4.1 Introduction:

Cu complexes are probably the most studied metal complexes having importance in a wide variety of fields such as catalysis, biomimetics, spectroscopy, magnetism, liquid crystal, basic coordination chemistry etc. Transition metal complexes such as Cu complexes as catalysts have received much attention because of their almost exclusive biological function in living systems. Cu (II) complexes have been known for their structural diversity and functional models for the active centres of copper containing redox enzymes [1-4]. The construction of a biomimetic sensor is a formidable challenge in the development of analytical procedures for the determination of various analytes [5, 6]. But reports on copper complexes as electrode modifying agents is very few due to the instability caused by the changed structure during redox process [7-10]. Hence discovering a new copper complex as electrode modifying agent for detection of analytes is a matter of importance.

Dopamine (DA) and ascorbic acid (AA) are compounds of great biomedical and neurochemical interest playing a potential role in human metabolism. DA is one of the most significant catecholamine, an excitatory neurotransmitter, plays an important role in several important physiological functions such as renal, central nervous, hormonal and cardiovascular [11, 12]. Deficiency of DA leads to neurological diseases like Parkinson’s disease, Alzheimer’s disease, Schizophrenia [13, 14] and is also believed to be related to HIV infection [15]. In mammalian brain DA and AA coexists in the extracellular fluids with AA in 100 to 1000 times in excess [16]. Thus the simultaneous determination of DA and AA is of critical importance in the field of biochemistry and medical treatment.

Among various determination methods like ultraviolet spectroscopy (UV) [17,18], high performance liquid chromatography (HPLC) [19,20] and electrochemical approaches [21-23], the later have obtained considerable attention for their high sensitivity and selectivity, especially for the in-vivo detection of neurotransmitter. Again electrochemical methods have more advantages than other methods because the electrodes can be made conveniently to sense the neurotransmitters in the living organism [24].
DA is electro active and therefore electrochemical sensors should be quite useful for DA detection. But the major problem encountered in electrochemical detection of DA is the interference from ascorbic acid (AA), which has overlapping oxidation potential with DA on solid electrodes [25]. Even in absence of AA also determination of DA is troublesome. The bare electrode very often suffers from fouling effect due to the accumulation of oxidised products of DA on the electrode surface [26], which results in rather poor selectivity and sensitivity.

In order to solve these two major problems, chemically modified electrodes are widely used in case of DA detection or determination for enhanced selectivity and to eliminate the interference of AA [27-30]. A wide variety of agents have been reported as electrode modifying agent in order to develop electrochemical sensor for DA in presence of AA, few of them are - didodecyl(dimethyl)amine bromide [31], poly(vinylalcohol) [32], composite polymers [33], dendrimers containing gold nanoparticles [28], polypyrrole/ferrocyanide film [34], gold nanoparticles distributed poly(4-aminothiophenol) [35], poly (acrylic acid) multi walled carbon nanotube composite [36], boron doped diamond[37], boronic acid functionalized multi walled carbon nanotube [38], self doped polyaniline/carbon nano tube composite[39] p-nitrobenzeneazo resorcinol [40 ] etc.

Encapsulation of metal complexes and organic molecules within the super cages of zeolites has been a topic of intense research as a result of the remarkable catalytic properties of the resulting materials. Zeolites are a family of crystalline aluminosilicates whose structure defines strictly uniform channels and cavities of molecular dimensions. Zeolite modified electrodes (ZMEs), a subcategory of the “Chemically modified electrodes”, became popular in mid 1980s and had drawn great attention of electrochemists [41, 42]. The interest behind developing ZMEs are – (i) it can distinguish small enough analytes because of unique molecular sieving property of zeolites, (ii) development of new electroanalytical devices with higher sensitivity than other chemically modified electrodes, (iii) possible use in electrocatalysis. Zeolites attract interest for electrocatalysis because they offer selectivity based on the size and the shape of the reactants, together with a three –dimensional lattice in a variety of support sites for various catalysts. Voltammetric biosensors based on ZMEs are known
for a number of analytes such as dopamine [43] glucose [44], phenols [45], ascorbic acid [46], catechol and 4-chlorophenol [47], epinephrine [49] metal ion [41] etc.

Cu (II) ion or its complexes have gained recent interest as electrode modifying agents for detection of DA. The surface properties of Cu particles modified electrode mainly depend on the electro deposition of copper at different applied potential [49]. Immobilization of cupric ions in poly (ethylene-co vinyl acetate) film on electrode surface had excellent selectivity for DA over AA [50]. Amperometric sensor for DA using a Nafion membrane doped with copper dipyridyl complex was also reported [51]. GC electrode modified electrochemically with bipyridine bridged bicopper complex with the ligand 2 [bis(2-amino-ethyl)amino]ethanol could distinguish DA and AA by cyclic voltammetry and differential pulse voltammetry [52]. There is also report on square-wave voltammetric detection of DA at Copper- (3-mercaptopropyl) triethoxy silane complex modified electrode [53].

In this chapter we report the synthesis and characterization of a new bimetallic copper(II) complex, \([LCu(II)(CH_3COO)_2Cu(II)L])(CH_3COO)_2\) where L is N, N bis(phthalimide)ethylenediamino. GC electrode surface on modification by this complex encapsulated ZSM-5 could voltammetrically distinguish between DA and AA, at \([DA]: [AA] = 1:100\) with an oxidation peak separation value 0.340 V, the detection limits were also found to be quite low. The complex was also tested for SOD scavenging activity.

4.2 Experimental:

Phthalic anhydride was purchased from Merck, ethylenediamine and L-Ascorbic acid were from Loba Chemie. Dopamine hydrochloride was purchased from SIGMA. Freshly prepared solutions of DA, and AA were used in all experiments. All the aqueous solutions were prepared using double distilled water.
4.2.1 Synthesis:

4.2.1.1 Synthesis of 2-(2-(1,3-dioxoisooindolin-2yl)ethyl)isoindoline-1,3-dione (L):

L was synthesised as per reported procedure [54]. Briefly: phthalic anhydride (2.96 g, 20 m mol) was dissolved in 5 mL tetrahydrofurane (THF). Ethylene diamine (0.60 g, 10 m mol) was added drop wise at room temperature with constant stirring. The reaction mixture was stirred for three hours till white precipitate was obtained. The product was filtered and recrystallized from water (yield 91%).

\[
\begin{align*}
\text{Scheme 4.1}
\end{align*}
\]

4.2.1.1.1 Characterization of L:

Melting point: 237 °C;

Elemental analysis: C 67.1% (calc. 67.5%), H 3.68% (calc. 3.75%), N 8.66 (calc. 8.75%);

FTIR analysis of L: An FTIR spectrum of L was recorded in KBr and has been shown in Fig. 4.1. Peaks were observed at 1708 cm\(^{-1}\) & 1769 cm\(^{-1}\) due to \(\nu_{\text{C}=\text{O}}\), 1060 cm\(^{-1}\) & 1170 cm\(^{-1}\) due to \(\nu_{\text{C-N}}\), 1450 cm\(^{-1}\) due to \(\nu_{\text{CH}_2}\), 1284 cm\(^{-1}\) due to \(\nu_{\text{C-H}}\) out of plane and 1284 cm\(^{-1}\) due to \(\nu_{\text{C-N}}\).

\(^1\)H NMR of L: \(^1\)H NMR spectrum of L was recorded in D\(_2\)O with TMS as internal reference. The spectrum has been shown in Fig. 4.2. Peaks were observed at \(\delta\) values 7.55 – 7.52 (m, 4H), 7.41 -7.39 (m, 4H), 3.19 (s, 4H).

LC-MS of L: LC-MS of L was also done and the spectrum has been shown in Fig.4.3. The molecular peak was observed at m/z value 320 together with peaks at m/z
values 56, 76, 146 and 246 the molecular fragments corresponding to these m/z values are included in Table 4.1.

Fig 4.1: FTIR spectrum of L in KBr
Fig. 4.2: $^1$H NMR spectrum of L in D$_2$O

<table>
<thead>
<tr>
<th>Fragmentor Voltage</th>
<th>Collision Energy</th>
<th>Ionization Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>0</td>
<td>ESI</td>
</tr>
</tbody>
</table>

+ESI Scan (0.802 min) Frag=70.0V BS-L3.020512-0004.d Subtract

Fig. 4.3: LC-MS spectrum of L in H$_2$O
Table 4.1: Assigned molecular fragments of L in LC-MS

<table>
<thead>
<tr>
<th>m/z value</th>
<th>Molecular fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>N-CH₂-CH₂-N</td>
</tr>
<tr>
<td>76</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>146</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>188</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>246</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

4.2.1.2 Synthesis of \([\text{LCu(II)(CH}_3\text{COO)}_2\text{Cu(II)L]}(\text{CH}_3\text{COO)}_2\]:

0.8 g (4.0 m mol) of \(\text{Cu(CH}_3\text{COO)}_2\cdot\text{H}_2\text{O}\) was dissolved in 10 mL water and a solution of L (0.64 g, 4.0 m mol) in water (10 mL) was added dropwise with stirring. The reaction mixture was stirred for three hours and a dark blue precipitate was obtained. The precipitate was washed with diethyl ether and dried in air.
4.3 Results and Discussion:

4.3.1 Characterization of $[\text{LCu}^{2+}(\text{CH}_3\text{COO})_2\text{Cu}^{2+}L](\text{CH}_3\text{COO})_2$:

4.3.1.1 UV/Vis spectroscopic study:

The UV/Vis spectrum of $[\text{LCu}^{2+}(\text{CH}_3\text{COO})_2\text{Cu}^{2+}L](\text{CH}_3\text{COO})_2$ was recorded in water and a broad band was observed at $\lambda_{\text{max}} = 620$ nm. This absorption maximum is a characteristic of d-d transition originating at Cu$^{2+}$ in its complexes.

![UV-Vis spectrum of [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$ in water](image)
4.3.1.2 FTIR spectroscopic study:

FTIR spectra of the complex recorded in KBr pallet showed peaks at 2922 cm$^{-1}$ and 2852 cm$^{-1}$ ($\nu_{\text{CH}_2}$), 1729 cm$^{-1}$ ($\nu_{\text{C}=\text{O}}$), 1057.4 cm$^{-1}$ ($\nu_{\text{C}-\text{N}}$), 1435 cm$^{-1}$ ($\nu_{\text{CH}_2}$) and 455 cm$^{-1}$ ($\nu_{\text{Cu-N}}$).

![FTIR spectrum of [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$ in KBr](image)

Fig. 4.5: FTIR spectrum of [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$ in KBr

4.3.1.3 Cyclic voltammetric study:

Fig. 4.6 shows the cyclic voltammogram of [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$ in water using platinum disc as working electrode and Ag/AgCl as the reference. The peak potential difference is 398 mV and redox
potential is calculated as 255 mV. This redox couple must be due to Cu\textsuperscript{II}/Cu\textsuperscript{I} redox process.

**Fig 4.6:** Cyclic voltamogram of [LCu\textsuperscript{2+}(CH\textsubscript{3}COO)\textsubscript{2}Cu\textsuperscript{2+}L](CH\textsubscript{3}COO)\textsubscript{2} in water,
WE: Pt disc, RE: Ag-AgCl, SE: TBAP

4.3.1.4 EPR studies of [LCu\textsuperscript{2+}(CH\textsubscript{3}COO)\textsubscript{2}Cu\textsuperscript{2+}L](CH\textsubscript{3}COO)\textsubscript{2}:

To understand more about the coordination geometry around the Cu\textsuperscript{2+} centre, the X-band EPR spectra of the complex was recorded as microcrystalline solid.

The solid state EPR spectrum has been shown in Fig.4.7. The figure showed a strong feature at $g_{\perp} = 2.07$ with a shoulder at 2.236 which has been assigned as $g_{\parallel}$. The observed $g$ values are in the range of tetragonal Cu\textsuperscript{2+} complexes with loosely bound axial ligand. The feature with $g_{\parallel} > 2.1 > g_{\perp} > 2.0$ confirm the tetragonal co-ordination environment for Cu\textsuperscript{2+}. 

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Fig 4.7: X-band EPR spectra of [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$
  as microcrystalline solid

All the spectroscopic results discussed above confirms the structure of
[LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$ as shown in Scheme 4.2 below.

Scheme 4.2
4.3.1.5 Mass Spectroscopy study for

\[ \text{[LCu}^{2+}(\text{CH}_3\text{COO})_2\text{Cu}^{2+}L](\text{CH}_3\text{COO})_2 : \]

The ESI-MS spectra recorded for the complex has been shown in Fig. 4.8. A number of peaks were observed which could be correlated to various fragments of the complex. The peaks with their assigned fragments have been shown in Table 4.2. No peak corresponding to the molecular mass of the complex was observed which may be due to the complete breakdown of the complex by high energy electron spray.

![Fig. 4.8: ESI-MS spectra of [LCu^{2+}(CH_3COO)_2Cu^{2+}L](CH_3COO)_2](image-url)
Table 4.2: ESI MS peaks and the corresponding assigned molecular fragments for 
\([\text{LCu}^{2+}(\text{CH}_3\text{COO})_2\text{Cu}^{2+}\text{L}]\)(\text{CH}_3\text{COO})_2

<table>
<thead>
<tr>
<th>Fragments</th>
<th>Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>121</td>
<td>\text{CH}_3\text{COO.Cu(II)}</td>
</tr>
<tr>
<td>168</td>
<td>[ \text{N} \quad \text{O=CH} \quad \text{N} \quad \text{C=O} ]</td>
</tr>
<tr>
<td>207</td>
<td>[ \text{C} \quad \text{O=CH} \quad \text{N} \quad \text{Cu} ]</td>
</tr>
<tr>
<td>329</td>
<td>[ \text{C} \quad \text{O=CH} \quad \text{O} \quad \text{CH}_3 \quad \text{O} \quad \text{CH} \quad \text{O} \quad \text{CH}_3 ]</td>
</tr>
<tr>
<td>455</td>
<td>[ \text{N} \quad \text{Cu} \quad \text{O} \quad \text{CH}_3 \quad \text{CH}_2 \quad \text{O} \quad \text{CH} ]</td>
</tr>
</tbody>
</table>
4.3.1.6 Magnetic moment measurement for 
[LCu$^{2+}$\((CH_3COO)_2Cu^{2+}L\)](CH$_3$COO)$_2$:

To investigate spin state of Cu$^{2+}$ in the complex, the magnetic susceptibility measurement was done at 298 K. The experimental value of magnetic moment was calculated to be 2.88 BM using the molecular weight corresponding to the structure shown in Scheme 4.1. The spin only value for magnetic moment by two Cu$^{2+}$ ions should be 2 x 1.732 BM = 3.464 BM. The lowered value of magnetic moment is due to antiferromagnetic coupling of the magnetic moment of individual Cu$^{2+}$ ions.

4.3.1.7 Thermogravimetric analysis (TGA) study for 
[LCu$^{2+}$\((CH_3COO)_2Cu^{2+}L\)](CH$_3$COO)$_2$:

[LCu$^{2+}$\((CH_3COO)_2Cu^{2+}L\)](CH$_3$COO)$_2$ was subjected to thermogravimetric analysis (TGA) and Fig 4.9 shows the % weight loss versus temperature curve. Two weight drops were observed with initiation temperatures 110 °C and 240 °C. The first weight loss observed was 11.19% which could be due to the loss of the two acetate anions. Since theoretically calculated weight losses due to two acetates being 11.78%. The second weight loss (71.85%) should be due to the loss of all the other ligands and formation of CuO, the calculated weight loss being 72.45%. Hence TGA analysis supports the structure of the complex as depicted in Scheme 4.1.
4.3.2 Application of \([\text{LCu}^{2+} \text{(CH}_3\text{COO)}_2 \text{Cu}^{2+} \text{L}_1 \text{(CH}_3\text{COO)}_2\) as electrode modifying agent to distinguish DA and AA:

4.3.2.1 Intercalation of \([\text{L}_1 \text{Cu}^{2+} \text{(CH}_3\text{COO)}_2 \text{Cu}^{2+} \text{L}_1 \text{(CH}_3\text{COO)}_2\) in ZSM-5:

0.5 g of ZSM-5 and 0.2 g of \([\text{LCu}^{2+} \text{(CH}_3\text{COO)}_2 \text{Cu}^{2+} \text{L}_1 \text{(CH}_3\text{COO)}_2\) were taken in 50 mL of distilled water and stirred for 36 hours at room temperature. A bluish green product was obtained which was filtered, washed with distilled water and dried.

The intercalation of the complex into ZSM-5 was characterised by FTIR spectroscopy (Fig.4.10). Presence of Cu-N stretching peak at 451.79 cm\(^{-1}\) indicates the intercalation of \([\text{LCu}^{2+} \text{(CH}_3\text{COO)}_2 \text{Cu}^{2+} \text{L}_1 \text{(CH}_3\text{COO)}_2\) in ZSM-5. The shift of the 1057.4 cm\(^{-1}\)
peak to 1097.51 cm\(^{-1}\) with increased intensity also supports the intercalation. Presence of ZSM-5 was characterized by the peak at 553.73 cm\(^{-1}\) [55].

![FTIR spectrum](image)

**Fig 4.10:** FTIR of \([\text{LCu}^{2+}(\text{CH}_3\text{COO})_2\text{Cu}^{2+}\text{L}\text{]}(\text{CH}_3\text{COO})_2\) loaded with ZSM-5

### 4.3.2.2 Preparation of the modified electrode:

50 mg of \([\text{LCu}^{2+}(\text{CH}_3\text{COO})_2\text{Cu}^{2+}\text{L}\text{]}(\text{CH}_3\text{COO})_2\) encapsulated ZSM-5 was powdered and dispersed in a solution of 0.3 mL THF and 0.2 mL styrene. The volume of styrene to be taken was standardised by making the film from a series of solutions containing 0.05 mL, 0.1 mL, 0.2 mL, 0.3 mL and 0.5 mL of styrene. It was found that

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when the film was prepared from solution containing less than 0.2 mL styrene, the copper complex leached out from the film. This was confirmed by taking UV/visible spectra of the solution in which the modified electrode was kept for 2 hours. The leaching effect is not observed when film was prepared from solutions containing 0.2 mL styrene or more, but the currents of the CV as well as SWV peaks were found to be maximum for the film prepared from solution containing 0.2 mL styrene. 25 μL of the prepared suspension was placed on the surface of the pre-cleaned GC electrode surface and allowed to dry in air for about 30 minutes. This modified electrode shall be designated as \([\text{LCu}^{2+}(\text{CH}_3\text{COO})_2 \text{Cu}^{2+}L](\text{CH}_3\text{COO})_2/\text{ZSM-5/GC}\) henceforth in this chapter.

4.3.2.2 Electrochemical characterization of the modified electrode:

Fig 4.11 shows the cyclic voltammogram of the \([\text{LCu}^{2+}(\text{CH}_3\text{COO})_2 \text{Cu}^{2+}L](\text{CH}_3\text{COO})_2/\text{ZSM-5/GC}\) electrode in phosphate buffer (pH 7.0) at different scan rates. At the scan rate 0.020 Vs\(^{-1}\) the oxidation and the reduction peaks were observed at -0.006 V and -0.214 V respectively. The redox potential value was calculated to be -0.110 V and the separation in peak potential was 0.208 V. With the increase in scan rate the oxidation peak current was found to increase with no change in peak position. The plot of peak current versus scan rate was found to be linear as shown in Fig. 4.11, inset. This linear dependence of peak current on the scan rate is a characteristic of surface adsorbed electrochemistry. Effect of scan rate on the reduction process was interesting as the peak potential gradually shifted towards negative direction with a decrease in currents. The redox potential obtained at scan rate 0.020 V s\(^{-1}\) was further confirmed by SWV.
4.3.2.2 Effect of pH on the electrochemical behaviour of the modified electrode:

Both cyclic voltammogram and SWV were recorded for the \([\text{LCu}^{2+}](\text{CH}_3\text{COO})_2\text{Cu}^{2+}\text{L}]^{2+}/\text{ZSM-5/GC}\) electrode at different pH values in the range 5.0 to 8.5 in PBS. The pH of the electrolytic medium did not affect either the currents of redox peaks or the redox potential. Hence, all the electrochemical experiments were performed at pH 7.0.
4.3.2.2.3 Determination of accumulation time of DA and AA onto the modified electrode:

SWV was recorded for the modified electrode in the potential range +0.600 V to -0.400 V, while the electrolytic medium contained 1.0 mM DA with 0.1 M NaNO$_3$ as supporting electrolyte. The voltammograms were recorded immediately after the insertion of the modified electrode in the electrolytic solution and at an interval of 30 seconds. A plot of current versus time showed that the current was highest at 90 seconds and remains constant within experimental error thereafter. Similar experiments with AA (1.0 mM with 0.1 M NaNO$_3$) yielded an accumulation time approximately 100 seconds. Hence in all our works the electrode was allowed to be in the solution for at least 180 seconds before any measurements were made.

4.3.2.2.4 Simultaneous determination of DA and AA:

Fig. 4.12A shows the square wave voltammogram of 0.1 mM DA and 1.0 mM AA mixture in PBS at pH 7.0 when a bare electrode was used. A peak at +0.130 V was accompanied by a shoulder at +0.220 V, hence the bare electrode could not distinguish the AA and DA. When the bare electrode was replaced by the modified electrode, two well separated square wave voltammetric peaks were obtained (Fig.4.12B). The peaks corresponding to DA and AA were identified by recording square wave voltammogram of the modified electrode in solution of DA or AA. The peak at +0.230 V was identified to be due to DA and the peak at -0.090 V was identified to be due to AA, the separation between the two peaks is 0.320 V. This separation is much higher than the reported 0.210 V separation in peak position for DA and AA [26] showing the effectiveness of this modified electrode. To further investigate the independency of the modified electrode towards one analyte (DA or AA) in presence of the other, square wave voltammogram was recorded at different added concentration of DA (from 0.1 mM to 1 mM) when the electrolyte contained 0.01 M AA (Fig.13A). An excellent linear relation ($i/\mu$A = 1.189[DA] – 0.2823) between current versus DA concentration with $R^2$ = 0.9617 confirmed that the modified electrode can independently detect DA in presence of AA. Fig. 6B shows the similar result for AA in presence of 0.01 M DA and the
linearity of current versus concentration plot was excellent ($i/\mu A = 1.462[AA] + 2.799$) with $R^2 = 0.9882$. Hence, the modified electrode is effective in determining DA in presence of AA and also in determination of AA in presence of DA.

![Graph showing current (I/\mu A) vs. voltage (E/V)](image)

Fig. A
Fig. 4.12: Square wave voltammogram of a mixture of 0.1 mM DA and 1.0 mM AA at a bare GC electrode (A) and at a [LCu(II)(CH$_3$COO)$_2$Cu(II)L] (CH$_3$COO)$_2$/ZSM-5/GC electrode (B).
Fig. 4.13: Square wave voltammogram at [LCu(II)(CH$_3$COO)$_2$Cu(II)L] (CH$_3$COO)$_2$/ZSM-5/GC electrode – (A) at different added concentration of DA (from 0.1 mM to 1 mM) when electrolyte contained 0.01 M AA; (B) at different added concentration of AA (from 0.1 mM to 1 mM) when electrolyte contained 0.01 M DA.
Fig. 4.14: Square wave voltammetric response of the [LCu(II)(CH$_3$COO)$_2$Cu(II)L] (CH$_3$COO)$_2$ / ZSM-5/GC electrode towards a mixture of DA and AA when concentration of both of them were increased simultaneously in the electrolytic medium.
Fig. 4.15: Plot of SWV current versus concentration of DA (A) and AA (B) when a mixture of both was added maintaining a [DA]:[AA] = 1:10.
Fig. 4.14 shows the square wave voltammetric response of the [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L]/ZSM-5/GC electrode towards a mixture of DA and AA when concentration of both of them were increased simultaneously in the electrolytic medium maintaining [DA]:[AA]=1:10. The separation in peak positions with increase in the concentrations of DA and AA increased marginally. This proves that the electrode’s response towards DA or AA is independent of each other even at a relatively high concentration. The currents of the square wave voltammetric peaks for both the DA and AA were found to increase linearly with an increase in their concentration in the electrolytic medium (Fig. 4.15). The linear relation between the concentration and current for DA is $i$/$\mu$A = 0.463[DA] + 0.560 ($R^2 = 0.983$) and for AA is $i$/$\mu$A = 0.768[DA] + 1.329 ($R^2 = 0.986$). This result confirmed the capability of the modified electrode in determining simultaneously DA and AA in their mixture. The relative standard deviation of concentration determination was 3.12% for DA and 3.35% for AA and is in close agreement with reported ones [55]. The dynamic range for DA detection was 0.7x10$^{-6}$ M to 3.0x10$^{-6}$ M and that for AA was 0.6x10$^{-5}$ M to 3.2x10$^{-5}$ M.

The detection limits of DA in presence of AA was determined and found to be 2.91x10$^{-7}$ M while that for AA in presence of DA was found to be 3.5 x 10$^{-7}$ M. This detection limit for DA is better than the detection limit of 1.4x10$^{-6}$ M for voltammetric sensor based on bimetallic copper complex [52]. GC electrode modified by Nafton membrane doped with copper dipyridyl complex showed detection limit of 9.0 x 10$^{-6}$ M which is also higher than the value reported in this paper [56]. The detection limit is slightly better than the 3.1x10$^{-7}$ M value reported for GC electrode modified by 5-hydroxytryptophan [57]. This detection limit is also found to be better than the detection limit (4.2x10$^{-7}$ M) obtained at amperometric sensor based on single walled carbon nano tube [58].

4.3.3 Superoxide dismutase (SOD) activity of [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$:

The SOD activity [LCu$^{2+}$(CH$_3$COO)$_2$Cu$^{2+}$L](CH$_3$COO)$_2$ was determined spectrophotometrically by the NBT assay employing KO$_2^-$ as the source of superoxide radical [59]. One unit of SOD activity was defined as the concentration of the test
substance required for 50% inhibition of NBT reduction by the superoxide (IC\textsubscript{50} value) anion. The SOD activity measured for the present copper complex by the method of NBT reduction yields a linear relationship between the concentration of the copper compound and the subsequent inhibition of superoxide ions. The IC\textsubscript{50} value for the copper compound is found to be 3.5x10^{-4} M, which is comparable with that found for the complex \([\text{Cu (aspirinite)}_2(\text{DMSO})_2]\) [60].

Fig 4.16: Percentage of inhibition of NBT reduction as a function of [LCu\textsuperscript{2+}(\text{CH}_3\text{COO})_2Cu\textsuperscript{2+}L](\text{CH}_3\text{COO})_2
4.4 Conclusion:

A new copper complex $[\text{LCu(II)(CH}_3\text{COO)}_2\text{Cu(II)L}](\text{CH}_3\text{COO})_2$ where L is N,N bis(pthalimide)ethylenediamino was synthesized and characterized. This complex was encapsulated in ZSM 5 with which GC electrode surface was modified. Thus modified GC electrode acts as a sensor for simultaneous determination of DA and AA in a concentration ratio $[\text{DA}]:[\text{AA}] = 1:100$. Compared to bare electrode, the modified one can separate the oxidation peaks of DA and AA by 0.320 V. The detection limits for DA in presence of AA is $2.91 \times 10^{-7}$ M while that for AA in presence of DA is $3.5 \times 10^{-7}$ M. The oxidation of DA is independent of the presence of AA and vice versa. Further a number of metal ions - Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Fe$^{2+}$; anions Cl$^-$, SO$_4^{2-}$; and Glucose has no effect on the determination of DA or AA by this new modified electrode.
4.5 References:

