UNIVERSITI PUTRA MALAYSIA

ENCAPSULATION OF TAMOXIFEN CITRATE CONJUGATED WITH MAGNETITE NANOPARTICLE

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ENCAPSULATION OF TAMOXIFEN CITRATE CONJUGATED WITH MAGNETITE NANOPARTICLE

By

EMMELLIE LAURA ALBERT

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ENCAPSULATION OF TAMOXIFEN CITRATE CONJUGATED WITH MAGNETITE NANOPARTICLE

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EMMELLIE LAURA ALBERT

May 2017

Chairman : Che Azurahanim Che Abdullah, PhD
Faculty : Institute of Advance Technology

Cancer chemotherapy drugs are not specific on their metabolic pathways to the cancer cell. Therefore, there is a need to overcome this disadvantages by applying targeted drug delivery using composite nanoparticles. Similarly, tamoxifen citrate (TAM) also suffer from this disadvantages. TAM is a drug used for breast cancer treatment. So, current investigations are proposing the usage of magnetite nanoparticles (MNP) as an anti-cancer drug carrier because of its biocompatibility, ultrafine size, and its superparamagnetic nature. In this study, poly (d,l-lactice-co-glycolide acid) (PLGA) were used to encapsulated both MNP and TAM to form a multifunctional nanoparticle which have both the superparamagnetic properties of MNP and therapeutic ability of TAM. MNP were synthesized via the co-precipitation method. Then, it was coated with oleic acid (OA) to reduce the aggregation and it was abbreviated as OAMNP. Formation of functionalized OAMNPs with TAM using PLGA (TAM-PLGA-OAMNP) was obtained by oil in water emulsion evaporation technique. The XRD pattern showed that crystalline phase of the MNP is inverse spinel cubic of Fe_3O_4. After modification, FTIR spectra revealed that the TAM were successfully encapsulated into the PLGA matrixes. By using TEM, the particles size is determine by 131 ± 28 nm for TAM-PLGA-OAMNP. The VSM analysis for TAM-PLGA-OAMNP showed no hysteresis loop indicating superparamagnetic characteristic. This projects also presents a discussion on the optimum condition for colloid stability for TAM-PLGA-OAMNPs based on the aggregation and sedimentation. Finally, TAM released behavior is studied. TAM-PLGA-OAMNPs followed a biphasic phase released.

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

TAMOXIFEN CITRATE DIKANDUNG BERSAMA MAGNETITE NANOPARTIKEL

Oleh

EMMELLIE LAURA ALBERT

Mei 2017

Pengerusi : Che Azurahanim Che Abdullah, PhD
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Ubat kemoterapi kanser adalah tidak spesifik dalam laluan metabolik ke sel kanser. Oleh itu, untuk mengatasi masalah ini, kaedah penyampaian ubat secara spesifik dengan menggunakan komposit nanopartikel digunakan. Tamoxifen citrate (TAM) juga menghadapi masalah yang sama. TAM adalah ubat untuk merawat kanser payudara. Oleh itu, TAM diperkenalkan dengan kaedah penyampaian ubat secara spesifik untuk mengatasi masalah tersebut dan juga meningkatkan ketepatan penyampaian TAM. Partikel magnetit (MNP) dicadangkan sebagai pembawa TAM kerana kelebihannya iaitu biokompatibiliti, saiz yang kecil dan juga ciri-ciri superparamagnetiknya. Dalam kajian ini, poly (d,l-lactice-co-glycolide asid) (PLGA) terkandung kedua-dua bahan iaitu MNP dan TAM untuk menghasilkan partikel bersaiz kecil yang mempunyai kebolehan supeparamagnetik, MNP dan kebolehan terapeutik, TAM. MNP dihasilkan melalui kaedah mendakan. Kemudian, ia disaluti dengan oleik asid (OA) untuk mengurangkan aggregasi dan ia dinamakan sebagai OAMNP. PLGA menyaluti kedua-dua OAMNP dan TAM dengan menggunakan kaedah emulsi minyak dalam air. XRD data menunjukan bahawa MNP mempunyai kubus spinel terbalik Fe_3O_4. Setelah modifikasi, spektra FTIR mendedahkan bahawa TAM berjaya di kandung oleh PLGA matriks. Dengan menggunakan TEM. Saiz partikel TAM-PLGA-OAMNP adalah 131 ± 28 nm. Analisis data VSM menunjukan tiada histeresis justeru TAM-PLGA-OAMNPs mempunyai kebolehan superparamagnetik. Walaubagimanapun, Ketepuaan magnetik bagi MNP berkurang daripada 57.923 emu/g ke 8.3096×10^{-3} emu/g disebabkan kandungan yang tidak magnetik iaitu PLGA dan TAM yang mengurangkan ketepuaan magnetik dan juga kandungan OAMNP yang sedikit. Projek ini juga membincangkan kondisi optimimum untuk stabiliti koloid TAM-PLGA-OAMNPs melalui aggregasi dan sedimentasi yang berlaku. Sifat pelepasan TAM daripada TAM-PLGA-OAMNPs menpunyai sifat dua fasa.

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I certify that a Thesis Examination Committee has met on 18 July 2017 to conduct the final examination of Emmellie Laura Albert on her thesis entitled "Encapsulation of Tamoxifen Citrate Conjugated with Magnetite Nanoparticle" 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 Master of Science.

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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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Supervisory Committee: Prof. Azmi Zakaria, PhD

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LIST OF ABBREVIATIONS

MNP	Magnetic Nanoparticle75
Nd-Fe-B	Neodymium Iron Boron76
TAM	Tamoxifen Citrate77
PLGA	Poly (D,L-Lactide-Co78-Glycolide Acid)
OA	Oleic Acid79
OAMNP	Oleic Acid Coated Magnetite Nanoparticle
TAM-PLGA-OAMNP	Tamoxifen Citrate Superparamagnetic Polymeric Nanoparticle
PLGA-OAMNP	Superparamagnetic Polymeric Nanoparticle
PBS	Phosphate Buffer Saline
CM	Complete Media For Cell Culture
CMWS	Cell Culture Media Without Serum
DLVO	Derjaguin, Landau, Verwey, And Overbeek Theory
FDA	Food And Drug Administration
BRCA1	Breast cancer susceptibility 1
BRCA2	Breast cancer susceptibility 2
DNA	Deoxyribonucleic Acid
lGF-1	Insulin Growth Factor-1
TGF-B	Transforming Growth Factor B
PEVA	Poly (Ethylene-Co- Vinyl Acetate)
PVA	Poly (Vinyl Pyrrolidone)
PEG	Poly (Ethyleneglycol)
PVA	Poly (Vinyl Alcohol)
PEMA	Poly (Ethylen-Alt-Maleic Acid)
O/W	Oil In Water
DLS	Dynamic Light Scattering
DCM	Dichloromethane
DI	Deionized Water
XRD	X-Ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
TGA	Thermalgravimetric Analysis
FESEM	Field Emission Scanning Electron Microscopy
EDS	Energy Dispersive Spectroscopy
TEM	Transmission Electron Microscopy
VSM	Vibrating Sample Magnetometer
PSA	Particle Size Analyzer
Vas	Asymmetric Vibration
Vs	Symmetric Vibration
Ms	magnetic saturation
Al	Aluminium
Cl	Chloride
Pt	Platinum
DTA	Derivative Weight
R^2	Correlation Coefficient
e	Extinction Coefficient
UV	Ultraviolet
Kα	K alpha emission

Lα	L alpha emission

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer chemotherapy drug is a powerful drug used to treat cancer. Many of these powerful drugs are lethal to the normal cell given that their dosage is high enough to cause undesirable effects such as cell death to the surrounding healthy cells. Cancer chemotherapy drugs are not specific on their metabolic pathways to the cancer cell. They suffer from poor tissue specificity and non-specific toxicity. Therefore, researchers around the world have begun to improve the delivery of chemotherapeutic agents to the cancer cells by applying targeted drug delivery using composite nanoparticles (Rahman & Hasan, 2015)

The composite nanoparticles used for the targeting chemotherapy drug are delivered via intravenous pathway to increase their effectiveness in treating the unhealthy tissue and thus reduce the general toxicity (Xu *et al.,* 2015). Additionally, they also help patients to be more comfortable during treatment by avoiding repetitive injection to improve favorable drug pharmacokinetics (Horcajada *et al.,* 2010)

In many cases, administering drugs in conventional dosage form, for example, using pills and tablets, must achieve successive doses to maintain the drug activity for a long period of time. Figure 1.1 shows the profile for the concentration of the drug dosage in the body as a function of time.

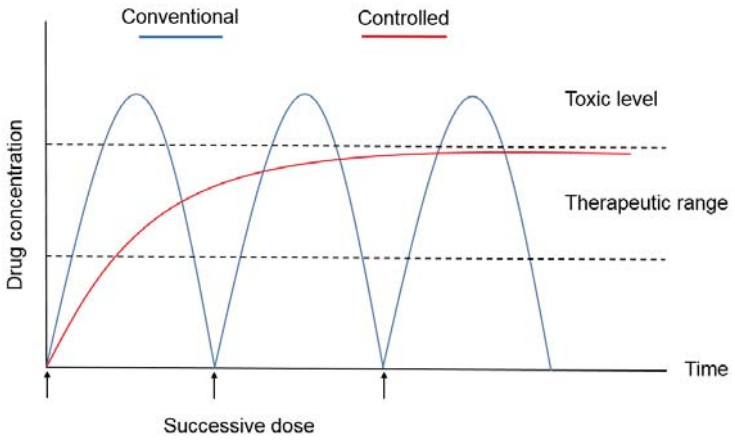


Figure 1.1: Comparison of drug concentration profiles versus time using conventional administration form and controlled drug delivery form

In Figure 1.1, the drug concentration in the human body follows a pattern where the initial drug concentration has a sharp increase and is eventually diminished as time passes. The sharp increase in the drug concentration is reached the toxic level where it is considered dangerous for humans. After some time, the concentration of the drug starts to decrease until it reaches below therapeutic range therefore a new dose of drug needs to be taken by the body. This situation can be overcome by controlled targeted drug delivery.

The main purpose of controlled release systems is to obtain a more effective therapy by avoiding large fluctuation in drug concentration and to reduce the needs of multiple (Pérez de Diego, 2005). In the late 1970, scientist have proposed the usage of nano- and micro-sized particles such as magnetic nanoparticles (MNP) as a drug carrier for targeted drug delivery (Mosbach & Schröder, 1979; Widder, Senyei, & Scarpelli, 1978). They had conjugated MNPs with cytotoxic drugs where they introduced the particles to the subject by intra-arterial or intravenous injection. External magnetic fields with high gradient are applied to the subject to lead and concentrate the drugs at a chosen site. Since then, MNPs have been continuously studied for the last 40 years *(Chomoucka et al.,* 2010). In addition, drug can be released at a desired site via the enzymatic activity or through changes of the pH, temperature, and osmolality causing the increased uptake of the drug to the desire sites such as tumor cell (Alexiou *et al.,* 2000).

It was discovered that it is a great challenge to move this technology from the animal studies to a successful clinical trials owing to the limited theoretical parameter, yet with a good theoretical technique and advancement in experiment design, it could prompt an achievement in targeted drug delivery by utilizing MNPs (Grief & Richardson, 2005).

In order to design a good MNPs suitable for targeted drug delivery, many factors must be taken into consideration such as the physical parameters including the field geometry and strength of the magnetic field applied, the size of the particles, and also the binding capacity of drug or genes (Neuberger *et al.*, 2005). In addition, the human physiological parameters, for instance, like the body weight, the vascular supply, the rate of the blood flow, and the depth of the target sites also affect the design of the MNPs. Yang *et al*., (2006) studies had discovered that the force of the blood pressure and the applied magnetics forces can affect the localization of the MNPs. Therefore, a strong permanent magnet such as neodymium iron boron (Nd-Fe-B) is used as the source of the magnetic field gradient, which is placed on a specific area outside the human body.

The drug carrier is usually introduced into the body through the circulatory system and remains at a chosen site only if the applied magnetic field can overcome the rate of the blood flow in the capillaries (0.05 cm/s) and arteries (10 cm/s). As it remains at the chosen site, the carried drug is then released

via an enzymatic process or through the changes to the physical environment (i.e. temperature, pH or osmolarity). Then, it will be absorbed by the endothelial cell of the specific tissue or the tumor cells thus killing the tumor cells (Mahmoudi *et al.,* 2011). So, these projects are aiming to attach MNPs to anti-cancer drug, so that it can be used for biomedical application such as targeted drug carrier.

1.2 Problem Statement

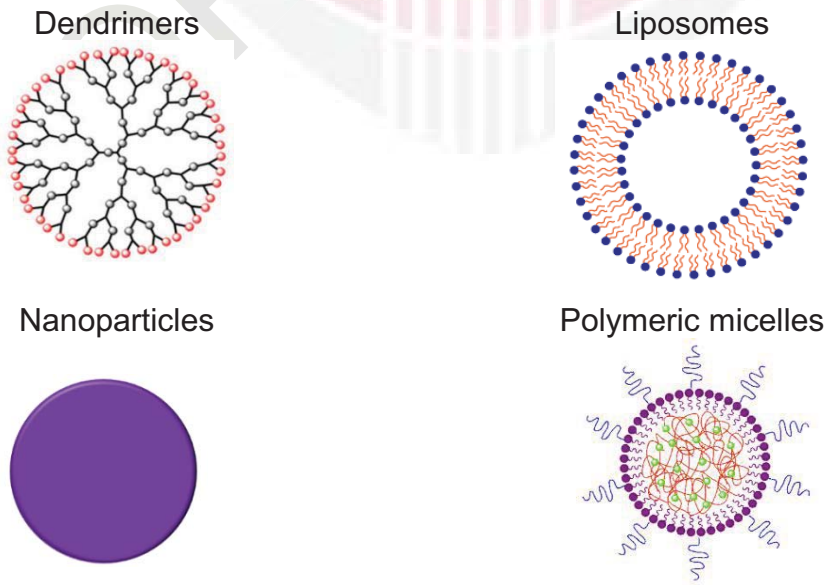
Cancer is one of the deadliest disease in the world. It is the second leading cause of death coming shortly after the heart disease. It is expected in this coming few years that cancer will be the number one cause of death in the world. Among women, breast cancer is the number one cause of death regardless of their ethnicity background. In 2015, about1.6 million new cases were diagnosed to have breast cancer and about 560 thousand will die from breast cancer. In every 19 women pick at random, one of them will develop breast cancer. These represents a terrifying picture of how common breast cancer is in Malaysia. Every year, around 5000 Malaysian women aged between 30-60 years are identified to have breast cancer. Besides the unclear causes of cancer, its treatment is remarkably daunting and challenging.

Conventional cancer treatments including radiotherapy and chemotherapy have their own devastating side effect to the immune system and dividing cells, which can cause nausea, vomiting, diarrhea, and anemia. Additionally, chemotherapy is very lethal to the normal tissues because of its inability to differentiate between the normal cells and cancer cells. Thus, damaging both the cancerous cells and the healthy cells. On the other hand, radiation therapy for cancer treatment is using a high energy beams to induce the death of cancer cells. The beam generated by the machine is focused on a specific point of the body. Subsequently, terminating the genetic material that controls the ways the cells grow and divide (Baskar *et al.,* 2012). There are many disadvantages of using conventional method to treat cancer, therefore, it is crucial to improve the current treatment of cancer. Novel therapeutic options should be developed in order to deal with these side effects.

It has been discovered that nanotechnology, especially nanoparticulate used for targeted drug delivery systems is a promising technique to overcome the disadvantage of the current treatment (Zhang & Chatterjee, 2007). Moreover, they stated that cancer treatment can be improved by using nanocarrier. For instance, lipophilic drugs can be stabilized in circulation and their circulatory duration is increased by controlling the drug release. Thus, the drug toxicity owing to the high concentration of the drug in periodic doses can be overcome. The idea here is to synthesize nanoparticles and functionalize it so that it can be applied in targeted drug delivery. Below is the objectives of targeted drug delivery system (Danhier, Feron, & Préat, 2010)

1. Increasing the drug concentration via passive and active targeting
2. Decreasing the drug concentration in normal cell to a safe level for human
3. Improving the pharmacokinetics and pharmacodynamics profiles
4. The drug solubility permits intravenous administration.
5. Reducing the drug release during delivery
6. Increasing the drug release at targeted tissue
7. Improving the stability thus reducing drug degradation
8. Internalization and intracellular delivery can be improved.
9. Biocompatible and biodegradable

There are several types of nanoscale drug delivery, which are presented in Figure 1.2. Nanoparticles have solid and spherical compounds. They can be categorized into nanocapsules and nanospheres. Nanocapsules are vesicular systems that entrapped the drug inside its membrane while nanospheres are matrix particulates with drugs spread all over the system (Mishra, Patel, & Tiwari, 2010). Polymeric micelles are amphiphilic block copolymers making a nanosized core/shell composition in an aqueous solution according to Cho *et al.,*(2008). The hydrophobic core is a place for hydrophobic drugs while the hydrophilic shell stabilizes the hydrophobic core hence creating water soluble nanoparticle. Park *et al.,* (2008) stated that liposomes are spherical self-closed structures consist of lipid bilayers with an aqueous phase inside. Liposomes can bind with both hydrophilic and hydrophobic drugs inside it. On the other hand, Balogh (2007) described dendrimers as a highly branched regularly in three-dimensional macromolecules. For intravenous administration, nanoparticles are considered a better agents than larger microparticles as they can easily aggregate. Furthermore, they can go over the smallest capillaries with 5-6 μm diameter.



Polymer-drug conjugates

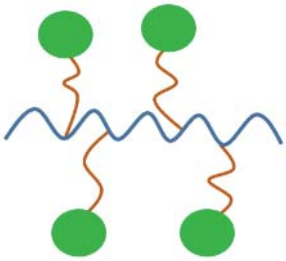


Figure 1.2:Types of nanoscale for drug delivery (Source: Danhier et al., 2010)

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1.3 Thesis Objectives

The main objective of the present project is to encapsulate oleic acid (OA) coated MNPs and anti-cancer drug, tamoxifen citrate (TAM), together using poly (d,l-lactide-co-glycolide acid) (PLGA) so that it can used for biomedical application.

1.4 Specific Objectives

This project is conducted specifically based on the following objectives:

1. To synthesize and evaluate the characteristic of MNPs by co-precipitation technique and encapsulate the MNPs using OA (OAMNP).
2. To synthesize superparamagnetic polymeric nanoparticle made up of OAMNPs and TAM through PLGA encapsulation also known as TAM-PLGA-OAMNPs and its blank nanoparticles abbreviated as PLGA-OAMNP and evaluate their properties.
3. To study the drug release behavior of TAM from TAM-PLGA-OAMNPs.
4. To assess the colloidal stability of the superparamagnetic polymeric nanoparticle in terms of different sonication time, temperature, and concentration of the nanoparticle inside phosphate buffer saline (PBS), complete media (CM) for cell culture, and cell culture media without serum (CMWS).

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