UNIVERSITI PUTRA MALAYSIA

PREPARATION AND OPTIMIZATION OF CARBOXYMETHYL SAGO STARCH HYDROGEL AS A POTENTIAL CARRIER FOR NEWCASTLE DISEASE VIRUS VACCINE

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By

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Carboxymethyl sago starch (CMSS) is an important water-soluble polysaccharide in many applications in food and non-food industries. When crosslinked, CMSS produce three-dimensional polymeric network hydrogel which has the ability to retain a large amount of solution inside its network. Newcastle disease (ND) is a highly contagious disease which causes devastating losses in poultry industries worldwide. This disease is caused by virulent strains of avian paramyxovirus and can be controlled by the used of Newcastle disease virus (NDV) vaccine. However, NDV vaccine is highly sensitive towards high temperature and need a proper storage chain in order to secure its efficacy. In this study, CMSS hydrogel was prepared by dissolving CMSS in HCl solution under vigorous stirring to form hydrogel. This CMSS hydrogel was then used to encapsulate NDV. Four parameters were studied to optimize the preparation of CMSS hydrogel, which were the effect of the percentage of CMSS, concentration of the HCl solution, reaction time and reaction temperature. The percentage of gel content and degree of swelling become the indicators studied in each parameter. 60% of CMSS in 2.0M HCl solution for 12 hours reaction time at room temperature were the optimum conditions for the preparation of CMSS hydrogel which resulted in 70.40 % of gel content with 50.79 (g/g) degree of swelling. The CMSS hydrogel was characterized by using Fourier transform infrared (FT-IR), thermogravimetric analysis (TGA), X-Ray diffraction (XRD) and scanning electron microscopy (SEM). FTIR spectrum of CMSS showed an additional absorption band at 1597 cm^{-1} indicating the substitution of $CH_2COO^-Na^+$ group on the starch molecular chain during carboxymethylation. While the spectrum of CMSS hydrogel showed an

additional sharp absorption band at 1723 cm^{-1} indicating that the Na in CMSS being exchanged to H from hydrochloric acid solution. TGA thermograms showed that carboxymethylation reaction shifted the maximum decomposition of sago starch to a lower temperature from 309.57 ºC to 295.33 ºC. However, the presence of crosslinkages in CMSS hydrogel gave better thermal stability when compared to CMSS which gave maximum temperature of decomposition at 330.22 ºC with 60.22 % major weight loss. X-ray diffraction pattern of CMSS showed that the semi-crystalline structure of sago starch completely destroyed after underwent carboxymethylation reaction. SEM image showed distorted and irregular shape of modified sago starch after carboxymethylation. Whereas, CMSS hydrogel showed a spongy surface with empty space called pores in structure and interconnected to each other to form networks. CMSS hydrogel exhibited pH-sensitive behavior as it showed highest swelling at PBS pH 7 but shrank at low pH and acidic solution. Two parameters were carried out in order to investigate the loading and release of encapsulated NDV vaccine in CMSS hydrogel which were the amount of CMSS hydrogel and loading time. 0.02 g of CMSS hydrogel at 30 minutes loading time were the optimum conditions in this study which gave 69.23% of loading and 86.26% of release of NDV vaccine. The stability study of NDV-CMSS hydrogel showed that CMSS hydrogel successfully able to protect the NDV vaccine and retained its stability when stored up to 30 days at room temperature. High thermal properties of CMSS hydrogel with porous structure has allowed NDV vaccine to be trapped inside its three-dimensional matrix. CMSS hydrogel successfully protected the NDV vaccine from deterioration due to high temperature of its surrounding. In conclusion, CMSS hydrogel showed an outstanding result as NDV vaccine carrier which gave ample protection to NDV vaccines. CMSS hydrogel can improve current NDV vaccine storage and delivery without depending on cold chain system.

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PENYEDIAAN DAN PENGOPTIMUMAN HIDROGEL KARBOKSIMETIL KANJI SAGU YANG BERPOTENSI SEBAGAI PEMBAWA VAKSIN VIRUS PENYAKIT NEWCASTLE

Oleh

NORMASTURA BINTI SULTA

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Karboksimetil kanji sagu (CMSS) adalah polisakarida larut air yang penting dalam pelbagai kegunaan khususnya industri makanan dan juga bukan makanan. Apabila berangkai silang, CMSS menghasilkan hidrogel dalam rangkaian polimer tiga dimensi yang mempunyai keupayaan untuk memegang sejumlah besar larutan dalam rangkaiannya. Penyakit Newcastle (ND) adalah penyakit tular yang menyebabkan kerugian besar dalam industri ternakan ayam di seluruh dunia. Penyakit ini disebabkan oleh strain virulen *paramyxovirus* dan boleh dikawal dengan menggunakan vaksin virus penyakit Newcastle (NDV). Walau bagaimanapun, vaksin NDV sangat sensitif terhadap suhu tinggi dan memerlukan rantaian penyimpanan sejuk yang betul untuk menjamin keberkesanannya. Dalam kajian ini, hidrogel CMSS telah disediakan dengan melarutkan CMSS dalam larutan HCl di bawah pengadukan kuat untuk membentuk hidrogel. Hidrogel CMSS yang terhasil ini kemudiannya digunakan untuk merangkum vaksin NDV. Empat parameter dikaji untuk mengoptimumkan penyediaan hidrogel CMSS, antaranya termasuklah kesan peratusan CMSS, kepekatan larutan asid, masa dan suhu tindak balas. Peratusan kandungan gel dan tahap pengembangan menjadi kayu ukur yang dikaji bagi setiap parameter. 60% CMSS dalam 2.0M larutan asid dengan mengambil masa selama 12 jam pada suhu bilik merupakan keadaan optimum untuk penyediaan hidrogel CMSS dengan menghasilkan 70.40% kandungan gel dan tahap pengembangan sebanyak 50.79 (g/g). Hidrogel CMSS dicirikan dengan menggunakan spektrometer infra-merah (FT-IR), analisis termogravimetrik (TGA), pembelauan sinar-X (XRD) dan pengimbasan mikroskop elektron (SEM). Spektrum FTIR menunjukkan satu jalur penyerapan tambahan pada

1597 cm^{-1} yang menunjukkan penggantian kumpulan $CH_2COO^-Na^+$ pada rantai molekul kanji semasa tindak balas karboksimetilasi. Manakala spektrum hidrogel CMSS menunjukkan tambahan jalur penyerapan tajam pada 1723 cm^{-1} yang menunjukkan bahawa Na dalam molekul CMSS ditukar kepada H dari larutan asid hidroklorik. Analisis terma (TGA) menunjukkan bahawa tindak balas karboksimetilasi mengalihkan penguraian maksimum kanji sagu kepada suhu yang lebih rendah daripada 309.57ºC kepada 295.33ºC. Walau bagaimanapun, kehadiran rangkai silang dalam hidrogel CMSS memberikan kestabilan haba yang lebih baik berbanding CMSS dengan memberikan penguraian maksimum pada 330.22 ºC dengan penurunan berat utama sebanyak 60.22%. Corak belauan sinar-X CMSS menunjukkan bahawa struktur semi-berhablur kanji sagu dimusnahkan sepenuhnya selepas melalui tindak balas karboksimetilasi. Imej SEM menunjukkan bentuk sagu yang terherot dan tidak teratur selepas mengalami tindak balas karboksimetilasi. Manakala, hidrogel CMSS menunjukkan permukaan yang penuh dengan ruang yang dipanggil liang-liang dalam struktur dan saling berhubungan antara satu sama lain untuk membentuk rangkaian. Hidrogel CMSS menunjukkan perilaku sensitif terhadap pH kerana ia menunjukkan pengembangan yang lebih tinggi pada larutan PBS pH 7 tetapi mengecut pada pH rendah dan dalam larutan asid. Dua parameter telah dijalankan untuk menyiasat kajian pemuatan dan pelepasan vaksin NDV yang terkandung di dalam hidrogel CMSS iaitu jumlah hidrogel CMSS dan masa pemuatan. 0.02 g hidrogel CMSS pada masa pemuatan selama 30 minit adalah keadaan optimum yang dikenal pasti dalam kajian ini yakni memberikan capaian sebanyak 69.23% pemuatan dan 86.26% pelepasan. Kajian kestabilan hidrogel CMSS-NDV menunjukkan bahawa hidrogel CMSS telah berjaya melindungi vaksin NDV dan mengekalkan kestabilannya apabila disimpan pada suhu bilik selama 30 hari. Ciri-ciri terma yang tinggi serta struktur hidrogel CMSS yang berliang telah membolehkan vaksin NDV terperangkap di dalam matrik tiga dimensinya. Hidrogel CMSS berjaya melindungi vaksin NDV daripada kemerosotan akibat suhu tinggi di sekelilingnya. Kesimpulannya, hidrogel CMSS menunjukkan hasil yang memberangsangkan sebagai pembawa vaksin NDV yang memberi perlindungan yang mencukupi kepada vaksin NDV. Hidrogel CMSS boleh menambahbaik sistem penyimpanan dan penghantaran vaksin NDV tanpa bergantung pada sistem rantaian sejuk.

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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:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AGU	Anhydrous Glucose Unit
APMV-1	Avian Paramyxovirus Type 1
CMC	Carboxymethyl Cellulose
CMSS	Carboxymethyl Sago Starch
DNA	Deoxyribonucleic Acid
DS	Degree of Substitution
DTG	Derivative Thermogravimetric Analysis
F	Fusion Protein
FTIR	Fourier Transform Infrared Analysis
HCl	Hydrochloric Acid
HN	Hemagglutinin-Neuraminiside
IFN	Interferon
LA-5	Lactobacillus Acidophilus
NaOH	Sodium Hydroxide
ND	Newcastle Disease
NDV	Newcastle Disease Virus
PBS	Phosphate-buffered Solution
PVP-in-situ PAAm	Polyvinylpyrrolidone-in-situ-PolyAcrylamide
SEM	Scanning Electron Microscopy
SMCA	Sodium Monochloroacetate
SPF	Specific Pathogen-Free
TGA	Thermal Gravimetric Analysis
TS-PDMS	3-(triethoxysilyl)-Propyl-terminated Polydimethylsiloxane
UTAR	Universal Attenuated Total Reflectance
UV-VIs	Ultraviolet-Visible Spectrophotometer
XRD	X-Ray Diffraction

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Polysaccharides are polymeric carbohydrate molecules that can be subjected to a wide range of chemical and enzymatic reactions. They have biocompatible and biodegradable properties with low immunogenicity. Polysaccharides can be easily obtained and found in living organism which make it an ideal candidate as a promising chemical resources in many fields including biomedical and drug delivery applications (Pushpamalar *et al*., 2016). Some of the examples of polysaccharides are starch, alginate, cellulose, hemicellulose including chitin which originated from animals. Within this class, starch is the most abundant storage polymer that can be found in variety plant organs and is widely explored in the pharmaceutical and other industries. Chemical modification of starch provides a range of physicochemical benefits (Masina *et al*., 2017). Sago starch is one of the examples that differ from any typical industrial starches that derived from cereal (corn, wheat, rice), tubers (potato), or roots (cassava). Sago starch derived from the stem of sago palm known as *Metroxylon Spp*. It is an abundant renewable raw material and available at low cost (Karim *et al*., 2008). Sago starch undergo modification process to improve its properties. Oxidation, esterification and etherification are the most reviewed methods for chemical modification of starch.

Carboxymethyl sago starch (CMSS) denotes one of the most well-studied starch derivatives. CMSS can be produced through etherification technique where carboxymethyl group from monochloroacetic acid substituting the hydroxyl group of starch backbone. The presence of new functional group in the modified starch makes it more stable and easily soluble in water at ambient temperature (Zainuddin, 2003), CMSS is environmentally safe and ratified for variety of applications such as cosmetics, food, pharmaceutical products as well as for a wide range of technical and environmental purposes (Zdanowicz *et al*., 2014). CMSS hydrogel can be produced through chemical modification. It can be manufactured with various chemical compounds act as crosslinking agent, such as epichlorohydrin, phosphoryl oxychloride, dicarboxylic acids, and anhydrides (Mulhbacher *et al*., 2006; Carmona-Garcia *et al*., 2009). CMSS hydrogel is potential materials of “smart” delivery systems, which are capable of releasing its substances, at the appropriate time and site of action. This hydrogel has been

used in wide applications either in waste water treatment as a heavy metal removal (Basri *et al*., 2016) and controlled release of drug delivery (Singh & Nath, 2013).

Newcastle disease (ND) is fatal and highly contagious disease affecting poultry and a wide range of wild birds worldwide that is caused by the infections of virulent strains of Newcastle disease virus (NDV). This disease causes devastating losses in both commercial and village chicken all over the world (Wajid *et al*., 2017). Newcastle disease is caused by virulent strains of avian paramyxovirus type 1 (APMV-1) of the genus *Avulavirus* which belongs to the family of *Paramyxoviridae*. This disease was first identified in Java, Indonesia in 1926 and being endemic in many countries since then. Some clinical signs can be observed upon infection, these include depression, hyperthermia, ruffled feathers, open mouth breathing, anorexia, and edema. High virulent isolates can result in rapid, high mortality of birds (Kapczynski *et al.*, 2013). There are no treatments available for this disease, but it can be controlled by the use of vaccine. Two most common vaccine strains used worldwide are B1 and LaSota (Senne *et al*., 2004).

Vaccination of animals as an infectious disease control method has been practiced for century with remarkable success. Vaccination is the prominent way to protect livestock in poultry industries to against poultry disease including ND. Newcastle disease virus (NDV) vaccine produced in an envelope virus that is highly sensitive to environmental factor such as pH condition, temperature and any other impurities, thus contribute to complexity in vaccine delivery system. A proper administration of efficacious vaccines is needed to ensure the effectiveness vaccination besides can improve its stability and efficacy. Biodegradable polymers such as starch, cellulose, and chitosan have gained huge attention as drug carriers for drug delivery systems due to their excellent properties. Previous studies showed that the physicochemical properties of these biopolymers can increase overall drug delivery system efficacy and applicability (Pushpamalar *et al*., 2016).

This study proposed a new approach by using carboxymethyl sago starch hydrogel which has never been used as a material to encapsulate NDV vaccine inside its three-dimensional network to protect the NDV vaccine against heat. Proper protection and delivery of NDV vaccine will help the poultry industries to sustain and increase the production of egg and chicken livestock.

1.2 Problem Statement

An average of 60 countries reporting ND outbreaks yearly from 2013 to 2015 and the increasing number of genotypes demonstrate the broadening of virulent NDV genetic diversity. Poor vaccination strategy may contribute to the effect of uncontrolled outbreak of NDV virulent. Over the past few years, a number of new vaccination program, technique, and initiatives have been developed in order to improve vaccine delivery system.

NDV vaccine must be kept in the refrigerator between 4 and 8 ˚C to ensure their activity and most of them deteriorate after exposure for one or two hours at room temperature. Thus, makes NDV vaccine unsuitable for use in villages where the vaccine may need to be transported for hours or days at ambient temperature. Sufficient and effective cold chain system is needed during vaccines transportation. Some studied suggested that these vaccine antigens have a need for alternative carrier molecules that capable of protecting them towards heat resistance (Gerdts *et al*., 2013). Therefore, there is need to continue the search for better, alternative vaccine protector together with an effective vaccination program.

To overcome the above problem, there have been attempts to improve the method of vaccine delivery by using biodegradable polymers for protection such as encapsulation. Previous studies proposed that biodegradable materials could be surface modified and protect antigen or DNA from damage (Sun *et al*., 2014). The use of biopolymers from polysaccharides such as chitosan (Zhao *et al*., 2012), cellulose (Dou *et al.*, 2007), carrageenan (Rodrigues & Emeje, 2012) and others as a drug carrier and vaccine encapsulation have been studied previously. Alternative material should be investigated in order to protect NDV vaccine and improve the vaccine delivery. CMSS hydrogel as one of biodegradable polymers is a promising material in the delivery of ND vaccine due to their compatibility, thermal stability and its degradation behavior.

1.3 Significance of the Study

Malaysia is one of the highest poultry consumers in the world. Chickens and eggs being the primary protein source of majority of Malaysian consumer. Nearly 1.8 million chickens and 2.8 million chicken eggs daily consumed by Malaysian. People prefer chicken as a source of protein because it is much cheaper compare to the others. However, poultry industry not only in Malaysia but around the world is facing tremendous threats, such as supply and the production cost of chicken feed, disease and its control, including the increasing

concerns about health and welfare of the chicken (Gerber *et al*., 2008). Newcastle disease is one of the diseases that has affected the growth of chicken and caused devastating losses in poultry industry. Vaccination is an effective control of Newcastle disease outbreak. However, vaccination of NDV vaccine encounters some hindrance caused by its sensitivity towards some environmental factors. High temperature affects the life and activity of NDV vaccine. Improper transportation conditions can cause rapid deterioration and poor vaccine delivery can reduce the efficacy of its distribution. Thus, these vaccines need protection to foster their stability against factors that contribute to low efficacy of NDV vaccine delivery and administration.

This research highlighted an investigation on CMSS hydrogel that can protect NDV vaccine and improve its stability towards surrounding temperature. This improvement could bring a solution especially in the dependency of NDV vaccine on cold chain system during transportation and storage. CMSS hydrogel possessed excellent properties with high thermal and low tendency to retrograde. Entrapment of NDV vaccine in CMSS hydrogel can prevent it from deteriorate at high temperature, thus makes it easily stored and distributed especially by the farmers.

1.4 Objectives of the Study

The aim of this research study was to investigate the ability of CMSS hydrogel to protect NDV vaccine and improve its stability towards surrounding temperature. Thus, the main objectives were;

1) To optimize the production of carboxymethyl sago starch hydrogel
2) To evaluate the loading and release of NDV vaccine in carboxymethyl sago starch hydrogel
3) To study the stability of NDV vaccine encapsulated by carboxymethyl sago starch hydrogel at different storage time and temperature.

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