UNIVERSITI PUTRA MALAYSIA

ENZYMATIC SYNTHESIS, CHARACTERIZATION AND ANALYTICAL APPLICATIONS OF FATTY HYDRAZIDES FROM PALM OIL

SHARIFAH MOHAMAD

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By

SHARIFAH MOHAMAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malayisa, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Chair: Professor Dato' Wan Md. Zin Wan Yunus, PhD

Faculty: Faculty of Science

Hydrazides with the general formula R-CO-$NHNH_2$, have received a lot of attention due to their applications in biological, organic synthesis and analytical chemistry fields. The preparation of fatty hydrazides from palm oil has been developed in this research. Fatty hydrazides (FH) and fatty phenyl hydrazides (FPH) were successfully synthesized from palm oil as a raw material by one-step lipase catalyzed reaction. FTIR and CHN elemental analyses were carried out to determine the presence of the hydrazides in the products. The method offers several advantages such as renewable and abundant of raw materials, simple reaction procedure and high yield of products.

The application of the products as a reagent was carried out based on the ability of the FH and FPH to form complexes with some metal ions. FH was successfully used as an extractant for extraction and separation of copper(II). The separation of Cu(II) is

possible from other metal ions such as Co(II), Cr(VI), Ni(II), Zn(II) and Fe(III) at pH 5.5-6.5. A preconcentration method was proposed for the determination of Cu(II) in water samples. It was shown that the extraction from aqueous phase containing Cu(II) with organic phase containing FH and then stripping the organic phase with 2 M of HCl solution give a solution of Cu(II) 10 fold in concentrations.

FH was also successfully used as an extractant for extraction and separation of Mo(VI). This metal successfully separated from other metal ions such as Ni(II), Co(II), Al(III), Fe(III) and Mn(II). Quantitative stripping of Mo(VI) ion from the organic phase can be carried out using 2 M ammonium hydroxide. This proposed method was applied for the recovery of Mo(VI) from synthetic mixture and the results showed that more than 90% recovery of Mo(VI) is achieved and the metal ion solution is free from the impurities.

FH and FPH were also evaluated as an extractant for extraction and separation of gold(III) from chloride media. This metal was extracted quantitatively from hydrochloric media at 0.001-0.1 M into the organic phase. Gold(III) was successfully separated from Cu(II), Ni(II), Zn(II), Co(II) and Fe(III) by using FH or FPH. Quantitative stripping of gold(III) from the organic phase can be carried out using 2.0 M thiourea in 1.0 M HCl solution. The extraction and separation of gold(III) by FH or FPH was applied to separate and recover pure gold(III) from synthetic mixtures.

FPH synthesized from palm olein was also successfully used as a new reagent for the determination of V(V) by spetrophotometric method. The method is based on the colored complex of vanadium(V)-FPH. The metal ion forms dark brown colored complex which has an absorption maximum at 405 nm. Beer's law is valid over the concentration range of 0.2-20 mg/L and the limit of detection of this method is 0.01 mg/L. The characteristics of this developed method are simple, good selectivity, high sensitivity and rapid.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

SINTESIS BERENZIM, PENCIRIAN DAN PENGGUNAAN ANALISIS HIDRAZIDA LEMAK DARIPADA MINYAK KELAPA SAWIT

Oleh

SHARIFAH MOHAMAD

Mei 2008

Pengerusi: Professor Dato' Wan Md Zin Wan Yunus, PhD

Fakulti: Fakulti Sains

Hidrazida dengan formula umumnya R-CO-$NHNH_2$, mendapat banyak perhatian kerana pengunaannya dalam bidang biologi, sintesis organik dan kimia analisis. Penyediaan hidrazida lemak daripada minyak kelapa sawit telah dimajukan di dalam penyelidikan ini. Hidrazida lemak (FH) dan hidrazida fenil lemak (FPH) telah berjaya disintesiskan daripada minyak kelapa sawit sebagai bahan asas dengan tindakbalas satu peringkat menggunakan enzim lipase sebagai mangkin. Analisis FTIR dan analisis unsur CHN telah dijalankan untuk menentukan kehadiran kumpulan hidrazida di dalam produk. Kaedah ini mempunyai beberapa kelebihan seperti bahan asas yang berterusan dan amat banyak, prosedur tindak balas yang mudah dan hasil produk yang tinggi.

Penggunaan produk sebagai reagen telah dijalankan berasaskan kebolehan FH dan FPH membentuk kompleks dengan beberapa ion logam. FH telah berjaya digunakan sebagai pengekstrak untuk pengekstrakan dan pemisahan kuprum(II). Pemisahan kuprum(II) adalah mungkin dari ion logam yang lain seperti Co(II), Cr(VI), Ni(II), Zn(II) dan Fe(III) pada pH 5.5-6.5. Kaedah pra-pemekatan dicadangkan untuk penentuan Cu(II) di dalam sampel air. Ini telah ditunjukkan dengan pengekstrakan fasa akueus yang mengandungi Cu(II) dengan fasa organik yang mengandungi FH dan penanggalan daripada fasa organik dengan larutan 2 M HCl memberikan pemekatan larutan Cu(II) sebanyak 10 kali ganda.

FH juga telah berjaya digunakan sebagai pengekstrak untuk pengekstrakan dan pemisahan Mo(VI). Logam ini telah berjaya dipisahkan dari ion logam seperti Ni(II), Co(II), Al(III), Fe(III) dan Mn(II). Penanggalan kuantitatif bagi ion Mo(VI) dari fasa organik boleh dijalankan dengan menggunakan larutan 2 M ammonia. Kaedah ini diaplikasi untuk perolehan semula Mo(VI) dari campuran sintetik dan keputusan menunjukkan lebih dari 90% perolehan semula Mo(VI) telah dicapai and larutan ion logam tersebut bebas dari ketidaktulenan.

FH dan FPH telah berjaya digunakan sebagai pengekstrak untuk pengekstrakan dan pemisahan emas(III) dari medium klorida. Logam tersebut telah diekstrak secara kuantitatif dari medium hidroklorik pada 0.001-0.1 M ke dalam fasa organik. Emas(III) telah berjaya dipisahkan dari Cu(II), Ni(II), Zn(II), Co(II) dan Fe(III) dengan mengunakan FH atau FPH. Penanggalan kuantitatif bagi emas(III) dari fasa organik boleh dijalankan dengan menggunakan larutan 2.0 M thiourea di dalam larutan HCl

1.0 M. Pengekstrakan dan pemisahan emas(III) dengan FH atau FPH telah digunakan untuk pisah dan peroleh semula emas(III) tulen dari campuran sintetik.

FPH disintesis dari olein minyak sawit telah berjaya digunakan sebagai reagen baru dalam penentuan V(V) dengan kaedah spektrofotometrik. Kaedah ini beasaskan warna bagi kompleks vanadium(V)-FPH. Ion logam tersebut membentuk kompleks berwarna perang kehitaman dengan penyerapan maksimum pada 405 nm. Hukum Beer adalah sah di dalam julat kepekatan 0.2-20 mg/L dan had pengesanan bagi kaedah ini adalah 0.01 mg/L. Ciri kaedah yang dimajukan ini adalah mudah, keselektifan yang bagus, kesensitifan yang tinggi dan cepat.

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I certify that an Examination Committee has met on **date of viva voce** to conduct the final examination of Sharifah Mohamad on her Doctor of Philosophy thesis entitled "Enzymatic Synthesis Characterization and Analytical Application of Fatty Hydrazides from Palm Oil**"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Examination Committee were as follows:

Chairman, PhD
Professor
Department of Chemistry
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Examiner 1, PhD
Associate Professor
Department of Chemistry
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Examiner 2, PhD
Department of Chemistry
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

External Examiner, PhD
Professor
Department of Chemistry
Faculty of Science
University Kebangsaan Malaysia
(External Examiner)

_____

HASANAH MOHD. GHAZALI, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment for the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Wan Md. Zin Wan Yunus, PhD
Professor
Department of Chemistry
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Md. Jelas Haron, PhD
Professor
Department of Chemistry
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohamad Zaki Abd Rahman, PhD
Associate Professor
Department of Chemistry
Faculty of Science
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 July 2008

DECLARATION

I declare that the thesis is my original work expert for quotations and citations which have been duly acknowledgment. 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 degree at Universiti Putra Malaysia or at any other institution.

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SHARIFAH MOHAMAD

Date: 18 June 2008

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LIST OF ABBREVIATIONS

DAGs	diacylglycerols
DBC	di-butylcarbitol
DEHPA	di-2-ethylhexylphosphoric acid
FAAS	flame atomic absorption spectroscopy
FFAs	free fatty acids
FH	fatty hydrazide
FPH	fatty phenyl hydrazide
FTIR	fourier transforms infrared spectroscopy
ICP-AES	inductively coupled plasma-atomic emission spectroscopy
MAGs	monoacylglycerols
NMR	nuclear magnetic resonance
PH	palmityl hydrazide
PPH	palmityl phenyl hydrazide
TAG	triacylglycerol
TAGS	triacylglycerols
TBP	tributyl phosphate
TBPO	tributyl phosphine oxide

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CHAPTER I

INTRODUCTION

1.1 Background of study

1.1.1 Hydrazide

Hydrazides with the general formula $RCONHNH_2$ have been thoroughly studied as ligands with non-bonded lone pair of electrons on the nitrogen of amino group and oxygen of the carbonyl group. It may coordinate with many metal ions monodentate or multidentate (Gudasi *et al.*, 2007; Becher *et al.*, 2006; Zhang *et al.*, 2006). The molecular structure of chelate-forming reagents must contain at least two donor atoms capable of bonding to the same metal atom. The complex formation with hydrazide ligands in aqueous solution demonstrated clearly that two atoms (O, N) bonding modes of the ligands are accessible to metal ions (Bontchev *et al.*, 1981; Ahmed and Chaudhuri, 1971).

The formation of metal complexes plays an important role in the enhancement of their biological activity (Singh *et al.*, 2000). In recent years, hydrazides have received a lot of attention due to their biological activity as tuberculostatic (Yadav *et al.*, 2005), antibacterial agent (Malhotra *et al.*, 1992), antitumor agent (Dodoff *et al.*, 1994) and anticancer agent (Zhang *et al.*, 2004). In addition, hydrazides have received considerable attention in analytical chemistry chelating reagents for metal extraction and determination (Jal *et al.*, 2001; Ahmed and Banoo, 1999; Liu *et al.*, 1999).