OPTIMIZATION OF APTAMER-BASED MANGANESE DOPED ZINC SULPHIDE/CHITOSAN AND CARBON DOTS/CHITOSAN AS DRUG NANOCARRIERS FOR CONTROLLED RELEASE OF MITOMYCIN C

By

FARIZA AINA BINTI ABD MANAN

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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This thesis wholeheartedly dedicated to my dearest mum (Norshida binti Wan Husain) and dad (Abd Manan bin Ros) for their eternal love, financial, emotional, spiritual, and physical support. Your encouragement played an integral role in my accomplishments. A special gratitude goes to my beloved husband, Zul Adlan bin Mohd Hir for his infinity love, inspiration, motivation, support of all my endeavours. Your love, partnership, steadfastness sustains me. Special dedication to our beloved daughter, Aafiyah Zahra binti Zul Adlan for all the sacrifices you have endured for me to pursue my dream. You have made me stronger, better, and more fulfilled than I could imagine. And lastly, I dedicated this thesis to my beloved brothers (Farizul Anuar and Faizal Amri), families and in-laws, my lecturers, teachers and friends for their support and encouragement.

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

OPTIMIZATION OF APTAMER-BASED MANGANESE DOPED ZINC SULPHIDE/CHITOSAN AND CARBON DOTS/CHITOSAN AS DRUG NANOCARRIERS FOR CONTROLLED RELEASE OF MITOMYCIN C

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Drug delivery system (DDS) using nanocarriers have gained immense acclamation as a new prospect in nanomedicine. Conventional free drug delivery is associated with low therapeutic windows and inadequate therapeutic efficiency. Nanocarriers is outlined as a platform to combat such hurdles by transporting and releasing the drug precisely at the target site with maximum efficiency and safety in controlled manner. Therefore, in the present work, DDS based on hybrid two types of quantum dots (QDs); manganese doped zinc sulphide (Mn:ZnS) and carbon dots (CDs); incorporated onto chitosan (CS) as drug nanocarriers for Mitomycin C (MMC), further conjugated with aminated aptamer (Apt02); MMC@Mn:ZnS/CS-Apt02 and MMC@CDs/CS-Apt02 have been successfully synthesized. The physicochemical characterizations of nanocarriers were assessed using field-emission scanning electron microscopy (FESEM), High Resolution Transmission Electron Microscopy (HRTEM), and Fourier Transform Infrared

Spectroscopy (FTIR). The morphological analysis shows that both DDS exhibited nearly spherical shape with average particle size ranging from 80 to 110 nm. Subsequently, FTIR results reveals that there is slight shifting in absorbance peak at 1646cm^{-1} in both DDS due to the formation of hydrogen bond between amide group in MMC with hydroxyl group in chitosan. The drug encapsulation efficiency and drug release studies were further evaluated using UV-Vis Spectroscopy at 362 nm. The highest encapsulation efficiency were obtained as 82.6 ± 2.2% and 83.7 ± 0.7% for MMC@Mn:ZnS-CS and MMC@CDs-CS, respectively. The cumulative drug release percentages at pH 5.5 were 82.86 ± 1.33, and 81.44 ± 2.41 for MMC@CDs-CS-Apt02, and MMC@Mn:ZnS-CS-Apt02, respectively. Preliminarily, the binding affinity of two sequences of aptamers, Apt01 and Apt02 towards Vascular Endothelial Growth Factor Receptor 1 (VEGFR1) have been assessed. From molecular docking and experimental analyses, Apt02 exhibit better binding affinity towards the VEGFR1. Hence, Apt02 has been used for the conjugation with TDDS. Finally, the interaction of DDS towards synthetic VEGFR1 were evaluated using fluorescence spectroscopy. An intense emission peak centered at 610 nm and 430 were observed for MMC@Mn:ZnS/CS-Apt02-VEGFR1 and MMC@CDs/CS-Apt02-VEGFR1, respectively. These DDS were used in proof-of-concept studies as a possible application in targeted drug delivery application.

Keywords: drug delivery system; chitosan-based nanocarriers, quantum dots, mitomycin c, carbon dots

SDG: GOAL 3: Good Health and Well-Being

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

PENGOPTIMUMAN ZINK SULFIDA TERDOP MANGAN/KITOSAN DAN TITIK KARBON/KITOSAN BERASASKAN APTAMER SEBAGAI PEMBAWA NANO UBAT UNTUK PELEPASAN TERKAWAL MITOMISIN C

Oleh

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Sistem penyampaian ubat (DDS) menggunakan pembawa nano telah mendapat sambutan yang hebat sebagai prospek baharu dalam bidang nanomedik. Penghantaran ubat rawak konvensional dikaitkan dengan tingkap terapeutik yang rendah dan kecekapan terapeutik yang tidak mencukupi. Pembawa nano digariskan sebagai platform untuk memerangi halangan tersebut dengan mengangkut dan melepaskan ubat tepat di tapak sasaran dengan kecekapan dan keselamatan maksimum dalam cara terkawal. Oleh itu, dalam kerja ini, DDS berdasarkan dua jenis titik kuantum (QDs); zink sulfida terdop mangan (Mn:ZnS) dan titik karbon (CD); digabungkan ke dalam kitosan (CS) sebagai pembawa nano ubat untuk membawa ubat Mitomycin C (MMC), seterusnya dikonjugasikan dengan aptamer (Apt02); MMC@Mn:ZnS/CS-Apt02 dan MMC@CDs/CS-Apt02 telah berjaya disintesis. Pencirian fizikokimia pembawa nano telah dilakukan menggunakan mikroskop elektron pengimbasan pelepasan medan (FESEM), Mikroskop Elektron

Transmisi Resolusi Tinggi (HRTEM), dan Spektroskopi Inframerah Transformasi Fourier (FTIR). Analisis morfologi menunjukkan bahawa kedua-dua DDS mempamerkan bentuk hampir sfera dengan saiz zarah purata antara 80 hingga 110 nm. Selepas itu, keputusan FTIR mendedahkan bahawa terdapat sedikit peralihan dalam puncak penyerapan pada 1646cm^{-1} pada kedua-dua DDS disebabkan oleh pembentukan ikatan hidrogen antara kumpulan amida dalam MMC dengan kumpulan hidroksil dalam kitosan. Kecekapan enkapsulasi ubat dan kajian pelepasan dadah dinilai selanjutnya menggunakan Spektroskopi UV-Vis pada 362 nm. Kecekapan enkapsulasi tertinggi diperolehi sebagai 82.6 ± 2.2% dan 83.7 ± 0.7% untuk MMC@Mn:ZnS-CS dan MMC@CDs-CS, masing-masing. Peratusan pelepasan ubat kumulatif pada pH 5.5 ialah 82.86 ± 1.33, dan 81.44 ± 2.41 untuk MMC@CDs-CS-Apt02, dan MMC@Mn:ZnS-CS-Apt02, masing-masing. Pada awalnya, pertalian ikatan dua jujukan aptamer, Apt01 dan Apt02 terhadap Reseptor Faktor Pertumbuhan Endothelial Vaskular 1 (VEGFR1) telah dinilai. Daripada dok molekul dan analisis eksperimen, Apt02 mempamerkan pertalian pengikatan yang lebih baik terhadap VEGFR1. Oleh itu, Apt02 telah digunakan untuk konjugasi dengan kedua-dua DDS. Akhirnya, interaksi DDS terhadap VEGFR1 sintetik dinilai menggunakan spektroskopi pendarfluor. Puncak pelepasan tertinggi berpusat pada 610 nm dan 430 diperhatikan untuk MMC@Mn:ZnS/CS-Apt02-VEGFR1 dan MMC@CDs/CS-Apt02-VEGFR1, masing-masing. DDS ini digunakan dalam kajian sebagai bukti konsep DDS yang mungkin digunakan dalam aplikasi penghantaran ubat yang disasarkan.

Kata Kunci: sistem penyampaian ubat; pembawa nano berasaskan kitosan, titik kuantum, mitomisin c, titik karbon

SDG: MATLAMAT 3: Kesihatan dan Kesejahteraan yang Baik

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

µm	Micrometer
3D	Three dimensional
AUA	American Urological Association
BC	Bladder cancer
BCG	Bacillus Calmette-Guerin
CDs	Carbon Dots
CIS	Carcinoma in situ
CNPs	Chitosan Nanoparticles
CS	Chitosan
CT	Computer tomography
DDS	Drug Delivery System
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
DOX	Doxorubicin
EAU	European Assiociation of Urology
EpCAM	Epithelial Cell Adhesion Molecule
EPI	Epirubicin
EPR	Enhanced Permeability and Retention
FDA	Food and Drug Administration
FGFR3	Fibroblast growth factor receptor 3
g	gram
GC	Gemcitabine and Ciplastin
HRTEM	High Resolution Transmission Electron Miscroscopy
IBCG	International Bladder Cancer Group
IDDS	Intravesical Drug Delivery System

L	Liter
LMWC	Low Molecular Weight Chitosan
mg	Milligram
MIBC	Muscle invasive bladder cancer
mL	Milliliter
MMC	Mitomycin C
Mn:ZnS	Manganese doped Zinc Sulphide
MPS	Mononuclear Phagocyte System
MRI	Magnetic Resonance Imaging
MTT	(3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide)
MUC1	Mucin-1
MVAC	Methothexate, Vinblastine, Doxorubicin and Cisplastin
NCCN	National Comprehensive Cancer Network
nm	Nanometer
NMIBC	Non-muscle invasive bladder cancer
NPs	Nanoparticles
PEG	Polyethylene Glycol
PSMA	Prostate Specific Membrane Antigen
PTEN	Phospatase and Tensin Homolog
QDs	Quantum Dots
RC	Radical Cystectomy
SELEX	Systematic Evolution of Ligands by Exponential enrichment
SEM	Field emission scanning electron microscopy
STPP	Sodium Tripolyphosphate
TDDS	Targeted Drug Delivery System
TNM	Tumour, Nodes and Metastasis
TURBT	Transurethal Resesection of Bladder Tumour

UV	Ultraviolet
UV-Vis	Ultraviolet-Visible
VEGFR1	Vascular Endothelial Growth Factor Receptor 1
WHO	World Health Organization
XRD	X-ray diffraction

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CHAPTER 1

INTRODUCTION

1.1 Research background

Bladder cancer (BC) has been listed as tenth most common diagnosed cancer worldwide according to statistical data provided by World Health Organization (WHO) in 2020. Based on continents, Asia recorded the highest case with greatest prevalence in Singapore, followed by Indonesia, Thailand, and Malaysia. The incidence rate is 2.7 per 100,000 individuals. Particularly, Malaysia has been listed as the third highest estimated crude fatality rate for bladder cancer among its neighbouring countries, after Singapore and Thailand (Kadir et al., 2022). BC can be subdivided into two categories based on the degree of invasion, namely muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC). NMIBC accounts for approximately 75-80% of incident cases of bladder cancer (Ho et al., 2021; Sylvester et al., 2021).

Transurethral resection of bladder tumours (TURBT), followed by intravesical immunotherapy or chemotherapy and serial surveillance with cystoscopy are the standard treatment for non-muscle invasive bladder cancer (NMIBC) (Yanagisawa et al., 2022). Intravesical chemotherapy exhibit impediments such as insufficient amount of drugs on the cancer cells, has the potential to acquire multidrug resistance (MDR), hence hindering the efficacy of the treatment (Karami et al., 2023).

Targeted drug delivery systems (TDDS) have profoundly revolutionized both in diagnosis and treatment of cancer. Myriad kinds of nanomaterials have been integrated into this system to provide sustained drug release, superior imaging, eliminate the drug resistance, and increased drug permeability. This technology has the potential to improve targeted cancer treatment by enhancing bioavailability, extending drug half-life, and reducing the necessary frequency of administration, concomitantly reduce the adverse effects (Lunawat et al., 2024).

Presently, immense fabrication of multimodal nanoparticles empolying quantum dots (QDs) and polymeric nanoparticles garnered substantial attention as a novel theranostic agents for concurrent cancer diagnosis and therapy. Among polymeric nanoparticles, chitosan (CS) has been widely utilized for multifaceted applications in DDS owing to their extraordinary features such as low toxicity, biocompatibility, biodegradability and low immunogenicity (Chadar & Kesharwani, 2021; Patnaik et al., 2021). Future theranostic approaches hold significant promise with the hybridization of QDs fluorescent labels as an indispensable nanomaterial in DDS.

This study entails the hybridization of chitosan nanoparticles with two types of quantum dots (QDs); manganese doped zinc sulphide (Mn:ZnS) quantum dots and carbon dots (CDs) derived from orange peels, encapsulation of anti-proliferative drugs Mitomycin C (MMC), and conjugating with aminated aptamer to actively targeting the vascular endothelial growth factor receptor 1 (VEGFR1). To the best of our knowledge, this presents a novel attempt on

MMC loaded in hybrid chitosan and quantum dots conjugated with aminated aptamer for drug delivery system.

1.2 Problem statement and research motivation

Intravesical chemotherapy for NMIBC suffers from lack of specificity of drugs and short residence time due to urine voiding, hence the drug will be easily washed off due to urine excretion (Mullapudi et al., 2022). MMC is one of the anti-proliferative drugs that has been used in this treatment modalities. MMC is an antimetabolite antibiotic drugs which has potential role to suppress the DNA synthesis by cross-linking with the DNA and inhibit the cellular mitosis, particularly in cancer cells (Sultana et al., 2020). Eventually, MMC also can inhibit the cellular mitosis in bone marrow, gastrointestinal tract cells and immune system throughout the body (Amin et al., 2023; Ruman, Fakurazi, Masarudin, & Hussein, 2020). Hence, emergence of drug pre-targeted delivery system has been addressed with high specificity and extended residence time for more effective treatment.

Targeted drug delivery systems have recently emerged as a smart platform, serving of both attribution in cancer diagnosis and therapy. In this regards, multimodal nanoparticle, chitosan incorporated with quantum dots, integrated with aminated aptamers has been engineered as a tracable drug delivery system. Chitosan has mucoadhesive properties, which can adhere to specific sites with extended residence time, thus enhanced drug absorption, impeding drug degradation, elevating bioavailability, leading to an enhancement in the drug delivery efficiency (Pornpitchanarong et al., 2022). Notably, quantum

dots exhibit distinctive optical characteristics including wide absorption and narrow emission spectra, bright fluorescence emission and photoresistance have prompted significant interest as an excellent potential candidates for bioimaging applications (Abrishami et al., 2024).

In this study, encapsulated MMC in hybrid chitosan quantum dots; MMC@Mn:ZnS-CS and MMC@CDs-CS were successfully synthesized using ionic gelation method. This established method was employed because it has the capability to yield high drug encapsulation and good dispersity (Wang et al., 2020). Other than that, ionic gelation is widely employed due to its ability to create mild conditions without compelled for hazardous organic solvents, heat, or vigorous agitation. This makes the procedure more cost-effective and safer (Galbiatti et al., 2019). Interestingly, chitosan-based nanomaterials synthesized by ionic gelation also have high tendency to bind with protein receptor (Sarkar et al., 2020).

A crucial strategy in this fabricated theranostics agents is the pre-targeting of aminated aptamer with synthetic VEGFR1. In this approach, aminated aptamer were integrated by covalent bond with amine group of chitosan in both MMC@Mn:ZnS-CS and MMC@CDs-CS, using glutaraldehyde (GLU) as the crosslinker. Next, the DNA nucleotides in aptamer will interact with amino acid residues in VEGFR1 by hydrogen bonding arise from the N–H···O type in aptamer-protein interactions. In future application, this designed drug delivery system will be administered directly into bladder using urinary catheter for

potential cancer diagnosis and drug transportation towards targeted cancerous sites.

1.3 Research objectives

The objective of this study is to synthesis chitosan-based nanocarriers for the delivery of MMC. Hence, five specific objectives were addressed:

i. To synthesis Mn:ZnS-CS, CDs-CS and CS nanocarriers for the encapsulation of MMC.
ii. To characterize the particle sizes of the synhesized MMC@Mn:ZnS-CS, MMC@CDs-CS and MMC@CS drug delivery system using TEM and DLS.
iii. To evaluate the drug encapsulation efficiency and drug release sustainability of MMC@Mn:ZnS-CS, MMC@CDs-CS and MMC@CS nanocarriers using UV-Vis spectroscopy.
iv. To investigate the binding affinity for two sequences of aptamers, Apt01 and Apt02 towards VEGFR1 in silico using molecular docking using Autodock Vina complement with experimental anaysis using Enzyme Linked Oligonucleotides Assay (ELONA).
v. To assess the interaction of the MMC@Mn:ZnS-CS-Apt02 and MMC@CDs-CS-Apt02 interaction with synthetic VEGFR1 using fluorescence spectroscopy.

1.4 Scope and limitation of the study

Firstly, the architecture of drug delivery system started with the synthesis of two fluorescence probes, manganese-doped zinc sulphide (Mn:ZnS) via co-precipitation method and synthesis of carbon dots (CDs) derived from orange peel using hydrothermal method. Next, three chitosan-based nanocarriers, which are manganese-doped zinc sulphide-chitosan (Mn:ZnS-CS), carbon dots-chitosan (CDs-CS) and chitosan (CS) were successfully synthesized using ionic gelation approach. The CS nanocarriers were used as the control. The optical properties of fluorescence probe were successfully accessed using UV-Vis and fluorescence spectroscopy. The next part involved the encapsulation of anticancer drugs of Mitomycin C (MMC) onto those three chitosan-based drug nanocarriers, followed by their drug release sustainability. Afterwards, Mn:ZnS-CS and CDs-CS were conjugated with aminated aptamer, Apt02 using glutaraldehyde as the crosslinkers. Finally, the functionalized Apt02/MMC@Mn:ZnS-CS and Apt02/MMC@CDs-CS TDDS interaction with VEGFR1 were investigated using fluorescence spectroscopy.

Generally, the present study only focuses on the fabrication of targeted drug delivery system (TDDS) using Apt02/MMC@Mn:ZnS-CS and Apt02/MMC@CDs-CS as assays for specific binding of VEGFR1. Consequently, there is a limitation for the targeting other types of receptors on the bladder cancer cells such as P-glycoprotein-1 (Pgp-1), Her2/Erb-b2 receptor tyrosine kinase 2, tumour-associated calcium-signal transducer 2 protein (TAC-STD2) . Nonetheless, it must be emphasized that the fabricated

Apt02/MMC@Mn:ZnS-CS and Apt02/MMC@CDs-CS has been done using the synthetic VEGFR1 as proof of the concept of TDDS performance.

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