems are simple devices with no special requirements in terms of cell geometry or sample pretreatment and without complex moving parts. Vibrating electrode systems have been employed previously in electroanalysis as hydrodynamic sensor [28] and for heavy metal stripping analysis [29].

Here, a 250 Hz laterally vibrating electrode (see Fig. 1) is employed for the measurement of the indigo content in plant-derived indigo samples. In order for this vibrating electrode system to be employed, the mass transport effects at the electrode surface have to be understood and calibrated (as a function of temperature). Therefore, initially mass transport conditions at the vibrating electrode were characterised with the well-known $Fe(CN)_6^{3-/4-}$ redox system. The peak or limiting currents for the one-

electron oxidation of ferrocyanide to ferricyanide (see Eq. 1) are measured at a range of different scan rates, for different vibrational amplitudes, and for different temperatures. Figure 2a shows typical voltammograms obtained for (i) high vibrational amplitude, (ii) low amplitude, and (iii) for no vibration (temperature 55 °C; scan rate 0.2 V s$^{-1}$).

$$Fe(CN)_6^{3-} + e^- \rightleftarrows Fe(CN)_6^{4-} \tag{1}$$
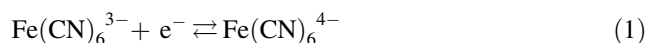
In the absence of electrode vibration, the measured anodic peak currents scale with the square root of the applied scan rate. From plots of the peak currents, $I_{peak}$, versus the square root of scan rate, $v$, the diffusion coefficients $D$ for $Fe(CN)_6^{4-}$ were calculated based on the appropriate Randles–Sevcik equation [30] (see Eq. 2).
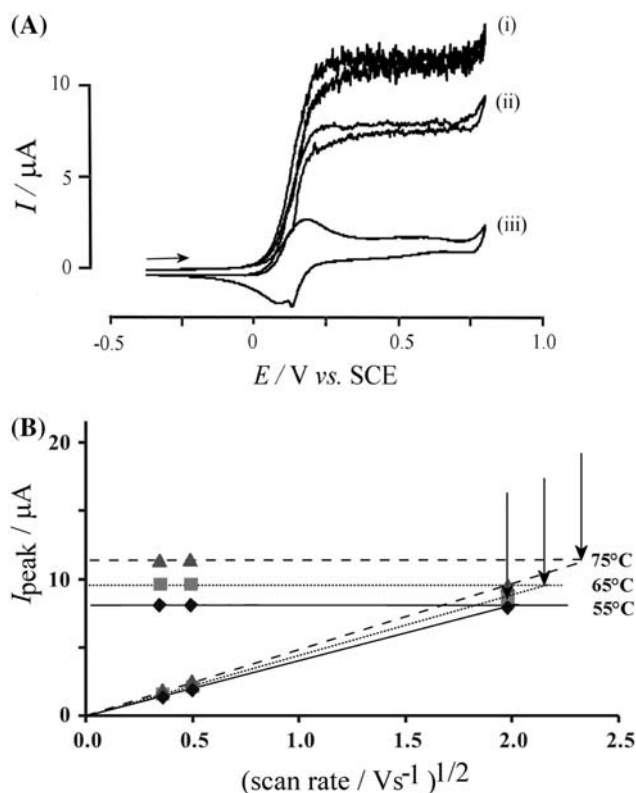


**Fig. 2** (a) Cyclic voltammograms (scan rate 0.2 V s$^{-1}$) for the oxidation of 2.4 mM $Fe(CN)_6^{3-/4-}$ in aqueous 0.1 M KCl at 55 °C at a 500 μm diameter gold disc electrode (i) with high vibrational amplitude, (ii) low vibrational amplitude, and (iii) no vibration. (b) Plot of peak/limiting current data versus the square root of scan rate for the oxidation of 2.4 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl at temperatures of 55, 65, and 75 °C. Extrapolated lines indicate peak and limiting currents at no and at low vibrational amplitude. An arrow indicates the transition point from steady state voltammetry at low scan rates to transient voltammetry at higher scan rates for each temperature

$$I_{peak} = 0.4463 \, nFAc \sqrt{\frac{nFDv}{RT}} \qquad (2)$$

In this equation, $n = 1$ is the number of electrons transferred per molecule diffusing to the electrode surface, $F$ is the Faraday constant, $A$ is the area of the electrode, $c$ is the bulk concentration of $Fe(CN)_6^{4-}$, $R$ is the gas constant, and $T$ is the absolute temperature.

Analysis of data in Fig. 2b based on Eq. 2 suggests approximate diffusion coefficients of $D^{55}_{Fe(CN)_6^{4-}} = 1.3 \times 10^{-9} \, m^2 s^{-1}$, $D^{65}_{Fe(CN)_6^{4-}} = 1.5 \times 10^{-9} \, m^2 s^{-1}$, and $D^{75}_{Fe(CN)_6^{4-}} = 1.7 \times 10^{-9} \, m^2 s^{-1}$ in good agreement with known literature values [31]. In the presence of vibration, the voltammetric responses at lower scan rates (below 4 $Vs^{-1}$) are considerably modified. Figure 2a shows typical mass transport controlled limiting currents and the plot in Fig. 2b demonstrates the transition from limiting (or steady state) to peak (or transient) current behaviour at a scan rate of typically 4–5 V s$^{-1}$. A simple model describing the mass transport controlled limiting current, $I_{lim}$, can be based on the average Nernst diffusion layer [32] (Eq. 3).

$$I_{lim} = \frac{nFDAc}{\delta} \qquad (3)$$

In this expression, the average diffusion layer thickness $\delta$ is employed to describe the thinning of the diffusion layer due to convection. Diffusion layer thickness values determined from the measured limiting currents are $\delta_{55} \approx \delta_{65} \approx \delta_{75} = 7.0 \, \mu m$ or $5.0 \, \mu m$ at low and high vibrational amplitude, respectively. The value for the diffusion layer thickness remains approximately constant with temperature due to the compensating effects of the increase in diffusion coefficient and the increase in mass transport limiting currents. From data in Fig. 2b it can be seen that a scan rate of less than approximately 4 V s$^{-1}$ will always result in analytically reliable steady state current responses. For the analysis of indigo in aqueous media higher scan rates are beneficial due to the precipitation of water-insoluble indigo onto the electrode and results obtained at 75 °C proved to be most reliable (*vide infra*).

## 3.2 The oxidation of leuco-indigo at a vibrating gold electrode

We recently reported [14] that glucose is effective in reducing indigo to leuco-indigo and that this process can be monitored voltammetrically. Here, the resulting methodology is further developed in order to provide a tool for the quantitative determination of leuco-indigo content or for the determination of indigo content in impure plant extracts. In alkaline media, indigo is reduced in the presence of glucose to give leuco-indigo in a two electron

process [33] (or oxidised in a two-electron process at more positive potentials; see Eq. 4).



$$(4)$$
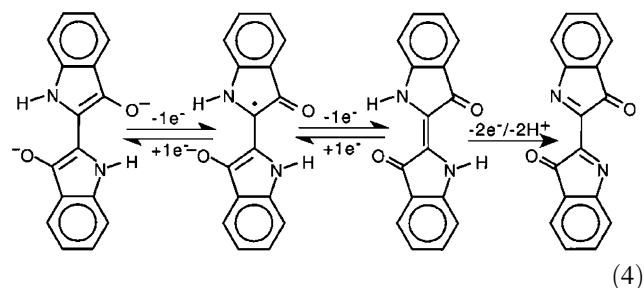
Indigo is highly water-insoluble and therefore only solid indigo directly immobilised at the electrode surface shows the reduction response [33]. However, leuco-indigo is water-soluble and the corresponding oxidation is observed in conventional solution phase studies [14]. Here a vibrating electrode system is introduced for this process to be observed under steady state conditions. Figure 3a shows a typical set of voltammograms obtained for the oxidation of 1.1 mM leuco-indigo in 0.2 M NaOH at 75 °C at a vibrating (low amplitude) 500 μm diameter gold electrode. The reduction of indigo to leuco-indigo was performed in situ with glucose and this has the advantage over dithionite as reductant in that glucose does not cause any interfering electrode process. Figure 3b shows a typical set of voltammograms obtained under similar conditions and for three different concentrations of leuco-indigo. Based on these data, Process 1 at a potential of −0.5 V vs. SCE is consistent with the mass transport controlled limiting current for the oxidation of leuco-indigo (Eq. 4, see also the grey zone in Fig. 3a). At more positive potentials a broad response is observed (Process 2) which is linked to the oxidation of glucose. Due to the formation of insoluble indigo at the electrode surface, blocking occurs with time and both the leuco-indigo oxidation (Process 1) as well as the glucose oxidation (Process 2) are affected. Figure 3a clearly demonstrates the effect of indigo blocking the electrode to increase as the scan rate decreases. After reversal of the scan direction a further peak response (cathodic) is observed at a potential of −0.9 V vs. SCE (see Process 3) consistent with the electrochemical "stripping" response of solid indigo adhering to the gold electrode surface. It can be concluded that in order to improve the analytical signal (Process 1) (i) the scan rate has to be high (up to 4 V s$^{-1}$, vide supra), (ii) the vibrational amplitude is preferred to be low (faster mass transport also leads to faster blocking), and (iii) the temperature has to be high (*vide infra*).

With the voltammetric technique, the chemical indigo reduction process (with glucose in 0.2 M NaOH) was monitored by measuring the mass transport controlled
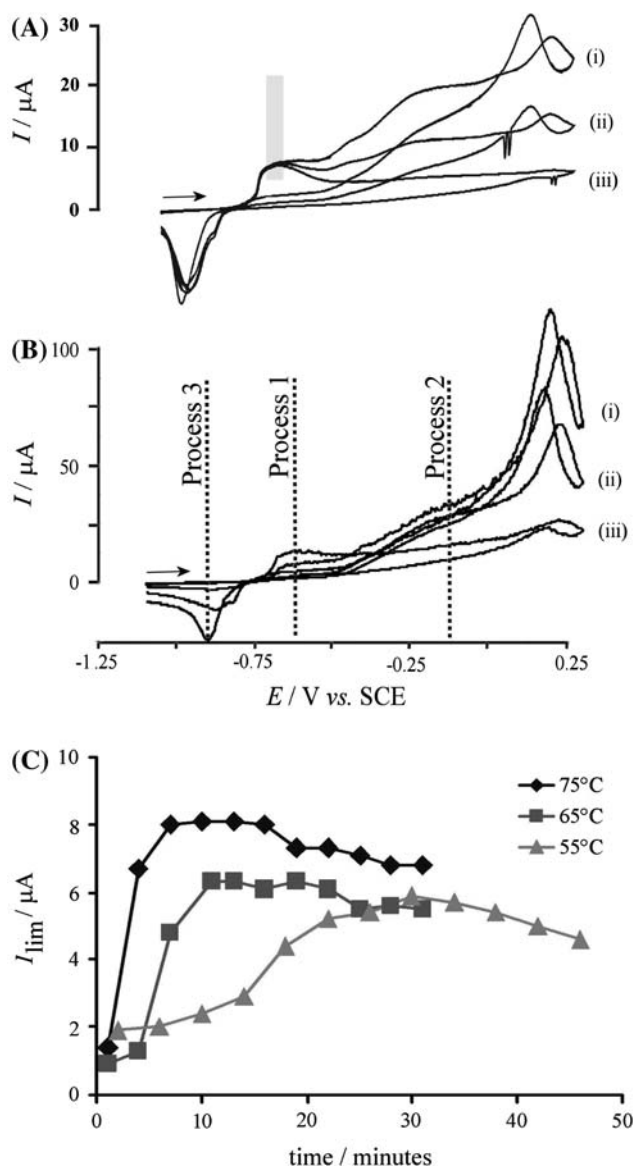
**Fig. 3** (**a**) Cyclic voltammograms (scan rate (i) 90, (ii) 60, and (iii) 50 mV s$^{-1}$) for the oxidation and re-reduction of 1.1 mM leuco-indigo (dissolved in 33 mM glucose in 0.2 M NaOH at 75 °C) at a vibrating (low amplitude) 500 μm diameter gold disc electrode. The grey zone indicates the steady state limiting current region. (**b**) Cyclic voltammograms (scan rate 200 mVs$^{-1}$) for the oxidation and re-reduction of (i) 0.34 mM, (ii) 1.1 mM, and (iii) 1.8 mM leuco-indigo in 0.2 M NaOH at 75 °C at a vibrating (low amplitude) 500 μm diameter gold disc electrode. (**c**) Plot of the limiting current versus time showing the formation of the leuco-indigo from 1.1 mM indigo (suspended in 33 mM glucose in 0.2 M NaOH) at temperatures of 55, 65, and 75 °C

limiting current (Process 1) for the oxidation of leuco-indigo every 3 min. Figure 3c shows the time dependence of the leuco-indigo concentration at three different temperatures: 55, 65, and 75 °C. The plateau reached at the highest limiting current shows that the reaction has gone to completion (simultaneously a colour change from dark

blue to yellow-brown occurred). The slow decrease of the plateau current at longer times (more than 15 min after the plateau is reached) can be attributed to a slow loss of the reducing power after glucose decomposition and possibly also traces of oxygen leaking into the cell. From the temperature effect on the slope of leuco-indigo formation versus time, it can be seen that the rate of indigo reduction is approximately doubled for each 10 °C increase in temperature (the approximate activation energy for the heterogeneous reduction of indigo is $E_A \approx 65$ kJ mol$^{-1}$). It would be possible to perform the indigo purity determination even faster at even higher temperatures, but the temperature effect on the seal in the PEEK mounting of the vibrating electrode prevented work at temperatures higher than ca. 75 °C.

Also the increase in the final limiting current (see Fig. 3c) is consistent with the faster rate of diffusion of the leuco-indigo molecules in a less viscous solution (at higher temperature). The diffusion coefficients for leuco-indigo can be estimated from the limiting currents (Process 1) based on the Fe(CN)$_6^{3-/4-}$ calibration data and Eq. 3 (vide supra). Assuming a two-electron oxidation, the estimated diffusion coefficients are $D_{leuco-indigo}^{55} = 0.9 \times 10^{-9}$ m$^2$s$^{-1}$, $D_{leuco-indigo}^{65} = 1.1 \times 10^{-9}$ m$^2$s$^{-1}$, and $D_{leuco-indigo}^{75} = 1.3 \times 10^{-9}$ m$^2$s$^{-1}$. These values are in good agreement with estimates for these diffusion coefficients based on the Wilke–Chang expression [34].

### 3.3 Determination of indigo content in plant-derived indigos at a vibrating gold electrode

The leuco-indigo and indigo determination method based on voltammetry at a vibrating electrode was used for samples of impure plant-derived indigo. A comparison
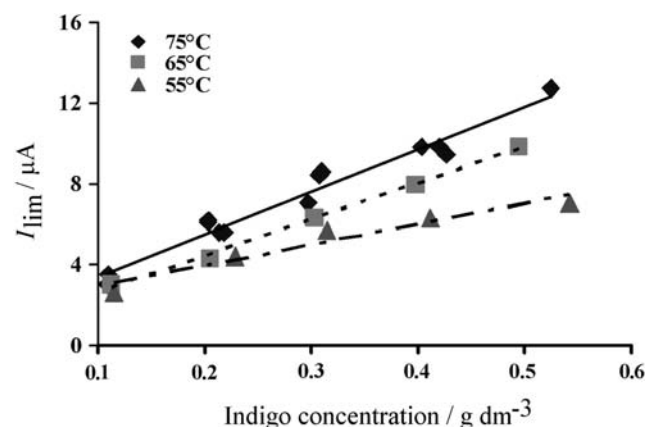


**Fig. 4** Calibration plots for the indigo determination at 55, 65 and 75 °C. The procedure was based on cyclic voltammograms (scan rate 200 mV s$^{-1}$) for the oxidation of leuco-indigo (formed from synthetic indigo) in 0.2 M NaOH after addition of 33 mM glucose and after an appropriate reaction time at a vibrating (low amplitude) 500 μm diameter gold disc electrode
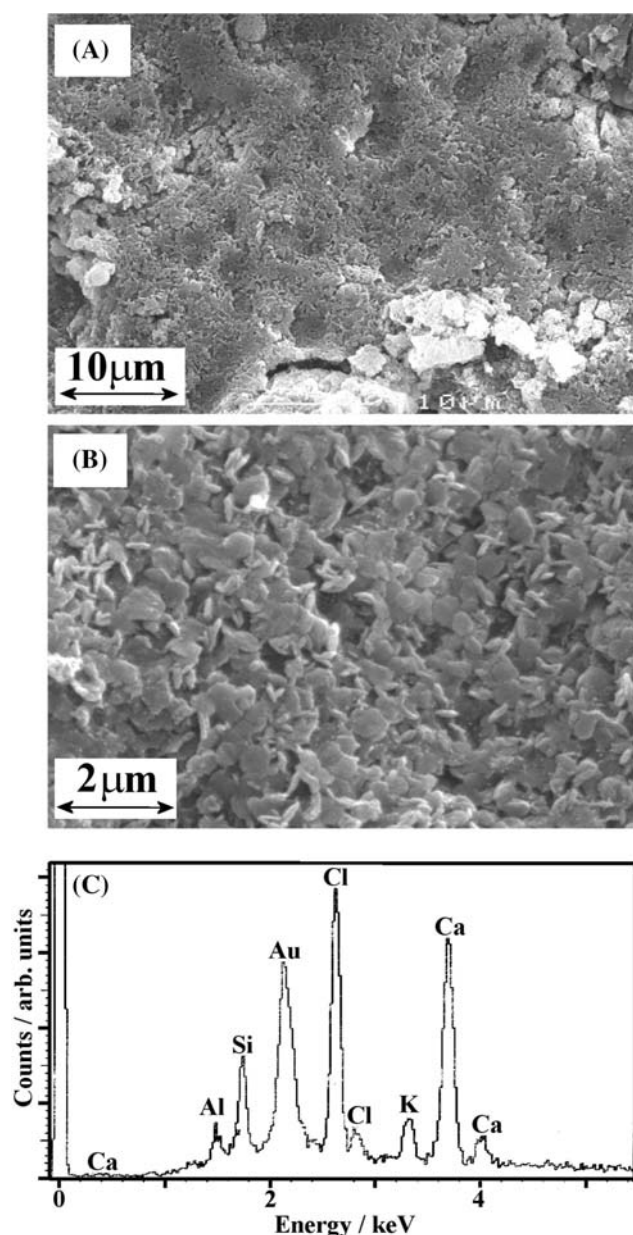
**Fig. 5** SEM data for plant derived indigo showing (**a**, **b**) secondary electron images for a typical sample at two different magnifications and (**c**) the energy dispersive atomic x-ray fluorescence analysis of the elemental composition. Samples were gold sputter coated before imaging

**Table 1** Determination of the indigo mass (mg) and purity (%) of natural indigo (sample mass, mg) by voltammetric (a) and spectrophotometric (b) methods

| Plant batch | Sub-sample | Sample mass (mg) | Indigo mass[a] (mg) | Purity[a] (%) | Mean purity[a] (%) | Purity[b] (%) | Mean purity[b] (%) |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 27.2 | 5.09 | 18.7 | 21 ± 5 | 37.4 | 41 ± 6 |
|   | 2 | 34.2 | 5.53 | 16.2 |        | 52.0 |        |
|   | 3 | 36.7 | 12.9 | 35.2 |        | 47.6 |        |
|   | 4 | 55.6 | 11.2 | 20.1 |        | 37.5 |        |
|   | 5 | 54.6 | 9.45 | 17.3 |        | 34.9 |        |
| 2 | 1 | 28.1 | 10.8 | 38.3 | 28 ± 5 | 27.0 | 36 ± 7 |
|   | 2 | 53.8 | 12.5 | 23.2 |        | 27.2 |        |
|   | 3 | 30.1 | 9.01 | 29.9 |        | 40.7 |        |
|   | 4 | 41.4 | 9.45 | 22.8 |        | 43.2 |        |
|   | 5 | 45.9 | 12.5 | 27.2 |        | 42.7 |        |
| 3 | 1 | 33.6 | 10.8 | 32.0 | 23 ± 5 | 55.9 | 42 ± 5 |
|   | 2 | 29.5 | 5.96 | 20.2 |        | 36.7 |        |
|   | 3 | 33.3 | 8.58 | 25.8 |        | 41.1 |        |
|   | 4 | 36.7 | 5.96 | 16.2 |        | 38.2 |        |
|   | 5 | 41.6 | 9.01 | 21.7 |        | 38.5 |        |
| 4 | 1 | 27.7 | 6.40 | 23.1 | 27 ± 4 | 39.8 | 39 ± 3 |
|   | 2 | 35.9 | 10.3 | 28.7 |        | 33.7 |        |
|   | 3 | 34.8 | 12.1 | 34.7 |        | 39.3 |        |
|   | 4 | 34.5 | 8.58 | 24.9 |        | 46.1 |        |
|   | 5 | 53.9 | 12.9 | 24.0 |        | 38.7 |        |
| 5 | 1 | 23.6 | 8.58 | 36.3 | 27 ± 8 | 23.0 | 32 ± 5 |
|   | 2 | 33.1 | 5.96 | 18.0 |        | 32.7 |        |
|   | 3 | 36.1 | 13.4 | 37.0 |        | 42.9 |        |
|   | 4 | 46.5 | 6.83 | 14.7 |        | 29.0 |        |
|   | 5 | 67.1 | 19.5 | 29.0 |        | 34.5 |        |

Mean indigo purities (%) are with standard deviation

[a] Determined voltammetrically (see experimental)

[b] Determined spectrophotometrically (see experimental)

Figure 5 shows typical SEM images and elemental analysis for the plant-derived indigo samples. At high magnification well-defined nanocrystalline indigo platelets are seen (see Fig. 5b) in between areas with inorganic impurities. It has been shown previously [6] by SEM-EDAX that impurities in plant-derived indigo are non-uniformly dispersed, and that soil and plant-derived particulates are responsible for persistent impurities in the final product.

The leaf material from which the indigo is extracted is not homogeneous and there are many parameters which affect the indigo yield as well as the dye quality. For example, the field conditions preceding harvest can significantly alter the indigo yield [35] and thus the impurity level [6]

Table 1 summarises the results from the voltammetric and spectrophotometric analyses of 25 different samples of plant-derived indigo. The voltammetric analyses consistently indicate a purity less than that indicated by the

shows that the voltammetric method produces reliable results even when considerable amounts of ill-defined impurities are present. The results are compared to those obtained by the more conventional spectrometry.

Initially, a calibration plot (limiting current versus indigo content) was obtained based on pure synthetic indigo samples. Figure 4 shows the linear plots for 55, 65, and 75 °C. Sufficiently reproducible data were obtained and the 75 °C conditions were selected for the determination of indigo in plant-derived indigo samples.
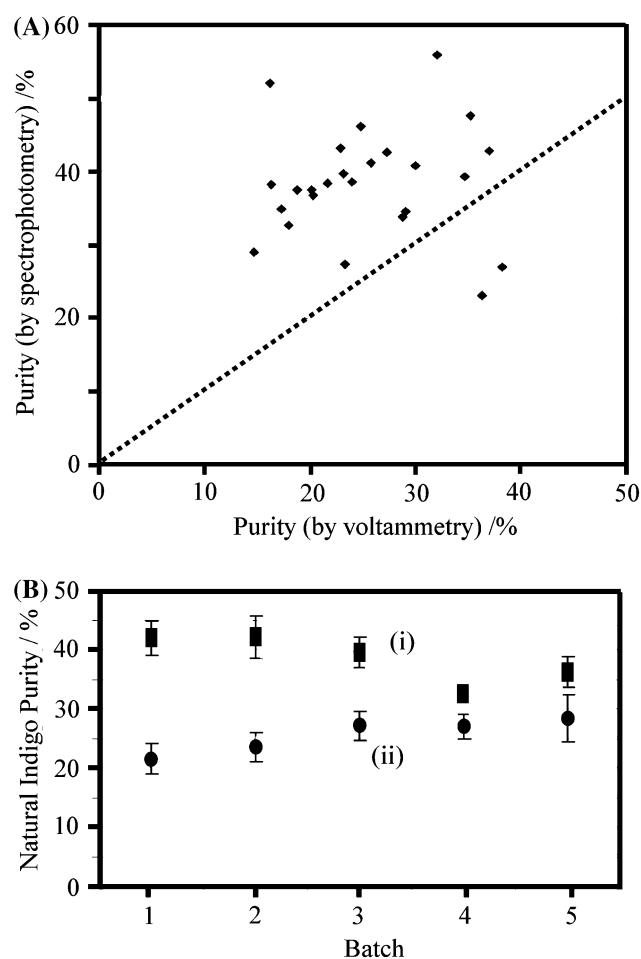
Fig. 6 (a) Plot of the purity determined by voltammetry versus the purity determined by UV/Vis spectrophotometry. The dashed line corresponds to matching purity levels. (b) Histogram of the mean purity (i, rectangles) determined by spectrophotometry and (ii, circles) determined by voltammetry for each batch of plant material

spectrophotometric method. The latter method is known to be affected by the state of aggregation of the indigo in the solvent [6], and it has been suggested [36] that impurities in natural indigo reduce the size of indigo aggregates in solvents. Thus when pure synthetic indigo (larger aggregates) is used as a standard, the determinations of the impure natural indigo samples (finer aggregates) will overestimate the real indigo content. On this basis we conclude that the voltammetric determinations of the indigo content of the natural indigo samples are the more accurate, and that the purity of the samples was typically 20–30%. A plot of the purity data obtained with spectrophotometry versus purity data obtained with voltammetry is shown in Fig. 6a. Only two samples show an increased content when measured voltammetrically compared to spectrophotometrically, and the deviation between the two methods is generally considerable. In support of our conclusion that the impurities are responsible for an

overestimation by the spectrophotometric method, we note that when the impurity content is higher, the discrepancy between the two methods is greater, and the purer the samples, the closer are the values obtained with the two methods (Batch 4 in Fig. 6b) Our present results suggest that the voltammetric method is experimentally reproducible and more reliable than the spectrophotometric method due to the complete dissolution of leuco-indigo and the reliable steady state voltammetric response at the vibrating electrode. Further work will be needed to determine the effect of indigoid impurities.

## 4 Conclusions

A novel voltammetric determination method for indigo and/or leuco-indigo in impure plant derived samples has been developed employing a vibrating (250 Hz) 500 μm diameter gold disc electrode. Hydrodynamic voltammetry at a temperature of 75 °C has been used to provide a well defined steady state current response for the quantitative determination of impure indigo samples. The reductive dissolution of indigo driven by glucose under alkaline conditions and at an elevated temperature of 75 °C allows leuco-indigo oxidation to be exploited and the limiting current to be used as a reliable measure of indigo content without faradaic interference from glucose.

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