UNIVERSITI PUTRA MALAYSIA

HIGH INTERNAL PHASE EMULSION AS A REACTION MEDIUM FOR FABRICATION OF BRUSHITE CRYSTAL

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By

LIM HONG NGEE

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

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To my husband for braving the many ups and downs with me during the trying times, steadily and stoically. You are indeed my pillar of strength.

To Ma and Pa for your unceasing love, support and faith in me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

HIGH INTERNAL PHASE EMULSION AS A REACTION MEDIUM FOR FABRICATION OF BRUSHITE CRYSTAL

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Chair: Anuar Kassim, PhD

Faculty: Science

This present work was aimed at fabrication of porous brushite crystals using oil-in-water high internal phase emulsion stabilized by synthesized palm-based nonionic surfactant as a reaction medium. This research work was divided into four categories. The first part of the work involved synthesizing palm-based nonionic surfactants. Palm oil derivatives, lauryl, palmityl and stearyl alcohols as renewable resources, were ethoxylated with an average of three, six (or eight or nine) and 100 moles of ethylene oxide. The critical micelle concentration of the synthesized surfactants was found to decrease with increasing ethylene oxide head groups due to intertwist amongst the head groups. This phenomenon enhances surfactant-surfactant interaction rather than surfactant-solvent interaction which increases the rate of micellization as proven by the Gibbs energy. The increase in the surfactant tail length had minimal effect on micellization. The second part of the work was to stabilize the high internal phase emulsion using the synthesized surfactants. The oil phase was vegetable oil, namely olive and olein oils. These

emulsions, with dispersed phase of more than 75 wt%, were easily prepared by one-pot homogenization. Due to the high oil volume fraction, the oil droplets were no longer spherical but were squeezed to take the shape of polyhedral. Light scattering results showed that the droplet size increased with increasing ethylene oxide chain length. The rheology of the emulsions was governed by droplet size and oil volume fraction. The emulsions exhibited high stability as indicated by the rheological measurements even after storage at 40^{o}C for three months. The third part of the work was on the fabrication of brushite crystals with high degree of porosity using the high internal phase emulsion as a reaction medium. The porosity of the crystals was manifested by precursor concentration, surfactant concentration, oil volume fraction, mixing method, mixing time, aging temperature, precursor type, mode of recovery and surfactant head group. Pore size of the brushite crystals was less than 5 μm. The mechanism for the formation of porous brushite crystals was postulated schematically based on the small angle x-ray scattering analysis. The fourth and final part of this work was related to the application of the porous brushite crystals as drug delivery devices. Prior to the controlled release study, the crystals were subjected to cytotoxicity test to ensure their compatibility with synoviocytes, which are cells that line the knee joints of rabbits. The crystals were found to enable cell growth for up to five days. Sodium ampicillin, a wide spectrum antibiotic, was successfully loaded into the pores of the crystals and subsequently released in vitro for 14 days. This work underlines the simplicity of using highly stable high internal phase emulsion as a reaction medium for the fabrication of porous brushite crystals, in which when loaded with drug, exhibited potential as localized bone treatment demonstrated by the promising controlled release rate.

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

EMULSI BERKEPEKATAN TINGGI SEBAGAI MEDIA TINDAK BALAS UNTUK PEMBENTUKAN HABLUR BRUSHITE

Oleh

LIM HONG NGEE

Disember 2009

Pengerusi: Anuar bin Kassim, PhD

Fakulti: Sains

Kajian ini bertujuan untuk menghasilkan hablur brushite berliang menggunakan emulsi berkepekatan tinggi minyak-dalam-air yang distabilkan oleh surfaktan nonionik berasaskan sawit sebagai media tindak balas. Kajian ini dipecahkan kepada empat bahagian. Bahagian pertama kajian melibatkan sintesis surfaktan nonionik berasaskan sawit. Terbitan minyak sawit, iaitu alkohol lauril, palmitil and stearil sebagai sumber guna semula, telah dietoksilasikan dengan purata tiga, enam (atau lapan atau sembilan) dan 100 mol etilena oksida. Kepekatan misel kritikal surfaktan yang disintesiskan menurun dengan peningkatan kumpulan kepala etilena oksida disebabkan pembelitan antara kumpulan-kumpulan tersebut. Fenomena ini menambahkan interaksi surfaktan-surfaktan berbanding dengan interaksi surfaktan-pelarut yang akan meningkatkan kadar permiselan seperti yang dibuktikan oleh tenaga Gibbs. Peningkatan dalam panjang ekor surfaktan memberikan kesan yang sedikit terhadap permiselan. Bahagian kedua kajian merangkumi penstabilan emulsi berkepekatan tinggi menggunakan surfaktan yang disintesiskan. Fasa minyak ialah minyak sayuran, khasnya minyak-minyak zaitun dan

olein. Emulsi ini, dengan fasa tersebar melebihi 75 wt%, dapat disediakan dengan mudah melalui penghomogenan satu-kali. Akibat daripada pecahan isipadu minyak yang tinggi, titisan-titasan minyak tidak lagi berada dalam keadaan sfera tetapi dihimpitkan kepada bentuk polihedral. Hasil penyerakan cahaya menunjukkan saiz titisan meningkat dengan penambahan rantai panjang etilene oksida. Reologi emulsi dikawal oleh saiz titisan dan pecahan isipadu minyak. Emulsi menonjolkan kestabilan yang tinggi berdasarkan pengukuran reologi walaupun selepas penyimpanan pada 40^{o}C selama tiga bulan. Bahagian ketiga kajian ini adalah berkaitan dengan penghasilan hablur brushite menggunakan emulsi berkepekatan tinggi sebagai media tindak balas. Keporosan hablur dipengaruhi kepekatan bahan pemula, kepekatan surfaktan, pecahan isipadu minyak, cara pencampuran, masa pencampuran, jenis bahan pemula, cara perolehan dan kumpulan kepala surfaktan. Saiz liang hablur brushite adalah kurang daripada 5 μm. Mekanisma pembentukan hablur brushite berliang dijangka secara skematik berdasarkan analisis penyerakan sinar-X bersudut kecil. Bahagian keempat dan terakhir kajian ini adalah berhubungan dengan penggunaan hablur brushite berliang sebagai alat penghantaran ubat. Sebelum kajian kawalan perlepasan, ujian ketoksikan dijalankan terhadap hablur tersebut untuk memastikan keserasiannya dengan sinoviosit, iaitu sel yang melapik sendi lutut arnab. Hablur itu didapati menggalakan pertumbuhan sel selama lima hari. Natrium ampisilin, antibiotik dengan spektrum yang luas, berjaya dimasukkan ke dalam liang hablur dan seterusnya, dilepaskan in vitro selama 14 hari. Kajian ini menyerlahkan kemudahan menggunakan emulsi berkepekatan tinggi sebagai media tindak balas untuk pembentukan hablur brushite berliang, apabila dimasukkan ubat, memaparkan potensi sebagai perubatan tulang setempat seperti yang ditunjukkan oleh kadar kawalan perlepasan yang memuaskan.

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It may sound cliché but my baby is my inspiration. His smile is enough to make me forget the darkest moment in my life. I love you and thank you for being a good baby when Mama was rushing to complete her assignments.

This chapter of my life opens my eyes and heart and makes me believe that this world is full of hope and kindness. The next chapter continues what promises to be an even more exciting and enticing journey. I look forward to more colourful sparks awaiting me.

Life rocks!

I certify that a Thesis Examination Committee has met on 24 December 2009 to conduct the final examination of Lim Hong Ngee on her thesis entitled "High Internal Phase Emulsion as a Reaction Medium for Fabrication of Brushite Crystal" in accordance with the Universities and University Colleges 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 Doctor of Philosophy Degree.

Members of the Thesis Examination Committee were as follows:

Md. Jelas Haron, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Taufiq Yap Yun Hin, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mahiran Basri, PhD
Professor
Centre of Foundation Studies for Agriculture Sciences
Universiti Putra Malaysia
(Internal Examiner)

Hamdan Suhaimi, PhD
Professor
Department of Chemical Sciences
Faculty of Science and Technology
Universiti Malaysia Terengganu
Malaysia
(External Examiner)

BUJANG BIN KIM HUAT, 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 of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Anuar Kassim, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Dzulkefly Kuang Abdullah, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Abdul Halim Abdullah, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohd. Ambar Yarmo, PhD
Professor
School of Chemical Sciences and Food Technology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(Member)

Yeong Shoot Kian, PhD
Head
Advanced Oleochemicals Technology Division
Malaysian Palm Oil Board
(Member)

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

Date: 11 February 2010

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.

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LIM HONG NGEE

Date: 22 February 2010

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BMP	Bone morphogenetic proteins
CMC	Critical micelle concentration
CPCs	Calcium phosphate cements
D	Deborah Number
DCPD	Bicalcium phosphate dehydrate or brushite
DSD	Droplet size distribution
EO	Ethylene oxide
FID	Flame ionization detector
FTIR	Fourier Transformed Infrared Spectroscopy
GC	Gas chromatography
HA	Hydroxyapatite
HIPE	High internal phase emulsion
HLB	Hydrophilie-lipophile balance
LVR	Linear viscoelastic region
MTX	Methotrexate
NMR	Nuclear magnetic resonance
O/W	Oil-in-water
OCP	Octacalcium phosphate
PBS	Phosphate buffer solution
PMMA	Poly(methyl-methacrylate)
SAXS	Small angle x-ray scattering
SEM	Scanning electron microscopy

TCP	Tricalcium phosphate
UV	Ultraviolet
W/O	Water-in-oil
XRD	X-ray diffractometry
σ	Stress
σ_0	Critical stress
ε	Strain
γ_c	Critical strain
δ	Phase angle
Ø	Volume fraction
A	Absorbance
a	Proportionality constant
c	Concentration
E_c	Cohesive energy
ΔG^o_{mic}	Standard free energy of micellization
G’	Storage modulus
G”	Loss modulus
G*	Dynamic modulus
k	Constant
l	Pathlength
M_{hg}	Molecular weight of the hydrophilic head group
M_s	Total molecular weight of the surfactant
M_w	Molecular weight

R	Universal gas constant
R	Aggregates diameter
R_g	Radius of gyration
T	Absolute temperature
V_c	Energy barrier for three particles
V_d	Energy barrier for two particles

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