UNIVERSITI PUTRA MALAYSIA

SYNTHESIS AND CHARACTERIZATION OF ION-IMPRINTED POLYMER FOR DETERMINATION OF IONIZED CALCIUM IN HUMAN BLOOD SERUM

RAFAH JASIM MOHAMMED AL-MAIBD

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By

RAFAH JASIM MOHAMMED AL-MAIBD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

To my lovely husband, Wissam, who has supported and encouraged me all the way to finish my Master study at Universiti Putra Malaysia

…I dedicate this research.

Thank you,
My love for you can never be quantified. Allah bless you.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman : Sazlinda Kamaruzaman, PhD
Faculty : Science

Calcium (Ca) is a vital element in the human body as it maintains the integrity of the bone system and acts as regulatory ion. Total calcium (tCa) circulates the human body in different forms with only ionized calcium (iCa) is the physiology active fraction. Direct measurement of tCa in human serum remains the most common way of assessing calcium status in patients. The majority of clinical laboratories do not have iCa analysers but instead predict iCa from the tCa value measured in the lab. However, one of the pressing issues is the potential of underdiagnoses of Ca deficiency due to the false prediction of iCa.

The adsorption properties of ion-imprinted polymers (IIPs) make them ideal for the selective removal of Calcium ions [Ca(II)] from human blood serum and the subsequent determination of iCa level. Ca(II)-IIP was developed from two naturally formed biopolymers, cellulose and sodium alginate. The polymers were dissolved in co-solvent and casted into films, Ca(II) ions were added to the polymer matrix using $CaCl_2$ bath, the polymer films were cross-linked using Epichlorohydrin (ECH) and then the template Ca(II) ions were extracted using EDTA. The final Ca(II)-IIP was in the form of white, porous film with high selectivity to Ca(II) ion.

Taguchi method was utilized to determine the optimum adsorption conditions of the Ca(II)-IIP, the resulted optimum conditions were pH 5.9, initial concentration (50 mg/l), dosage (300 mg) and 90 min contact time. The effects of pH, dosage, initial concentration on the Ca(II) adsorption process were investigated. The kinetic study of Ca(II) adsorption fitted well with the pseudo-first-order while, the adsorption isotherm is well fitted with Langmuir isotherm model.

The Ca(II)-IIP has a good selectivity towards Ca(II) in the presence of one or more competing ions. The removal percentage of Ca(II) remains high in the presence of one competing ion but showed a lower removal percentage of Ca(II) ion in the presence of multiple competing ions. The developed Ca(II) exhibits good reusability which can be recycled for 5 times before its efficiency starts to degrade significantly.

The Ca(II)-IIP was successfully applied for the determination of iCa in human blood serum. The optimum adsorption conditions (determined by Taguchi analysis) to achieve the best prediction of iCa level in human blood serum are IIP to serum ratio (60 mg/mL), dilution times (10) and dilution mixing time (30 min).

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

SINTESIS DAN KARAKTERISASI POLIMER TERKINI ION UNTUK PENGHASILAN KALSIUM IONISASI DALAM SERUM DARAH MANUSIA

Oleh

RAFAH JASIM MOHAMMED AL-MAIBD

Januari 2021

Pengerusi : Sazlinda Kamaruzaman, PhD
Fakulti : Sains

Kalsium (Ca) adalah elemen penting dalam tubuh manusia kerana ia mengekalkan keutuhan sistem tulang dan bertindak sebagai ion pengawal selia. Kalsium total (tCa) beredar dalam tubuh manusia dalam pelbagai bentuk dan hanya kalsium terion (iCa) wujud sebagai pecahan aktif fisiologi. Pengukuran secara langsung tCa dalam serum manusia merupakan kaedah paling umum bagi menilai status kalsium pesakit. Sebilangan besar makmal klinikal tidak memiliki penganalisis iCa, sebaliknya akan meramal iCa berdasarkan nilai tCa yang diukur di dalam makmal. Namun begitu, antara masalah mendesak yang mungkin timbul ialah diagnosis tersasar tentang kekurangan Ca akibat ramalan iCa yang salah.

Sifat penjerapan polimer tercetak ion (ion-imprinted polymers, IIP) menjadikannya sesuai untuk penyingkiran selektif ion Kalsium [Ca(II)] daripada serum darah manusia dan menentukan tahap iCa yang seterusnya. Ca(II)-IIP telah dihasilkan daripada dua biopolimer yang terbentuk secara semula jadi, iaitu selulosa dan natrium alginat. Polimer-polimer ini dilarutkan dalam pelarut bersama dan dituang menjadi filem nipis, dan ion Ca(II) ditambah ke dalam matriks polimer menggunakan rendaman CaCl2. Filem polimer yang terhasil diikat silang menggunakan epiklorohidrin (epichlorohydrin, ECH) dan kemudian, templat ion Ca(II) diekstrak menggunakan EDTA. Ca(II)-IIP yang terhasil adalah dalam bentuk filem putih yang berliang dengan kepilihan ion yang tinggi terhadap ion Ca(II).

Kaedah Taguchi digunakan bagi menentukan syarat penjerapan Ca(II)-IIP yang optimum. Syarat optimum yang diperoleh adalah pH 5.9, kepekatan awal (50 mg/L), dos (300 mg) dan tempoh sentuhan selama 90 minit. Kesan pH, dos, dan kepekatan awal terhadap proses penjerapan Ca(II) dikaji. Kajian kinetik penjerapan Ca(II) padan dengan tertib pseudo pertama, sementara isoterma penjerapan padan dengan model isoterma Langmuir.

Ca(II)-IIP memiliki kepilihan ion yang baik terhadap Ca(II) dengan kewujudan satu atau lebih ion bersaing. Peratusan penyingkiran Ca(II) kekal tinggi dengan kewujudan satu ion bersaing, namun menunjukkan peratusan penyingkiran ion Ca(II) yang lebih rendah dengan kewujudan pelbagai ion bersaing. Ca(II) yang dihasilkan menunjukkan sifat kebolehgunaan semula yang baik dan ia boleh diguna semula sebanyak 5 kali sebelum kecekapannya mula menurun dengan ketara.

Ca(II)-IIP berjaya digunakan bagi menentukan kandungan iCa dalam serum darah manusia. Syarat penjerapan optimum (ditentukan melalui analisis Taguchi) untuk memperoleh ramalan terbaik tahap iCa dalam serum darah manusia adalah nisbah IIP kepada serum (60 mg/mL), pengulangan pencairan (10) dan tempoh pengadunan pencairan (30 minit).

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

Sazlinda Kamaruzaman, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Nor Azowa Ibrahim, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Intan Nureslyna Samsudin, MB Bch Bao, MPath
Associate Professor (Medical)
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Norhazlin Zainuddin, PhD
Senior Lecturer
Faculty of Science
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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LIST OF ABBREVIATIONS

4-VP	4-Vinylpyridine
AAS	Atomic absorption spectrophotometry
AIBN	2,2'-azobisisobutyronitrile
ANOM	Analysis of mean
ANOVA	Analysis of variance
APS	3-amino propyl tri-methoxy silane
b	Langmuir adsorption constant
BET	Brunauer Emmett Teller
BPO	Benzoyl peroxide
C_0	Initial concentration
Ca	Calcium
Ca(II)-IIP	Calcium ion imprinted polymer
CE	Cellulose
C_e	Final concentration
Cf	Final concentration
C_F	Final Ca(II) concentration in diluted serum after IIP treatment
C_i	Initial concentration
COVID-19	Coronavirus disease
C_R	Ca(II) concentration of the reference sample (serum without IIP treatment)
DEM	2-(diethylamino) ethyl methacrylate
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DoE	Design of Experiment
DVB	Bi-vinylated monomer divinylbenzene
ECH	Epichlorohydrin

EDMA	3,4-Ethylenedioxy-N-methylamphetamine
EDTA	Ethylenediaminetetraacetic acid
FESEM	Field emission scanning electron microscopy
FMHS	Faculty of medicine and health sciences
FTIR	Fourier transform infrared spectroscopy
HEMA	2-hydroxyethyl methacrylate
iCa	Ionized (free) calcium
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma - optical emission spectrometry
IIP	Ion imprinted polymer
IPT	Imprinted polymer technology
ISE	Ion-selective electrodes
K'	Effectiveness of imprinting factor
k_1	Pseudo-first-order rate constant
k_2	Pseudo-second-order rate constant
k_3	Intraparticle transport rate constant
K_F	Freundlich constant
MAA	Methacrylic acid
MBA	N,N0-methylene bis (acrylamide)
MCO	Movement control order
MI	Molecular Imprinting
MIP	Molecular imprinting polymer
MIT	Molecular imprinting technology
n	Freundlich exponent
NIIP	Non ion imprinted polymer
PETRA	Pentaerythritol triacrylate
PPE	Percentage prediction error
Q_e	Total adsorption capacity

q_e	Amount of Ca(II) ions removed at equilibrium
Q_{max}	Maximum adsorption capacity
q_t	Amount of Ca(II) ions removed at specific contact time (t)
R^2	Slope
R_L	Separation factor
S	Selectivity coefficient
SA	Sodium alginate
SNR	Signal to Noise Ratio
SPE	Solid-phase extraction
SR	Swelling ratio
Sr	Selectivity ratio
t	Contact time
tCa	Total calcium
TGA	Thermogravimetric analysis
THF	Tetrahydrofuran
TRIM	Trimethylolpropane trimethacrylate
UPM	Universiti Putra Malaysia
V	Volume of the solution
V_f	Final volume
V_i	Initial volume
WIIP	IIP dosage
Wt.%	Weight percentage
XRD	X-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Calcium is the fifth most abundant element in Earth's crust and critical prevalent cation present in the human body. Calcium has a vital role in the mineralization and maintenance of the skeletal system of the human body. It also plays an essential role in blood coagulation, nerve signal transmission, the excitability of cardiac muscle and the preservation of cell membrane integrity and permeability [1]. Calcium exists in the human extracellular fluid (including serum or plasma) in soluble form as 0.1 wt.% of the body's total calcium mass [2]. Serum or plasma calcium exists in the human body in three fractions: ionized calcium (iCa), complexed or reacted calcium, and protein-bound calcium. Around 40 % of the total circulating calcium in human blood is bound to protein (mainly albumin), 10 % is complexed with different ions with the residual 50 % circulates as free or ionized calcium [2].

Different type of illness causes the concentration of iCa to be disrupted, and the body will be no longer able to supply and maintain the desired levels of calcium for the intracellular functions. The disruption of calcium levels in the body is the leading cause of hypercalcemia or hypocalcaemia to develop [3]. Hence, in most large hospitals, calcium is monitored daily for inpatients with calcium disorder related illness. iCa is commonly tested for patients with low total calcium (tCa) [4].

It is increasingly evidenced that iCa represents the physiologically active fraction of calcium that significantly affects the body's health. The direct measurement of iCa provides a more accurate picture of the calcium level in the human body. Nowadays, tCa can be measured using different modern methods such as spectrophotometry, atomic absorption spectrophotometry [5], and inductively coupled plasma mass spectrometry [6]. In contrast, iCa is mainly measured by potentiometry with ion-selective electrodes (ISEs) in some clinical laboratory [7]. Other methods that depend on mathematical formulas based on statistical data to adjust tCa from albumin is commonly used in clinical laboratories [8].

The development of portable ISE clinical analysers marks the next milestone in the measurement of iCa in clinical laboratories. These analysers utilize disposable cartridges that contain impregnated iCa biosensors for use with whole heparinized blood [9]. Despite further advancement in the ISEs, cost remains a disadvantage derived from frequent equipment maintenance, electrode replacement, and associated downtime [10]; thus, the clinical application of this technique is still limited due to the relatively higher maintenance cost of analysis, high probability of errors from the likely CO_2 losses during sample handling and the subsequent impact on pH and iCa measurement accuracy. Meanwhile, albumin adjusted formulas remain the commonly used methods in the majority of the clinical laboratory to estimate iCa quickly but less accurately.

1.2 Problem Statement

Measurement of tCa in human blood serum is a routine test for assessing calcium status in patients. Many laboratories do not have iCa analysers but instead utilize published formulas to predict iCa from the tCa value measured in the lab. One of the pressing issues is the potential of underdiagnoses the calcium deficiency due to the false prediction of iCa.

In a recent study, ion imprinting polymer (IIP) was reported as a suitable candidate for the removal of Ca(II) ions from aqueous water samples [11]. It showed adequate adsorption capacity, high selectivity to Ca(II) ion. Unfortunately, the study has not provided full adsorption study to determine the effect of some critical factors such as pH, initial concentration and dosage on the removal of the Ca(II) ions.

Given above, IIP was proposed as an alternative approach for the removal of calcium ions (iCa fraction) from human blood serum for the subsequent determination of iCa level. The present work proposes the synthesis and characterization of IIP in the form of porous film for the initial removal of Ca(II) from aqueous solution and then apply it to human blood serum for the determination of iCa level.

Finally, Taguchi design of experiments (DoE) was utilized in the current study for designing and optimizing all adsorption and application experiments.

1.3 Objectives of the Current Research

The general objective of the current research is to prepare calcium ion-imprinted polymer [Ca(II)IIP] for the determination of iCa level in human blood serum. The following specific objectives are derived to achieve the general aim of the study:

i. To prepare and characterize Ca(II)-IIP for the selective removal of Ca(II) ion from Ca(II) aqueous solutions.
ii. To utilize Taguchi DoE for the optimization of the IIP synthesis, adsorption and application processes.
iii. To carry out adsorption, kinetic, isotherm, reusability and selectivity studies on the Ca(II)-IIP.
iv. To apply Ca(II)-IIP in the removal of Ca(II) ions from an actual sample (human blood serum) and study the predictivity of iCa level using Ca(II)-IIP.

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BIODATA OF STUDENT

Rafah Al-maibd was born in Basra (Iraq) on the 16th May 1972. She started her primary education at Al-Fayhaa primary school in Basra. Then, she completed her secondary education at Al- Basra school secondary. She obtained her Baccalaureate level in 1990. Later, she completed her Bachelor of Science (Hons) degree in Chemistry in 1995 from Basra University.

In 1997, she worked as chemist in the quality control department at the Advanced pharmaceutical industry in Amman, Jordan. In 2012, Rafah was offered a government job in Al-Fayhaa general hospital in Basra as Chemist in AL-FAIHA SPECIALIZED DIABETES ENDOCRINE AND METABOLISM CENTER in which she was in charge of the daily analaysis of daily patient's samples using automated clinical biochemistry analyzers. She was responsible of trouble shooting and maintaining the analayzers at the laboratory.

Rafah has enrolled as a full time Master student in September 2018 at Universiti Putra Malaysia under the supervision of Dr. Sazlinda Kamurzamman.

LIST OF PUBLICATIONS

Peer review papers:

Rafah AL-Maibd, Wissam Al-Ashaq, Norhazlin Zainuddin, Nor Azowa Ibrahim, Intan Nureslyna Samsudin, Sazlinda Kamaruzaman, *Synthesis and optimization selective ion□imprinted polymer for the elimination of Ca II ions using Taguchi design",* Journal of Polymer Research, Vol 28, Issue 3, Pages 1-16, Springer, (March 202)

Conferences

Rafah AL-Maibd and Sazlinda Kamurzamman, *Optimization of the synthesis and application of Novel Ca(II) Ion-Imprinted Polymer (IIP) using Taguchi Method*, The International Virtual Conference on Fundemnatl and Applied Scinces (IFSC 2020), Kasetsart University, Thailand.

Rafah AL-Maibd, 11th International Fundamental Science Conference (iFSC 2019), Palm Garden Hotel, Putrajaya, Malaysia (October 2019)

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