STUDY ON THE ADSORPTION OF DNA ON β-CYCLODEXTRIN COATED Fe_3O_4 NANOPARTICLES

By

GOH YING HWA

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (TRANSFUSION SCIENCE)

ADVANCED MEDICAL AND DENTAL INSTITUTE
(AMDI)
UNIVERSITI SAINS MALAYSIA
JUNE 2017

DECLARATION

I hereby declared that the dissertation is based on my original work except for the quotations and citations which have been duly acknowledged. I also declared that this project has not been previously or concurrently submitted for other degree of Master of Science at Universiti Sains Malaysia or any other institutions. With that, this research might be used for consultation and can be photocopied as reference.

_____

GOH YING HWA

(920907086204)

ACKNOWLEDGEMENT

First and foremost, I am grateful to my supervisor, Dr Muggundha Raoov A/L Ramachandran, whose expertise, understanding and generous guidance made it possible for me to work on the project of interest. It was a pleasure working with him.

I would like to express my gratitude to my co-supervisor, Dr Emmanuel Jairaj Mosses who find time to assist me in the study and for always giving his invaluable advice and guidance regarding the project.

I would also like to express my appreciation to my colleagues of program of Master of Transfusion Science especially to Diyana Mazuin, Lina alSharif, Lim Wen Siew, and Ong Chiew Wai for being a constant source of motivation. My gratitude goes out especially to Tey Lee Hung and Ong Si Min who is with me through day and late-night working in the laboratory.

The gratitude goes out to all the staff who have directly or indirectly involved and given their kind assistance in succeeding this project.

Last but not least, I would like to express my gratitude to highly indebted family members, for without their supports, I would not be able to accomplish what I have been thus far. Beyond words can my gratitude be expressed.

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF SCHEMES

Schemes **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

AC	Alternating current
ALL	Acute lymphoblastic leukaemia
ALCL	Anaplastic large cell lymphoma
β-CD	Beta-cyclodextrin
BKV	Polyomavirus BK
C_e	Concentration of adsorbate at equilibrium in liquid phase
CGTase	Cyclodextrin glycosyltransferase
DMF	Anhydrous N,N-dimethylformide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
Fe^{2+}	Ferrous ions
Fe^{3+}	Ferric ions
$FeCl_2.4H_2O$	Ferrous chloride tetrahydrate
$FeCl_3.6H_2O$	Ferric chloride hexahydrate

Fe_3O_4	Ferroferric oxide
FT-IR	Fourier transform infrared
ΔG°	Gibb's free energy
ΔH°	Changes in enthalpy
ΔH_a	Adsorption affinity enthalpy
ΔH_d	Dehydration enthalpy
ΔH_m	Molecular conformation enthalpy
HIV	Human immunodeficiency virus
K	Kelvin (temperature unit)
k_d	Equilibrium constant ($m^3 mol^{-1}$)
K_F	Freundlich equilibrium constant($(mg\ g^{-1})(L\ mg^{-1})^{\frac{1}{n}}$)
k_i	Intra-particle diffusion rate constant ($mg/g\ min^{1/2}$)
K_L	Langmuir constant($L\ mg^{-1}$)
k_1	Pseudo-first order equilibrium rate constant(min^{-1})
k_2	Pseudo-second order equilibrium rate constant($g\ mg^{-1} min$)
MNP	Magnetic nanoparticle
MRD	Minimal residual disease
MRI	Magnetic resonance imaging
N_2	Nitrogen
NH_4OH	Ammonium

$\mathbf{q_{cal}}$	Calculated adsorption capacities(mg g^{-1})
$\mathbf{q_e}$	Concentration of adsorbate on adsorbent surface at equilibrium state(mg g^{-1})
$\mathbf{q_{exp}}$	Experimental adsorption capacities(mg g^{-1})
$\mathbf{q_m}$	Maximum binding capacity of adsorbate(mg g^{-1})
R	Universal gas constant (8.314 4621kJ $mol^{-1}K^{-1}$),
RNA	Ribonucleic acid
$\mathbf{r_{sp}}$	Superparamagnetic radius
$\Delta \boldsymbol{S}°$	Changes in enthropy
$\Delta \mathbf{S_a}$	Adsorption affinity entropy
$\Delta \mathbf{S_d}$	Dehydration entropy
$\Delta \mathbf{S_m}$	Molecular conformation entropy
siRNA	Small interfering ribonucleic acid
TDI	Toluene diisocyanate
$\mathbf{T_B}$	Blocking temperature
VSM	Vibrating sample magnetometer

KAJIAN TERHADAP PENJERAPAN DNA KE ATAS PERMUKAAN NANOPARTIKEL MAGNET BERSALUT β-SIKLODEKSTRIN

ABSTRAK

Tujuan utama kajian ini adalah untuk menyiasat penjerapan asid deoksiribonukleat ke atas permukaan nanopartikel magnet bersalut β-siklodekstrin. β-siklodekstrin bersalut nanopartikel magnetik digunakan untuk menjerap dan mengasingkan asid deoksiribonukleat, dari fasa cair hingga fasa pepejal. Jumlah pemisahan asid deoksiribonukleat terserap diukur menggunakan spektrofotometer pada 258 nm. Parameter yang berbeza yang mempengaruhi penjerapan asid deoksiribunukleat ke nanopartikel dipelajari dalam projek ini untuk mendapatkan keadaan penjerapan optimum untuk nanopartikel magnet bersalut permukaan oleh β-siklodekstrin. Model kinetik, isoterma dan termodinamik digunakan untuk mengkaji dan menggambarkan tingkah laku penjerapan asid deoksiribonukleat ke atas nanopartikel. Permukaan nanopartikel magnet bersalut β-siklodekstrin, terbukti dengan kajian lebih berkesan daripada nanopartikel magnetik dengan kecekapan penjerapan sebanyak 67%. Nanopartikel magnet dan nanopartikel magnet bersalut permukaan oleh β-siklodekstrin dicirikan menggunakan peranti inframerah transformasi fourier (FT-IR) dan magnetometer sampel bergetar (VSM). Spektrum ciri nanopartikel magnet bersalut permukaan oleh β-siklodekstrin ditunjukkan pada puncak ciri pada 1657 cm^{-1} yang disebabkan oleh dematerialisasi kumpulan isosianat yang pada asalnya dijumpai pada toluene-2,4-diisocyanate (TDI)

dengan kemunculan hubungan karbamat (NHCO) pada puncak yang sama 1657 cm^{-1}. Kehadiran kaitan karbamat menandakan penyempurnaan pempolimeran antara TDI dan β-siklodekstrin. Momen magnetik nanopartikel magnet bersalut permukaan oleh β-siklodekstrin (~ ± 40 emu / g) adalah lebih rendah daripada momen magnetik nanopartikel magnet (~ ± 60 emu / g) apabila kedua-dua nanopartikel disubjek kepada medan magnet luar disebabkan oleh bahan salutan semasa yang mencetuskan penyerapan dan mengurangkan tenaga permukaan yang besar. Parameter kerja yang optimum dari nanopartikel magnet bersalut permukaan oleh β-siklodekstrin didapati berada pada pH 6, 298 K, dengan proses penjerapan mencapai keseimbangan pada 20 minit, dan kepekatan asid deoksiribonukleat awal yang berkesan adalah 10 mg /L. Kesan kekuatan ionik yang dihasilkan oleh natrium klorida pada julat 0 hingga 0.40 mol /L memberi kesan minimum ke atas kapasiti penjerapan nanopartikel magnet bersalut permukaan oleh β-siklodekstrin. Kecekapan penjerapan nanopartikel magnet bersalut permukaan oleh β-siklodekstrin didapati berfungsi pada paras 70 ± 5% selepas dikitar semula selama tiga kali. Isotherma adsorpsi dan penjerapan asid deoksiribonukleat ke nanopartikel magnet bersalut permukaan oleh β-siklodekstrin didapati paling tepat digambarkan oleh isoterma Freundlich, kinetik urutan pseudo-kedua. Perubahan entropi dan entalpi asid deoksiribonukleat ke nanopartikel magnet bersalut permukaan oleh β-siklodekstrin adalah masing-masing -12.1567 J/Kmol dan -3.7371 J/mol. Perkembangan tenaga bebas Gibbs dari nilai negatif kepada nilai positif menunjukkan bahawa proses penjerapan adalah termodinamik, dilaksanakan, secara spontan dan dikawal secara kimia. Penemuan dari kajian penjerapan mencadangkan bahawa interaksi antara penjerap berlaku melalui kompleks inklusi, interaksi hidrofobik, ikatan hidrogen dan daya Van der Waals.

STUDY ON THE ADSORPTION OF DNA ON β-CYCLODEXTRIN COATED Fe_3O_4 NANOPARTICLES

ABSTRACT

The primary purpose of this study is to investigate the adsorption of deoxyribonucleic acid onto surface coated magnetic nanoparticles (MNP) by β-cyclodextrin. β-cyclodextrin coated magnetic nanoparticles, β-CD-MNP-TDI were used to adsorb and isolate deoxyribonucleic acid, DNA from liquid to solid phase. The amount of adsorbed DNA was measured using spectrophotometer at 258 nm. Different parameters affecting the adsorption of DNA onto nanoparticles were studied in this project to obtain the optimum adsorption condition for β-CD-MNP-TDI. Different kinetic, isoterms and thermodynamic models were used to study and described the adsorption behaviour of DNA onto nanoparticles. Surface-coated magnetic nanoparticles β-CD-MNP-TDI, in evident with study attested to work more efficient than magnetic nanoparticles with adsorption efficiency at 67%. The MNP and β-CD-MNP-TDI was characterized using fourier transform infrared (FT-IR) device and vibrating sample magnetometer (VSM). The characteristic spectra of β-CD-MNP-TDI was shown in the characteristic peak at 1657 cm^{-1} which is attributed by the dematerialization of isocyanate group which was originally found on toluene-2,4-diisocyanate (TDI) with the emergence of carbamate linkage (NHCO) at peaks 1657 cm^{-1}. The presence of carbamate linkage signifies the completion of polymerization between TDI and β-cyclodextrin. The magnetic moment of β-CD-MNP-TDI (~ ±40 emu/g) was lower than MNP magnetic moment (~ ±60 emu/g)

when both nanoparticles were subjected to the same amount of external magnetic field due to the present of the coating material which suggestively chaperone and reduced the inherently large surface energies. The optimum working parameter of β-CD-MNP-TDI was found to be at pH 6, 298 K, with adsorption process reaching equilibrium at 20 minutes, and efficient initial DNA concentration being 10 mg/L. Ionic strength effect created by sodium chloride at range of 0 to 0.40 mol/L have minimal effect on the adsorption capacity of β-CD-MNP-TDI. The adsorption efficiency of β-CD-MNP-TDI was found to perform on a par of 70 ± 5 % after being recycled for three times. The adsorption isotherms and kinetics of DNA onto β-CD-MNP-TDI was found to be best described by Freundlich isotherm, pseudo-second order kinetics. The entropy and enthalpy change of DNA on β-CD-MNP-TDI were -12.1567 J/Kmol and -3.7371 J/mol respectively. The progression of Gibbs free energy from negative value to positive value indicates that the adsorption process is thermodynamically feasible, spontaneous and chemically controlled. The findings from the sorption studies suggest that the interaction between adsorbent and adsorbate occur through inclusion complex, hydrophobic interaction, hydrogen bonding and Van der Waals force.

Chapter 1 Introduction

1.1 Study Background

Fight against cancer has been proven to be determined by several factors, one of them is the early detection of malignant growth with adequately efficient and specific treatment (Minetto et al., 2015). Conventional treatments and diagnosis methods to date are lacking in sensitivity and specificity as the chemotherapeutic agents are not designed to distribute specifically to evade tumour cells but normal cells too. This results in suboptimal treatment caused by cytotoxicity in normal tissue. The treatment in latter stages of cancer treatment is often intense with high dosage, as the tumour cells developed resistance to the chemotherapeutics agent, which lead to more severe toxicities to patient. With the increasing number of cancer reported over the centuries (Siegel et al., 2016), efforts in minimizing the side effect of cancer treatment should be emphasized, to improve the possibilities and chances of the cancer patient to relive an all-round healthy life as before cancer development should be taken into consideration as well. Thus, there is need to develop methods that are highly specific and sensitive to detect early stage of malignant growth and a treatment method that is specific enough for target delivery of drug precisely to malignant site, and minimize the toxicity to non-target cells.

Haematological cancers draw our attention the most as per Siegel et al. in Cancer Journal 2016, leukaemia's account for the second highest incidence amongst youngster under 20 years old, with acute lymphoblastic leukaemia (ALL) dominating the chart among other haematological cancer (Siegel et al., 2016). Leukaemia's are a group of disorders caused by accumulation of malignant leukocytes in the bone marrow and blood. The abnormal accumulation is usually due to the failure of bone marrow and infiltration of organs (Hoffbrand and Moss, 2011).

Conventional diagnosis of acute leukaemia involve microscopic examination, immunophenotyping, cytogenetic and molecular analysis (Olsen et al., 2008, Hoffbrand and Moss, 2011). Microscopic examination remains as the most practiced method as it is one of the easiest and cost saving method (Olsen et al., 2008, Shook et al., 2009), however, it is relatively subjective based on haematologist's experience and interpretation. Immunophenotyping is utilized to detect the immunological marker present on surface of blast cell to determine the lineage of blast cell, as well as to localize the stage of cellular differentiation. Immunophenotyping and molecular analysis are utilized when cytochemical stains can no longer serve as a method to differentiate the morphology of blasts (Olsen et al., 2008, Hoffbrand and Moss, 2011). The downside of immunophenotyping is the limitation of the antibodies used to detect the surface protein, as the type of antibodies are not all-round and may not be able to detect all molecular event in track of malignancy events. In addition, immunophenotyping and molecular analysis often come in higher cost (Samir et al., 2015).

The pursue in diagnostic and treatment of cancer was aggressive and tremendous over the last half century. This leads to a more ameliorate treatment and diagnostic method, and betterment in risk stratification as well as supportive care. As nanoparticles technology arise, it arises as the new game changer in medicine field. Nanomedicine is the application of nanotechnology in clinical field (Tatar et al., 2016). It is gaining rising attention as the concept of nanoscale size polymerized carrier makes the delivery of drugs or gene to the targeted cells or tissues possible by obsoleting nano-sized biological barrier (Jiang et al., 2008, Samir et al., 2015). To date, wide variety of nanoparticles have been developed to suit wide range of application and to suit specific condition of patient. Nanoparticle can be altered by changing its size, shape, and physico-chemical properties, through surface modification by functionalizing the nanoparticle surface with different

type of precursor, thus ample amount nanoparticles can be fabricated to fit different user requirement (Chen, 2008, Siddiqi et al., 2014, Yang et al., 2014). In targeting the cancerous cells, both active and passive targeting methods have been employed to increase the specificity and to prolong the retention of chemotherapeutic drug in tumour cells. In addition, the synthesis of nanoparticles can be done at expenses of low capital (Sahoo and Liu, 2015). Nanoparticle can be used either as standalone application or works in conjunction with conventional diagnosis and treatment methods. Nanoparticles in leukaemia's treatment are surface modified to recognize specific immunomarker on the malignant tissues and cells to efficiently delivered to target site (Yang et al., 2014). The use of nanoparticle to carry drugs increases the bioavailability of drug to the target site thus reduces the frequency of drug intake and dosage (Li et al., 2007a, Samir et al., 2015, Shen et al., 2016). Nanoparticles in gene therapy for cancer treatment involve the delivery of small interfering ribonucleic acid (siRNA) to silence the oncogene to stop the growth and to induce apoptosis of malignant cell, eg. anaplastic large cell lymphoma (ALCL) (Abdou et al., 1997, Zhao et al., 2011, Aranda et al., 2013, Vecsernyés et al., 2014). To conclude from the above discoveries and properties of nanoparticles in leukaemia's treatment and diagnosis, nanotechnology has the potential in unfolding new possibilities in nanomedicine. Thus, this triggered our interest to develop and to research on nanoparticles.

Our aim in this research is to study the adsorption of deoxyribonucleic acid, DNA onto newly synthesized nanoparticles. The study of DNA adsorption on this newly synthesized nanoparticle might also contribute to the advancement in nucleic acid extraction using surface modified nanoparticles as the bio-identifier. Nucleic acid extraction has been recognized as the most significant preceding step in a vast majority of molecular biology applications. Downstream analytical processes such as gene

modification, amplification, and cloning would be unattainable without the isolated nucleic acid as a source (Wink, 2013). Handcrafted methods of nucleic acid extraction and purification often involve the use of hazardous organic solvents such as phenol and chloroform (Ghaemi and Absalan, 2014) with labor intensive repeated centrifugation steps which produce low yield of nucleic acid with less satisfactory quality (Tan and Yiap, 2009). Thus, there is an increasing shift from conventional methods towards the use of commercial extraction kits thereafter the invention of a less labor intensive nucleic acid extraction kits (Lever et al., 2015). However, there is no universal extraction kit that has claimed to be able to perform well in extraction of every single type of sample exist (Martin-Laurent et al., 2001). The reason behind the futility in the design of universal kit is the diversity of the properties of sample (Tagliavia et al., 2016). Thus, to date small population of scientists still practiced handcrafted extraction method that facile fine tuning due to selection of sample that is less popular as study subject (Ogram et al., 1987, Tagliavia et al., 2016). Hence, there is a need in finding an alternative that is less laborious.

Nanomaterials can be fabricated to suit specific usage requirement (Wang et al., 2015) due to its small physical and large surface area which in turn facilitate high-throughput processes (Chen et al., 2012, Maccarini et al., 2014). Utilization of nanoparticles to adsorb nucleic acid is a more promising approach as it can be synthesized with limited use of chemicals utilizing a less laborious procedure and reduce the cost of extraction. Therefore, there is potential that newly synthesized nanoparticles will unfold a new method of nucleic acid extraction. One such instance is the native magnetic nanoparticles, MNPs eg. Fe_3O_4 or surface modified magnetic nanoparticles eg. β-Cyclodextrin-MNPs (Ghaemi and Absalan, 2014, Sahoo and Liu, 2015) .

Magnetize nanoparticles have the unique properties of superparamagnetism which makes it easily magnetized and demagnetized by applying or removing external

magnetic force (Huber, 2005). To date MNPs have been widely use in medical radio-imaging (Lee et al., 2012), biosensors (Li et al., 2016), drug delivery (Li et al., 2007a) and much more. However, there are shortcomings of MNPs, it aggregates easily due to its magnetic attractive forces with inherently large surface energies and leads to possible loss of superparamagnetism. Hence there is a need in employing surface coating agent to confer colloidal stability, to prevent cell toxicity, and to render the MNPs with solubility in water (Tao et al., 2008).

Cyclodextrin is a cyclic oligosaccharide is used in this study as the surface coating agent. This group of cyclic oligosaccharide can form solid inclusions (host-guest complex) with its properties of hydrophobic internal cavity that can incorporate small molecules by hydrophobic interactions, hydrogen bonding and electrostatic interactions (Freeman et al., 2009). The external structure consists of large amount of hydroxyl group which makes it water soluble (Abdou et al., 1997). The hydroxyl constitute can be manipulated to enhance the interaction between cyclodextrin and its adsorbate for instance nucleic acid with capability of electrostatic interaction (Schofield et al., 2012). Therefore, the unique properties of cyclodextrin render it as a functional adsorbents for a wide range of compounds and an efficient carrier in enveloping drug and nucleotide thus enhancing disease treatment (Aranda et al., 2013).

To date, albeit low there are several types of surface modified and native nanoparticles that have demonstrated efficiency in nucleic acid adsorption (Chen et al., 2012, Ghaemi and Absalan, 2014, Sun et al., 2016). However, to the best of our knowledge, the combination of organic to inorganic, β-CD-MNP-TDI application in nucleic acid adsorption has yet to be reported in any studies. Therefore, the establishment of new nucleic acid adsorption method from this research might contribute to the

development of new nucleic acid extraction methods and specific therapeutic nucleic acid vector through nucleic acid adsorption which is less laborious with high-throughput.

1.2 Research Justification

To the best of our knowledge, the combination of organic to inorganic, β-CD-MNP-TDI application in nucleic acid adsorption has yet to be reported in any studies. The establishment of new nucleic acid adsorption method from this research might contribute to the development of new nucleic acid extraction methods and specific therapeutic nucleic acid vector.

1.3 The desired objectives of this research are summarized as below:

Objective:

- To study the adsorption of deoxyribonucleic acid (DNA) on β-cyclodextrin coated magnetic nanoparticles (β-CD-MNP-TDI).

Sub-objectives:

- To optimize and compare the parameters from batch adsorption studies, such as the effects of pH, concentration, time, and temperature, using β-CD-MNP-TDI.
- To study the adsorption mechanisms by using different types of kinetic, isotherm and thermodynamics models.

1.4 Hypothesis

The synthesized MNPs and β-CD-MNPs will efficiently adsorb DNA.

1.5 Outline of thesis/study

The thesis is presented in five chapters. Chapter one composed of the brief introduction of magnetic nanoparticles and cyclodextrin coated magnetic nanoparticles and the recent advances in nanomedicines oncology field, as well as the possible application of magnetic nanoparticle in nano-medical field. Study objectives and the organization structure of the dissertation were also included in chapter one. Literature review regarding the study was presented in chapter two. In chapter three, the material and method applied in the study of parameters as well as the characterization technique were presented. Chapter four composed of the results and the discussion of the characterization of the nanoparticles and the optimized parameters for sorption studies between adsorbent adsorbate. The mechanism of adsorption between adsorbent and adsorbate was described using the best adsorption kinetic and thermodynamic model. Chapter five was composed of the overall conclusion of the study inclusive of the prospect and future direction of the study.

Chapter 2 Literature Review

2.1 Magnetic Nanoparticles

At the end of 19th century, nanotechnologies breakthrough brought paramount significant transformations affecting technology in different field. Medical and biological field has thus achieved rapid evolution from many contacts of nanotechnologies and biological as well as medical field. One of the nanotechnologies widely studies being magnetic nanoparticles.

The idea of manipulating the magnetize material in nano-structure through external magnetic field was a breakthrough in recording field, as such, videotape, audiotape, and in many of data storage device that is being used to date. Apart from that, in 1960s it has brought significance advancement in medical fields, such as medical diagnostic tool, radio imaging field, magnetic resonance imaging (MRI), ameliorates cancer treatment method, radio-frequency hyperthermia, and drug delivery systems to targeted tissue through conjugation with a variety of biological or chemical agents (Ito et al., 2005, Nikalje, 2015).

2.2 Properties of Magnetic Nanoparticles (MNP)

Comprehension of the nature of behaviour of magnetic nanoparticles is foremost important in designing and optimization of magnetic properties of MNPs. In order to synthesize MNPs that suits user needs, size and shape factors of nanomaterial are of utmost important. The modulation of magnetic parameters of MNPs depends primarily on the modulation of MNPs size and shape. One of the most interesting features of MNPs being its superparamagnetism which is size dependent (Pershina et al., 2014).

Alteration of iron oxide sizes alter the magnetic anisotropy or the coercivity as the energy barrier (blocking temperature, T_B, the transition temperature for the transition of magnetize vector to go from ferromagnetic to superparamagnetic state) to magnetize the vector changes accordingly based on the sized of nanoparticles as illustrated in Fig. 2.1. The reduction of nanoparticles size beyond the so-called superparamagnetic radius (r_{sp}), and in association with the thermal energy, magnetic anisotropy will be altered from ferro- or ferrimagnetism to become superparamagnetism. Bulk iron oxide which exist as multi-domain ferromagnetic material cannot change its magnetic direction due to the insufficiency in thermal energy fluctuation to allow free spins rotation, thus it is exist in a permanently magnetized state without the need of external magnetic field (Faraji et al., 2010). For an iron oxide that is sufficiently small enough, in size ranging from 20-30 nm, will contain only a single magnetic domain that has only single magnetic moment. Hence the nanosized particles will exhibit superparamagnetism due to the energy from thermal fluctuation is sufficient to overcome the energy barrier and to trigger a fast spin rotation. This spin rotation will reset the internal magnetic dipoles to random and leads to zero net magnetization. The zero coercivity property plays paramount role in spontaneous recovering of the initial paramagnetic phase (a phase of randomize magnetic dipoles that can be aligned along the direction of external magnetic field) of zero net magnetic moment when external magnetic field is removed, thus a second source of energy is no needed to demagnetize the nanomaterial. This makes magnetized nanoparticles an interesting subject of research of modern science and medicine (Faraji et al., 2010, Pershina et al., 2014).

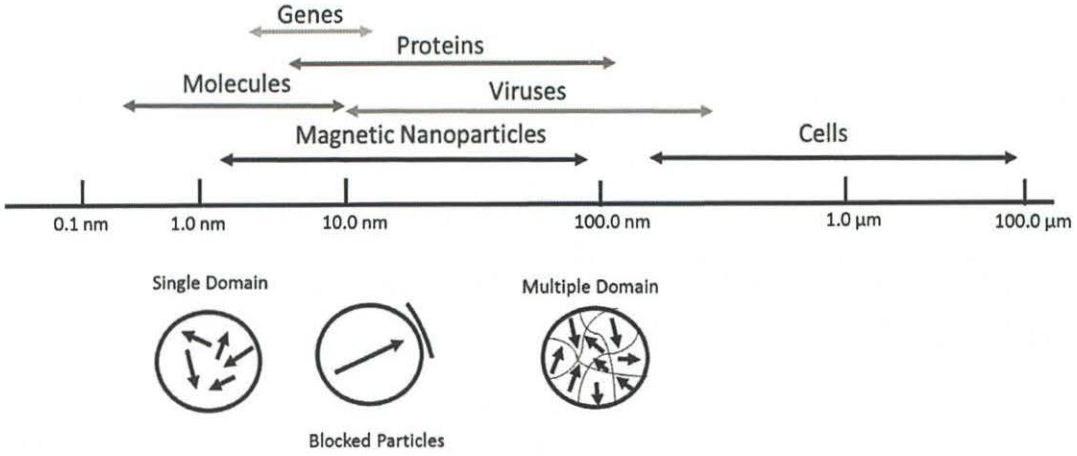

Fig. 2.1. Illustration on the sizes of magnetic nanoparticles.

Iron oxide nanoparticles despite having large specific surface are and magnetic dipole interaction they are highly susceptible to oxidation and agglomeration. Oxidation process on the surface of iron oxide nanoparticles occurs especially rapid when they exist under ambient conditions. Some of the unwanted side effect attributed by the oxidation includes, the oxidative damage of cells and tissue from the liberation of free radical and the formation of thin oxide layers (Donaldson et al., 1996, Xia et al., 2006, Singh et al., 2010). The formation of oxide layers leads the alteration of the magnetic properties of the magnetic nanoparticles. Thus efforts have been devoted over the past few decades in developing different coating materials to passivate the surface oxidation of the iron oxide nanoparticles in order to preserve their magnetic properties and halt the agglomeration (Faraji et al., 2010).

2.3 β-Cyclodextrin Magnetic Nanoparticles (β-CD-MNP-TDI)

2.3.1 Cyclodextrin

Cyclodextrins are enzyme derived products from starch using cyclodextrin glycosyltransferase (CGTase). As illustrated in Fig. 2.2., cyclodextrin is a cyclic α-1,4-

glucans constitute of six units of glucopyranose and above, with a few widely used to date, α-(6 glucopyranose), β-(7 glycopyranose), γ-(8 glycopyranose). The glucopyranose of cyclodextrin structure is arranged in such a way that it forms a truncated cone with hollow centre. The truncated structure of the cyclodextrin with free hydroxyl group deposited exterior of the structure renders it readily hydrophilic, while the hydrocarbon groups with glycosidic oxygen ring deposited within the structure provides a hydrophobic internal cavity, as showed in Fig. 2.3. Ergo the structure can act as an envelope to form host-guest inclusion complexes with different type of hydrophobic molecules (Li et al., 2007b). This unique property of cyclodextrin can be further made to order by the complexion of different functional groups have led to the invention of new technology in wide range of application fields (Li et al., 2007b, Badruddoza et al., 2013, Chen et al., 2014)

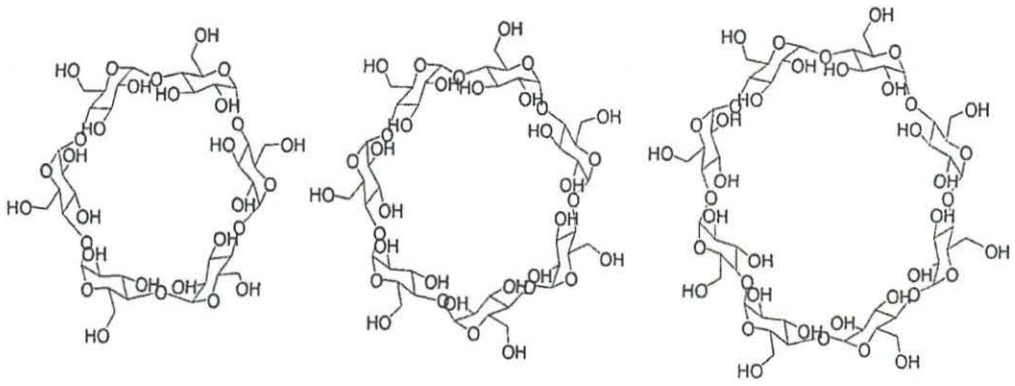

Fig. 2.2. Cyclodextrin illustration adapted from study done by Gnaim and Athamma (Gnaim and Athamna, 2010).

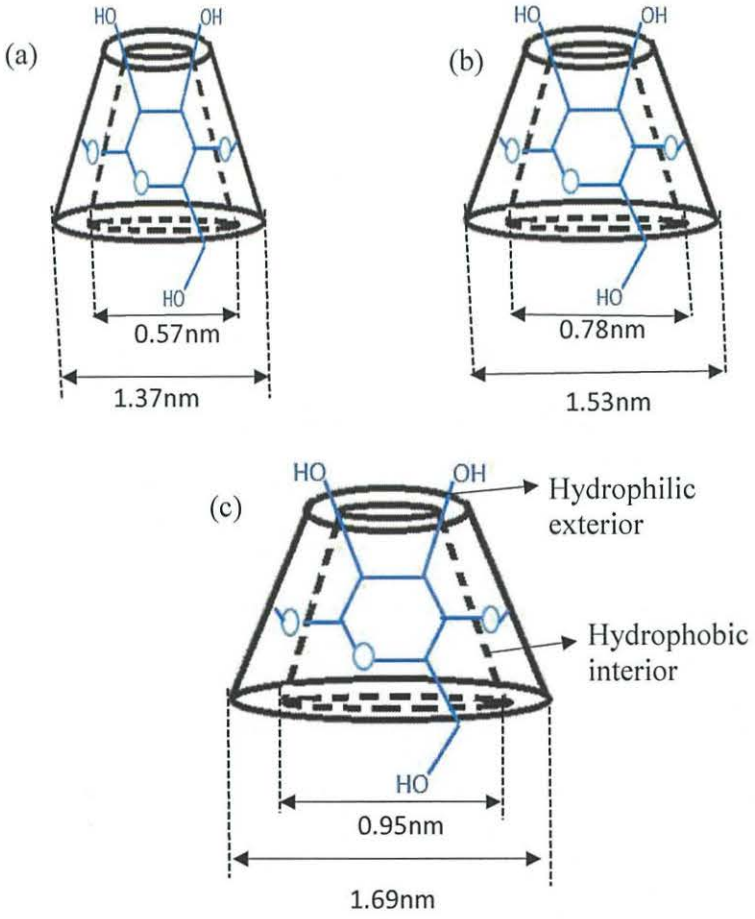

Fig. 2.3. Illustration of truncated structure of cyclodextrin. (a) α-cyclodextrin (b) β-cyclodextrin (c) γ-cyclodextrin

2.3.2 Cyclodextrin Inclusion Complexes

The unique property of cyclodextrin inclusion complex can only be made practical if it is capable to selectively include guest molecules of interest into the complex (Chernykh and Brichkin, 2010). In order to form a host and guest inclusion, the physicochemical properties of the guest molecules, the polarity of the solvent that hold the guest molecules and thermodynamics interaction between host and guest molecules plays an important role. Owing to the hydrophobic property of the internal cavity and hydrophilic property of the structure exterior, molecules that can form stable complexes

with cyclodextrin must be able to form van der Waals, and or hydrogen bonds with the hydrophobic molecules and or hydroxyl groups on the exterior of the truncated structure, respectively. In addition, the guest molecules must be less polar compared to the solvent in order to replace the solvent molecules from hydrophobic cavity, as the hydrophobic bond established between guest molecules will be more stable and energetically favourable compare to the solvent. An interaction that is favourable in the sense of thermodynamic will drive the drawing of guest molecule into the hydrophobic cavity of cyclodextrin (Badruddoza et al., 2010, Chernykh and Brichkin, 2010). The formation of stable and fit host and guest inclusion also dependent on the precision in sizes of the cavity to guest molecules. Incompatibility in the size of the guest molecule will result in both less fitting and less stability of the inclusion complex (Del Valle, 2004).

2.3.3 Application of cyclodextrin and magnetic nanoparticle

The usage of cyclodextrin and magnetic nanoparticle either as complexation or as auxiliary conglomerate has bring about significance changes in wide range of field. Cyclodextrin can be seen apply in pharmaceuticals, and medical diagnostic field, by utilizing the host and guest inclusion property of the cyclodextrin to modify the physical and chemical properties in order to enhance the bioavailability of the poorly soluble drugs (Pershina et al., 2014). For instance, a low polarity drugs which have been surface modified through the formation of inclusion complex will allow the engulfment of low polarity drug into the hydrophobic cavity through hydrophobic and van der Waals interaction. The cyclodextrin exterior being hydrophilic, ergo more soluble in aqueous system, thus the drugs can be retained in the body system for a longer time, with higher bioavailability, and with higher chances of delivering of drugs to targeted tissues (Challa et al., 2005).

On the strength of the review done by Dreyfuss and Oppenheimer which described the effect of interaction of cyclodextrin with cell membrane detailed that lipid rich cell membrane, for instance cholesterol and sphingolipid when it is in close proximity cyclodextrin will results in the efflux or extraction of lipid constituent from cell thereby lysing the cell (Ohtani et al., 1989, Dreyfuss and Oppenheimer, 2011) or resulting in modulation of efflux transporter. This property is used targeting to solve the issues of poor bioavailability of drugs due to the existence of biological barrier such as endothelial and epithelial barriers that maintain the dynamic homeostasis of body system through restriction entry of drugs. The extraction of cholesterol by cyclodextrin from brain capillary endothelial cells has shown to inhibit the P-glycoprotein activity, a type of protein associated with multidrug resistance (Cordon-Cardo et al., 1989). The inhibition of P-glycoprotein through alteration of the overall lipid packing and raft association of the glycoprotein results in longer retention of drugs in plasma when the drugs are not efflux out of the cells (Tilloy et al., 2006, Gil et al., 2009). Others well characterized effect of complexation of cyclodextrin on drugs in enhancing the drugs bioavailability include the subsequent diminishing of tight junction proteins and integrity through the depletion of cholesterol by cyclodextrin (Lambert et al., 2007, Deli, 2009).

The cholesterol depleting effect of cyclodextrin has also been described useful against microbial infection. Pathogens such as human immunodeficiency virus (HIV), influenza, bacteria such as *Eschericia coli, Salmonella* are some of the pathogens that was susceptible to the inhibition of cell adhesion ensue the disruption of lipid rafts of cells membrane following the cholesterol depletion by cyclodextrin. In addition, the depletion of platelet membrane cholesterol by β-cylodextrin will prevent platelet aggregation which indicates the therapeutic potential in acquired or congenital disorder induced by abnormal platelet aggregation, for instance atherosclerosis (Toyama et al., 2007). In cancer

treatment, cholesterol depletion property of cyclodextrin leads to the reduction in expression of proteins in signalling pathway, cell proliferation, and angiogenesis. The lack of expression of the protein will inhibit the metastasis and development of tumour, as shown in mice model (Li et al., 2006, Zhang et al., 2006, Raghu et al., 2010, Onodera et al., 2014).

Table 2.1. Application of magnetic nanoparticles and different surface coating in medical field.

Magnetic Nanoparticles	**Surface coating**	**Function**	**References**
Iron oxide	Polysaccharide	Magnetic Resonance Imaging (MRI) contrast agents	(Babes et al., 1999)
Iron oxide	-	Tissue-specific therapeutic drug administration	(Byrne et al., 2004) (Osaka et al., 2006)
Iron oxide	Ionic liquid	Isolation and purification of deoxyribonucleic acids	(Ghaemi and Absalan, 2014)
-	α-cyclodextrin	Treatment of peripheral arterial occlusive disease	(Vecsernyés et al., 2014)
-	SBE-β-CD	Treatment of multiple myeloma	(Vecsernyés et al., 2014)

2.4 Deoxyribonucleic acid

Nucleic acid, the basic yet fundamental unit that made up the template inherited for the continuing of life. These units code for every single information needed for the regeneration cells and maintenance of homeostasis, as well as the information needed to cause catastrophe in one's life (Passarge, 1995, Voet et al., 1999).

2.4.1 Structure and Properties

Nucleotide composed of three integral parts, which are phosphates, pentose sugars, and purine or pyrimidine bases (Fig. 2.4). The different composition pentose sugars derived from the present or absent of oxygen in the 2' carbon of pentose sugars, give rise to ribonucleic acid, RNA (**D**-oxyribose) and deoxyribonucleic acid, DNA (2'-deoxy-**D**-ribose). The differences in the presence of double bond or side chain or both in nitrogenous bases structure give rise to different purine and pyrimidine. The phosphate group in the structure gives the acidic properties to the structures at physiological pH. The combination of all these unique structures give rise to eight different nucleotides, in which the phosphate group is linked to the pentose sugar by phosphodiester bond at the 5' carbon position, while at the 1'carbon position of the same pentose sugar is linked to the N9 atoms and N1 atoms in purines and pyrimidines nitrogenous bases respectively. The unique template that code for living cells begin when all these subunits are polymerized by the means of phosphodiester bond between 3' carbon of the extending nucleotide to 5' carbon of the upcoming nucleotide. Thus, linear sequence of nucleotide is usually written from left, 5' end to right, 3' end. As the polymeric nucleotides structure extend, the physical properties (ion charges) varies according to the composition of the individual units in the polymeric nucleotide structure in evident of Chargaff's rules which described the equal numbers of adenine and thymine residues in pairs, and equal numbers

of guanine and cytosine residues in pairs. However, the composition of each residues in pairs varies widely in different individuals. Ultimately two strands of polynucleotide chains that is complementary to each other will form a helix with each strand running in antiparallel pattern. The nitrogenous bases interact with its complementary pair with hydrogen bonds, leaving the pentose-phosphate backbone winding around the periphery of the molecules. The winding of the pentose-phosphate backbone around the periphery exposed the polyanions structure, and rendering the double helix negatively charges in overall (Passarge, 1995, Voet et al., 1999).

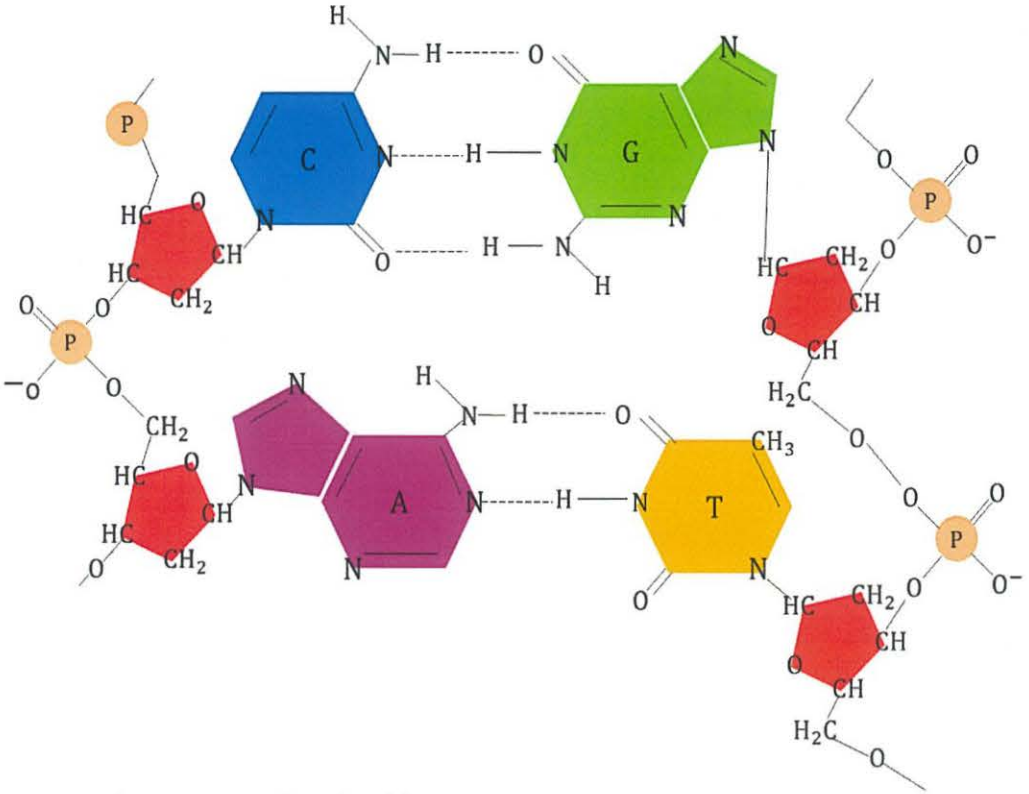

Fig. 2.4. Basic structure of nucleotides.

2.5 Salmon testes DNA

DNA obtained from salmon testes was chosen as the source of DNA in this study as the DNA synthesis process is continuous in the developing testes of salmon until it reaches maturation stage. In other words, the DNA synthesis is in rapid growth spurt

manner, thus this DNA source is generally assumed to be nuclear and have relatively lower protein content (Creelman and Tomlinson, 1959, Dixon and Smith, 1968). The lower protein content indicates better DNA purity, and lower protein interference to the adsorption study.

2.6 Application of the surface modified MNP with DNA

Superparamagnetism of magnetic nanoparticles enable it for ready isolation, concentrate and detection of analyte of interest. These unique properties give a good prospect in application in medical and biological field.

The complexation of magnetic nanoparticle with suitable surface coating allows its wide application in different field. For instance, the complexation of β-cyclodextrin onto the surface for a more stable adherence of DNA onto the surface through higher degree of bond interaction. The adhered DNA on the surface are found to be useful in studies in separation of biomolecules, genomic and plasmid DNA as nucleic acid can adhere with high specificity to its complementary strand. Hence, with specifically designed DNA sequence, it can be fabricated through adherence to the surface of nanoparticles for extensive application. For instance, the isolation of DNA from urine samples. DNA content in urine has been reported to be low and deteriorate at fast rate, hence it is conventionally not considered to be suitable source of DNA, however to obtain DNA from urine source is less invasive and easy. As per study done by Shan et al., the detection of genomic DNA was made possible with magnetic nanoparticles that has been carboxylated at DNA concentration of 50×10^{-6}. This study is especially important when it comes to the detection for DNA from pathogen in patient (Shan et al., 2012). For example, the detection of polyomavirus BK through kidney biopsy which is regards as highly invasive procedure has been practiced as gold standard in accessing the tendency

of graft loss in patient with kidney transplantation due to BK virus infection post kidney transplantation. However, upon the positive detection of BKV nephropathy, the patient would have suffered irreversible late-stage renal injury. Thus, molecular detection of viral load is suggested to be able to give early and perhaps better prognosis and higher tendency in reversal of the catastrophe effect (Bechert et al., 2010). The utilization of DNA-magnetic nanoparticles complex has also seen apply in detection of herpes simplex virus DNA at picomolar level through the complexation of magnetic nanoparticles with riboflavin-5'-monophosphate and fluorescence, followed by the quenching of fluorescence, this result in intensify fluorescence intensity, ergo the successfulness in measurement (Lin and Chen, 2011).

Precise drug delivery using magnetic nanoparticles was first proposed in 1978 (Widder et al., 1978). It was deemed possible based on the nano-sized particles which obsolete the biological membrane and allows the drugs or biomolecules it carry to be delivered to the target site without the risk of embolism. In addition, the complexation of the surface with specific oligonucleotide, aptamer or protein allow the specific recognition by the cells to be uptake, or to avoid the metabolism of the drugs or DNA through the complexation of certain peptide on the surface nanoparticles-DNA complex to prevent the recognition by P-glycoprotein (a drug efflux transporter that pumped out drug from intracellular and results in development of cell resistance to drug) in order to maintain the highest possible therapeutic dose. On top of that, the monitoring of target drugs administered with magnetic nanoparticle might be able to be carried out using magnetic resonance imaging. The therapeutic application of magnetic nanoparticles in term of gene therapy is none other than the delivering of genomic nucleic acid and enhance transfection of the nucleic acid into the malignant cells. It is in hope that the transfected nucleic acid can change the expression of gene, leading to the initiation or

suppression of protein expression. In addition, the usage of nanoparticles-DNA/RNA complex as an alternative to viral vector can reduced the occurrence of unwanted side effect, such as the infection and elicitation of immune response due to the presence of alloantigen on the vector (Alcantara et al., 2012, Cheng et al., 2012, Pershina et al., 2014, Busquets et al., 2015).

Gene therapy has been a sought-after alternative in providing a permanent alleviation in disease caused by defective gene, such as thalassemia, sickle cell anaemia (substitution of valine in beta chain with glutamic acid) (Serjeant, 1997), myeloid leukemia (nucleotide insertion, mutation in the form of internal tandem duplication) (Tanaka et al., 1995), and other types of cancer. Therapeutic genes can theoretically be used in complexation with nanoparticles can be used to deliver the substitute nucleotide for the missing or deleted sequence in lymphoblastic leukemia; it can be used to deliver small regulatory non-coding antisense RNAs for the silencing of gene and alteration of immunogenicity in tumours. The hallmark of cancer always involved the alteration of genes such as tumour suppressor gene, TP53 and oncogenes. The alteration results in failure of cell to suppress the malignant cells from excessive proliferation, through the obstruction in initiation of apoptosis and DNA repair mechanism. In the alteration of oncogenes, the alteration of genes results in uncontrollable, excessive and inappropriate expression of malignant cells (Morales et al., 2002, Mesri et al., 2014). The combination of the alteration results in organs aplasia which subsequently leads to more severe downstream side effect such as major organs failure especially kidney and liver.

2.7 Adsorption and desorption

Adsorption process is regards as efficient and economically practicable separation method for its simplicity in functionality and bulk production (Raoov et al., 2013). Hence

magnetic nanoparticles with such adsorption features are chosen to be studied in order to fully utilized the features for betterment of readily available technology. Magnetic nanoparticles equipped with superparamagnetism in conjunction to separation technique bring about major changes in different application field. Magnetic nanoparticles in size of micron means that it has large surface area adequate to accommodate the adsorption of wide range of analyte of interest when it is complexed with different coating agent (Faraji et al., 2010, Pershina et al., 2014). Upon reaching adsorption equilibrium, the magnetic nanoparticle can be separated, removed from aqueous phase simply through the application of external magnetic force. Desorption can be done subsequently to recover the analyte from nanoparticles. In order to comprehend the adsorption and desorption property of nanoparticles with regards to the analyte of interest, the investigation of adsorption equilibrium, kinetics, mechanisms of action, and parameters affecting the adsorption and desorption of analyte onto and off the nanoparticles are utmost important.

2.8 Adsorption equilibrium

2.8.1 Langmuir model

The Langmuir isotherm model assumes that the adsorption of adsorbate occurs in monolayer pattern over the fixed number of sites of homogenous adsorbent surface. Each of the site of adsorption existing on the adsorbent surface is identical and is equal of adsorption energy with equal accessibility for adsorption until the equilibrium being reached. At equilibrium state, a state where all the adsorbent sites are saturated, and no further adsorption can take place. The Langmuir isotherm model is described by the equation follows:

$$q_e = \frac{q_m C_e}{K_L + C_e} \qquad (1)$$

The equation can be integrated to linear form represented as follows:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m K_L} \quad (2)$$

C_e (mg L^{-1}) represents the concentration of adsorbate at equilibrium in liquid phase, q_e (mg g^{-1}) represents the concentration of adsorbate at adsorbent surface, q_m (mg g^{-1}) represents the maximum binding capacity of adsorbate, and K_L (L mg^{-1}) represents the Langmuir constant, the binding affinity of adsorbate to adsorbent. The value of K_L and q_m can be obtained from the slope and intercept of linear plot of $\frac{C_e}{q_e}$ against C_e (Badruddoza et al., 2010, Ghaemi and Absalan, 2014).

2.8.2 Freundlich model

The Freundlich isotherm assumes that the adsorption of adsorbate onto adsorbent with heterogenous surface occurs in multilayer patterns dependent on the interaction between adsorbate and different classes of adsorption site on adsorbent. The adsorption process is reversible and occurs with uniform energy. The model assumes the process of adsorption will occurs continuously with increasing solute concentration until maximum saturation at the adsorption site is reach. Upon reaching maximum saturation at the adsorption site on the adsorbent, the adsorption energy will decrease exponentially. The Freundlich isotherm model is described by the equation as follows:

$$q_e = K_F C_e^{1/m} \quad (3)$$

The equation can be integrated to linear form represented as follows:

$$lnq_e = \left(\frac{1}{n}\right) lnC_e + lnK_F \quad (4)$$

$K_F((\text{mg g}^{-1})(\text{L mg}^{-1})^{\frac{1}{n}})$ and n represent the Freundlich equilibrium constant and heterogeneity factor respectively. The values of K_F and $\frac{1}{n}$ can be obtained from the slope and intercept of linear plot of lnq_e against lnC_e (Badruddoza et al., 2010, Raoov et al., 2013, Ghaemi and Absalan, 2014).

2.9 Adsorption kinetics

Adsorption kinetics models have been widely utilized in testing experimental data in modelling of adsorption mechanism. It is of utmost important to know and to describe the adsorption mechanism and its rate at which the adsorbates are removed from liquid phase, in order to utilize the adsorption mechanism of the nanoparticles in different setting. The two main models that are focused in this study are pseudo-first order equation, and pseudo-second order equation.

2.9.1 Pseudo first order

The pseudo- first-order model assumes that there is directly proportional relationship between the rate of change of uptake of solute with time and the difference in concentration of saturation together with the amount of adsorbate adsorbed with time. The pseudo-first order is described as follows:

$$\frac{dq_t}{d_t} = k_1(q_e - q_t) \quad (5)$$

The equation can be integrated when t=0 to t=t and q=0 to q=q_t, shown as follows:

$$\log(q_e - q_t) = logq_e - \frac{k_1}{2.30}t \quad (6)$$

q_e (mg g^{-1}) and q_t (mg g^{-1}) refer to the adsorption capacity at equilibrium state and the adsorption capacity at any given time, t (min), respectively, k_1 (min^{-1}) represents the