CHAPTER 1

Introduction

Supraparamagnetic iron oxide (SPIOs), possesses nanoscale magnets differ from bulk materials due to their high surface-to-volume ratios and exhibit superparamagnetic property at room temperature [1]. The magnetic anisotropy energies of superparamagnetic nanoparticles (SNPs) are smaller than their thermal energies, and their magnetic moments freely flip in any direction [2]. Superparamagnetism occurs in nanoparticles is single-domain which is the summation of all the individual magnetic moments carried by the atoms of the nanoparticle. The magnetic property of SPIOs is depended on appropriate synthesis methods: 1. size, 2. shape, 3. composition and 4. Shell-core design.

In biological application, the nanoparticles need to be biocompatible, non-toxic and stable at physiological pH. The criteria of SPIOs for biological management must have (1) high magnetization and homogeneity in size [3] and (3) surface modification with biocompatible material to reduce aggregation and toxicity or specific biomarker probe increase specific uptake on target [4-6]. Normally, SPIOs were uptake by the mononuclear phagocyte system (MPS) or reticuloendothelial system (RES) after intravenous administration made it possible at the end of the 1980s to use SPIOs in preclinical and clinical diagnostic MRI of liver and spleen [7]. SPIOs can be accumulated by passive or active targeting mechanism. In passive targeting, the SPIOs are concentrated in the phagocytic cells that are related to cleared the nanoparticle from the body [8]. When the SPIOs were placed in a magnetic field, they affected on the microenvironment of magnetic field yielding an inhomogeniety of the magnetic field. And depending on the nature of molecule coated on their surface, the SPIOs-modifiedd surface can bind to the water molecule found nearby the particles decreasing of mobile water concentration. Thus the presence of SPIOs-modified surface in the homogeneity

magnetic field will cause : (1) an inhomogeniety of microenvironment that will effect directly the relaxation mechanism of proton by shortening its relaxation time and (2) while the affinity of water molecules to SPIOs affected the yield of proton signal loss. The overall proton signal intensity recorded by the MRI instrument was clearly depend on the two events mentioned above. The SPIOs-base specific probe imaging should be designed in order to optimize the two parameters. Since the SPIOs were specifically accumulated in a specific microstructure that cause the generated differential microenvironment of the tissue/organ.

SPIOs can be designed to be multiple functions in cancer theranostics, such as drug carriers, specific MRI contrast agent for tumors imaging [9]. SPIOs has high potential development by combined with specific molecule such as antibodies, dyes, chemotherapeutic agents and nucleic acids to have multiple functions, specific uptake on target and increase their properties. Now a day, SPIOs were most frequently investigated for biomedical applications, because they are generally stable under air and highly biocompatible [10-11]. But these nanoparticles exhibit chronic cytotoxic effects and were easily aggregated and chemical erosion which limited their successfully use in biomedicine. In order to prevent aggregation, chemical erosion, and chronic cytotoxic effects, surface coatings are needed during synthesizing these nanoparticles [12]. The coated SPIOs nanoparticles have been investigated for a large range of biomedical applications, such as hyperthermia, targeted drug delivery and molecular, cell separation [13-15] and diagnosis imaging. Since coated SPIO nanoparticles exhibited superparamagnetic property that made possible to measure small amount of SNPs distribution and accumulation on the targeted tissue *in vivo* situation using MRI scanner. Indeed, SNPs should be considered as MRI tracer thus its biodistribution and molecular interaction can be analyzed by using a tracer-based technology that might allow producing MRI images of sub-mm resolutions and micromolar-level sensitivity.

Currently, MRI contrast agents based on SPIOs, have become commercially available and can be acquired off-the-shelf [16-18]. Dextran is used as biocompatible molecule for modifying the surface of SPIOs that use in clinical diagnosis imaging. The dextran coat has shown to considerably improve the stability of particles at neutral pH. Another advantage of dextran is that it is nontoxic and biodegradable. When taken up

by cells the SPIOs accumulate within the lysosomes, and are eventually broken down, causing no detrimental effect to the cells [19-20]. Dextran is already used for stabilisation of the FDA approved SPIONs Endorem® EU (Ferridex USA; Guerbet/Berlex lab) and Resovist® (Schering AG), furthermore, Sinerem EU (Combidex USA; Guerbet/Advance Magnetics), which is currently in phase III trial (see section: Application of SPIONs in MRI for more detail on these SPIOs).

The SPIOs-coated dextran is currently used for liver cancer MRI imaging. AMI-25, the SPIOs have hydrodynamic sized was approximately 150 nm and non-specifically taken up by Kupffer cells in the normal liver tissue, but in malignancies tumor area was absent Kupffer cell cause the hyper intensity more than the normal area which has accumulation of SPIOs distinguish the healthy area from diseased area [21-22].

Mitochondria play an important role in cell functioning, for example in cell calcium signaling. But possibly the greatest interest has been in the emerging role of mitochondria as regulators of the cell life–death transition, in both necrotic and apoptotic forms of cell death [23-25] Changes in mitochondrial membrane potential ($\Delta\Psi_m$) are integral to the cell life–death transition [26] Overexpression of ATP-binding cassette (ABC) drug transporters, such as P-glycoprotein (P-gp or ABCB1), breast cancer resistance protein (BCRP or ABCG2) and/or multidrug resistance-associated protein (MRP1 or ABCC1), confers an acquired MDR due to their capabilities of transporting a broad range of chemically diverse anticancer drugs.[27] There is research show that when MDR cells were deprived of ATP, even partially, it would block the P-glycoprotein and MRP1 pump activity, leading to an increase in cellular drug accumulation [28-29]. It was report that drug sensitive carcinoma cells need less cellular ATP contents than MDR cells that of their corresponding due to the addition of the ATPase activity of Pglycoprotein and MRP1 protein [30-31]. For mitochondrial membrane potential mornitoring, Fluorescent characteristic of Rhodamin 123 (figure 1a.) when incubate with living cell related to mitochondrial membrane potential [32-33] and Rhodamine B (figure 2b.) molecule was use in experiment as the mitochondrial membrane potential probe in electronic emission spectroscopic technique (as scheme 1)

in human small lung carcinoma (GLC4) and its multidrug resistance cell line (GLC4/adr) [34].

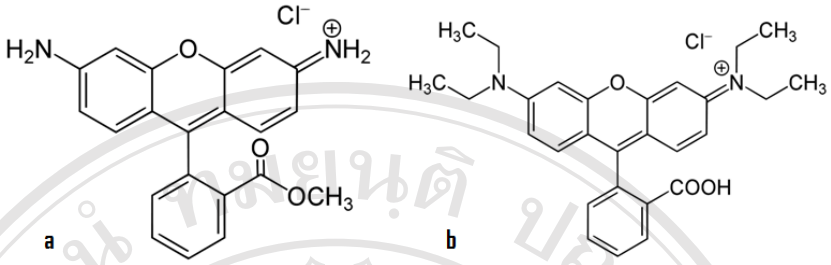
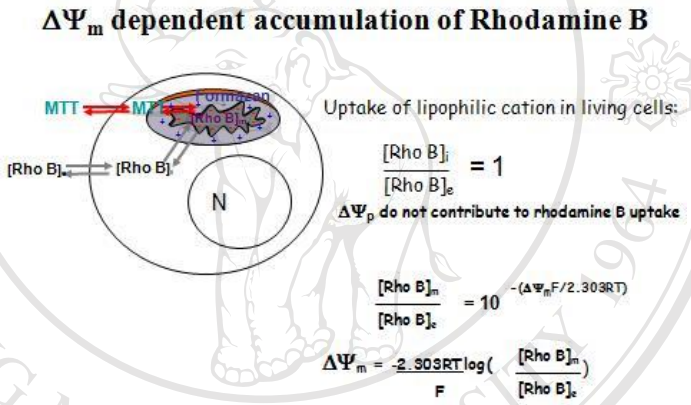


Figure 1. Chemical structure of (a) Rhodamine 123 and (b) Rhodamine B



Scheme 1. concept for determiantion mitochondrial membrane potential by Rhodamine in electronic emission spectroscopy

Researchers attempted to modify the chemical structure of dextran to yield an oxidizing form then conjugated with linkers in particularly diamino hexane. The conjugated dextrans were used as polymer for synthesis of SNPs in co-precipitation method. The coated SNPs can covalently bind with pharmacophors such as, positive charge molecule, antibody and/or aptamer which will allow molecular imaging a lesion or illness by the detection of a single molecule specific for this illness. The rational design of SPIOs can improve potential to MRI as a powerful medical imaging modality in biomedical areas, such as cardiovascular, oncology and cell labeling [35-36].

Objective

The studys aimed to synthesize SPIOs coated with dextran-diamino hexane conjugated with rhodamine B in order to obtain a permanent positively charges of biocompatible nanoparticles. The objectives of the thesis were :

1. To synthesize and characterize SPIOs coated with dextran-diaminohexane conjugated with Rhodamine B.

2. To determine its cellular uptake and distribution in hepatocellular carcinoma cells with transfected with hepatitis virus B (HepG2.2.15) compared with hepatocellular carcinoma (HepG2) and Breast carcinoma (MCF7)