Electrochemical Analysis of Curcumin Indole-3-Carbinol, Aloe-emodin and β-Carotene in plant extract and Pharmaceutical Formulations
ELECTROCHEMICAL ANALYSIS OF CURCUMIN, INDOLE-3-CARBINOL, ALOE-ENODIN AND BETA-CAROTENE IN PLANT EXTRACT AND PHARMACEUTICAL FORMULATIONS

Electrochemical methods (analytical and preparative) and parameters can be widely used in medicinal chemistry, especially because they furnish an enormous amount of evidences regarding the mechanisms of biological electron-transfer processes. There are still a vast scope of using modern polarographic and voltammetric methods. However, the most important impetus for further development and more frequent practical applications would be the enthusiasm of innovative electrochemists.(1)

The versatility of electrochemical methodology allows to mimic the multitude of biological environments: the conditions can be widely varied in the attempt to resemble them. Different ranges of pH, oxygen content in the electrochemical cell and solvents of diverse properties can be used. However, standardization is urgently required in terms of methods, electrodes, supporting electrolyte etc, to allow a more general use of the already available data.

In electrochemistry, considerable progress has recently been made in the development of new and rather sophisticated techniques. The goal of the present study is to analyse drugs obtained from natural origins and pharmaceutical formulations by DCP and DPP (direct current and differential pulse polarography).

Polarographic analysis of curcumin:

Curcumin [1,7-bis (4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione] is the major yellow pigment extracted from turmeric(2) a commonly used spice derived from Rhizome of herb Curcuma longa Linn. Curcumin has shown antineoplastic, anti-inflammatory properties.(2-4)
The authentic curcumin sample in 1M Ammonium Tartrate at pH 8.1±0.1 produced a well defined direct current polarogram (Fig. 3A1) with half wave potential, $E_{1/2} = -1175 \text{ mV vs SCE}$. Where as the differential pulse polarogram (DPP) of the solution resulted in two well defined peaks (Fig. 3A2) with peak potential ($E_p$) = - 1125 mV and -1275 mV vs SCE.

The developed procedure was therefore applied for the analysis of curcumin in the extracted samples. The samples showed two well defined DPP peaks at -1125 mV and -1275 mV vs SCE at pH. 8.1±0.1. The results indicating the presence of curcumin in turmeric extract. Method of standard addition was used for quantitative analysis of curcumin in the extracted mass of Turmeric. The resulting DPP curves of spiked analyte showed two peaks with no change in peak potential ($E_p$) value but peak height was increased. Thus, confirming the presence of curcumin in the extracted sample and also enabling the possible use of the developed procedure for an accurate quantitative analysis of curcumin in natural origin extracts. The results of External spiking of the test sample have been reported in Table 3A1. The percentage recovery was found to be more than 99% in each case.

**Table 3A1. Differential pulse polarographic analysis of curcumin in extracted sample from turmeric.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracted sample (ml)</th>
<th>Amount of curcumin (mg/ml)</th>
<th>Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found*</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
<td>0.415</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.184</td>
<td>0.590</td>
</tr>
</tbody>
</table>

*Average of four determinations

**Analysis of curcumin in pharmaceutical formulation:**

The developed procedure was applied for the analysis of curcumin in a pharmaceutical formulation, Turmeric Force™ at pH 8.1±0.1.
Fig. 3A1: DCP of 0.0012 M Curcumin in 1 M Ammonium Tartrate at pH 8.1 ± 0.1

Fig. 3A2: DPP of 0.0012 M Curcumin in 1 M Ammonium Tartrate at pH 8.1 ± 0.1
The resulting direct current polarogram showed only one curve at \( E_{1/2} = -1275 \text{ mV} \) at pH 8.1±0.1 and 2 peaks were obtained by using differential DPP (differential pulse polarography) mode with \( E_p \) values = –1125 mV and –1275 mV vs SCE at the same pH.

Method of external spiking was used to validate the presence of curcumin in the formulation, Turmeric Force\textsuperscript{TM} at pH 8.1±0.1. The results of the analysis are tabulated in Table 3A2. The concentration of curcumin was found 0.35 mg/ml which is in excellent agreement with that reported by the manufacturer.

The standard deviation and coefficient of variance never were exceeded. The recovery was over 99.9% for curcumin in natural sample and pharmaceutical formulation. Thus, confirming the reliability of the observed data.

The observed results were also supplemented by the FTIR study on the extracted and pharmaceutical formulation samples. FTIR spectra of authentic curcumin (Fig. 3A3) clearly shows 6 characteristics signals at 3508 cm\(^{-1}\) (phenolic OH), 1640 cm\(^{-1}\) (β diketone), 1489 cm\(^{-1}\) (>C=C), 1387 cm\(^{-1}\) (C-H), 856 cm\(^{-1}\) (Aromatic C-H), 1153-1260 cm\(^{-1}\) (C-O, C-C-C)(5-6) respectively corresponding to the presence of curcumin extracted from turmeric powder and pharmaceutical formulation Turmeric Force\textsuperscript{TM} also showed 6 signals at the same waver numbers. Thus, confirming the presence of [1,7—bis (4, hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione] in the extracted sample and pharmaceutical formulation.

**Table 3A2 : Differential pulse polarographic analysis of curcumin in pharmaceutical formulation Turmeric Force\textsuperscript{TM}.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Pharmaceutical formulation (ml)</th>
<th>Amount of curcumin (mg/ml)</th>
<th>Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found*</td>
</tr>
<tr>
<td>1</td>
<td>3 ml</td>
<td>0.184</td>
<td>0.213</td>
</tr>
</tbody>
</table>

*Average of four determinations
Fig. 3A3 : IR spectra of Curcumin
Proposed reduction mechanism of curcumin at DME:

Curcumin contains three double bonds and a keto group which are easily reducible at DME surface (pH 8.1±0.1) in Ammonium Tartrate as supporting electrolyte. According to the literature(7-8) when an electro reducible group conjugated with olefinic group, the double bond was reduced before the carbonyl group. Curcumin involves two electrons reduction process at pH 8.1±0.1. The two double bonds which are by the side of the OH and C = O groups are reduced to give a doublet in its differential pulse polarogram. The scheme for the reduction of curcumin is shown as under.

Electro chemical reduction of curcumin in ammonium tartrate at pH 8.1 ± 0.1

Polarographic analysis of Aloe-emodin:

Aloe-emodin, 1,8, dihydroxy, 3-(hydroxymethyl) 9,10-anthracenedione(9) is present in aloe-vera leaves.(10) Aloe-emodin is the antitumor drug which shows toxicity for malignant tumor such as neuroblastoma cells, Ewing’s Sarcoma cells etc.(11)

The goal of the present study is to authenticate direct current polarographic (DCP) and differential pulse polarographic (DPP) methods for the analysis of Aloe-emodin in phytochemicals and industrial samples.
Aloe- emodin produces a well-defined polarographic wave/peaks (Fig 3A4 DCP, Fig 3A5 DPP) in 1 M Ammonium Tartrate at pH 5.6±0.1 with \( E_{1/2} = -1120 \) mV and \( E_p = -1120 \) and -1350 mV vs SCE. Its wave height/peaks height \( (i_0/i_p) \) was found to be proportional to Aloe- emodin concentration.

**Analysis of Aloe- emodin in Aloe-vera leaves extract:**

The Aloe-vera leave extract when subjected to DPP study also produced two well defined peaks similar to that observed with authentic Aloe-emodin with \( E_p \) values -1120 mV and -1350 mV vs saturated calomel electrode (SCE) in ammonium tartrate as supporting electrolyte at pH 5.6±0.1. However, its DC polarogram produced only one polarographic wave with \( E_{1/2} = -1120 \) mV.

The external spiking method was used to validate the DCP and DPP methods for the analysis of Aloe-emodin in the natural extract. The concentration of Aloe-emodin in the extract was determined by standard addition method. It was noted that there was no change in \( E_{1/2} \) (DCP) and \( E_p \) (DPP) values of the observed polarogram with increasing Aloe-emodin concentration. Thus, authenticating the qualitative as well as quantitative use of the developed electrochemical procedure for the analysis of Aloe-emodin in different samples. The results have been depicted in Table 3A3. The percentage recovery was always above 98.8.

**Table 3A3 : Polarographic analysis of Aloe-emodin in Aloe-vera leaves extract.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample (ml)</th>
<th>Amount of Aloe-emodin (mg/ml)</th>
<th>Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found*</td>
</tr>
<tr>
<td>1</td>
<td>15 ml</td>
<td></td>
<td>23.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.0</td>
<td>48.4</td>
</tr>
</tbody>
</table>

*Average of four determinations
Fig. 3A4: DCP of 0.0015 M Aloe emodin in 1 M Ammonium Tartrate at pH 5.6 ± 0.1

Fig. 3A5: DPP of 0.0015 M Aloe emodin in 1 M Ammonium Tartrate at pH 5.6 ± 0.1
Analysis in pharmaceutical formulations of Aloe-emodin:

Pharmaceutical formulations Kumaraysava (A) and green gel (B) contain Aloe-emodin. The developed procedure was used for the determination of Aloe-emodin in pharmaceutical formulations but pH was adjusted to 2.3±0.1 and 4.7±0.1 for its analysis in Kumaraysava and green gel samples respectively. The differential pulse polarogram of test samples of Kumaraysava and green gel shows two peaks at $E_p = -1120 \text{ mV}$ and $-1350 \text{ mV}$ vs SCE. The method of standard addition was used for qualitative and quantitative analysis of Aloe-emodin in pharmaceutical formulations. The results have been tabulated in Table 3A4.

The observed results were also supplemented by the FTIR study of Aloe-emodin extracted from plant origin and pharmaceutical formulations. FTIR spectra of authentic aloe-emodin clearly shows (Fig. 3A6) five characteristic signals at 3490 cm$^{-1}$ (alcoholic OH), 1352 cm$^{-1}$ (phenolic OH), 1673 cm$^{-1}$ (quinone) and 740 cm$^{-1}$ (substituted aromatic ring).(5) Peaks with similar frequencies (wave number) were observed in the FTIR spectra of the extracted Aloe vera leaves and the two pharmaceutical formulations under studies.

Table 3A4 : Polarographic analysis (DPP) of Aloe-emodin in pharmaceutical formulations (A) Kumaraysava and (B) Green Gel.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample (ml)</th>
<th>Amount of Aloe-emodin (mg/ml)</th>
<th>Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found*</td>
</tr>
<tr>
<td>1</td>
<td>Kumaraysava (A)</td>
<td>-</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>1 ml</td>
<td>2.42</td>
<td>4.26</td>
</tr>
<tr>
<td>2</td>
<td>Green gel (B)</td>
<td>-</td>
<td>4.03</td>
</tr>
<tr>
<td></td>
<td>4 ml</td>
<td>2.42</td>
<td>6.42</td>
</tr>
</tbody>
</table>

* Average of four determinations.
Fig. 3A6: IR spectra of Aloe-emodin
Effect of pH:

The pH of test solution is a key factor to get better polarographic waves. In the present experiment the pH 5.6±0.1 of the test solutions has been used for the determination of Aloe- emodin in authentic and natural origin samples.

At this pH of the test solution the observed polarogram/peaks were well defined and $i_d/E_p$ of the polarogram was found to be proportional to the Aloe- emodin concentration. However, in the analysis of pharmaceutical formulations the matrix effect was found to be predominant at pH 5.6±0.1. As such, the determination of Aloe- emodin in pharmaceutical formulations were done at pH 2.3±0.1 in Kumaraysava and green gel at 4.7±0.1, at these pH values the matrix effect was found to be minimized and very well defined polarographic signals were observed.

Proposed reduction mechanism at DME:

Aloe-emodin contains diquinone group. The electrochemistry of quinone has been reported in the literature.(12,13) The electrochemical reduction of Aloe- emodin at pH 5.6±0.1 can be represented by the following mechanism.

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{OH} & \quad \text{O} \\
\text{CH}_2\text{OH} & \quad \text{OH} \\
\text{CH}_2\text{OH} & \quad \text{OH}
\end{align*}
\]

1,8-dihydroxy-3-(hydroxy methyl)-9,10-anthracenedione

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{CH}_2\text{OH} & \quad \text{OH} \\
\text{CH}_2\text{OH} & \quad \text{OH}
\end{align*}
\]

1,2,3,7-tetrahydroxy, 5-(hydroxymethyl), anthracene

In this process two (C=O) groups are reduced using two electrons in acidic medium at pH 5.6±0.1 to produce two well defined DPP signals.
Polarographic analysis of β-carotene:

Carotenoids are a family of conjugated polyenes, found largely in fruits and vegetables, which possess antioxidant activities. β-carotene have a range of diverse biologic functions and action and have an essential role in human health. β-carotene also appear to have a variety of chemo-preventive actions. In particular they have antioxidant potential and capable of immuno enhancement. It can reduce chromosome aberrations, inhibit the formation of premalignant lesions, reduce cell proliferation and transformation.(14-16)

The authentic beta-carotene sample in 1M ammonium tartrate at pH 4.6±0.1 produced well define direct current polaarogram (DCP) (Fig. 3A7) with half wave potential $E_{1/2} = -260 \, \text{mV}, -660 \, \text{mV}, -860 \, \text{mV}$ and $-1300 \, \text{mV}$ vs SCE. The differential pulse polarogram (Fig. 3A8 and 9) of beta-carotene clearly shows four conjugated peaks and its peak potential are $E_p = -260 \, \text{mV}, -660 \, \text{mV}, -860 \, \text{mV}$ and $-1300 \, \text{mV}$ vs SCE. The peak height of all the four peaks was increased found to be proportional to the concentration of β-carotene.

Analysis of β-carotene in carrot extract:

As mention earlier β-carotene was extracted from carrots. The developed procedure was therefore applied for the analysis of Beta-carotene in carrots extract. The Direct current polarogram showed four signals and their $E_{1/2}$ values were the same as obtained with authentic β-carotene at pH 4.6±0.1 in ammonium tartrate as supporting electrolyte. The extracted sample was also analysed using differential pulse polarography under similar experimental conditions. The resulting polarogram also produced four signals with $E_p$ values are $-260 \, \text{mV}, -660 \, \text{mV}, -860 \, \text{mV}$ and $-1300 \, \text{mV}$ vs SCE at pH 4.6±0.1.

The method of standard addition was used to validate the presence of Beta-carotene in extracted sample of plant-origin. The results of analysis have been tabulated in Table 3A5. The percentage recovery was found to be over 99.5 in each case.
**Fig. 3A7:** DCP of 0.0015 M β-carotene in 1 M Ammonium Tartrate at pH 4.6 ± 0.1

**Fig. 3A8:** DPP of 0.0015 M β-carotene in 1 M Ammonium Tartrate at pH 4.6 ± 0.1

**Fig. 3A9:** DPP of 0.0015 M β-carotene in 1 M Ammonium Tartrate at pH 4.6 ± 0.1
Table 3A5: Polarographic analysis (DPP) of β-carotene in Carrot extract sample.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracted sample (ml)</th>
<th>Amount of β-carotene (mg/ml)</th>
<th>Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found*</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>-</td>
<td>4.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.03</td>
<td>6.06</td>
</tr>
</tbody>
</table>

*Average of four determinations

Analysis of Beta carotene in pharmaceutical formulations:

Iozeal and carofit are the pharmaceutical formulations of β-carotene. In 1M Ammonium Tartrate at pH 5.4±0.1 Iozeal producing four polarographic waves/peaks (Fig. 3A7,8 and 9) well defined direct current-polarogram (DCP) and differential pulse polarogram (DPP). The peak potentials are $E_p^1 = -260$ mV, $E_p^2 = -660$ mV, $E_p^3 = -860$ mV, $E_p^4 = -1300$ mV, vs SCE. The $E_{1/2}$ values were also closed to the above values. External spiking method was applied for the quantitative analysis of β-carotene in Iozeal. It was noted that there is no change in $E_{1/2}$ (DCP) and $E_p$ (DPP) values of the observed polarogram with increasing concentration of β-carotene.

The developed polarographic method was also applied in the analysis of β-carotene in carofit sample. For the polarographic (DCP and DPP) determination of β-carotene in carofit (pharmaceutical formulation) in the ammonium tartrate, the pH was adjusted to 3.7±0.1. The resulting DCP and DPP curves produced four signals each as have been observed using authentic sample of β-carotene. The spiking method was used for qualitative as well as quantitative analysis of β-carotene in pharmaceutical formulation. The results of the analysis are tabulated in Table 3A6. The found β-carotene concentration in each of the two pharmaceutical formulations under study was found to be in excellent agreement with that reported by the manufacturer of carofit pharmaceutical formulation.
Table 3A6 : Polarographic analysis of Beta-carotene in pharmaceutical formulations Iozeal (A) and carofit (B).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample (ml)</th>
<th>Amount of β-carotene (mg/ml)</th>
<th>Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found*</td>
</tr>
<tr>
<td>1</td>
<td>Iozeal (A)</td>
<td>-</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>5 ml</td>
<td>1.55</td>
<td>3.07</td>
</tr>
<tr>
<td>2</td>
<td>Carofit (B)</td>
<td>-</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td>5 ml</td>
<td>1.55</td>
<td>2.93</td>
</tr>
</tbody>
</table>

*Average of four determinations

It is a well recognized fact that pH plays a significant regulatory role in the analysis of β-carotene obtained from pharmaceutical formulations and carrot extract samples.

The author has performed the polarographic analysis of β-carotene in authentic and natural origin (from carrots) sample in ammonium tartrate as supporting electrolyte at pH 4.6±0.1. At this pH of the test solution the observed polarograms/peaks were well defined and \( i_d/E_p \) of the polarogram was found to be proportional to the β-carotene concentration. However, in the analysis of pharmaceutical formulations the matrix effect was much more predominant at pH 4.6±0.1. As such, the determination of β-carotene was done at pH 5.4±0.1 and 3.7±0.1 in Iozeal and carofit respectively to minimize the matrix effect.

The FTIR study was done in extracted and pharmaceutical formulation of β-carotene. The FTIR spectra of authentic β-carotene (in chloroform phase) clearly shows (Fig. 3A10) three signals at 1513 cm\(^{-1}\) (C=C), 3051 cm\(^{-1}\) (C-H) and 1185 cm\(^{-1}\) (C-C)(6) corresponding to the presence of β-carotene extracted from carrot and pharmaceutical formulations Iozeal and carofit. Thus, confirming the presence of β-carotene in the test samples.
Fig. 3A10: IR spectra of Beta carotene
Proposed reduction mechanism at DME:

\( \beta \)-carotene consists of a polyene chain with nine conjugated double bonds and two \( \beta \)-ionone rings. \( \beta \)-carotene in 1.0 M Ammonium Tartrate as supporting electrolyte at pH 5.2 \pm 0.1 produced four very well defined polarographic waves/peaks with \( E_{1/2}/E_p \) = -260 mV, -660 mV, -860 mV and -1300 mV vs SCE. The electrochemistry of \( \beta \)-carotene has been reported in the literature(17,18). The above reduction may be explained in the basis of the fact that under the given experimental conditions, \( \beta \)-carotene molecule presents four double bonds in conjugation available for reduction. The height of each wave/peak was found to be proportional to the \( \beta \)-carotene concentration.

![\( \beta \)-carotene](image)

Thus, producing 4 peaks in its differential pulse polarogram) at pH 4.6±0.1.

Polarographic analysis of Indole-3-carbinol:

The literature reveals that many natural products are available as chemoprotective agents against commonly occurring cancers occurring worldwide. Indole-3-carbinol is the one of the natural drugs obtained from (Cruciferous vegetable broccoli, cauliflower, cabbage) that is used in the treatment of breast cancer and lung cancer(19).

The authentic Indole-3-carbinol sample in 1M ammonium tartrate at pH 6.1±0.1 produced a well defined Direct current polarogram DC curve (Fig. 3A11) with half wave potential \( E_{1/2} \) = -1324 mV vs SCE, where as the differential pulse polarogram (DPP) of the solution resulted in two well defined peak at (Fig. 3A12) with peak potential \( E_p \) = -1210mV and -1324 mV Vs SCE at pH 8.1±0.1.
Fig. 3A11: DCP of 0.0015 M Indole-3-carbinol in 1 M Ammonium Tartrate at pH 8.1 ± 0.1

Fig. 3A12: DPP of 0.0015 M Indole-3-carbinol in 1 M Ammonium Tartrate at pH 8.1 ± 0.1
Polarographic analysis of Indole-3-carbinol in cauliflower extract:

The developed procedure was applied for the analysis of Indole-3-carbinol in the extracted sample from Cauliflower. 5 ml extracted sample of produced two well defined DPP peaks at -1210 mV and -1324 mV vs SCE in 1M ammonium tartrate at pH 8.1±0.1. Thus reveling the presence of Indole-3-carbinol in Cauliflower extract. Method of spiking the analyte with authentic Indole-3-carbinol was used for the quantitative analysis in cauliflower extract.

The resulting DPP curve of spiked analyte showed two peaks with no change in peak potential ($E_p$) value but peak height was found to be increased by increasing the concentration of Indole-3-carbinol. Thus, confirming the presence of Indole-3-carbinol in the extracted sample and also enabling the possible use of the developed procedure for an accurate qualitative as well as quantitative analysis.

The results of analysis have been tabulated in Table 3A6. The percentage recovery was found to be more than 96 in each case.

### Table 3A6: Polarographic analysis (DPP) of Indole-3-carbinol in extracted sample.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracted Sample (ml)</th>
<th>Amount of Indole-3-carbinol (mg/ml)</th>
<th>Percentage Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Added</td>
<td>Found*</td>
</tr>
<tr>
<td>1</td>
<td>5 ml</td>
<td>-</td>
<td>3.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.85</td>
<td>7.65</td>
</tr>
</tbody>
</table>

*Average of four determinations

The observed results were supplemented by the FTIR study of Indole-3-carbinol in extracted from plant origin. The FTIR spectra of Indole-3-carbinol clearly shows (Fig. 3A13) characteristics signals at 3387 cm⁻¹ (NH secondary), 3059 cm⁻¹ (aromatic ring joint with five membered ring) and 3288
cm\(^{-1}\) (O-H group) respectively corresponding to the presence of Indole-3-carbinol. (20) The FTIR spectra of cauliflower extract also showed three signals at the same wave members. Thus, confirming the presence of Indole-3-carbinol in the Cauliflower extract.

**Proposed reduction mechanism at DME:**

Indole-3-carbinol contains one Indole group and a CH\(_2\)-OH group. The two groups are easily electrochemically reducible at pH 8.1±0.1. As such, the electrochemical reduction of Indole-3-carbinol may be proposed as under:

\[
\text{Indole-3-carbinol} \rightarrow \text{3-hydroxy-2-oxindole}
\]

**Electro chemical reduction of Indole-3-carbinol at pH 8.1±0.1 in ammonium tartrate**

In this reaction Indole-3-carbinol is converted into 3 hydroxy-2-oxindole by using 2 electrons in basic medium at pH 8.1±0.1 which gives a doublet in DPP mode. The literature also support the above finding. (21-22)
Fig. 3A13: IR spectra of Indole-3-Carbinol
References:

17. Kuta EJ, Science; 1964, 144, 1130-1131.
22. Moto MC, Rua ML, Ferro E, Physiol Plant; 1988, 72, 84.