# Recent development of amino acids and peptides in metal ions detection: An overview

**MOHD. RASHIDI ABDULL MANAP, NOR AZAH YUSOF\*,
SITI MARIAM MOHD. NOR and FAUJAN B.H. AHMAD**

Department of Chemistry, Universiti Putra Malaysia 43400 UPM Serdang, (Malaysia).

## ABSTRACT

Little work has been reported on the development of solid state metal ion sensor based on the use of amino acids and peptides. This review covers literature on the use of amino acids and peptides (short peptide, oligopeptide and cyclic peptide) as a recognition molecule for metal detection system. Amino acids and peptides offer a high degree of selectivity, good limit of detection and high sensitivity towards detection of metal ion.

**Key words:** Amino acid, Peptides, Metal ions detection.

## INTRODUCTION

Certain heavy metals can pose potential health risks when consumed by humans due to their high toxicity. Heavy metals such as $Hg^{2+}$, $Pb^{2+}$ and $Cd^{2+}$ can be released into the environment through industrial use and erosion of natural deposits. Once it released to the environment, it can contaminate water, soil and air. These metals are very harmful to humans and have negative impact on the environment[1].

Abundant references concerning the determination of metal with chemical sensors were available in literature. Table 1 showed some chemosensors used for metal ion detection. Some of the chemosensors have a low stability of a metal complex over time, high solubility only in organic solvent and also difficulty during response measurement.

Amino acids and peptides promise a bright chance for metal ion detection because it is usually soluble in aqueous medium and it can easily interact with metal. Peptides can be effective for metal ion sensor due to specificity and because they contain of potential donor atoms through the peptide backbone and also on the amino acid side chains. The donor's atoms can be carbonyl oxygen or amide nitrogen and terminal amine. Polymeric character of peptide permits polydentate chelation which can provide strong binding and fast kinetic with ion. Selectivity and sensitivity were related with side chain of the amino acid in the peptide and can be optimized by further amino acid replacement during peptide synthetic strategy.

The aim of this review is to explore the use of amino acid, short peptide, oligopeptide and cyclic peptide for metal detection. Limit of detection and selectivity/interference of the sensors will be discussed. One letter abbreviation of amino acids were used in this review.

### Amino acids

Amperometric method was used in order to trace $Hg^{2+}$ in water[7]. The research focused on the usage of platinum electrode (high positive

potential) on the effect of the presence of mercury ions on the current due to oxidation of amino acid L-Tyrosine. The detection limit was 0.014 mM with relative standard deviation (RSD) 2.2%. The system used 20 µM L-Tyrosine, applied potential + 0.75V, 0.1 M phosphate buffer at pH 7. During interference studies, different metal have been used, among them are $Zn^{2+}$, $Ag^+$, $Cu^{2+}$, $Ni^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Co^{2+}$, $Fe^{2+}$ and $Fe^{3+}$. Only $Co^{2+}$ showed a significant interference at a concentration higher than that of $Hg^{2+}$.

An optical approach (fluorescence study) was reported in development of a water soluble sensor L-Cysteine-capped ZnS Quantum Dots(QD) for detection of $Cu^{2+}$ [8]. The sensor developed was not sensitive towards $Co^{2+}$ and $Mn^{2+}$. QD have various advantages, it can overcome problem from organic dyes molecule, have a high photochemical stability and resistant to photo degradation. As the pH increase, the deprotonation of thiol group in the L-Cysteine occurred and resulting fluorescence intensity increased. As a result, the bond strength between Zn and L-Cysteine molecule become high. But at too high pH, fluorescence decrease due to the formation of $Cu(OH)_2$. Detection limit of this probe was $7.1 \times 10^{-6}$ M. The luminescence intensity of the L-Cysteine-capped ZnS QDs is minimally affected by $Fe^{2+}$ and $Ag^+$, but at very low ratio 1:100 ($Cu^{2+}$ : Interference Ion).

Voltammetric study for detection of $Cd^{2+}$ was carried out using mercury electrode in the presence of cysteine[9]. 0.1 M $KClO_4$ has proven to be a good supporting electrolyte for the $Cd^{2+}$ detection system. The system developed is able to detect 5.62 mg/ml of $Cd^{2+}$. Heterocyclic thiophene and benzoxazole attached with alanine has been synthesized and characterized as a fluorescent probe[10]. The fluorescent probe developed did not show any change in the absorption and emission upon addition of $Na^+$, $Ca^{2+}$, $Zn^{2+}$, $Cu^{2+}$ and $Ni^{2+}$. Deprotection of ester group at carboxylic has caused the probe to respond towards $Cu^{2+}$, $Ni^{2+}$ and $Hg^{2+}$. This selective deprotection increased the coordination stability of metal complex. The S atom from the thiophene was believed to have complexation with $Hg^{2+}$. The removal of protecting group on N terminal has caused the amine group become protonated and addition of $Cu^{2+}$, $Ni^{2+}$ and $Hg^{2+}$ decreased the fluorescence intensity.

Metallothionein (MT), a family of cysteine-rich was used as a biosensor for $Pd^{2+}$ [11]. 0.05 M KCl was found as the best supporting electrolyte for determination of $Pd^{2+}$. The sensitivity of the biosensor not only depends on the adsorptive transfer stripping (AdTS) but also on affinity of MT to $Pd^{2+}$. The ability of the biosensor was tested on human urine and human blood sample with limit of detection of 0.8 µM $Pd^{2+}$.

### Short peptide
A fluorescent peptide consists of 9-carbonylantracene (AN) as fluorophore for the tripeptide Glycyl-Histidyl-Lysine (GHK) has been synthesized[12]. It was selective towards $Cu^{2+}$. Little or no change in the fluorescence emission can be seen upon addition of 0.1 mM of $Fe^{2+}$, $Co^{2+}$, $Ni^{2+}$ and $Zn^{2+}$. In the presence of $Cu^{2+}$ at a much lower concentration ($10^{-6}$M), the fluorescence of GHK-AN was quenched down to 34% of its original intensity.

Hepel *et al* [13] investigated the immobilized film of tripeptide glutathione Glycyl-Seryl-Histidine (GSH) on a cysteamine-SAM (CA-SAM) formed on Au piezoelectrodes by adsorptive dissociation of a disulphide, cysteamine. By using electrochemical quartz cystal nanogravimetry (EQCN), voltammetric analyses of $Hg^{2+}$ at Au/CA and Au/CA-GSH have

**Table 1: Some of chemosensors for metal ion detection**

| Chemosensors | Selectivity | Disadvantages |
|---|---|---|
| 8-hydroxyquinoline[2] | $Cu^{2+}$ | Unstable complex |
| Conjugated polymer[3] | $Mg^{2+}$ | Solubility in THF |
| SNS[4] | $Cr^{3+}$ | Solubility in THF |
| Calix[4]crown[5] | $Pb^{2+}$ | Solubility in $CH_3CN$ |
| Metal ion templated resin[6] | $Pb^{2+}$ | Difficult measurement |

been analyzed. For Au/CA-GSH piezosensor, a single mercury electrooxidation peak was observed. Meanwhile for bare Au and Au-CA piezosensors, multiple peaks were observed. This was a possibility of extensive interaction of mercury species with various groups of this multifunctional film. Mass to charge ratio plots indicate predominant ingress/egress of $Hg^{2+}$ to/from the film. Electronic structure in form of $(CA)_2Hg^{2+}$ revealed that two CA molecules adsorbed on Au in near-vertical and two nitrogen from CA attached to $Hg^{2+}$. The functional groups (free sulfhydryl, amine, carboxylate) introduced to the film with tripeptide glutathione can be utilized in designing a sensor.

Tripeptide Glycyl-Glycyl-Histidine (GGH) modified electrode was reported based on its analytical performance[14,15]. Tripeptide electrode was modified by self-assembling mercaptopropionic acid (MPA) onto the gold electrode followed by covalent attachment of the tripeptide to the self assembled monolayer using carbodiimide coupling. The peptide modified electrodes were found to exhibit high sensitivity to $Cu^{2+}$ in the range of 0 to 30 pM of $Cu^{2+}$ [14] and 5 nM of $Cu^{2+}$ [15]. In analytical performance aspect, the sensor could be regenerated for twenty times. The tripeptide electrode was used to determine 0.12 ppm of $Cu^{2+}$ in real sample. The interference of $Ni^{2+}$ to this modified electrode was only minor.

Chow *et al* [15] also reported on the characterization of the tripeptide modified electrode. The

characterization was focused on the transformation from bare Au surface to the MPA-GGH peptide. The sensor surface was modified via the formation of mercaptopropionic acid self-assembled monolayer (MPA SAM) followed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) activation and attachment of the peptide. It was found that, the response time was short (less than 5 minutes) for this sensor. MPA-GGH modified electrode selective towards $Cu^{2+}$. $Pb^{2+}$ and $Cr^{3+}$ were identified as significant interfering ions based on Plackett-Burman experimental design. The significant interfering could be related to the hard or soft atom properties of the ions. Fig.1 showed the fabrication of MPA-GGH gold electrode and complexation with $Cu^{2+}$.

High resolution differential surface plasmon resonance (SPR) was combined with highly selective cysteamine-modified peptide as molecular recognition element where it can specifically sense $Cu^{2+}$ and $Ni^{2+}$ ion[16]. Two immobilization methods were used which are 11-mercaptoundecanoic acid, (MUA)-NHS activation method and chemisorption through thiol bond for cysteamine. Combination of $His_6$ with MUA-NHS activation monolayer was able to detect $Ni^{2+}$ as low as 10.4 ppt, however chemisorption offered the highest sensitivity as low as 2.4 ppt $Ni^{2+}$.

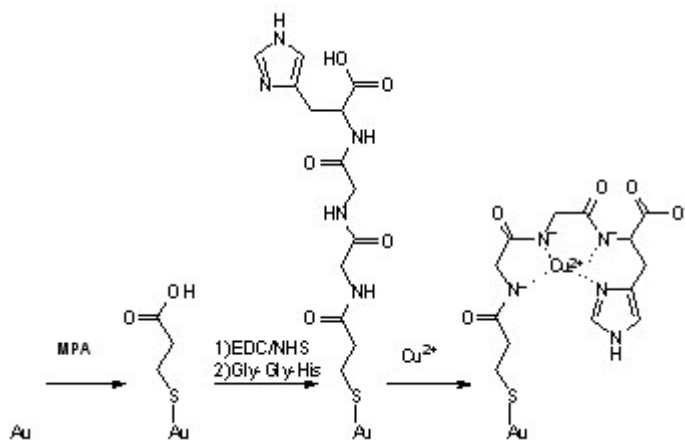Meanwhile for GGH modified SPR sensor (chemisorption method) was able only to detect 0.1



**Fig. 1: Fabrication of MPA-Gly-Gly-His gold electrode and complexation with Cu²⁺ Source: Chow *et al* [15]**

ppb of $Cu^{2+}$. MUA-NHS activation of GGH was able to detect until 2.0 ppt of $Cu^{2+}$. Selectivity study showed that $His_6$ is selective only towards $Ni^{2+}$ but not for GGH. Meanwhile GGH is selective for both $Ni^{2+}$ and $Cu^{2+}$. GGH cysteamine modified electrode reported to have better dynamic range. It has been applied for determination of $Cu^{2+}$ from tap water with detection limit of 0.34 ppm.

### Cyclic peptide

*N, N', N''*-Trisubstituted-cyclo-triglycines were synthesized and exploited by Hioki *et al*[17]. They studied on potentiometric ion selectivity coefficient of the electrodes. Only two electrodes of these showed a great affinity towards $Ca^{2+}$ over other cations. Molecular modeling showed one or two benzylic oxygens are outside cavity in some rotational isomers of amide linkage. Detection limit of the modified electrode was
$1 \times 10^{-5}$ M of $Ca^{2+}$.

Incorporating different soft and hard atoms in the chelating ring promoting the stability and selectivity of the resulting complex. Copper poly(vinyl chloride) matrix membrance sensors based on cyclic tetrapeptide ionophores were prepared and characterized[18]. Two ionophores were used, Ionophore **I** is hydrophobic with less ring size and Ionophore **II** is more polar due to the presence of asymmetric substitution group, an ester, as shown in Fig 2. The sensors exhibit fast response, wide working pH range, high sensitivity, long-term stability and good selectivity. Different plasticizers affect the detection limit of the ionophores. The sensors have a detection limit of 0.05-0.13 $\mu gml^{-1}$. The sensors

have been applied for determining $Cu^{2+}$ in ores and industrial wastewater.

### Oligopeptide

Polyalanine peptide consists of twenty alanine residues for metal detection has been reported[19]. The oligopeptide can interact with monovalent ($Li^+$, $Na^+$, $K^+$, $Cs^+$ and $R^+$) and dwivalent ($Mg^{2+}$, $Sr^{2+}$ and $Ba^{2+}$) ion, but not with trivalent ion ($In^{3+}$, $Sc^{3+}$ and $Y^{3+}$). In the unsolvated complex, the polyalanine peptide adopts a helical conformation that which stabilized by coordination of the metal ion to the C-terminus. Polyalanine peptide was helical in the $Ala_n$ + $M^{2+}$ complexes, but substantial disruption at the C-terminus where the metal ion bound. The interaction between the unsolvated peptide and metal ion increase as the charge ion increase. But not in the case of trivalent metals where it has high hydration energy and may not recovered by interaction with peptides, thus resulting no complex formation with peptide.

Conducting polymer was attractive because it can directly convert the binding event into an electrical signal. Aguilar *et al* [20] developed a sensor that was sensitive to $Cu^{2+}$ and $Ni^{2+}$ in ppt range by fabrication of polymer nanojunction on Si attached with peptide. GGH and $His_6$ peptides were incorporated into the polymer nanojunctions. Nanojuntion is formed by bridging a pair of nanolectrodes with electrodeposited peptide-modifed polyaniline (PANI). The system work based on the change in the nanojunction conductance when polymer conformational changes during peptide metal interaction. For poly (GGH-ANI)
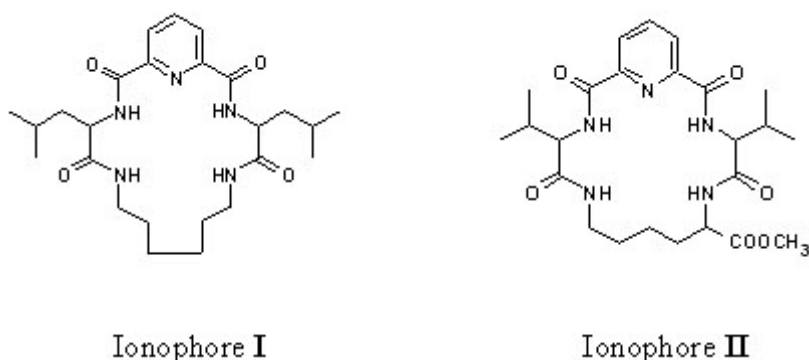


Fig. 2: Structure of Ionophore I and Ionophore II. Source: Hassan *et al* [18]
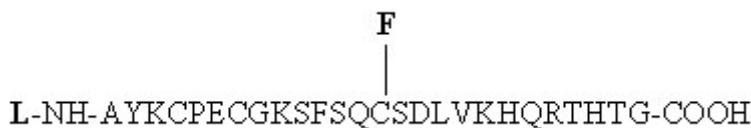
nanojuntion, decrease in current was seen upon addition of $Cu^{2+}$. The detection limit was found to be 4 ppm of $Cu^{2+}$. For the unmodified PANI, only little changes were observed during the process. Strong metal ion-peptide binding on the polymer nanojunction is due to the low dissociation constant[16]. This method has a high sensitivity and fast response. The nanojunctions are stable at room temperature at least six week and can be regenerate. Sensor was applied for detection of $Cu^{2+}$ in drinking water sample.

White *et al.,* [21] using nano magnetic material of $\gamma\text{-}Fe_2O_3$ and coated/immobilized with poly-L-Cysteine with 20 cysteine residues for chelation of $As^{3+}$, $Cu^{2+}$, $Cd^{2+}$, $Ni^{2+}$, $Pb^{2+}$ and $Zn^{2+}$. Poly-L-Cysteine-nano was inexpensive and showed strong highest binding towards $Cu^{2+}$ and the lowest for $Pb^{2+}$. Addition of poly-L-Cysteine increased the binding capacity towards these metals compared with no addition of peptide. The binding between poly-L-Cysteine –nano to metal increased with the increase of pH.

Another type of fluorescent probe based on the zinc finger consensus peptide (CP) that consists of twenty six linear amino acid arrangements was shown in Fig 3. This peptide showed a great response to $Zn^{2+}$ [22]. Modified CP with two fluorescent dyes, flourescein (F) as donor and lissamine (L) as acceptor group were used to "visualize" zinc binding. In the absence of $Zn^{2+}$, the peptide was unfolded and the dyes were relatively far from each other.

The fluorescence emission spectrum contains two peaks (521 and 596 nm) that likewise correspond to fluorescein and lisamine. Upon addition of $Zn^{2+}$, the fluorescence spectrum shift to a new wavelength, thus making the probe is ratioable.

Another example of peptidyl chemosensor was developed using combination fifteen residues of gylcine and aspartic acid in it sequence[23]. It consists of 2 flurophores, trytophan (Trp) as a donor and 5-(dimethylamino)naphthalene-1-sulfonyl (Dns) as acceptor for the probe. The probe had capability of Free Resonance Energy Transfer (FRET) between the Trp and Dns. Lack of a change in FRET efficiency indicates that the conformation of the peptide in this system was not changed as metal concentrations in the solution were altered. It was found that $Cu^{2+}$ binding causes no change in FRET efficiency. This chemosensor was selective towards $Cu^{2+}$ and detection limit was 32 µg $L^{-1}$. Selectivity towards ions were tested with interfering ions such as $Cd^{2+}$, $Mn^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Zn^{2+}$ and $Cu^{2+}$. Fluorescence intensity decrease only upon addition of $Cu^{2+}$ and this bring significant towards selected metal compared to other metals.

Another fluorescent peptide probe developed based on ratiometric and selective towards $Pb^{2+}$ [24]. Two tetrapeptides were developed, Glutamyl-Cysteinyl-Glutamyl-Glutamic Acid (ECEE) and Glycyl-Glycyl-Glycyl-Glycyine (GGGG) Each probe consists of a fluorescent dye of dansyl group. Upon binding Dns-ECEE with $Pb^{2+}$, the polarity of the environment surrounding changes and caused a concomitant shift in the fluorescence emission spectrum but not with Dns-GGGG. Ratiometric study was conducted by developing a calibration graph based on the ratio of fluorescence emission intensity before (557 nm) and after addition of metal to the system (510 nm). Shift in the emission spectrum only occurred in the system Dns-ECEE, but not in the Dns-GGGG. This probe behaves reversibility by addition of ethylenediaminetetraacetic acid (EDTA). Selectivity phenomena were observed during addition of $Ca^{2+}$, $Zn^{2+}$ or $Cd^{2+}$. It was shown by the researchers that the probe was selective only towards $Pb^{2+}$ even tough in the presence of either equimolar $Zn^{2+}$ or equimolar $Ca^{2+}$.

F
|
L-NH-AYKCPECGKSFSQCSDLVKHQRTHTG-COOH

**Fig. 3: CP consists of amino acid residues and fluorescent dyes of L and F. Source: Godwin and Jeremy [22]**

Bi *et al.,* [25] reported the preparation and application of oligopeptide-modified single crystal silicon nanowire (SiNW) arrays as multichannel metal ion sensor. Surface of SiNW cluster was modified with different oligopeptides. Peptides were immobilized onto the SiNW clusters. Cysteinyl-Aspartyl-Arginyl-Valyl-Tyrosyl-Isoleucyl-Histidyl-Prolyl-Pheneylalanyl-Histidyl-Leucine (CDRVYIHPFHL) oligopeptide was reported to be sensitive towards $Pb^{2+}$ meanwhile GGH was sensitive towards $Cu^{2+}$. Selectivity towards $Pb^{2+}$ could be explained by possibilities of $Pb^{2+}$ to bind with Histidine residues and two adjacent carbonyl groups. High selectivity of GGH for $Cu^{2+}$ can be attributed to the formation of three fused chelate rings and flat 4N coordination around $Cu^{2+}$. By modifying each SiNW cluster with a different oligopeptide, it can be used as a multichannel metal ion sensor.

Joshi *et al.,* [26] synthesized three florescent peptide probes for the detection of metal ion which is easily prepared through solid phase peptide synthesis (SPPS). The peptide consists of natural amino acid of histidine (H), cysteine (C), glutamic acid (E), proline (P) and glycine (G),  Dansyl group acted as a flurophore for the probe. The probes were PG2 Dns-CPGHPGE-NH$_2$, PG1 Dns-CGGHPGE-NH$_2$ and GG2 Dns-CGGHGGE-NH$_2$. The strategy was to develop prolyl-glycine (PG) sequence to stabilize a turn structure in the peptide and glycyl-glycine (GG) sequence was to adopt a random coil in the peptide structure. Results showed that the secondary structure played important role for selectivity and pre-organized secondary was not required for selective detection of $Cu^{2+}$ but not for the detection of $Zn^{2+}$. A GG2 probee was selective towards $Cu^{2+}$ due to the two GG sequences in the peptide. Meanwhile for PG1, it was selective towards $Zn^{2+}$ and $Cu^{2+}$ because of one PG sequence in the peptide.

Dan-Glycyl-Glycyl-Histidyl-Glycine (Dns-GGHG) peptide was synthesized by Zheng *et al.,*[27]. The developed chemosensor was selective only towards $Cu^{2+}$. Chemosensor was reported to interact with $Cu^{2+}$ in the system at concentration as low as 1.0 μM. During the addition of $Fe^{2+}$, $Fe^{3+}$, $Zn^{2+}$, $Co^{2+}$ and $Ni^{2+}$ to the chemosensor, the fluorescent intensity remained unchanged as like the chemosensor itself.

## CONCLUSION

It seem that the development of amino acids and peptides as metal ion sensor promised a better detection system with abilities for low detection limit in the range of ppb and ppt. Peptide base sensors have been applied on real sample and the efficiency are comparable with other sensors. With high degree of selectivity and good sensitivity, peptides are indeed a promising ligand for multiple metal ions detection.

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