SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE/MONTMORILLONITE NANOCOMPOSITE FOR DRUG DELIVERY SYSTEM

By

NUR AKMA BINTI ABDUL HALIM

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

My late pa, Abdul Halim Mohamad, my late ma, Razmah Epit, my beloved husband, Shahrizan Shahdan, my beautiful and cute daughter, Jasmine Suraya Shahrizan and my siblings, Qalam Abdul Halim, Rabiatul Adawiyah Ismail, Kamil Abdul Halim, Nuratiqa Suhaimi, Fitriyanie Abdul Halim, Nur Ain Nafeesa and Uwais Al-Qarni who always give me support along my study journey. I love all of you so much.

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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Chair : Prof. Mohd Zobir Hussein, PhD
Institute : Nanoscience and Nanotechnology

The low mechanical strength of hydroxyapatite restricts its use as a synthetic bone scaffold in bone tissue engineering applications. In this research work, hydroxyapatite was successfully incorporated with montmorillonite via a conventional sintering technique. Due to the insertion of montmorillonite, the specific surface area of hydroxyapatite was decreased from 76 m^2/g to 73 m^2/g and 68 m^2/g for 80%/20% and 50%/50% of hydroxyapatite/montmorillonite nanocomposite, respectively which subsequently enhanced the mechanical strength nanocomposite. The nanocomposites were undergone a sintering process in order to further ameliorate their mechanical strength. Consequently, the mechanical strength of 80%/20% hydroxyapatite/montmorillonite nanocomposite reached a maximum strength of 421 MPa. Meanwhile, the strength of 50%/50% of hydroxyapatite/montmorillonite merely reached 225 MPa after undergoing the sintering process. FTIR study showed that the absorption band of the resulting nanocomposite was comprised of both characteristics of hydroxyapatite and montmorillonite which witnessed the successful incorporation. XRD assayed revealed that new peakshave appearedat the incorporation of nanocomposite which was evidenced bythe formation of whitlockite and anhydrite. The nanocomposite was examined to deliver anti-inflammatory drugs for bone tissue engineering namely cloxacillin and fusidic acid. 2% w/w of cloxacillin and fusidic acid were encapsulated into nanocomposite in different temperatures viz. 37°C, 50°C and 70°C. The amount of drug encapsulated was found to be high in 80%/20% hydroxyapatite/montmorillonite nanocomposite for cloxacillin and fusidic acid at 37°C. Besides that, the drug adsorption in fusidic acid occurred due to an exothermic reaction.Further studies on in vitro drug delivery of nanocomposites were done at fixed conditions. The drug-encapsulated nanocomposites at 37°C showed high cumulative drug release for both drugs.The drug release mechanism for cloxacillin and fusidic acid wasfound to follow pseudo-second-order kinetic models with R^2 above 0.98. An apatite layer was formed on the

surface of the nanocomposite indicating the bioactivity of hydroxyapatite increased as the amount of montmorillonite increased. This is toward the upconversion of hydroxyapatite using a cheap material for dual purposes; bone tissue engineering and drug delivery.

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

SINTESIS DAN PENYIFATAN NANOKOMPOSIT HIDROKSIAPATIT/MONTMORILLONIT UNTUK SISTEM PENYAMPAI UBAT

Oleh

NUR AKMA BINTI ABDUL HALIM

Mei 2023

Pengerusi : Prof. Mohd Zobir Hussein, PhD
Institut : Nanosains dan Nanoteknologi

Kekuatan mekanikal hidrosiapatit yang rendah menyekat penggunaannya sebagai implan tulang dalam aplikasi kejuruteraan tisu tulang. Dalam penyelidikan ini, hidrosiapatit telah berjaya digabungkan dengan montmorillonit dengan menggunakan teknik pensinteran konvensional. Disebabkan oleh kemasukan montmorillonit, luas permukaan BET hidroksiapatit telah menurun daripada 76 m^2/g kepada 73 m^2/g dan 68 m^2/g untuk masing-masing 80%/20% dan 50%/50% nano komposit hidrosiapatit/montmorillonit, dimana seterusnya meningkatkan kekuatan mekanikalnya. Nanokomposit tersebut telah menjalani proses pensinteran untuk meningkatkan lagi kekuatan mekanikal nanokomposit. Akibatnya, kekuatan mekanikal 80%/20% hidroksiapatit/montmorillonite nanokomposit mencapai kekuatan maksima iaitu 421 MPa. Sementara itu, kekuatan 50%/50% hidroksiapatit/montmorilonit hanya mencapai 225 MPa selepas melalui proses pensinteran. Kajian FTIR menunjukkan bahawa jalur penyerapan nanokomposit yang dihasilkan terdiri daripada kedua-dua ciri hidrosiapatit dan montmorillonit memberi bukti kejayaan penggabungan tersebut. Ujian XRD mendedahkan bahawa jalur baharu telah wujud yang membuktikan pembentukan whitlokit dan anhidrit. Nanokomposit tersebut diuji untuk menyampaikan ubat anti radang untuk kejuruteraan tisu tulang iaitu kloksasillin dan asid fusidic. 2% berat kloksasillin dan asid fusidik telah terkapsul di dalam nano komposit dalam suhu yang berbeza iaitu 37°C, 50°C and 70°C. Ubat terkapsul nanokomposit didapati tinggi dalam 80%/20% hidroksiapatit/montmorillonite untuk kloksasillin dan asid fusidik. Selain itu, penjerapan asid fusidikberlaku disebabkan oleh tindak balas eksotermik. Kajian lanjut mengenai in vitro penyampai ubat telah dijalankan pada keadaan tetap.Ubat terkapsul nanokomposit pada suhu 37°C telah menunjukkan pelepasan ubat kumulatif yang tinggi bagi kedua-dua jenis antibiotik darinanokomposit. Mekanisma pelepasan ubat untuk kloksasillin dan asid fusidik didapati mengikut model kinetik pseudo orde kedua dengan nilai R^2 melebihi 0.98. Tompokan apatit telah muncul diatas permukaan nanokomposit menunjukkan aktiviti bio hidrosiapatit meningkat apabila bilangan montmorillonit

meningkat. Ini adalah kearah penukaran hidrosiapatit menggunakan bahan murah untuk dua tujuan; kejuruteraan tisu tulang dan sistem penghantaran ubat.

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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:

Mohd Zobir bin Hussein, PhD
Professor
Institute of Nanoscience and Nanotechnology
Universiti Putra Malaysia
(Chairman)

Che Azurahanim binti Che Abdullah, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

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

Date: 12 October 2023

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LIST OF ABBREVIATIONS

IUPAC	International Union of Pure and Applied Chemistry
XRD	X-ray diffraction
MSSA	Methicillin-susceptible staphylococcus aureus
SEM	Scanning electron microscopy
MIC	Minimal inhibitory concentration
PBS	Phosphate buffer solution
FDA	Food drug additive
FTIR	Fourier transform infrared spectroscopy
ATR	Attenuated total reflectance
FESEM	Field emission scanning electron microscopy
UV-Vis	Ultraviolet-Visible spectrophotometer
BET	Brunauer-Emmet-Teller
UTM	Universal testing machine
FWHM	Full width at half maximum
SSA	Specific surface area
MRSA	Methicillin-resistance S. aureus
EF-G	Elongation factor G
GDP	Guanosine diphosphate
ΔH	Enthalpy change
ΔS	Entropy change
ΔG	Gibbs free energy change
K	Kelvin
R	Universal gas constant
R2	Coefficient of determination

CHAPTER 1

INTRODUCTION

1.1 Research background

Nanotechnology is defined as the technology that utilizes molecules or structures in nanometer size ranging between 1nm and 100nm, where the uniqueness entitle novel application in broad-spectrum, from chemistry, physics and biology to engineering, medical and electronics. In 1959, the lecture entitled “There’s Plenty of Room at the Bottom” by Richard Feynman, predicted that humans will utilize things at the atomic level. Feynman’s hypotheses have been proven practical, and for this reason, he is known as the father of modern nanotechnology. After almost two decades, a scientist from Japan named Norio Taniguchi coined the term “nanotechnology” as; the separation, consolidation and deformation of materials by one atom or one molecule (Javed et al., 2019). In the medical field, nanotechnology is extensively applied for bone disease treatment in order to combat the limitations of conventional treatment. Unfortunately, the current golden standard of autologous bone grafting is hampered by inadequate supply, donor site morbidity, variable resorption and low passing rate in certain sites. These limitations brought an idea of the nanomaterials formulation, especially for the regeneration of bone tissue. At the present time, research focused on the application of nanomaterials as an implant material in bone tissue engineering field. Bone tissue engineering is an emerging field where chemical, physical and biochemical signal like genetically fabricated materials, growth factor and drugs are carried through a nano-biomaterial-based scaffold to improve bone regeneration in the desired site of human body (Mottaghitalab et al., 2015; Samorezov and Alsberg, 2015). In numerous cases, the man-made bone scaffold itself was not able to complete bone regeneration. To address this limitation, the idea to formulate bone scaffold made from bio-nanocomposite incorporating drug to accelerate bone tissue regeneration is emerged.

The porous biodegradable nano-size materials are preferred due to their ability to provide mechanical support during the repair and regeneration of damaged or infected bone. Based on the International Union of Pure and Applied Chemistry (IUPAC), nanoporous materials are grouped as macroporous ($d \geq 50nm$), mesoporous ($2nm \leq d < 50nm$) and microporous ($d < 2nm$). However, it is quite challenging to produce a nano-biomaterial with good mechanical strength. The high mechanical strength can be achieved by scarifying the porous structure as the porous structure is important for cell adhesion and proliferation. Besides that, the porous structure is also useful as a controlled drug delivery vehicle owing to several features such as high surface area, tunable pore sizes and stable uniform pore structure. These features empower them to adsorb and release drugs consistently and in a predictable manner.

Nowadays, research focused on fabricating nanocomposite to mimic the original bone. This fact was owed to the unique character of nanocomposite including high wear resistance and chemical inertness with great mechanical strength which can reduce the aforementioned limitations of conventional therapies. In general, the nanocomposite is comprised of a matrix material incorporated with nanomaterials like nanoclay, nanotubes, nanorod and nanowires, etc. Nanocomposites are tailored to suit their intended application, which is the reason behind their classification. These nanocomposite materials are classified depending on the nature of matrix phase material namely ceramic matrix nanocomposite, metal matrix nanocomposite and polymer matrix nanocomposite. Aside from being widely applied in the medical field, nanocomposites also find applications in a variety of sectors of human society such as in biotechnology (e.g. gene delivery devices), agriculture (e.g. nanopesticides and nanofertilizers), automotive manufacturing (e.g. automobile parts like car compartment), construction manufacturing (e.g. material for buildings and bridges, etc) (Garcés et al., 2000);(Sahoo & Tripathy, 2017);(Tripathy, 2017);(Hossain et al., 2020);(Kumar et al., 2021). Nanocomposite has been used, is still in use and will continue to be used to satisfy the material demand of such industries, at least until a more sustainable alternative is coined or invented.

1.2 Problem statement

Modern medicine is formulated to treat certain health problems in a short period of time. In modern medicine, drug was widely used and applied in treating illness and injury. Drug is a chemical substance that is used as a remedy to prevent and cure various diseases. Conventional drug administration, while extensively applied such as tablets, syrups, capsules and ointments endure from poor bioavailability, poor absorption from desired site, instability level in body plasma and high dose discard. The conventional drug administration methods also showed ineffective antibiotics localization and an excessive amount of drugs may cause the risk of systemic toxicity. This therapy often failed when bacteria form a biofilm and adhere to the bone implant in bone regeneration process.

A well-known nano-biomaterials, hydroxyapatite (HA) is a type of calcium phosphate and is recognized to be biocompatible due to its chemical similarities to the mineral constituent of bones or teeth. Consequently, HA is usually used as a drug carrier material in drug delivery sytem to aid in bone tissue regeneration. Nevertheless, HA has low mechanical strength as low as 12 MPa even after being sintered at 1000°C (Komur et al., 2016) which limit its application as drug carrier in bone tissue engineering. Figure 1.1 showed the chemical structure of hydroxyapatite.

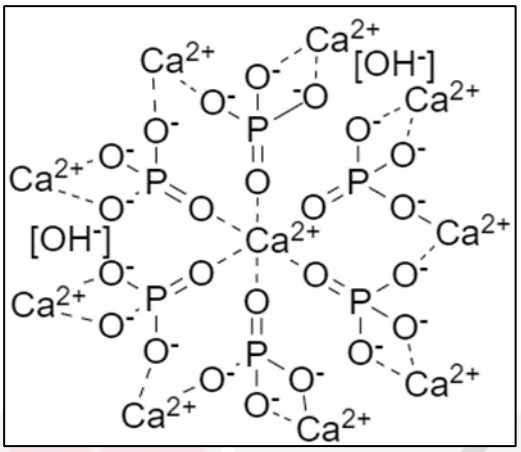

Figure 1.1: Hydroxyapatite chemical structure

In this research, the nanocomposite-based hydroxyapatite was formulated by incorpating clay named montmorillonite to achieve better mechanical strength. The addition of montmorillonite into the nanocomposite is expected to improve the mechanical strength of nanocomposite-based hydroxyapatite by tuning the pores structure of hydroxyapatite. In addition, the pores structure of hydroxyapatite/montmorillonite nanocomposite could make the nanocomposite as a good candidate for drug carrier.

1.3 Objectives

There are a few objectives of this research. Essentially, the main goal of this research is to synthesis and characterizes the hydroxyapatite-montmorillonite nanocomposite and its controlled release properties as drug carrier.

In particular, the objectives of this research are as follows:

1. To design, synthesis and optimize the hydroxyapatite-montmorillonite nanocomposite.
2. To study the physicochemical properties of hydroxyapatite-montmorillonite nanocomposite at different temperatures by using fourier transform infrared spectroscopy (FT-IR), x-ray radiation diffraction (XRD), universal testing machine (UTM), Brunauer-Emmet Teller (BET) and field emission electron microscopy (FESEM).
3. To study the adsorption mechanism and controlled release properties of hydroxyapatite-montmorillonite nanocomposite of cloxacillin and fusidic acid by using uv-vis spectrophotometer.

1.4 Significance of study

The present studies were carried out in order to formulate a novel ceramic-based nanocomposite by using hydroxyapatite. Hydroxyapatite was incorporated with clay named montmorillonite through a conventional powder sintering technique. This synthesis method used is simple, cost-effective, eco-friendly and economical of scale method. These formulations were proved can improve the mechanical strength and pore structure of hydroxyapatite. Furthermore, the presence of montmorillonite was found to enhance the bioactivity of nanocomposite by increasing the apatite formation. In drug delivery studies, these formulations were capable to sustain the release of the drug adsorbed onto the pore structure of nanocomposite. Survey of the recent literature indicates that no such research studies were done in biomedical applications, especially for drug delivery systems.

1.5 Scope of study

The scope of this study is to investigate the physiochemical properties of hydroxyapatite/montmorillonite nanocomposite and drug delivery studies. In this study, hydroxyapatite/montmorillonite nanocomposite was synthesized by conventional powder sintering technique atvarious temperatures. Subsequently, the sintered hydroxyapatite/montmorillonite nanocomposite was used as a carrier for a drug delivery system with two types of drugs viz. cloxacillin and fusidic acid.

In Chapter 4, the study covers the synthesis and characterization of hydroxyapatite/montmorillonite nanocomposite and the drug delivery study of cloxacillin. A cloxacillin adsorption was studied at a certain temperature to investigate the effect of montmorillonite addition onto nanocomposite towards the drug adsorption process. The release profile of cloxacillin from nanocomposite(s) was studied in phosphate buffer solution (PBS) at pH 7.0 to mimic the human body fluid. In chapter 5, the scope is involving the physicochemical properties of hydroxyapatite/montmorillonite nanocomposite loaded fusidic acid, the adsorption behavior of fusidic acid onto hydroxyapatite/montmorillonite nanocomposite in different temperature and their drug delivery study.

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