SOLUTION PHASE SYNTHESIS AND CHARACTERIZATION OF PEPTIDYL CHEMOSENSORS FOR Pb(II) AND As(III) DETECTION

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science (Analytical Chemistry)

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SOLUTION PHASE SYNTHESIS AND CHARACTERIZATION OF PEPTIDYL CHEMOSENSORS FOR Pb(II) AND As(III) DETECTION

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July 2012

Chairman: Nor Azah Yusof, PhD

Faculty: Faculty of Science,UPM.

A new fluorescent dipeptide Dansyl-Ala-Val-OMe (**5**) has been synthesized and characterized. Peptide **5** was synthesized based on sulfonation reaction between dipeptide and 5-(dimethylamino)naphthalene-1-sulfonyl chloride (Dns-Cl) with molecular weight of *m/z* 435.2. Peptide **5** was obtained as a greenish solid in 80% yield. This compound was in a form of diastereomer in a 1:5 mixture ratio and it was not separated. Peptide **5** was characterized by melting point, IR, NMR, UPLC, GC-MS and HR-MS. Meanwhile another fluorescent dipeptide, Dansyl-Ala-Gly-OH (**6**) was purchased. The fluorescence intensity of peptide **5** was measured at pH 5 with excitation and emission wavelengths of 289 nm and 487 nm, respectively where as the excitation and emission wavelengths of peptide **6** at pH 12 were 331 nm and 524 nm, respectively. Fluoresence difference measurement was used for measurement. Effect of amount of reagent, different pH, dynamic range, reproducibility, interference and validation were studied. 4.0 µM of peptide **5** in a mixture of methanol:water (4:1) was observed to be the optimum concentration for detection of Pb(II). Meanwhile 1.5 µM of peptide **6** in 100% aqueous solution was needed for optimum detection of As(III). The working

medium of peptide **5** is at pH 5, meanwhile peptide **6** at pH 12. Limit of detection (LOD) for detection of Pb(II) and As(III) were calculated to be 0.08 μM and 1.86 μM respectively. The RSD of the reproducibility were 2.03% and 9.66% for 4.00 μM Pb(II) and 7.84 μM As(III). The presence of foreign ions at 1:1 molar ratio of interfering ion: studied metals did not affect the determination of Pb(II) and As(III) based on the low percentage of interference. The developed detection systems were validated with the established method of inductively coupled plasma optical emission spectrometry (ICP-OES).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SINTESIS FASA LARUTAN DAN PENCIRIAN PEPTIDA PENDARFLUOR PENGESAN KIMIA UNTUK PENGESANAN Pb (II) DAN As(III)

Oleh

MOHD RASHIDI ABDULL MANAP

Julai 2012

Pengerusi: Nor Azah Yusof, PhD

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Satu dipeptida pendarfluor baru iaitu Dansyl-Ala-Val-OMe (**5**) telah disintesis dan dicirikan. Peptida **5** telah disintesis berdasarkan tindak balas persulfonan antara dipeptida dan 5 - (dimetilamino) naftalena-1-sulfonil klorida (Dns-Cl) dengan berat molekul *m/z* 435.2. Peptida **5** telah diperoleh sebagai pepejal kehijauan dalam 80% hasil. Peptida **5** adalah dalam bentuk diastereomer dalam nisbah campuran 1:5 dan ia tidak diasingkan. Peptida **5** dicirikan menggunakan takat lebur, NMR, UPLC, GC-MS dan HR-MS. Sementara itu, satu lagi dipeptida pendarfluor, Dansyl-Ala-Gly-OH (**6**) telah dibeli. Keamatan pendarfluor peptida **5** adalah diukur pada pH 5 dengan pengujaan dan pelepasan jarak gelombang 289 nm dan 487 nm, masing-masing di mana panjang gelombang pengujaan dan pancaran peptida **6** pada pH 12 ialah 331 nm dan 524 nm, masing-masing. Perbezaan pengukuran pendarfluor telah digunakan untuk pengukuran. Kesan jumlah reagen, perbezaan pH, julat dinamik, kebolehulangan gangguan dan kajian pengesahan telah di kaji. Untuk jumlah reagen, 4 µM peptida **5** dalam campuran metanol: air (4:1) adalah kepekatan optimum untuk mengesan

Pb(II). Sementara itu, hanya 1.5 µM peptida **6** dalam 100% larutan akueus diperlukan untuk pengesanan optimum As(III). Medium kerja peptida **5** ialah pH 5 dan peptida **6** ialah pH 12. Had pengesanan (LOD) untuk pengesanan Pb(II) dan As(III) telah ditentukan; 0.08 µM dan 1.86 µM masing-masing. RSD kebolehulangan gangguan ialah 2.03% dan 9.66% untuk 4.00 µM Pb(II) dan 7.84 µM As(III). Kehadiran ion asing pada nisbah 1:1 molar ion penganggu atau Pb(II) dan peptida **6**-As(III) ion penganggu tidak menjejaskan penentuan Pb(II) dan As(III) berdasarkan peratusan rendah gangguan. Sistem pengesanan yang dibangunkan telah disahkan dengan kaedah induktif plasma berganding aruhan spektroskopi pemancaran optik (ICP-OES) yang sedia ada.

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I certify that a Thesis Examination Committee has met on 30 July 2012 to conduct the final examination of Mohd Rashidi bin Abdull Manap on his thesis entitled "Solution Phase Synthesis and Characterization of Peptidyl Chemosensors for Pb (II) and As (III) Detection" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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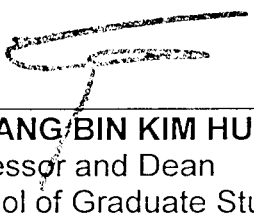

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

MOHD RASHIDI ABDULL MANAP

Date: 30 July 2012

LIST OF ABBREVIATIONS

app d	apparently doublet (spectral)
dd	double of doublet (spectral)
Boc	*tert*-butoxycarbonyl
br	broad
br s	broad singlet
^0C	degrees Celsius
cm^{-1}	wavenumber(s)
COSY	correlation spectroscopy
δ	chemical shift in parts per million downfield from tetramethylsilane
d	doublet (spectral)
dansyl	5-(dimethylamino)-1-naphtalenesulfonyl
DCC	*N,N'*-dicyclohexylcarbodiimide
DCM	dichloromethane
DEPT	distortionless enhancement by downfield from tetramethylsilane
EDTA	ethylenediaminetetraacetic acid
equiv	equivalent
FT	Fourier transform
g	gram(s)
GC	gas chromatography
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
Hz	hertz
IR	infrared
J	coupling constant (in NMR)
L	liter(s)
LOD	Limit of detection
LOQ	Limit of quantification
µ	micro
m	multiplet (spectral); mili
M	molar (moles per liter)
M^+	parent molecular ion
Me	methyl
MHz	megahertz
mL	mililiter
mM	milimolar (milimoles per liter)
mol	mole(s); molecular (as in mol wt)
mp	melting point
MS	mass spectrometry
MW	mol wt molecular weight
m/z	mass-to-charge ratio
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
ppb	part(s) per billion
ppm	part(s) per million

rt	room temperature
s	singlet (spectal)
t	triplet (spectral)
temp	temperature
TLC	thin-layer chromatography
TMS	trimethylsilyl; tetramethylsilane
UPLC	Ultra-performance liquid chrmoatography
t_R	retention time (in chromatography)
UV	ultraviolet
vis	visible

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CHAPTER 1

INTRODUCTION

Research Problem

Heavy metal ions such as As(III), Pb(II), Zn(II), Ni(II) and Hg(II) are easily found in the environment especially in industrial waste, agriculture and mining industry. These inorganic chemicals are usually present at low concentration in the environment either free or complexed with organic or inorganic ligand (Pesavento et al., 2009).

Unlike organic contaminants, these inorganic chemicals have high solubility in the aquatic environments. As a result, it can easily penetrate the food chain and stored in living organism. Moreover, these inorganic chemicals are non-biodegradable in nature, and on top of that it has long biological half life for elimination from body. It results in negative major problems to living organisms (Chen et al., 2008). Fu and Wang (2011) reported on a few heavy metal ions such as Zn(II), Ni(II), Cd(II) and Pb(II) which affect human's health such as vomiting, kidney failure and skin dermatitis.

Electronic configuration of metal ions are different from each other based on its oxidation state. Based on this properties, some of the metals can give spectroscopic due to its electronic configuration. However, some of them can not give spectroscopic signal.

For example, Zn(II) can not be detected directly using common analytic techniques such as UV-Vis spectroscopy or nuclear magnetic resonance (NMR) due to its $3d^{10}4s^{0}$ electronic configuration (Jiang and Guo, 2004). Thus the fluorescence method stands out as a method of choice. Fluorescent method offers high sensitivity compared to other methods.

Dansyl groups were widely used as fluorophore due to its intense absorption in the UV region and emits in the visible range with high fluorescence quantum yield. In addition, the dansyl molecule can undergo sulfonation reaction and it is suitable for the synthesis of peptide derivative (Chao et al., 2011). There were many fluorescent peptides that have been synthesized *via* Solid Phase Peptide Synthesis (SPPS) and used as a chemosensor (Joshi et al., 2007; White and Holcombe, 2007). Solution phase offers fast preparation of a small sequence of peptide and fast synthesis, meanwhile SPPS is most suitable for bulky and large scale of peptide.

It has been described that peptide formed complex with metal ion (Chekmeneva et al., 2006). There are a great number of potential donor atoms through the peptide backbone such as on nitrogen atom, oxygen atom, side chain, carbonyl on terminal or amide hydrogen. Owing to its great structural variety, peptide is suitable for complex formation. Metal binding is achieved with a high degree of selectivity using peptide motifs. Glycine and valine were chosen because of the non polar side chains. Glycine side chain is weakly hydrophobic meanwhile

valine side chain is strongly hydrophobic. This properties make the chemosensor works in different medium of solvents.

Therefore, the idea of this research is to synthesize a peptide which consists of a fluorophore which acts as a recognition molecule in metal ions detection. The fluorescent peptide will be synthesized *via* solution phase method.

Two different fluorescent peptides (synthesized and purchased) were tested as recognition molecule for analysis in different mediums (mixture solvent and single solvent). Several parameters were studied such as selectivity, pH, reagent concentration, dynamic range, reproducibilty, interference and validation.

Objectives

The objectives of this research are:

1)To synthesize a fluorescent dipeptide *via* solution phase method

2)To characterize the synthesized fluorescent dipeptide by spectroscopic methods

3)To characterize fluorescent dipeptides for metal ion detection

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