CHAPTER 4

Discussion

The study aimed to synthesize SPIOs that coated with known material like dextran that efficiently by conjugated with rhodamine B, the mitochondrial probe [34] Since coated SPIOs exhibited superparamagnetic property, that should make possible to measure small amount of SPIOs distribution and accumulation on the targeted tissue *in vivo* situation using MRI scanner. Indeed, SPIO 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 [41].

We had successfully conjugated the 1,6-diaminohexane, the so-called ‘linker” on the hydroxyl group dextran by which allow attaching the SPIOs to the biophore like Rhodamine B. The results of this study showed that the yield and quality of SPIOs-Modified dex was dependent on the temperature of the working system; the heterogeneity of size of SPIOs-Modified dex was found when the synthesis temperature was fixed to 70°C and this heterogeneity was decreased when the temperature was at 90°C. The density of the nanoparticle (core size = 10 nm) of the series synthesis at 90°C was significantly higher than those of the series of synthesis at 70°C. Due to its density (series of synthesis at 90°C), it was difficult to prepare the mono-dispersed SPIOs-Modified dex. As can be seen in Figure 1d, two populations of SPIOs-Modified dex were determined. One group, the SPIOs-Modified dex has diameter equal to 90 nm and the other group has diameter equal to 280 nm. In our conditions of experiments, the obtained SPIOs-Modified dex of both series was almost the same nature.

The cellular distribution of SPIOs-Modified dex was elucidated in this study. It should be noted that the uptake of SPIOs-Modified dex was clearly observed by HepG2 and Hepg2.2.15 cells but slightly in MCF-7 cells. This was a good point that the SPIOs exhibited differential uptake in hepatocyte and breast cancer cells. However, there was the similar typical cellular distribution among these cells.

In order to obtained differential uptake of SPIOs-Modified dex by HepG2 and HepG2.2.15 cells, the nanoparticles were conjugated with a lipophilic cation "Rhodamine B". The rhodamine B is a mitochondrial probe in use for measuring the electrical difference of mitochondrial membrane in our research group (Reungpatthanapong et al. 2002) As we previously reported that the mitochondrial energetic state of multidrug resistance cells was found in higher degree of magnitude than those of its corresponding parental cells. In particularly, the HepG2.2.15 cell which infected by Hepatitis B virus was characterized to be overexpressed MDR transporters [42-43] thus the mitochondrial energetic state might be found higher level than those of its corresponding parental HepG2 cells.

The overall results of this study suggested that SPIOs-Modified dex-RhoB, the permanent positive charge nanoparticle, was accumulated in higher degree of concentration in the hepatocellular carcinoma transfected with whole genome of hepatitis B virus (HepG2.2.15) compared with the two other cells lines. Since its intracellular target was found as peri-nuclear zone and lysosome, the SPIOs-Modified dex-RhoB should be sequestrated and digested, causing no detrimental effect to the cells. The SPIOs-Modified dex-RhoB might be useful as targeted contrast agent of MRI for imaging of liver cancer especially the hepatocellular carcinoma induced by hepatitis B virus.