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DEVELOPMENT OF HALAL PLANT-BASED HYDROCOLLOIDS ENCAPSULATION FOR TARGETED DELIVERY OF BOVINE SERUM ALBUMIN

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By

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DEVELOPMENT OF HYDROCOLLOIDS-BASED ENCAPSULATION FOR TARGETED DELIVERY OF BOVINE SERUM ALBUMIN

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Advances in biotechnology over the past few years have driven the production of various clinically useful protein and peptides. Till recent, parenteral route (injection) is the most common way for administering protein drugs. However, the patient compliance with injection regimens is very poor, particularly for disease like diabetes. Thus, oral route remains as the most preferable route to deliver protein drugs due to ease of administration. However, administration of protein and peptide drug through oral route is quite challenging in terms of controlled delivery, targeting formulations and controlled manner. One way to overcome this problem is by using encapsulation technique or incorporating the protein into microcapsule made of biodegradable polymers. The potential of using encapsulation method to develop controlled release matrices for protein delivery during passing through the gastrointestinal tract was investigated in this study. Konjac glucomannan and gum Arabic were chosen as the potential polysaccharides to be combined with sodium alginate as encapsulating matrices and bovine serum albumin (BSA) as model protein. The study was accomplished through the following approaches: 1) optimization of encapsulating matrices to produce controlled-release formulation and improve encapsulation yield; 2) determination of protein release activities based on swelling rate (%) and in-vitro release during exposure to simulated gastric (SGF) and intestinal fluid (SIF); 3) determination of protein-polysaccharide interaction within the beads and bead morphology by using Fourier-Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy, respectively. Statistical modeling based on the Face Centered Central Composite Design (FCCD) was employed for the optimization of encapsulating matrices. The optimum concentration for alginate-konjac glucomannan was predicted at 4% (w/v) and 0.6% (w/v), respectively. Whereas, in the case of alginate-gum Arabic, combination of alginate at concentration 3% (w/v) and 2% (w/v) of gum Arabic was predicted to produce optimum responses. Through verification step, experimental data of alginate-konjac glucomannan and alginate-gum Arabic remained close value to the predicted value with low error for all the response. IR spectra of alginate-konjac glucomannan beads showed that electrostatic interaction and hydrogen binding exist between alginate and konjac glucomannan. In addition,

significant characters of BSA were observed in IR spectrum which suggesting there was no interaction between the protein (BSA) and the polymer used (alginate and konjac glucomannan). In the case of alginate-gum Arabic beads, there was also no interaction between BSA and encapsulating matrices (alginate and gum Arabic). The SEM photograph of these beads showed spherical shape with a rough surface. Cracks and wrinkles also were seen on the beads surface which might occur during drying process. The performances of optimized alginate-konjac glucomannan and alginate-gum Arabic beads as sustained-release beads were determined. Five groups encapsulating matrices were evaluated (1: optimized alginate-konjac glucomannan, 2: optimized alginate-gum Arabic, 3: alginate-hydroxypropyl methylcellulose (HPMC), 4: alginate alone, 5: free protein). Low protein encapsulation efficiency was observed in group 4. On the other hand, group 2 showed the highest protein encapsulation efficiency. Slow swelling rate was observed during exposure to acidic medium (SGF) by groups 1 and 2 while groups 3 and 4 has demonstrated advanced swelling in 2h of exposure. The releases of protein occur when the beads disintegrate and these were observed through the release activity analysis. Positive performance in releasing protein into intestinal region was shown by groups 1, 2 and 3. The *in vitro* dissolution of these beads showed prolonged release of BSA for almost 4 h. Encapsulations of both konjac glucomannan and gum Arabic with alginate combination have successfully improved the survival and protein release to target area which is the small intestine. Therefore, these biodegradable materials could potentially be useful as alternative for halal capsule, instead of HPMC. Furthermore, by using these formulations, the oral delivery of protein drugs for the treatment of pediatric patients is now possible. Thus, the pain and discomfort due to frequent injections in everyday treatment can be avoided.

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

PENGHASILAN ENKAPSULASI BERASASKANHIDROKOLOID UNTUK PENGHANTARANYANG DISASARKAN BOVINE SERUM ALBUMIN

Oleh

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Kemajuan dalam bidang bioteknologi dalam masa beberapa tahun lepas telah meningkatkan penghasilan pelbagai protein dan peptide yang berguna sebagai ubat-ubatan. Sehingga kini, suntikan merupakan cara yang paling biasa digunakan untuk memasukkan protein ke dalam tubuh pesakit. Tetapi, toleransi pesakit terhadap cara ini adalah sangat rendah terutamanya untuk pesakit yang menghidap diabetes. Oleh sebab itu, pesakit lebih gemar memilih cara oral kerana ianya lebih mudah. Walau bagaimanapun, pengendalian protein dan peptide melalui cara oral adalah agak mencabar dari segi penghantaran berterusan, sasaran formulasi dan cara kawalan. Salah satu cara untuk mengatasi masalah ini adalah dengan menggunakan kaedah enkapsulasi atau memasukkan protein ke dalam mikro kapsul yang diperbuat daripada polimer biodegradasi. Oleh itu, potensi kaedah enkapsulasi dalam menghasilkan kapsul yang mampu melindungi protein semasa melalui sistem pencernaan telah dikaji. Konjak glukomanan dan gam Arabik telah dipilih untuk digabungkan dengan alginatsebagai bahan enkapsulasi, manakala bovine serum albumin (BSA) pula sebagai protein model. Kajian ini melibatkan beberapa peringkat seperti: 1) mengoptimasikan kombinasi bahan enkapsulasi yang digunakan bagi menghasilkan formulasi penghantaran berterusan dan meningkatkan kadar enkapsulasi protein (%); 2) mengkaji hubungan antara protein dan bahan enkapsulasi dengan menggunakan spektroskopi transformasi fourier inframerah (FT-IR) serta memerhati perubahan bentuk kapsul dengan menggunakan mikroskop imbasan electron (SEM); 3) mengkaji aktiviti perlepasan protein ketika di dalam SGF dan SIF berdasarkan kadar pembengkakan kapsul (%) dan analisis perlepasan protein.Model statistic berdasarkan *Face Centered Central Composite Design* (FCCD) digunakan untuk mengoptimasikan bahan enkapsulasi. Titik optima konsentrasi untuk alginat-konjak glukomanan adalah pada 4% (w/v) dan 0.6% (w/v). Manakala, alginat-gam Arabik ialah pada 3% (w/v) dan 2% (w/v) untuk kesan yang optima. Berdasarkan verifikasi, nilai eksperimen bagi kapsul yang dioptimakan tidak menunjukkan perbezaan besar dengan nilai ramalan. Spektra FTIR alginat-konjak glukomanan menunjukkan tarikan elektrostatik dan ikatan hidrogen wujud di antara alginat dan konjak glukomanan.

Tambahan pula, ciri-ciri penting BSA yang telah dikenal pasti dalam spektra tersebut menunjukkan tiada interaksi di antara protein dan bahan enkapsulasi iaitu alginat dan konjak glukomanan. Hasil ujian yang sama telah diperoleh dalam kes alginat-gam Arabik, iaitu tiada interaksi antara BSA dan polimer yang digunakan sebagai bahan enkapsulasi. Gambar rajah SEM menunjukkan kapsul berbentuk bulat dengan permukaan yang kasar. Keretakan dan kedutan yang terdapat dipermukaan kapsul mungkin terhasil semasa proses pengeringan kapsul. Tahap perlindungan bagi protein dengan menggunakan bahan enkapsulasi yang dioptimasikan telah dikaji. Lima formulasi bahan enkapsulasi telah dikaji (1:alginat-konjak glukomanan, 2: alginat-gam Arabik, 3: alginat-hidroxipropil metilselulos (HPMC), 4: alginat sahaja dan 5: protein bebas). Kadar enkapsulasi protein yang rendah diperolehi daripada kumpulan 4. Manakala, kumpulan 2 menunjukkan kadar enkapsulasi protein yang paling tinggi. Kadar pembengkakan yang rendah dilihat dari kumpulan 1 dan 2, manakala kumpulan 3 dan 4 menunjukkan pembengkakan yang cepat setelah 120 minit dalam SGF. Pembebasan protein berlaku apabila kapsul mula pecah dan ini boleh dilihat menerusi analisa pembebasan aktiviti. Kesemua kumpulan kecuali kumpulan 4 menunjukkan kesan positif dalam pembebasan protein ketika di dalam SIF. Enkapsulasi menggunakan konjak glukomanan dan gam Arabik dengan gabungan alginat dapat menambahbaikan tahap kehidupan dan pembebasan protein di dalam usus dan berpotensi untuk menggantikan penggunaan HPMC sebagai kapsul halal. Tambahan lagi, dengan menggunakan formula ini, penghantaran protein melalui cara oral boleh diaplikasikan. Maka, kesakitan dan ketidakkeselesaan yang disebabkan oleh suntikan kerap dalam rawatan harian dapat dielakkan.

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I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of Hajaratul Najwa Mohamed on her thesis entitled "Development of Hydrocolloid-based Encapsulation for Targeted Delivery of Bovine Serum Albumin" 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.

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LIST OF ABBREVIATION

3D	3 Dimension
ALG	Alginate
ANOVA	Analysis of variance
AR	Analysis Grade
β	Beta
BSA	Bovine serum albumin
°C	Degree centrigrade
-COOH	Carboxylic Acid group
$CaCl_2$	Calcium chloride
Ca^{2+}	Calcium ion
Cu^{2+}	Copper ion
DF	Degree of freedom
DNA	Deoxyribonucleic acid
Et al.	et cetera (and other)
FCCD	Face centered composite design
FT-IR	Fourier transform infrared spectroscopy
g	Gram
GA	Gum Arabic
gL^{-1}	Gram per liter
GHRH	Growth hormone releasing hormone
h	Hour
HCl	Hydrochloric acid
KGM	Konjac glucomannan
HPMC	Hyroxypropyl methylcellulose
HBsAg	Hepatitis B surface antigen
KBr	Potassium bromide
kV	Kilo volt
LOF	Lack-of Fit
mg	Milligram
min	minute
mM	milimolar
mm	Millimeter
MW	Molecular weight
NaCl	Sodium chloride
-OH	Hydroxyl group
o/o	Oil/oil
o/w	Oil/water
p	Probability
Pb^{2+}	Lead ion
PBS	Phosphate buffer saline
PEE	Protein encapsulation efficiency
pH	Power of hydrogen
PLA	Polylactic acid
PLG	Poly(lactide-co-glycolide)
R^2	Coefficient of determination
rpm	Revolution per minute
R_{2h}	Protein release at 2 h in SGF

RCOOH	Carboxylic acid group
$RCOO^-$	Carboxylate ion
RNA	Ribonucleic acid
RSM	Response surface methodology
± SD	Standard deviation
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
S_w	Swelling index
TA	Texture Analyzer
T_r	Time taken for 100% of BSA release in SIF
µL	Microliter
µM	Micromolar
UV-VIS	Ultra Violet-Visible
w/o/w	Water/oil/water
w/v	Weight per volume
x	Factor
Y	response
Zn^{2+}	Zinc ion

CHAPTER 1

INTRODUCTION

In recent times, the most common option for administering protein and peptide drugs is injections (i.e. intramuscular, intravenous or subcutaneous route). However, the patient compliance with this method is generally poor due to the discomfort during drug administration treatments. Subsequently, it will severely constrain the therapeutic value of the drug, especially for disease like diabetes (Rick, 2005). The alternate paths that have been tried for the time being are the oral, intranasal (Torres and Peppas, 2000), transdermal (Banga and Chien, 1993), buccal (Sayani and Chien, 1996), pulmonary (O'Hagan and Illum, 1990), rectal (Burgess, 1993) and ocular (Lee and Yalkowsky, 1999). All of these routes have varying degree of success and the most convenient as well as preferable approach to deliver drug is oral route. This is because oral route offers particular advantages to any other drug delivery. By using oral drug delivery, patients could avoid pain and discomforts related with injections and reduce the possibility of infections that caused by inappropriate use or recycle needles. In addition, the manufacturing cost for oral formulations is less expensive since they do not need to be prepared under sterile conditions (Salama, Eddington and Fasano, 2006).

Nevertheless, designing and formulating protein drug delivery system possess several challenges due to the critical physicochemical properties of proteins. These unfavorable properties are includes short plasma half-life, proneness to enzymatic degradation, large molecule size, immunogenicity, ion permeability and high possibility to undergo aggregation, denaturation and adsorption (Saffran, Kumar, Savariar, Burnham,Williams and Neckers, 1986). As a result, the bioavailability levels of most proteins and peptides are affected which are reported to be less than 1%. Therefore, the biggest challenge in protein drug delivery system is to increase the oral bioavailability to at least 30-50% (Vincent, Satish, George and Werner, 1991).

Various pharmaceutical approaches have been studied for improving oral protein and peptide bioavailability such as chemical modification, the use of enzyme inhibitors as well as absorption enhancer, encapsulation and mucoadhesive polymeric system (Shaji and Patole, 2008). Among all the methods, encapsulation becomes a promising technology to improve the bioavailability of protein drug delivery system (Johnson and Tracy, 1999). By definition, encapsulation is a technology which solid, liquid or gaseous material can be packaged or coated in small, sealed capsules that are able to release its content at controlled rates, affected by certain conditions (Anal, Stevens and Remuñán-López, 2006; Anal and Stevens, 2005; Kailasapathy and Masondole, 2005).

Among the various ionic biopolymers, sodium alginate is commonly used as the encapsulating material due to its unique property of forming hydrogel beads through ionotropic gelation (Patil *et al.,* 2010; Racovita *et al.,* 2009). Numerous attempts of producing sustained release beads by using sodium alginate have been carried out and many drugs have been successfully encapsulated with different drug release profiles (Morshad, Mallick, Nath, Uddin, Dut'ta, Hossain and Kawsar, 2010; Smrdel, Bogataj

and Mrhar, 2008). Even though alginate beads can be produced through a simple and mild ionotropic gelation method, it has main constraint due to drug loss during beads preparation through the beads pores (Singh, Sharma and Chauhan, 2010). For that reason, several modifications have been studied in which another biodegradable polymer was incorporated into the system to combine with alginate (Nayak, Das and Maji, 2012; Wang and He, 2002; Aral and Akbuğa, 1998). These new combinations of polymers were expected to improve the capsule physical properties including size, mechanical strength, wall thickness, permeability and surface characteristics (Wang, Lacik, Brissova, Anikumar, Prokop, Hunkeler, Green, Shahrokhi and Powers, 1997).

In this new era, hypromellose(HPMC) capsules are found to be a good alternative of gelatin capsule due to its plant source. To date, biodegradable gelatin has been used extensively in pharmaceuticals as drug carrier due to its excellent membrane-forming ability, amphoteric characteristics and biocompatibility (Wenrong and Griffiths, 2000). Most of pharmaceuticals capsules that available in market are made of porcine gelatin. However, porcine-derived gelatin is prohibited in Islam and forbidden for vegetarian. Thus, HPMC capsule have been produced as a vegetarian alternative to gelatin and already available commercially for nearly 10 years. HPMC capsule shells are made of hydroxypropyl methylcellulose which is also known as hypromellose. Various studies have been carried out in order to investigate the performance of HPMC capsule in term of drug release (Kumar, Sood, Rana and Singh, 2012; Akhgari, Abbaspour, Rezaee and Kuchak, 2011; Moawia, 2010; El-Malah, Nazzal and Bottom, 2007; Cole, Scott, Connor, Wilding, Petereit, Schminke, Beckert and Cadé, 2002).

HPMC possess unique characteristics such as fast gel formation to control initial release, high swelling, low erosion and formation of strong, viscous gel to control drug release. For these reasons, HPMC has been chosen by most formulators for preparation of hydrophilic matrix system and used in oral drug delivery (Siepmann and Peppas, 2001). Nochos and colleague (2008) have successfully encapsulated bovine serum albumin (BSA) in alginate – HPMC beads by using ionotropic gelation technique. In this study, all the encapsulating materials that have been used were plant based. Therefore, for comparison purpose, alginate – HPMC bead was set as a control. Although sodium alginate is widely used as encapsulation matrix, so far no studies have been conducted on the use of sodium alginate with the combination of konjac glucomannan or gum Arabic to encapsulate protein drug candidate, bovine serum albumin by using ionotropic gelation method. Therefore, this study was carried out with the main objectives to encapsulate bovine serum albumin using sodium alginate - konjac glucomannan/gum Arabic matrix. The specific objectives were:

1. To screen, optimize and validate the range of composition of alginate-konjac glucomannan and alginate-gum Arabic as encapsulating matrix for bovine serum albumin based on protein encapsulation efficiency (%) and protein release after being exposed to gastrointestinal fluid.

2. To investigate the excipients-protein interaction using Fourier Transform Infrared (FT-IR) spectroscopy and analyze the beads morphology using Scanning Electron Microscopy (SEM).

3. To determine the protein release profiles, swelling behaviour and stability of optimized alginate-konjac glucomannan/Arabic in different pH media, in comparison with alginate-HPMC based beads.

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