Performance enhancement of betanin solar cells co-sensitized with indigo and lawsone: A Comparative Study

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Extraction and Purification of Betanin, Indigo and Lawsone and its effect on photovoltaic performance in the DSSC

1. EXTRACTION AND PURIFICATION OF BETANIN

The procedure described by Sengupta et al. 2015 was modified and followed in all further experiments. For the extraction of betanin, 20 g of beetroot was sliced up and pulverized using a blender. The beetroot pulp was heated in the hot air oven at 50 °C for 3 h and mixed with ethanol for further use.

The purity of the pigments extracted was calculated as follows:

\[
\% \epsilon = \frac{m'}{m} \times 100 \quad (1)
\]

where \( m' \) is the assumed mass of pure complex, \( m \) is the measured mass of the actual sample and \( \% \epsilon \) is the percentage purity of the pigments extracted. The mass is calculated by measuring the absorbance and determining the concentration from the standard calibration curve of betanin at 535 nm. Ethanol was used as the solvent. Based on the purity of the extracted betanin, the factors: solvent, time and temperature of extraction were optimized. First, the purity of betanin extracted was checked for three of the most suitable solvents for betanin: ethanol, deionized water, and acetone. Ethanol was found to give the best results as mentioned in literature 1, and observed in Table S1(a).

| Solvent  | %Purity |
|----------|---------|
| DI Water | 27.92   |
| Ethanol  | 33.50   |
| Acetone  | 22.84   |

Table S1 (a): Optimization of factors for extraction of betanin: (a) solvent (b) time of extraction and the (c) temperature of extraction

Next, betanin was extracted using ethanol at an extraction temperature of 40 °C and the time was varied from 1-5 h, and the purity of the extracted betanin was checked at each condition. Best results were obtained at an extraction time of 3 h, as observed in Table S1 (b). Finally, the purity of betanin extracted using ethanol was checked for various extraction temperatures (time was kept constant at 3 h) (Table S1(c)). Best results were obtained at an extraction temperature of 50 °C for 3 h giving a purity of 59%. The time of extraction was also verified again to be 3 h at a 50 °C temperature of extraction.
Aggregation of unwanted compounds is detrimental to the performance of the device. In order to ensure that the impurities present in the extracted pigments do not affect the performance of the device, the dyes were purified using column chromatography. Betanin was purified by column chromatography using methods described in Gonalves et al. 2012. The methodology followed is shown in Chart S1.

**CHART S1** Column chromatography for purification of betanin from the raw extract

1. Prepared stationary phase of the column chromatography set-up with silica gel and glass wool.
2. Prepared silica gel slurry and allowed to stand for 8 h.
3. Eluent and solvent → ethanol:water at 8:2 with 1% v/v glacial acetic acid.
4. Washed the column with the solvent and allowed to stand to check for leaks.
5. Loaded the raw extracted pigment and allowed to stand for 1 h.
6. Collected the eluate and measured the absorption spectrum.

Though other betalain compounds have been removed, both raw and purified samples show peaks corresponding to betaxanthin at 488 nm apart from the absorption peak of betanin at 535 nm (seen in Figure S1(a) and (b)). The presence of betaxanthin is inhibitory to the DSSC performance since it competes with betanin for binding sites in TiO$_2$ and its properties are not suitable for application in DSSC. In order to completely remove the betaxanthins, the eluent from this step was subjected to chromatography again aimed at removing betaxanthins (Chart S2).

**CHART S2** Column chromatography for the removal of betaxanthins

1. 1$^{st}$ run by mobile phase of 90% water and 10% methanol to elute betaxanthins
2. 2$^{nd}$ run with methanol:water (9:1) to elute betanin
3. Multiple samples were tested

**FIGURE S1** Absorption spectra of (a) raw betanin extract (b) betanin extract purified through column chromatography (containing betaxanthin impurities (c) betanin extract after specific column treatment for separating and removing betaxanthins
The removal of betaxanthin and purification of betanin can be observed from the absorption spectra shown in Figure S1(c). The eluent was run through the column multiple times to get the best results in the solar cell performance and the purity was calculated after each run. The values are shown in Table S2.

| No. of runs | %Purity |
|------------|---------|
| Raw extract| 59.39   |
| 1 total run| 75.89   |
| 2 total runs| 84.01  |
| 3 total runs| 84.26  |

Betanin solar cells were fabricated using the purified extracts obtained from column chromatography by performing multiple runs and their performance was compared with each other as well as with the raw extract. The IV curves of the solar cells are shown in Figure S2. Enhanced performance can be observed after 2 runs of chromatography. Efficiency increased from 0.401% to 0.537% for the betanin solar cells.

2. EXTRACTION AND PURIFICATION OF INDIGO

50 g of *Indigo tinctoria* leaves were pulverized and left to stand for fermentation after adding 150 mL of distilled water to 235 g at room temperature for several (a period of 1, 2, 3, 4 and 5 days) days. The container was flipped over at the end of each day, during the period of fermentation. Subsequently, the fermented blue mass was filtered and the filtrate was centrifuged at 3000 rpm for 20 min to obtain a crude paste of the pigment which was re-dissolved in water for further use. The indigo leaves contain glucosides known as indican which gets hydrolyzed to indoxyl by the action of enzymes and subsequently oxidized to form indigo blue. The purity of the indigo extracted was calculated using Equation (1).

Based on the purity of the extracted indigo, the solvent, time and temperature of extraction were optimized. First, the purity of indigo extracted was checked for four of the most suitable solvents for DI water, ethanol, methanol, and acetone. DI water was found to give the best results as observed in Table S3(a). Next, indigo was extracted using DI water at room temperature of 33 °C and the time was varied from 6 h - 72 h and the purity of the extracted indigo was checked at each condition. Best results were obtained for a fermentation time of 60 h, as observed in Table S3(b). Finally, the purity of indigo extracted using DI water was checked for various extraction temperatures (time was kept constant at 60 h). Best results were obtained at an extraction temperature of 70 °C for 60 h giving a purity of ~ 72% (Table S3(c)). The time of extraction was also verified again to be 60 h at a 70 °C temperature of extraction. Aggregation of unwanted compounds is detrimental to the performance of the device. In order to ensure that the impurities present in the extracted pigments do not affect the performance of the device, the dyes were purified using column chromatography shown in Chart S3.
TABLE S3 Optimization of factors for extraction of indigo: (a) solvent used for extraction, (b) time of extraction and the (c) temperature of extraction

| Solvent   | %Purity |
|-----------|---------|
| DI Water  | 29.49   |
| Ethanol   | 21.54   |
| Methanol  | 21.29   |
| Acetone   | 22.15   |

(b)

| Time (h) | %Purity |
|----------|---------|
| 6        | 46.51   |
| 12       | 49.88   |
| 24       | 52.63   |
| 36       | 55.99   |
| 48       | 58.44   |
| 60       | 60.09   |
| 72       | 60.09   |

(c)

| Temperature (°C) | %Purity |
|------------------|---------|
| 30               | 60.09   |
| 40               | 60.89   |
| 50               | 63.95   |
| 60               | 65.21   |
| 70               | 71.60   |
| 80               | 71.29   |

CHART S3 Column chromatography for purification of indigo from the raw extract

Prepared stationary phase of the column chromatography set-up with silica gel and glass wool. Prepared silica gel slurry with hexane and allowed to stand for 8 h.

Washed the column with the solvent and allowed to stand to check for leaks.

Loaded the raw extracted pigment and allowed to stand for 1 h.

Eluent - chloroform-hexane-methanol (7:4:1 v/v/v) was added

Collected the eluate and measured the absorption spectrum

The characteristic peaks at 390 nm and 625 nm observed (Figure S3(a)) match closely with the observed spectra reported in the literature\textsuperscript{4,5}. The eluent was run through the column multiple times to get the best results in the solar cell performance and the purity was calculated after each run (the results are listed in Table S4).
The purified indigo was subsequently used to fabricate the solar cells and their performances were assessed. Enhanced performance was observed after two runs of chromatography. However, subsequent runs to do not increase the efficiency any further. The J-V curves of the solar cells fabricated with the purified indigo compared against that prepared using the raw extract are shown in Figure S3(b). The efficiency of the indigo solar cells increased from 0.04% to 0.06% following purification.

| No. of runs     | %Purity |
|-----------------|---------|
| Raw extract     | 71.60   |
| 1 total run     | 81.73   |
| 2 total runs    | 85.99   |
| 3 total runs    | 85.68   |

**TABLE S4** Effect of column chromatography on the purity of the indigo

3. EXTRACTION AND PURIFICATION OF LAWSONE

For the extraction of lawsone pigment from *Lawsonia inermis* leaves, various procedures were tested. The following procedure was found to be the best: Fresh lawsone leaves were ground with acetone in a pulverizer and the mixture was ultrasonicated further for 1 h at 50 °C. The purity was calculated using Equation (1).

Based on the purity of the extracted lawsone, the solvent, time and temperature of extraction were optimized. First, the purity of lawsone extracted was checked with three of the most suitable solvents for DI water, ethanol, and acetone. Acetone was found to give the best results as observed in Table S5(a). Next, lawsone was extracted using acetone at room temperature of 33 °C and the time was varied from 30 – 120 min and the purity of the extracted lawsone was checked at each condition. Best results were obtained for an ultrasonication time of 60 min, as observed in Table S5(b). Finally, the purity of lawsone extracted using acetone was checked for various extraction temperatures (time was kept constant at 60 h). Best results were obtained at an extraction temperature of 50 °C for 1 h giving a purity of ~ 76% (seen in Table S5(c)). The time of ultrasonication was also verified again to be 1 h at a 50 °C temperature of extraction.

Purification of lawsone was done through column chromatography by the methods described in the literature (depicted in Chart S4).
TABLE S5 Optimization of factors for extraction of lawsone: (a) solvent used for extraction, (b) time of ultrasonication and the (c) temperature of extraction

| (a) Solvent   | % Purity |
|---------------|----------|
| DI Water      | 49.39    |
| Acetone       | 62.56    |
| Ethanol       | 55.97    |

| (b) Time (min) | % Purity |
|----------------|----------|
| 30             | 62.56    |
| 60             | 73.43    |
| 90             | 73.19    |
| 120            | 65.53    |

| (c) Temperature (°C) | % Purity |
|----------------------|----------|
| 30                   | 73.43    |
| 40                   | 74.09    |
| 50                   | 76.06    |
| 60                   | 72.77    |
| 70                   | 69.48    |

CHART S4 Column chromatography for purification of lawsone from the raw extract

Prepared stationary phase of the column chromatography set-up with silica gel and glass wool.
Prepared silica gel slurry with 100% acetone and allowed to stand for 8 hours.

Eluent of acetone: DI water at 60:40 to extract lawsone.

Collected the eluate and measured the absorption spectrum.

TABLE S6 Optimization of column chromatography for purification of lawsone: (a) solvent system, (b) the ratio of the solvents in the solvent system and the (c) number of runs performed in the column.

| (a) Solvent systems   | %Purity |
|-----------------------|---------|
| Acetone: Water        | 83.96   |
| Ethanol: Water        | 75.73   |
| Methanol: Water       | 72.44   |

| (b) Acetone: Water (% Acetone) | %Purity |
|--------------------------------|---------|
| 40%                            | 79.03   |
| 50%                            | 82.32   |
| 60%                            | 85.61   |
| 70%                            | 82.32   |

| (c) No. of runs | % Purity |
|----------------|---------|
Of the three solvent systems tested, the system of acetone: water at a ratio of 60:40 gave the best results (seen in Table S6(a) and S6(b)). The characteristic peaks at 338 nm and 410 nm were observed (seen in Figure S4(a)) matching the spectra observed in the literature. The eluent was run through the column multiple times to get the best results in the solar cell performance. It was observed that increasing the number of runs increased the purity of the pigment up to two runs, however, it did not have any effect for a higher number of runs (Table S6(c)).

The purified pigments were then used in the fabrication of solar cells. The solar cell fabricated from the purified pigment and its performance was then compared against that of the solar cell prepared using the raw dye. The lawsone solar cell fabricated using the purified extract showed enhanced performance as observed from the J-V curves shown in Figure S4(b). The efficiency of the lawsone solar cells increased from 0.1% to 0.3% following purification.

| Run          | Efficiency |
|--------------|------------|
| Raw extract  | 83.64      |
| 1 total run  | 85.28      |
| 2 total runs | 85.94      |
| 3 total runs | 85.61      |

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FIGURE S4 (a) Absorption spectra of the raw and purified lawsone extracts (b) J-V curves of the solar cells fabricated using the raw and purified lawsone extracts
