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# Rapid Electrochemical Analysis of Plant and Synthetic Indigo Dyes

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Plant indigo was a very important dye historically, but it was later replaced by synthetic indigo. In recent years, plant indigo has been recognized for its artistic value and reduced environmental impact. However, the price of plant indigo is much higher than that of synthetic indigo. In this work, we propose an electrochemical method for quickly distinguishing plant indigo from synthetic indigo. This method uses an electrochemical fingerprint technique. Using the different fingerprints of plant indigo and synthetic indigo, corresponding indexes were developed for identification. In addition, this technology can also distinguish plant indigo and synthetic indigo mixed in different ratios. This rapid technology requires no large-scale instruments and has great potential for dye analysis.

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**Keywords:** Electroanalysis; Indigo dyes; Fast identification; Fingerprints; Differential pulse voltammetry

## 1. INTRODUCTION

Dyes have been used for much of human history, and plant dyes were used far earlier than synthetic dyes. However, plant dyes have some disadvantages, such as a poor color fastness and limited variety. In the 1860s, synthetic dyes gradually replaced plant dyes because of their greater color range, brightness, washability and sunlight resistance [1,2]. In recent years, and due to the increased awareness of environmental impacts and the importance of human health, plant dyes with nontoxic, harmless, pollution-free, elegant and beautiful color features have once again attracted attention. Related research has led to the development of new planting, production, processing and dyeing technologies [3,4].

Indigo is a natural blue dye with a long history. Indigo exists in the stems or leaves of bluegrass and has the chemical formula $C_{16}H_{10}N_2O_2$. Indigo was the first dye used by human beings. Before the commercialization of synthetic indigo in the 21st century, plant indigo was extracted from various plants, including *Baphicacanthus cusia (Nees) Bremek, Indigofera tinctoria L, and Isatis tinctoria L* in tropical and temperate areas [5,6]. Plants that can be used for the preparation of indigo do not contain indigo but accumulate cardanol derivatives and sugar [7]. These molecules can be transformed into indigo in the extraction process.

Plant indigo and synthetic indigo have the same dyeing effects and similar chemical compositions, but there is a significant difference in their prices. In recent years, increasing attention has been given to the adverse impacts of synthetic dyes on the environment, ecology and human health, which arise during the processes of production, use and waste disposal [8–10]. Because they are natural, green, safe, biodegradable, and exhibit other advantages, plant dyes are again favored by users. However, due to the price difference between plant and synthetic indigo, synthetic indigo has been mixed into plant indigo to generate high profits. Therefore, it is necessary to develop a way to quickly identify synthetic indigo and plant indigo.

Physical identification is the most commonly used method. Synthetic indigo dye is produced industrially. The product is pure, dark and bright. Regardless of its form, either powder or liquid, the dispersion is uniform, and there is no obvious smell. Because of its natural source and the preparation technology for plant indigo, its color is slightly dark and gray blue [11,12]. At the same time, powder particles of plant indigo easily agglomerate, and in liquid form, the dye easily clots. The dye has a slight smell of grass. The production method is convenient and fast and does not need testing equipment or operations, but it does require considerable industrial experience. Oxidative coloration has been used as a chemical identification method. Indigo pigment can be oxidized by concentrated nitric acid and then displays a yellow color, but indirubin is not oxidized by concentrated nitric acid. Because plant indigo contains indirubin pigment, nitric acid can be used to distinguish indigo from indirubin as a result of the differences in their antioxidant capabilities [13,14]. In the process of identification, the characteristic color is directly related to the content and uniformity of indirubin in plant indigo. If the purity of the plant indigo is relatively high, then the color change is not obvious and it is difficult to characterize the sample. Because of these problems, development of a fast and simple recognition method is required to overcome these shortcomings. The electrochemical fingerprinting we investigated is a technique for the detection of small molecules with electrochemical activity in the liquid phase. This technique has been successfully applied to the detection of soft drinks [15], wine [16], soy sauce [17], tea [18] and vinegar [19]. In this work, we considered the different properties of synthetic indigo and plant indigo and developed a technology based on electrochemical fingerprints that can be used to identify the dyes. In addition, this technology can also be used to distinguish different proportions of plant indigo and synthetic indigo. Therefore, this technology has great potential in the dye and textile industry.

## 2. MATERIALS AND METHODS

Plant indigo and synthetic indigo were supplied by Nanjing Botanic Garden. All chemicals were of analytical grade and used without purification. All electrochemical fingerprint determinations were conducted using a CHI760 electrochemical workstation. A commercial glassy carbon electrode (GCE), a Ag/AgCl electrode and a Pt electrode were used as the working electrode, reference electrode and counter electrode, respectively.

Differential pulse voltammetry (DPV) was used for fingerprint recording. The GCE was first polished using an alumina slurry after a water wash. Then, the three-electrode system was inserted into 10 mL of a solution of either plant indigo or synthetic indigo (dissolved in a 0.1 M acetic acid buffer solution). DPV was conducted in the range 0–1.4 V, with a pulse amplitude of 50 mV, a pulse width of 0.05 s and a pulse period of 0.5 s.

## 3. RESULTS AND DISCUSSION

Figure 1A shows digital photos of plant indigo and synthetic indigo. It can be seen that compared to synthetic indigo, the color of plant indigo is deeper. However, there is a strong relationship between the color of plant indigo and the production process used, so color cannot be the standard for distinguishing whether a sample is synthetic or comes from plants. Furthermore, it is difficult to use color to identify mixed samples with different proportions of plant indigo and synthetic indigo. Figure 1B shows digital photos of indigo solutions with various ratios of natural and synthetic indigo.

Electroanalysis is a widely studied analytical technique for the detection and identification of analytes *in vivo* or *in vitro* [20–25]. Figure 2 shows the DPV curves of plant indigo and synthetic indigo. Plant indigo has three distinct oxidation peaks at 0.4V, 0.83 V and 1.08 V (Figure 2A). These oxidation peaks may be due to the electrochemical oxidation of indigo molecules or the oxidation of electrochemically active molecules in the plant extract. Figure 2B shows the DPV curves of synthetic indigo. There are only two obvious electrochemical oxidation peaks at 0.53 V and 1.09 V. Therefore, the oxidation peak at 1.09 V can be used as a characteristic oxidation peak for indigo molecules. However, the shift of the oxidation peak between synthetic and plant indigo may be due to the slight difference in their chemical structures. Another possible explanation for the shift of the oxidation peak is that there are other substances in the plant indigo sample that accelerate the transfer of electrons on the electrode surface, thus reducing the overpotential [26]. These compounds could be flavonols [27], phenolic acids [28], procyanidins [29], alkaloids [30] and pigments [31]. We did not conduct a cathodic scan because it provides much less information than an anodic scan. Moreover, dissolved oxygen can produce a peak if we extend the potential window. In addition, we recorded the fingerprints of each sample three times. The fingerprint scans exhibited very good reproducibility with no obvious peak shifts or current changes.

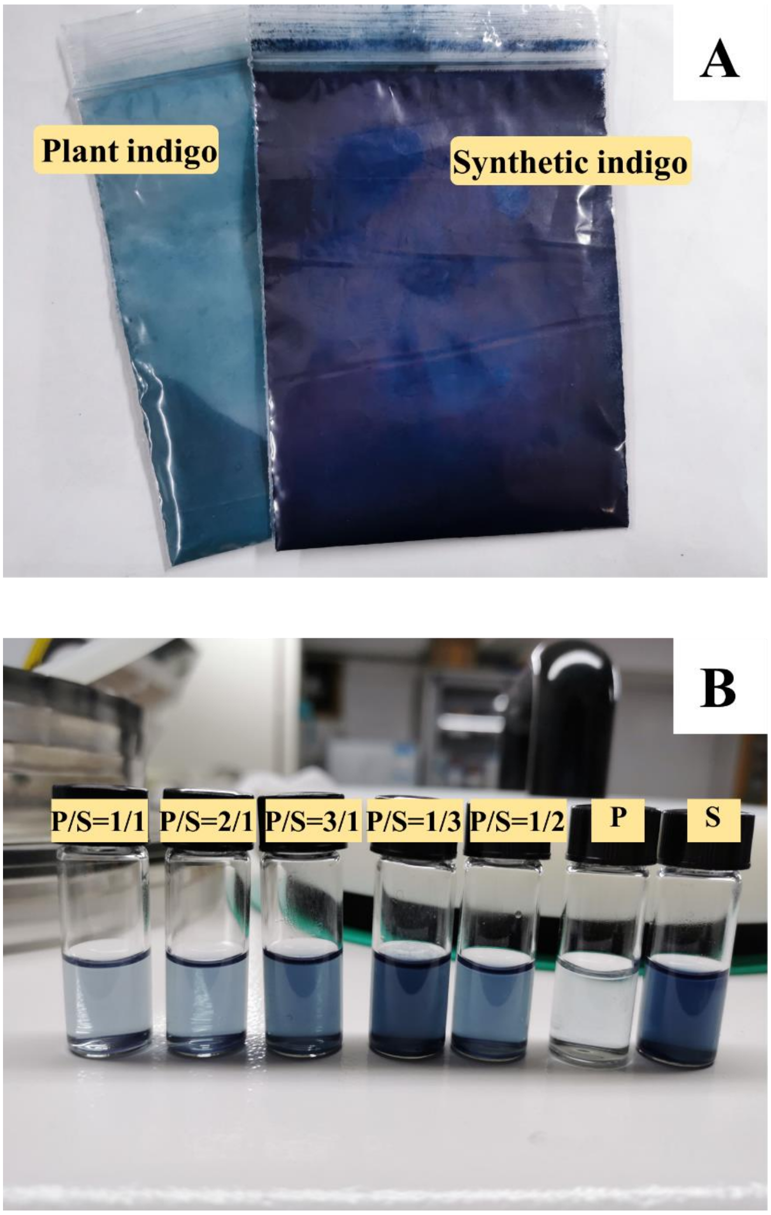


**Figure 1.** (A) Digital photos of plant indigo and synthetic indigo. (B) Digital photos of plant indigo, a synthetic indigo solution and a mixed solution with the same concentrations.

Figure 3 shows the results of the DPV studies of different proportions of plant indigo and synthetic indigo. The DPV scan of the mixed indigo combines the characteristics of plant indigo and synthetic indigo. The oxidation peak at 0.4 V is still very obvious, regardless of the ratio of plant indigo and synthetic indigo. We did not observe another oxidation peak at 0.53 V, which proves that the oxidation processes can be completed simultaneously in the mixed sample. The electrochemical oxidation peak of plant indigo at 0.83 V was also obvious in all samples. This peak increased significantly with the increasing proportion of plant indigo, so it is a very good indicator for the proportion study. In contrast, the oxidation peak of plant indigo at 1.08 V was not so obvious when the mixture contained a small plant indigo content. This peak could only be observed when the ratio of plant indigo to synthetic indigo was greater than or equal to 2. Therefore, this oxidation peak can also be used as an indicator to prove whether too much synthetic indigo has been added to plant indigo. This method overcomes the drawbacks of the chemical identification method based on oxidation coloration [32].

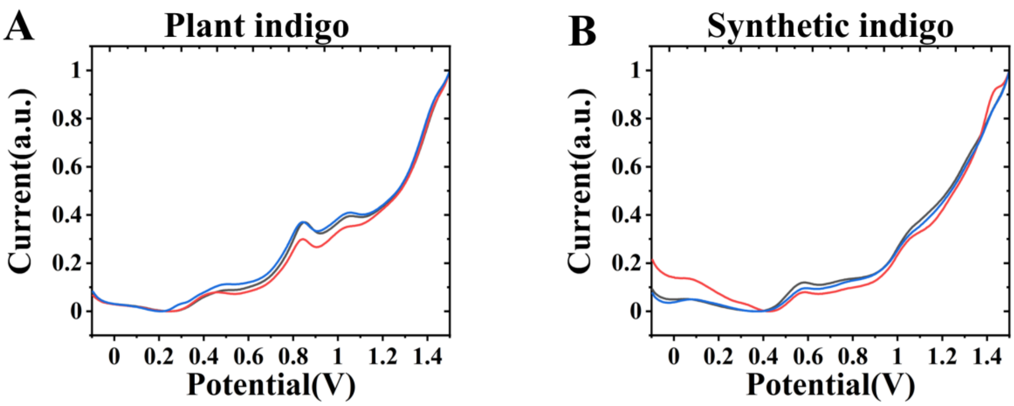


**Figure 2.** DPV curves of (A) plant indigo and (B) synthetic indigo.

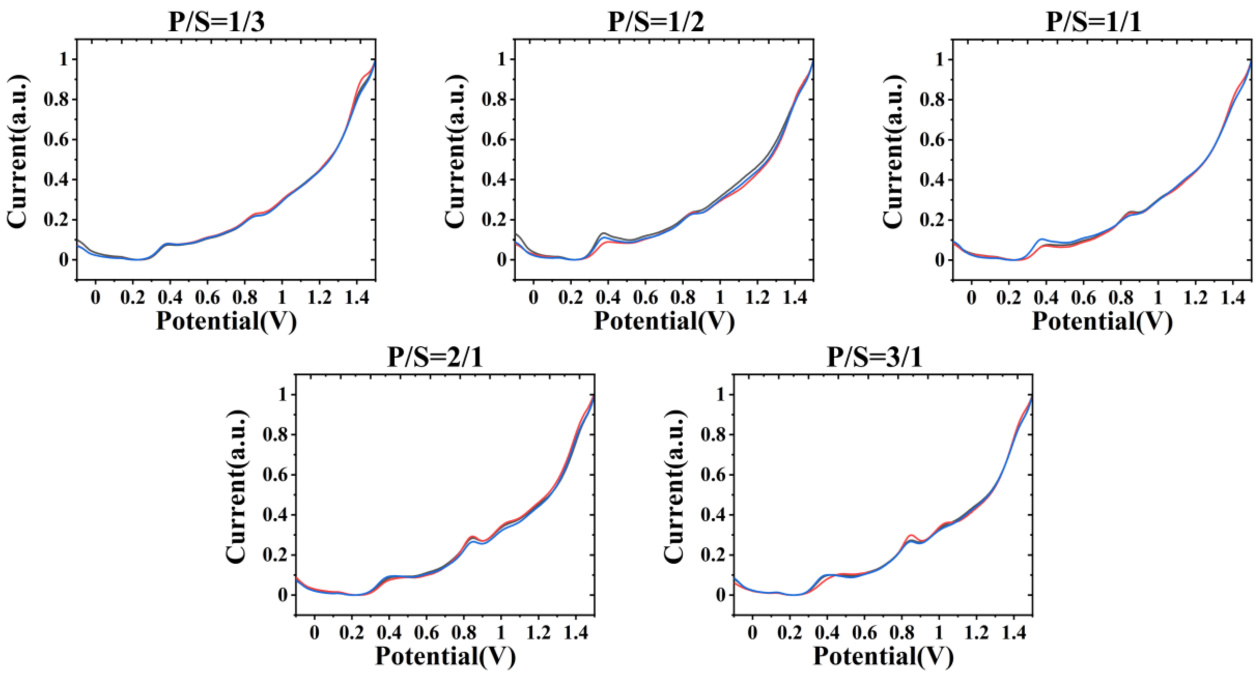


**Figure 3.** DPV curves of mixtures of plant indigo and synthetic indigo at different ratios.

We further calculated the ratios of the peak values of mixed samples. As shown in Figure 4, the current ratios of the 0.83 V and 1.08 V oxidation peaks are 0.655, 0.713, 0.742, 0.802 and 0.837, corresponding to ratios of plant indigo to synthetic indigo of 1:3, 1:2, 1:1, 2:1 and 3:1, respectively. The ratio of the currents for the peaks at 0.83 V and 1.08 V can also be used for characterizing the mixed sample.

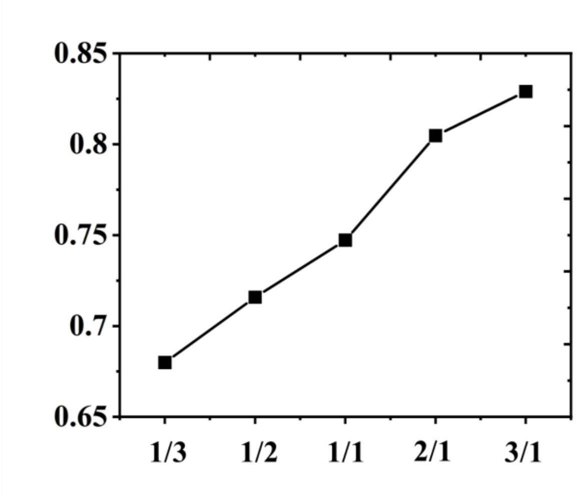


**Figure 4.** Current ratios of the 0.83 V and 1.08 V peaks for ratios of plant indigo to synthetic indigo of 1:1, 1:2, 1:3, 2:1 and 3:1.

According to the above results, electrochemical fingerprinting is a very fast way to distinguish plant indigo from synthetic indigo. We further explored the detection limit of this technology. Figure 5 shows the DPV curve of indigo at different concentrations. We can see that the electrochemical signal gradually decreased with decreasing concentration, but there was still a signal at a concentration of 0.125 g/L indigo. Therefore, this method can detect very low concentration samples, so it has a very broad application range.

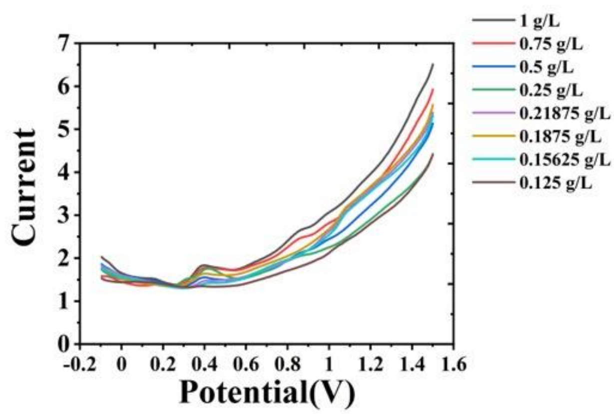


**Figure 5.** DPV curves of plant indigo at concentrations of 1 g/L, 0.75 g/L, 0.5 g/L, 0.25 g/L, 0.21875 g/L, 0.1875 g/L, 0.15625 g/L and 0.125 g/L.

## 4. CONCLUSION

In this work, we propose a technique based on an electrochemical fingerprint for the identification of plant indigo and synthetic indigo. There are three distinct oxidation peaks in the anodic scan of indigo because there are many electrochemically active substances in indigo. In contrast, synthetic indigo has only two oxidation peaks because it contains a single component. Based on the potentials and current ratios of the different oxidation peaks, we can use this technique to identify different proportions of mixed samples. In view of the huge price difference between plant indigo and synthetic indigo, this technology has great potential for use in dye analysis.

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