Research Paper

Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

Investigations on Etching, Morphology and Biological Activity of Metal ions doped L-Aspartic acid Single Crystals

Chemistry

KEYWORDS : Doping; Etching; X-ray diffraction; Crystal Growth.

V. Renuga Department of Chemistry, National College, Trichy, Tamil Nadu.

ABSTRACT

The single crystals of alkali, alkaline earth and transition metal ions doped L-aspartic acid were grown from slow evaporation solution growth method. By this method the transparent crystal of metal ions doped L-aspartic acid were prepared and they were characterized by FTIR, UV, Powder X-ray diffraction analysis, Etching and antimicrobial analysis. The results are discussed in detail.

INTRODUCTION

Aspartic acid naturally occurs as an L- or D-form. D-Aspartic acid is a D-form enantiomer of aspartic acid which naturally occurs also as an L-form aspartic acid. Like many other chemicals, aspartic acid demonstrates a varied biological activity dependent on which enantiomer it is in a pair [1]. L-Aspartic acid (L-Asp) belongs to endogenous human amino acids involved in peptides and proteins formation which also partakes in purine and pyrimidine synthesis.

Aspartic acid is one of the 20 amino acids commonly found in animal proteins. Aspartic acid is the carboxylic acid analog of Asparagine. Aspartic acid is alanine with one of the β-hydrogen molecules replaced by a carboxylic acid group. Aspartic acid is a part of organic molecules containing an amino group, which can combine in linear arrays to form proteins in living organisms. Its acidic side chain adds a negative charge and hence a greater degree of water-solubility to proteins in neutral solution and has been shown to be near the active sites of some enzymes. Aspartic acid is a non-essential amino acid having an acidic carboxyl group on its side chain which can serve as both an acceptor and a donor of ammonia. It is converted to l-Asparagine by binding with ammonia. The stronger bonding of calcium to a peptide with Asp residues next to each other has analogy with organic diacids that have nearby carboxylic groups and have lower pKa1's than those with remote functional groups [2, 3]. The close proximity of one carboxyl group to another enhances the acidity of the latter. It is of great interest the knowledge of the affinity of aspartic acid for other metal ions and the structure which shows the position of functional groups in M-Asp complexes [4]. This would help researchers to understand such reaction mechanism much better.

Aspartic acid (L-Aspartic acid) (Fig. 1) and glutamic acid play important roles as general acids in enzyme active centers, as well as in maintaining the solubility and ionic character of proteins.

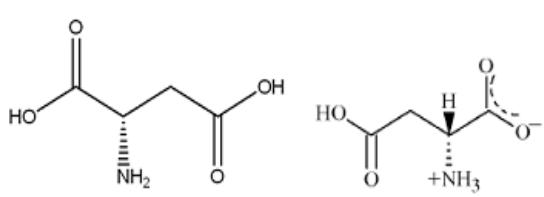

Fig.1Structure of L-Aspartic acid

Aspartic acid can help protect the liver from some sorts of drug toxicity and the body from radiation. Aspartic acid can also help to form the ribo-nucleotides that assist the production of DNA and RNA, and assists in energy production from carbohydrate metabolism. Aspartic acid may also help to improve the functionality of the immune system, and may play a role in protecting against toxins, and neural and brain disorders. Previous reports show that aspartic acid helps treat chronic fatigue. Aspartic Acid can easily be converted to glucose when demand for glucose exceeds supply [1,5]. Calcium ion is an intercellular messenger in many eukaryotic signal transducing pathways, such as vision [6], the phosphoinositide cascade, and the regulation of muscle contraction.

Despite the importance of aspartic acid only few studies have been done it. In order to find out the biological importance of L-Aspartic acid the present study involves the doping of alkali, alkaline earth and transition metal ions on L-Aspartic acid.

MATERIALS AND METHODS

SOLUBILITY STUDY OF PURE AND METAL IONS DOPED L-ASPARTIC ACID SINGLE CRYSTALS

Commercially available L-aspartic acid and metal sulphates (AR grade) were used for the solubility measurements and crystal growth. The solubility study is generally carried out to know the amount of the material available for the growth and hence defines the size of the crystal that can be grown. The solubility curves for pure and metal ions doped L-aspartic acid samples in water at different temperatures ranging from 30 to 50°C are shown in Fig.2. From the graphs, it was observed that solubility increases with increase in temperature for both the compounds and it was found to be more for the transition metal ions than for alkali and alkaline earth metal ions doped L-aspartic acid.

It is obvious that for the metal sulphate-doped sample, the solvent water is capable of accommodating a slightly increased amount of solute for saturation at a particular temperature. Since the solubility of the compounds increases with increase in temperature, the samples of this study have positive temperature coefficient of solubility. Hence pure and metal ions doped L-aspartic acid crystals can be grown from aqueous solution by slow solvent evaporation method.

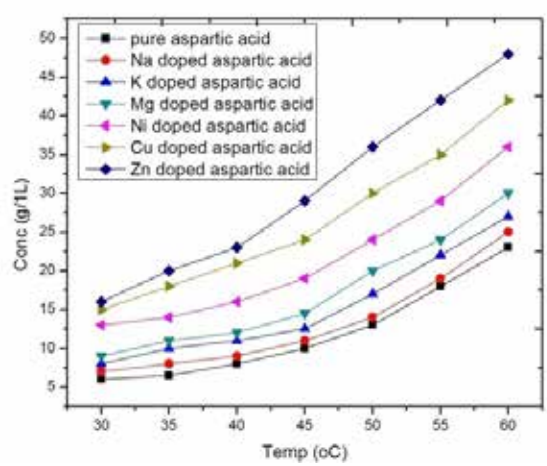

Fig.2 Solubility curve of pure and metal ions doped L Aspartic acid single crystals

The photographs of the prepared pure and metal ions doped L-aspartic acid are shown in Fig.3

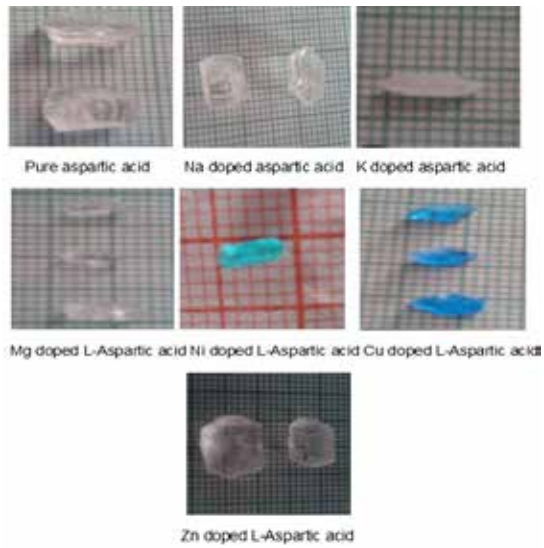

Fig.3 As grown good quality *L*-Aspartic acid single crystals.

SYNTHESIS OF PURE L-ASPARTIC ACID SINGLE CRYSTAL

The pure L-aspartic acid single crystal was synthesized by dissolving 10mmol of L-aspartic acid in 20ml of aqueous NH_3 (9:1) at room temperature. The reactants were stirred well for nearly 6 h using a magnetic stirrer in order to obtain a uniform mixture of the solution over the entire volume. The solution was finally filtered twice using micro-Whatmann filter papers to eliminate unwanted impurities. The filtered solution was then kept in crystal growth vessels followed by slow evaporation at room temperature. Transparent crystals were obtained in a period of 60 days.

SYNTHESIS OF METAL IONS DOPED L-ASPARTIC ACID SINGLE CRYSTALS

The metal sulphates doped L-Aspartic acid single crystals were synthesized by dissolving metal sulphate (Na^{2+},K^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) solution containing aqueous NH_3 (9:1) of L-aspartic acid at room temperature. The reactants were stirred well for nearly 6hrs using a magnetic stirrer in order to obtain a uniform mixture of the solution over the entire volume. The solution was finally filtered twice using micro-Whatmann filter papers to eliminate unwanted impurities. The filtered solution was then kept in crystal growth vessels followed by slow evaporation at room temperature. Transparent crystals were obtained in a period of 60-70 days.

RESULTS AND DISCUSSIONS

FT-IR SPECTRAL ANALYSIS OF PURE AND METAL IONS DOPED L-ASPARTIC ACID SINGLE CRYSTALS

The FTIR spectra were obtained using a Perkin Elmer spectrometer (Perkin Elmer,). Each sample was ground gently with dry potassium bromide (KBr) powder (1% w/w) in an agate mortar to avoid polymorphic transition during grinding and milling. Then, a manual press was used to form the pellet. The pellet was scanned 8 times with a resolution of 2 cm^{-1} in the region of 4000-400 cm^{-1}. FTIR was used to identify the metal ions present in aspartic acid crystals and is presented in fig.4.

In the infrared spectra, all the crystals have a strong and broad absorption appearing at ν =3011-3423 cm^{-1}, which is typical of molecules of high NH_2^+ content. A peak around 3505 cm^{-1} assigned to the stretching of OH was not observed in any of the compounds; the peak is probably obscured by the broad water absorption.

Metal coordination results in appreciable shifts of the asymmetric stretching frequency of the carboxylate moiety of the amino acids from 1605 to about 1590 cm^{-1}. The symmetric carboxylate stretching frequency shifts from 1402 to 1433 cm^{-1}. Strong and characteristic CN stretching frequencies between 1084-1118 cm^{-1} and 854-724 cm^{-1} are observed for the CH_2 rocking in all the doped crystals.

More over the presence of metal ions on L-aspartic acid exhibited corresponding characteristic frequencies. All these observations confirm the formation of dopant on L-aspartic acid.

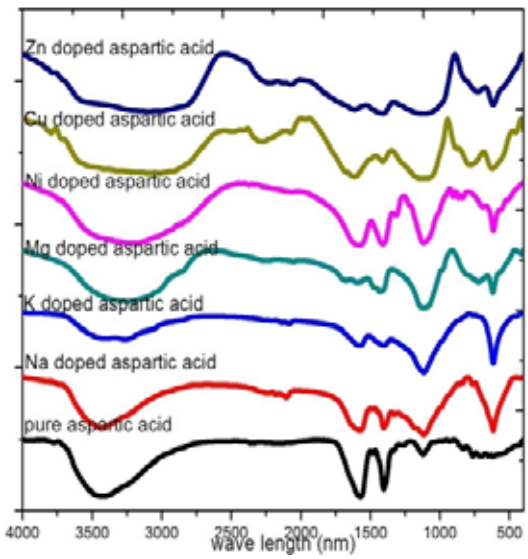

Fig.4 FT-IR Spectra of pure and metal ions doped L-Aspartic acid single crystals.

UV SPECTRA OF PURE AND METAL IONS DOPED L-ASPARTIC ACID SINGLE CRYSTALS

Optical absorption spectra of pure and metal ions doped L-Aspartic acid single crystals were recorded in the range of 200-800 nm and shown in figure 5. It is observed from the spectra that the maximum absorption is for Cu doped L-aspartic acid. Absorption intensity increases with the increase in dopant nature, such as from alkali to transition metal ions. In general, the high conjugation and delocalized π bonding orbitals of Cu^{2+} ions are responsible for the high intensity absorption in UV-region, due to this it shows double hump in the spectrum. But, in the case of Ni dopant the zwitter ionic property increases due to interaction between opposite charge ends of L-aspartic acid. This in turn reduces the delocalization of π bonding orbital. As a result, the electron jump takes high energy and hence absorption occurs in the shorter wavelength which is also evident from the spectrum.

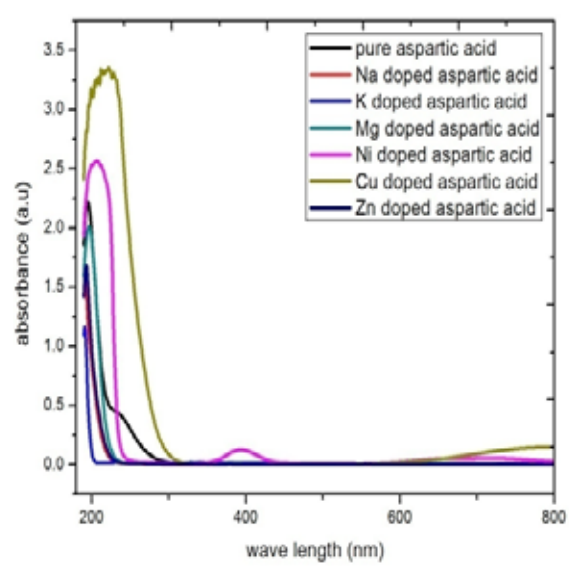

Fig.5 UV Spectra of pure and metal ions doped L-Aspartic acid single crystals

Volume : 3 | Issue : 6 | June 2014 • ISSN No 2277 - 8179

POWDER XRD ANALYSIS OF PURE AND METAL IONS DOPED L-ASPARTIC ACID

The grown crystals of L-aspartic acid and metal ions doped L-aspartic acid were subjected to powder X-ray diffraction studies to find the lattice parameters. Powder XRD study carried out using CuKα (λ=1.540598 Å) radiation revealed that pure L-aspartic acid crystallized in monoclinic system and that for most of the metal ions doped L-aspartic acid crystallizes in orthorhombic system with lattice parameters a=9.647Å, b=9.748 Å, c=10.855 Å. Comparisons of unit cell parameters of grown crystals along with metal ions doped are shown in table-1.

Table 1:
Comparison of unit cell parameters of grown crystals along with metal ions doped L-aspartic acid

	Pure L-Aspartic acid	Na doped L-Aspartic acid	K doped L-Aspartic acid	Mg doped L-Aspartic acid	Ni doped L-Aspartic acid	Cu doped L-Aspartic acid	Zn doped L-Aspartic acid
hkl	(120) (-121) (240)	(102) (201) (411) (145)	(200) (211) (240)	(011) (202) (404)	(100)(-111) (020)(-210) (-121)(012) (-302) (-220) (411)(231)	(111)(202) (131)	(100)(011) (200)(120) (002)(012) (202)(411)
System	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic	Orthorhombic	Monoclinic
Space Group	$P2_1(4)$	$P2_12_12_1$ (19)	$P2_12_12_1$ (19)	$P2_12_12_1$ (19)	$P2_1(4)$	$P2_12_12_1$ (19)	$P2_1(4)$
Cell Parameters	a=7.617 b=6.982 c=5.142	a=9.647 b=9.748 c=10.855	a=9.647 b=9.748 c=10.855	a=9.647 b=9.748 c=10.855	a=7.617 b=6.982 c=5.142	a=9.647 b=9.748 c=10.855	a=7.617 b=6.982 c=5.142
Interfacial angles	α=γ=90° β=99.84°	α=β=90° γ=90°	α=β=90° γ=90°	α=β=90° γ=90°	α=γ=90° β=99.84°	α=β=90° γ=90°	α=γ=90° β=99.84°
JCPDS Card No.	39-1523	37-1927	37-1927	37-1927	39-1523	37-1927	39-1523

Fig.6 shows powder XRD pattern of L-aspartic acid. Sharp peaks of XRD pattern indicate high degree of crystalline structure of grown crystals. When compared with XRD pattern of pure L-aspartic acid with metal ions doped L-aspartic acid it is seen that metal ions doped L-aspartic acid possess a different pattern. The reason may be due to the acidic nature of L-aspartic acid, and the incoming metal ions may have chance to bind with two carboxylate ions and in that process the seating of metal ions will be in different form than the undoped counterpart. Powder XRD pattern of grown crystals was used to calculate lattice parameters using unit cell software and the values are found to be in good agreement with single crystal XRD values and they are shown in table-1.

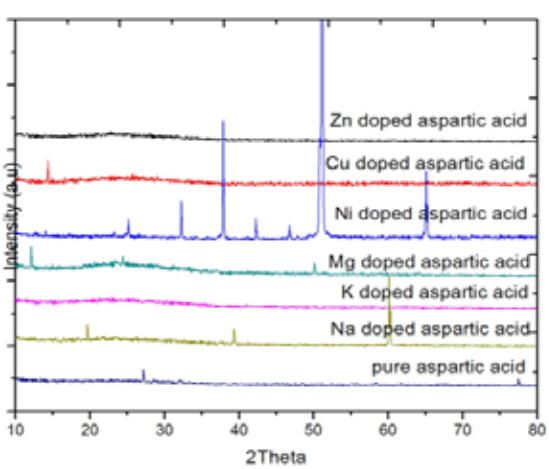

Fig.6 Powder XRD analysis of pure and metal ions doped L-aspartic acid

ETCHING STUDIES OF PURE AND METAL IONS DOPED L-ASPARTIC ACID

Etching property is used as the most convenient method for the visualization of defects. Etching studies were carried out on the (100) plane of crystals grown by conventional methods. In the present work water and methanol were used as etchants. Etching of the conventionally grown L-aspartic acid crystal with water for 5 seconds produces well defined diamond shaped etch pits and is shown in Fig -7. On successive etching for 15 sec and 25 sec the diamond etch pits enlarge in size, but retain their geometrical shape and do not disappear, suggesting that the etch pits are due to dislocations.

Surface appearance of L-aspartic acid and metal ions doped L-aspartic acid have a non-structured surface on a small scale, shown in Fig-7. Investigation with optical microscope shows that the etched samples exhibit a structured surface, shown in Fig-7. Grain boundaries are observed in conventionally grown aspartic crystals. Similar observations were reported on (100) faces of Na and K doped aspartic acid crystals (Sangwal and Zaniewska, 1984)[7]. The number of etch pits in the conventional slow evaporation solution technique(SEST) grown crystals of alkaline earth metal ions doped crystals are larger than the undoped grown crystals.

Multi crystalline alkaline doped aspartic acid etched wafers have very different formation depending on what grain is investigated. Two appearances that were observed on these metal ions doped L-aspartic acid crystals are shown in Fig 7, and it shows a grain with a textured surface with the surface appears to have regular linear features, although the surface on a small scale is smooth compared to the structures in undoped L-aspartic acid. This type of linear features is also found on grown crystals. In the Cu doped L-aspartic acid etch pits with various shapes are clearly visible. The etch pits did not disappear upon continuous etching, suggesting that the pits were due to dislocations [8].

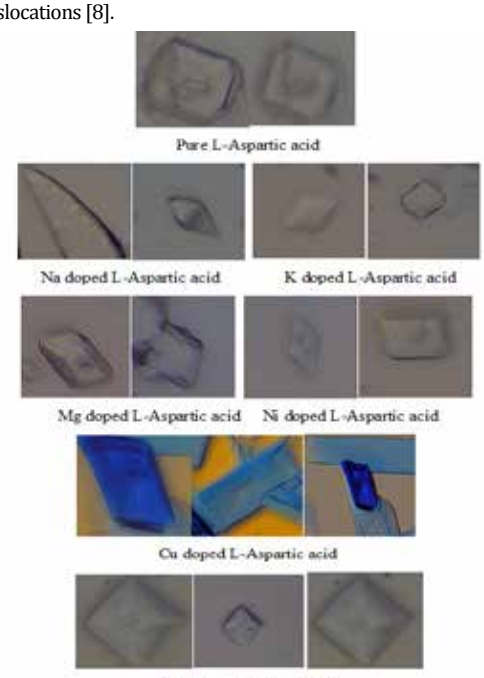

Fig.7 Surface revealing of etched pure and metal ions doped L-Aspartic acid single crystals

ANTIMICROBIAL ACTIVITY OF PURE AND METAL IONS DOPED L-ASPARTIC ACID SINGLE CRYSTALS

Antibacterial activity of amino acid L-aspartic acid and metal ions doped derivatives of L-aspartic acid had been studied in vitro against Enterobacter, Proteus, Klebsiella and Strptococcus by using agar diffusion method (Shank et al., 1979; Larry et al., 1981) [9]. The prepared, both pure and doped L-aspartic acid derivatives showed antibacterial activity with inhibition areas as shown in fig 8. Metal ions doped L-aspartic acid has more antibacterial activity against Strptococcus than the pure L-aspartic acid. The zone of inhibition is higher for transition metal ions than the alkali and alkaline earth metal ions. The reason may be due to weak resonance interaction taking place in the case of alkali and alkaline metal ions than the transition metal ions. Again in the case of transition metal ions Cu2+ exhibit

maximum zone of inhibition than the other transition metal ions counterpart like Ni and Zn, may be due to hole formylation nature of Cu2+ which has maximum electronic transition that may prevent the growth of micro organism on the crystal medium.

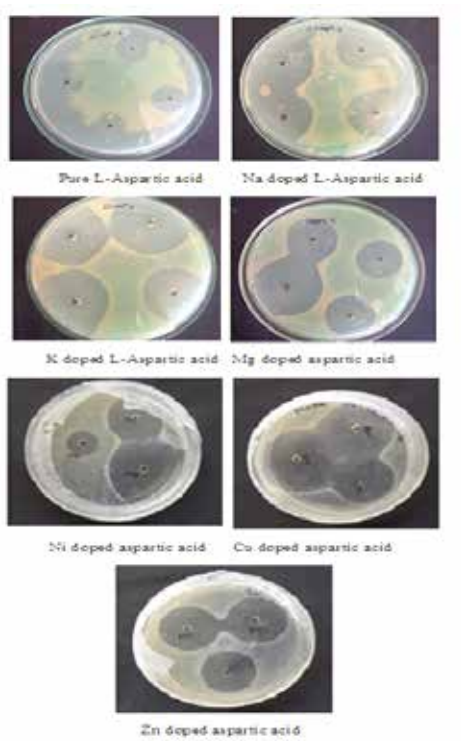

Fig.8 Antimicrobial activity of pure and metal ions L-Aspartic acid single crystals

The sensitivity of the microorganisms against the pure and metal ions doped L-aspartic acid are represented as bar diagram and is shown in Fig 9.

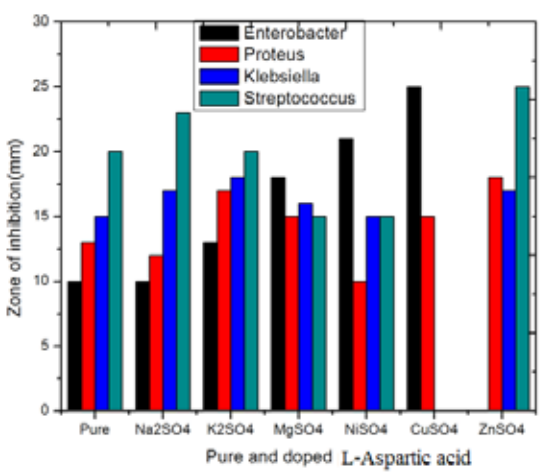

Fig.9 Zone inhibition areas of pure and metal ions doped L-Aspartic acid single crystals

CONCLUSION

The present study deals with growth of L-Aspartic acid (acidic) single crystals and alkali, alkaline earth and transition metal ions doped above said amino acid by using conventional slow evaporations solution growth method. The functional groups present in the grown crystals were identified by recording FTIR spectra.

It is observed from the UV Visible spectra of L-aspartic acid that the maximum absorption is for Cu doped L-aspartic acid than the pure and other metal ions doped L-aspartic acid. It is due the high conjugation and delocalized π bonding orbitals of Cu^{2+} ions are responsible for the high intensity absorption in UV-region.

The powder XRD of pure L-aspartic acid revealed that pure L-aspartic acid crystallized in monoclinic system and that for most of the metal ions doped L-aspartic acid crystallized in orthorhombic system.

Multi crystalline alkaline doped aspartic acid etched wafers have very different formation depending on what grain is investigated. Two appearances that were observed on these metal ions doped L-aspartic acid crystals of which one that shows a grain with a textured surface and the other on the surface, appears to have regular linear features, although the surface on a small scale is smooth compared to the structures in undoped L-aspartic acid.

The prepared both pure and doped L-aspartic acid derivatives showed antibacterial activity. The zone of inhibition is higher for transition metal ions than the alkali and alkaline earth metal ions doped L-aspartic acid. The reason may be due to weak resonance interaction taking place in the case of alkali and alkaline metal ions than the transition metal ions.

ACKNOWLEDGEMENT

This work was financially supported by the University Grants Commission, New Delhi.

REFERENCE

1. IUPAC-IUBMB Joint Commission on Biochemical Nomenclature. Nomenclature and Symbolism for Amino Acids and Peptides. Recommendations on Organic & Biochemical Nomenclature, Symbols & Terminology etc. Retrieved on 2007-05-17. | 2. Philip E. Chen, Matthew T. Geballe, Phillip J. Stansfeld, Alexander R. Johnston, Hongjie Yuan, Amanda L. Jacob, James P. Snyder, Stephen F. Traynelis, and David J. A. Wyllie. 2005. Structural Features of the Glutamate Binding Site in Recombinant NR1/NR2A N-Methyl-D-Aspartate Receptors Determined by Site-Directed Mutagenesis and Molecular Modeling. Molecular Pharmacology. Volume 67, Pages 1470-1484. | 3. Dunn, M. S.; Smart, B. W. "DL-Aspartic Acid"Organic Syntheses, Collected Volume 4, p.55 (1963). | 4. Nelson, D. L.; Cox, M. M. "Lehninger, Principles of Biochemistry" 3rd Ed. Worth Publishing: New York, 2000. ISBN 1-57259-153-6. | 5. Martel, A.E; Critical Stability Constants of Metal Complexes 26 (2006). | 6. Stryer, L; Biochemistry, 4th Edition, W.H. Freeman and Company New York, (1995). | 7. K. Sangwal, G. Zaniewska "Influence of impurities on the etching of NaCl crystals" Journal of Materials Science, Volume 19, Issue 4, pp 1131-1144, 1984. | 8. Petersson, A.M; Persson, H; Roos, A; Boström, T; "Optical Characterization Of Crystalline Silicon Surfaces For Solar Cell Applications" 24th European Photovoltaic Solar Energy Conference, 21-25 September 2009, Hamburg, Germany method (Shank et al., 1979; Larry et al., 1981) | 9. Shank, R.C., & Marmion, D.G. (1979). Microbiology.(12thedn.) , Churchill, Living Stone Edinburg, London, | | 10. Larry M, Judy R (1996). Microbial Essential and Applications, 2 nd Edition, McGraw-Hill, pp 376, 587-588. |