Studies on the Oxidation of α-Amino acids by Peroxomonosulphate Catalysed by Biopolymers Stabilized Copper Nanoparticles – Effect of Stabilizers

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Abstract
Copper nanoparticles (Cunps) are synthesized using biopolymeric stabilizers such as polyethylene glycol (PEG) and carboxymethyl cellulose (CMC) and synthetic polymer poly N-vinyl pyrrolidone (PVP) stabilizer using wet chemical method, using hydrazine hydrate as the reductant. The particles are size characterized using UV-VIS SPR spectra, FE-SEM and HR-TEM, and the values are found to be 6nm, 12nm and 15nm±1nm respectively. The as synthesized Cunps are used as catalyst for the oxidation of free α-amino acids by peroxomonosulphate (PMS) in aqueous medium. The time variances in the UV spectra are used for kinetic parameter determination. Cyclic voltammetry of α-amino acids and PMS with and without using the Cunp catalyst are measured to study the role of Cunp interactions. The products N-hydroxylated aminoacids are identified using TLC and FT-IR spectra. For any free α-aminoacid, PEG stabilised Cunps have more catalytic efficiency than CMC stabilised Cunps which are more efficient than PVP stabilised Cunps. Keeping the Cunp catalyst constant the trend in the oxidation of free α-amino acids is alanine>glycine>leucine>valine>phenylalanine>Serine. For oxidation of biomolecules biopolymer stabilised metal nanoparticles perform better than the synthetic stabilizers.

Key words: copper nanoparticles, polyethylene glycol, carboxymethyl cellulose, poly N-vinyl pyrrolidone, α-amino acids, peroxomonosulphate.

1. Introduction
Oxidation reactions of α-amino acids (AA) are one of the most relevant biochemical reactions because, such reactions serve as models for protein oxidations [1-3]. Also, uncharacterized oxidation reactions of α-amino acids involving a wide range of oxidants are of particular concern in biotechnology and medicine. Simple amino acids present in municipal waste waters cause serious eutrophication in water bodies. Pharma industries dealing with biochemicals and tanneries are some of the major sources of waste waters containing amino acids [4]. It is essential to remove or oxidatively degrade the dissolved amino acids from waste waters. Non enzymatic model oxidation reactions involving α – amino acids and a wide variety of oxidants are reported plenty in literature [5-10]. Some selective oxidation reactions are reported involving metal catalysts. Transition metal ions of Ag, V, Fe, Ru, Mn etc. are reported to act as catalyst for some of the α – amino acid oxidations[11-15].

With the emergence of metal nanoparticles possessing appreciable stability and high surface area per particle, their potential use as catalyst for organic biochemical relevant reactions stands well documented in recent years [16 -17]. The bio polymers stabilized mono metal nanoparticles of cheap, cost effective and abundant transition metals like Cu, Ni, Fe etc. when used as catalysts are expected to produce ecofriendly process enroutes [18-22]. In the present work the bio polymers such as poly ethylene glycol (PEG) and carboxymethyl cellulose (CMC) are used in the preparation of Copper nanoparticles (Cunps) and the subsequent catalytic activities are compared with the similarly synthesized Cunps having poly N-vinyl pyrrolidone (PVP) which is a synthetic polymer, as the stabilizer. PVP is a widely used metal nanoparticle stabilizer in numerous mono and bi metal nanoparticle preparations. α- amino acids such as glycine (gly), phenyl alanine (phe), leucine (leu), valine (val) and serine (ser) are chosen for the oxidation with sodium permonosulphate, an hydrophilic oxidant. Pseudo first order rate coefficient values are used to investigate the catalytic activity of Cunps, having three different stabilizers.

2 Experimental Sections
2.1 Reagents
CuCl₂, H₂O₂, NaBH₄, α-amino acids listed in Table 1 were analytical grade reagents and supplied by SRL, India. The samples were used as such without further purification. The molecular weights of PEG, CMC and PVP are 57 to
63KD, 90KD and 40KD respectively and are purchased from SRL, India. The reducing agent hydrazine hydrate and ammonia solutions are purchased from Qualigens, India. Milli-Q deionized water was used wherever necessary. Ethylene glycol (EG) and ethanol (EtOH) solvents were freshly distilled before use.

2.2 Synthesis of Cunps with PEG, CMC and PVP stabilizers

Copper nanoparticles are synthesized using CuCl₂,2H₂O as precursor, hydrazine hydrate as the reducing agent and PEG, CMC and PVP as the capping agents. Equal volumes of 0.01M metal salt, 0.01M reducing agent and 3% stabilizing agent solutions are mixed drop wise with constant stirring in a three necked flask having a total volume of 250mL. After the addition, 10mL of 5.0M aqueous ammonia solution was added to maintain basic medium. Stirring was continued for two hours. The surface plasmon resonance peaks of copper nanoparticles in EG-EtOH medium are taken using UV-VIS Spectrometer to confirm the formation of nanoparticles.

2.3 Particle Size Characterization

The UV-VIS surface plasmon resonance (SPR) spectra of the three types of copper nanoparticle solutions having PEG, CMC and PVP stabilisers are presented in Fig. 1. The SPR peaks at 421nm, 571nm and 580nm correspond to the copper nanoparticles having PEG, CMC and PVP as stabilizers. The larger wavelength peak samples show higher nanosize range [23-25]. Using rotovapour the nanoparticles are recovered from the solution and analyzed by powder XRD using Philips PW 1050/37 model diffractometer, operating at 40 kV and 30 mA. Cu Kα radiation with wavelength of 1.54Å and a step size of 0.02° in the 2θ range, 10–80° was used. Fig. 2 presents the XRD profiles of Cunps. FESEM and HR-TEM of the nanoparticles are measured using SU6600, HITACHI model operating at an accelerating voltage of 100kV and FEI-TECNAI G², model-T-30-S-twin operating at an accelerating voltage of 300kV instruments respectively. The FESEM and HR-TEM photographs are shown in Fig. 3. The morphology and the size of the nano crystallites are measured from HR-TEM images. The values coincided with those calculated from Scherrer formula using XRD data. The sizes of Cunps are given and the nano particles are found to be spherical and nearly monodisperse in nature. From the HRTEM photographs shown in Fig. 3, the average particle size was determined by counting 300 particles and found to be 6nm, 12nm and 15nm corresponding to PEG, CMC and PVP stabilized copper nanoparticles respectively. In Fig. 3 FESEM photographs are given and the nano particles are found to be spherical and nearly monodisperse in nature. From the HRTEM photographs shown in Fig.3, the average particle size was determined by counting 300 particles and found to be 6nm, 12nm and 15nm corresponding to PEG, CMC and PVP stabilized copper nanoparticles respectively.

2.4 Catalytic Oxidation

In a typical experiment, double walled three necked 100ml glass round bottomed flask was used into which 50ml of fresh aqueous solution of 10mM sodium peroxo-monosulphate was added drop wise.10mL solution of the 1mM α- amino acid and 5mL of Cunps solution diluted to 30mL aqueous solution are added drop wise from the each neck of the flask simultaneously with continuous stirring. The temperature was maintained at 30°C by circulating thermo stated water. The absorbance spectra of the solution are recorded at regular time intervals immediately after the addition of the catalyst. In the absence of Cunps, it took more than 8 hours to initiate the oxidation of amino acids and proceeded very slowly for several hours under the same conditions. In the presence of Cunps, the reaction completed within 2-3 hours time. The product was recovered by EtOH solvent extraction. The completion of the reaction was ensured by UV-VIS spectra where the absorbance decreased to baseline. The time of completion of the reaction was noted during this process. If any unreacted reactant remained, from the absorbance values the concentrations are noted and the % substrate reacted within the time of reaction was calculated. From the absorbance variation with time intervals, the progress of the reaction was monitored. Pseudo first order conditions were maintained by adding excess of oxidant with respect to the reactants. Kinetic plots constituting logODt/OD0 versus time are plotted and the slope values when multiplied by 2.303 produced the rate coefficient (k) values. ODt and OD0 are the values of absorbances at time equal to zero and at any time ‘t’ respectively.

2.5 Cyclic Voltammetry

A fully computerized electrochemical system (model PGSTAT-12) was used along with the three electrode system cell under nitrogen purging. Glassy carbon electrode (purchased from Sinsil International) as the working electrode, Ag/AgCl as the reference electrode and platinum wire as the counter electrode are used. 1M KNO₃ aqueous solution served as the supporting electrolyte. Milli-Q deionised water was used throughout.

The glassy carbon electrode (GCE) was polished with fine alumina powder (lower than 0.3micron grain size) on a wet polishing cloth and washed repeatedly with Milli-Q water. This procedure was repeated until GCE surface was a shiny black mirror. The cell was filled with the desired volume (20mL) of the analyte solution and nitrogen purged. nitrogen (analar grade) gas was used for purging.

3. Results and Discussion

3.1 Morphology and Nanoparticle size Analysis

The Cu nps formation pathway in the presence of hydrazine hydrate reductant can be considered as follows:

\[2\text{Cu}^{2+} + 4\text{H}_2\text{O} \rightarrow 2\text{Cu} + 4\text{H}_2\text{O}^{+}\]

The ammonia addition neutralizes the H⁺ ions. The peak patterns in the XRD patterns of the Cunps shown in Fig.2 are assigned according to JCPDS data file [26-28]. Applying the Scherrer formula on the peak with highest intensity, the sizes are found to be 6nm, 11.5nm and 15nm corresponding to PEG, CMC and PVP stabilized copper nanoparticles respectively. In Fig. 3 FESEM photographs are given and the nano particles are found to be spherical and nearly monodisperse in nature. From the HRTEM photographs shown in Fig.3, the average particle size was determined by counting 300 particles and found to be 6nm, 12nm and 15nm corresponding to PEG, CMC and PVP stabilizers. These values agree with the XRD results. The polydispersity of the sample was found to be narrow and within 8% of the particles with differed sizes. The trend in the sizes of Cunps increases as PEG<CMC<PVP. That is, the biostabilisers produce smaller nanoparticles than the synthetic polymer PVP, under similar conditions.

3.2 Catalytic oxidation of α – amino acids

A combination of Cunps and the oxidant PMS are found essential to bring out the oxidation reaction of α – amino acid at 30°C. All the oxidations are carried out with excess of the oxidant for pseudo kinetic conditions. The products
Fig 1. Typical UV – VIS spectra of Cunps having a) PEG, b) CMC and c) PVP as stabilizers in EG – EtOH medium, at 25°C are isolated from solvent extraction technology after the completion of the reaction. Using TLC method, the oxidized products are sorted out and by doing so, only single spots are obtained. In scheme 1, the overall oxidation reaction studied in the present work has been put forth. PMS acts as an oxidant through its oxygen atom transfer. Hence the reaction under mild thermal effects, involves amine group transformation to N-hydroxyl amino group. This forms the initial stage of oxidation of amino acids and may be so in case of proteins comprising these α – amino acids. Presences of N-hydroxyl amino groups are ascertained using FT-IR.

Scheme 1. The overall oxidation of α – amino acid by an oxygen atom transfer from peroxomonosulphate in aqueous medium

In Fig 4, the typical UV – VIS spectra of the α – amino acids under various catalyst conditions are presented. The absorbance – time variance spectra for the six α – amino acids in presence of the three types of Cunps are presented in Fig.5. The corresponding kinetic plots for the rate coefficient values determination by plotting log OD₀/ODₜ versus time, are presented in Fig 6. When the slope values of the linear plots are multiplied by 2.303, the

Fig 2. XRD patterns of a) PEG-Cu b) CMC-Cu and c) PVP-Cu nanoparticles

Fig 3. HRTEM and FESEM images of a,d) Cu-PEG b,e) Cu-CMC and c,f) Cu-PVP nanoparticles with 6nm, 12nm and 15nm (±1nm) sizes respectively.
rate coefficient (k) values are obtained. In Table 1, the reaction parameters such as %substrate reacted; time of reaction and the pseudo first order rate coefficient values for the three types of Cunps employed as catalyst are given. In Table 1 based on the k values, it may be seen that Cunps stabilized with PEG catalyse better than the Cunps containing CMC and PVP. The catalytic activity of the Cunps seems different when the organic stabilizer nature was changed such as PEG, CMC and PVP. Although there are no particular chemical interaction between the organic stabilizer and the respective aminoacids, the difference in catalytic activity can be attributed to the size variations in the resulting Cunps. Interestingly it was found that keeping other conditions constant, the catalytic activity depends on the sizes of nanoparticles. The trend in the size of the nanoparticles being PVP>CMC>PEG. The trend in the rate coefficient values being PEG>CMC>PVP. This effect can be attributed to the nanosizes of the particles, that as size decreases surface area increases and the active centers proportionately increase too. Upon comparing the nanoparticle sizes, PEG – Cunps are smaller than CMC – Cunps which in turn is smaller than PVP – Cunps. Because of the larger surface area to volume ratio value per particle, and for the PEG and CMC polymers having more oxy (-O) and hydroxyl (-OH) functional groups that efficiently help in the binding of the oxygen from the peroxomonosulphate ion through H-bonding, the above trend seem to be followed. PEG and CMC possibly show greater extents of H-bonding forces in addition to the capping and stabilizing effects of Cunps than the PVP stabilized Cunps. Keeping the PEG-Cunps in constant amounts, the reactivity trend set among the six α – amino acids investigated here has been found to be alanine>glycine>leucine>valine>phenylalanine>Serine. Also, the overall trend determined correlates to the structural effects of the α – amino acids. The rate coefficient values observed are comparable with the reported values of analogous oxidation reactions of α – amino acids reported[29]. Hence Cu being a cheap and abundant metal element when converted as nanoparticles using polymeric stabilizers, it has been found to act as a good catalytic agent. It is also encouraging to note that Cunps having greener stabilizers like PEG and CMC prove to be effective catalysts than those with synthetic polymer(PVP) as stabilizer.

The effect of catalyst mass in the feed was studied with the view of monitoring the overall reaction periods and % yield. When time of completion was noted for the AA system listed in Table 1, long durations are noticed. Therefore to reduce the reaction periods to 5 minutes catalyst mass control was adopted instead of lowering of reactant concentrations. Optimization studies showed that when 7.0mg of the nano Cu-PEG catalyst was loaded into the reaction mixture 80% conversion was achieved within 5 minutes of the reaction period. Keeping the catalyst mass constant as 7.0mg, depending on the AA nature slightly longer periods were detected as in the case of leu to ser in Table 1. Since catalyst mass variations produced significant results for 5.0mg of the catalyst loading, the yield % of the
product was monitored. In Table 1, the effect of constant catalyst mass (5mg) on the six AAs studied show that ala produced the highest 57% yield and a decrease effect was found leading to ser producing the least 24% as yield. When nano Cu-CMC and nano Cu-PVP catalyst are used the catalyst mass effect for 7.0mg showed the reaction periods near 5 minutes with longer durations for ser type AAs. The % yield for 5.0mg of the catalyst when kept constant, lower % yield than 50% only was recorded. The optimization results indicate that to achieve a minimum of 80% conversion near 5 minutes reaction period, 7.0mg of the catalyst was required. Also 5.0mg of the catalyst mass when kept constant PEG-Cu nps produced near 50% yield while CMC-Cu nps and PVP-Cu nps and other AAs combined systems produced lower than 50% yield (Table 1).

3.3 FT–IR spectral studies
After completion of the reaction when the contents are analyzed through TLC, single and bigger spots are extracted out using organic solvents like EtOH. The end products are recovered from the extractions and subjected to FT–IR analysis. In Fig.7, the FT–IR of N-hydroxylated α-amino acid products are presented. The highest frequency peaks are observed around 3650cm⁻¹ in the products from all the amino acids which are identified with the O-H stretchings. Identification of strong peaks around 3300cm⁻¹ can be assigned for N-H stretchings. Around 895cm⁻¹

**Fig 5** Absorbance – time dependence plots for the oxidation of α-amino acids by sodium peroxomonosulphate, with a) PEG - Cu, b) CMC – Cu and c) PVP – Cu nanoparticles as catalysts at 25°C

**Fig. 6** Kinetic plots for the rate coefficient determination of the oxidation of α-amino acids with sodium peroxomonosulphate catalysed by a) PEG - Cu, b) CMC – Cu and c) PVP – Cu nanoparticles

**Fig. 7** FT-IR Spectra of N-hydroxylated α-amino acids.
Fig. 7 FT-IR spectra in KBR pellets at 25°C of the N-hydroxylated products from the oxidation reactions of aminoacids a) glycine; b) leucine; c) valine; and d) phenylalanine.

region strong peak is observed which is attributed for N-O stretching. The IR peaks at 1625cm⁻¹, 1357cm⁻¹ and 1115cm⁻¹ are attributed to ONH, NOH and HNO bending and asymmetric modes respectively. The skeletal vibrations and the overall fingerprint region all remain same with the parent compound which indicates that the amino group is selectively hydroxylated.

3.4 Cyclic Voltammetric studies

In Fig.8, the cyclic voltammograms of PEG-Cunps, α-amino acid (AA) and PMS added into the analyte solution in different sequences are presented. As a representative case, leucine has been considered. In Fig 8a.1, blank cyclic voltammogram of the supporting electrolyte 1M KNO₃ at pH=7.0 (maintained by 0.01M buffer solution) has been given. When Cunp solution is added, the first anodic peak at -120mV corresponding to Cu(0) to Cu(I) and the second anodic peak at +209mV for Cu(I) to Cu(II) are seen in Fig.8a.2. The respective cathodic peaks for Cu(II) to Cu(I) occur at +149mV and Cu(I) to Cu(0) at -180mV respectively. As soon as PMS was added,(Fig.8a.3) the first anodic peak intensity at -120mV decreased to very low value while the intensity of the second anodic peak at +209mV shot up. This effect indicates that, all the Cu(I) formed from Cu(0) is being instantly oxidized to Cu(II) while the cathodic peak remains the same. When leucine was added to the Cunp and PMS containing electrolyte solution, (Fig.8a.4) the cyclic voltammogram showed the development of a new peak at -5.2mV due to the

Fig. 8 Cyclic voltammograms of the α-Leucine, PMS and PEG-Cunps with three different sequential additions. (a) 2–KNO₃+Cunps, 3–KNO₃+Cunp+PMS; (b) 2–KNO₃+Cunps, 3–KNO₃+Leu+PMS; (c) 2–KNO₃+Leu, 3–KNO₃+Leu+Cunps; a,b,c 1 – KNO₃, 4 – KNO₃+Leu+Cunps+PMS respectively at 25°C.
Cu(I) – leucine complex formation as Cu(I) is formed from Cu(0). Presence of AA induces the complex formation so that the first anodic peak at -120mV is not seen. On the other hand the second anodic peak at +209mV shifts a little at 212mV showing that the interaction between Cu(II) and leucine is insignificant. In the cathodic region, in addition to +149mV and -180mV there is a new peak at -65.3mV which is the reversible cathodic peak corresponding to anodic peak at -5.2mV. In Fig 8.b, the sequence of addition of Cunps and AA are interchanged and the cyclic voltammograms of the blank, blank with leucine, blank with leucine and PMS show that there is no noticeable electro activity under the chosen conditions. However as soon as Cunps are added, the cyclic voltammogram in Fig 8b.4 similar to Fig 8a.4 is found. That is, no anodic peak at -120mV for Cu(0) to Cu(I) is seen. Presence of -5.3mV anodic peak refers to Cu(I)-leu complex and in the cathodic region the corresponding reductions at +149mV for Cu(II) to Cu(I) in the complexed state and at -65.3mV for the reduction of Cu(I)-leu complex and at -180mV for remaining Cu(I) to Cu(0) transformations are detected. In Fig 8.c.1 and 2, the cyclic voltammograms of supporting electrolyte and the AA show no peaks. When the Cunps are added the cyclic voltammograms showed medium intensity peaks at -5.3mV, +209mV in anodic region while +149mV, -65.3mV and -180mV in the cathodic region respectively. To analyze mixture when PMS was added the cyclic voltammogram similar to Fig 8a.4 and 8b.4 with higher intensity of all the peaks are reproduced. All the α-AA chosen in present work are not electroactive by themselves and the initial interaction is between PMS and AA is weak. The transformation of the α-amino group in AA to N-hydroxyl amine occurs in presence of PMS only upon addition of Cunps while PMS is converted to hydrosulphate ion. The plausible mechanism in support of the observed kinetics and electrochemical observations is given in Scheme 2.

\[
\begin{align*}
\text{HSO}_4^- + Cu^0 & \rightarrow Cu^{2+} + HSO_4^- + O_2^- \\
Cu^{2+} + O_2^- + AA & \rightarrow \text{AANHOOH + Cu}^0 \\
\text{HSO}_4^- + AA & \rightarrow HSO_4^- + \text{AANHOH}
\end{align*}
\]

Scheme 2. The plausible enroute of Cunps catalyzed oxidation of α-amino acids.

4 Conclusions

In this study a range of six simple α – amino acids are oxidized to the respective hydroxylamine derivatives using Cunps as the catalyst and PMS as the oxidant. Smaller sized PEG – Cunps exhibit high catalytic activity compared to CMC and PVP stabilized Cunps in terms of time of reaction, % substrate reacted and the overall pseudo first order rate coefficient values. Within the α-amino acids investigated the trend with decreasing reactivity during oxidation is found as alanine > glycine > leucine > valine > phenylalanine > Serine.

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