CHAPTER IV
RESULTS AND DISCUSSION

4.1 Particle Analysis

4.1.1 Characterization of Hydrophobic Magnetite

Oleic acid (OA) coated magnetite nanoparticles were successful synthesized by a coprecipitation method. FTIR spectroscopy was used to investigate oleic acid coated magnetite particles. The symmetric and symmetric CH_2 stretches at 2924 and 2853 cm^{-1} occurred in the FTIR spectra of OA-coated Fe_3O_4, as shown in Fig. 1, were resulted from not only the nanoparticles were surrounded by hydrocarbon molecules in a crystalline state, but also, the layer of surfactant molecules was adsorbed on to the magnetite nanoparticles surface. Moreover, The C=O stretch, which occurred at 1,713 cm^{-1}, was almost disappear in the spectrum of OA-coated Fe_3O_4. The pieces of these evidence could confirm the incorporation of OA-coated Fe_3O_4 (Bootdee *et al*, 2012). The composition of oleic acid and magnetite nanoparticles was determined by TGA. In Figure 2 shows magnetite nanoparticles were coated by oleic acid. In this graph, the oleic acid on the surface of magnetite nanoparticles was approximately 23 percentage points.

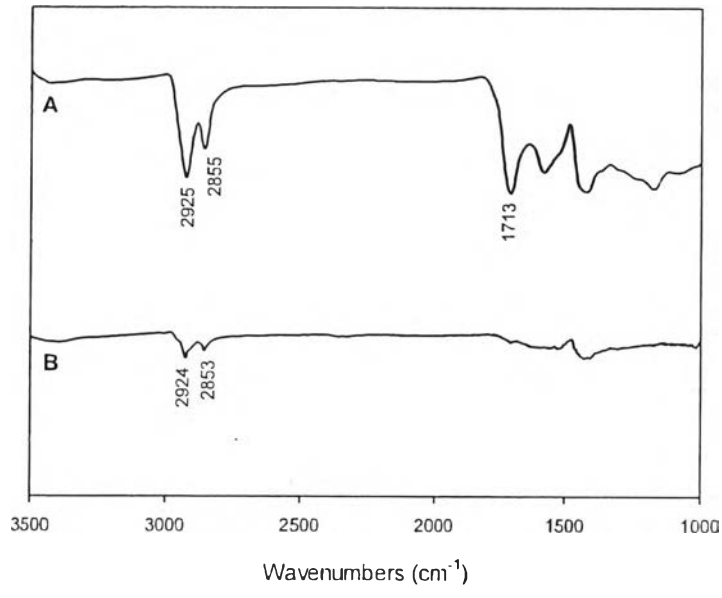

Figure. 4.1 FTIR spectra of (A) pure OA; (B) Fe_3O_4 coated with OA.

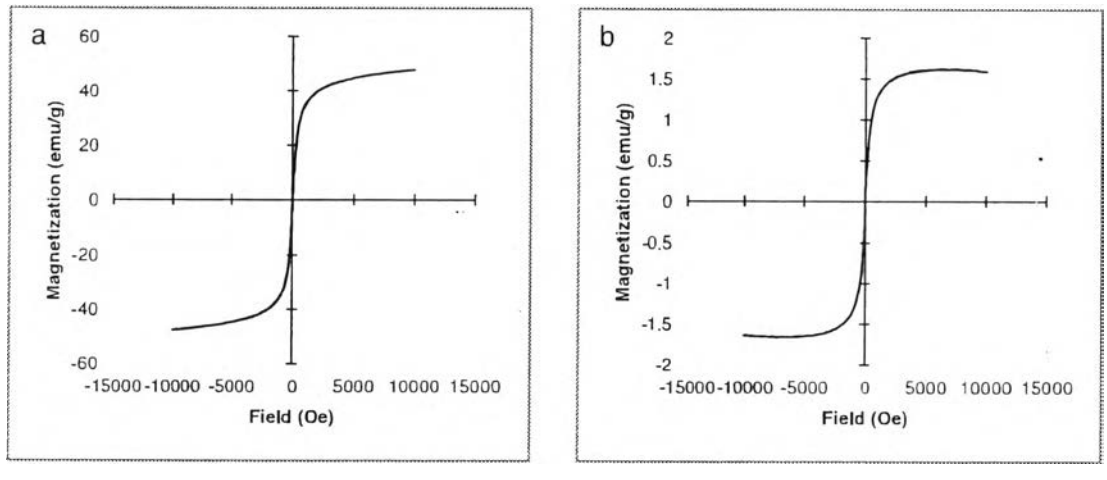

Figure. 4.2 Plot of magnetization, (a) pure magnetite nanoparticles. (b) PLGA nanoparticles loaded magnetite nanoparticles.

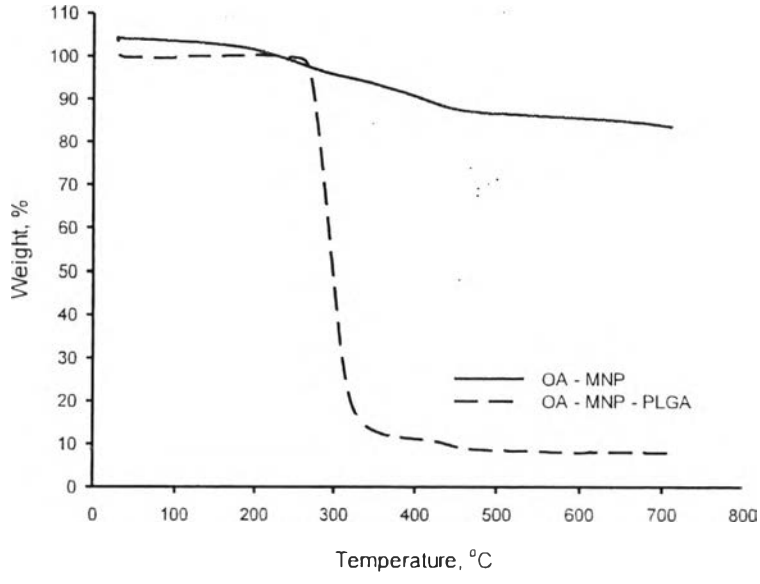

Figure. 4.3 Thermo-gravimetric curve of OA-coated magnetite and PLGA encapsulated magnetite

4.1.2 Characterization of PLGA Nanoparticles

Magnetite loaded PLGA nanoparticles were investigated by TGA. The ratio of magnetite to PLGA nanoparticles was roughly 7 % as shown in Figure 2. Besides, the superparamagnetic behaviors of pure magnetite nanoparticles and magnetite loaded PLGA nanoparticles were shown in Figure 3. Both of them exhibited superparamagnetic properties because a hysteresis loop did not appear in that graph. However, there were different in the values of magnetization. The pure magnetite nanoparticles presented the saturation magnetization around 48 (emu/g) In contrast, the magnetite nanoparticles loaded PLGA nanoparticles showed lower value of the saturation magnetization around 1.6 (emu/g). The ratio of PLGA nanoparticles to magnetite nanoparticles was the crucial factor playing a role in the decrease of the saturation magnetization. From pervious work, the saturation magnetization of the nanocomposites decreased with increasing PLGA concentration (Bootdee *et al*, 2012)

The average hydrodynamic particle sizes of low and high molecular weight were 348.4 and 414.3 nm, respectively, as determined by DLS. Figure 4 shows TEM images of the PLGA nanoparticles loaded with magnetite coated oleic acid. In

particular, the images exhibit the homogeneous distribution of the magnetite coated oleic acid in PLGA nanoparticles. The reason for this phenomenon was the polarity of magnetite coated oleic acid and PLGA nanoparticles were alike. Consequently, magnetite coated oleic acid were well dispersed in PLGA matrices.

- The zeta potential was determined in order to investigate the stability of the colloidal suspension. The zeta potential values of the low and high molecular weight PLGA nanoparticles loaded with SPION coated oleic acid were -19.9 ± 3.84 and 20.70 ± 4.34 respectively. Therefore, these values indicated that the molecular weight of PLGA had little or no effect on the zeta potential.

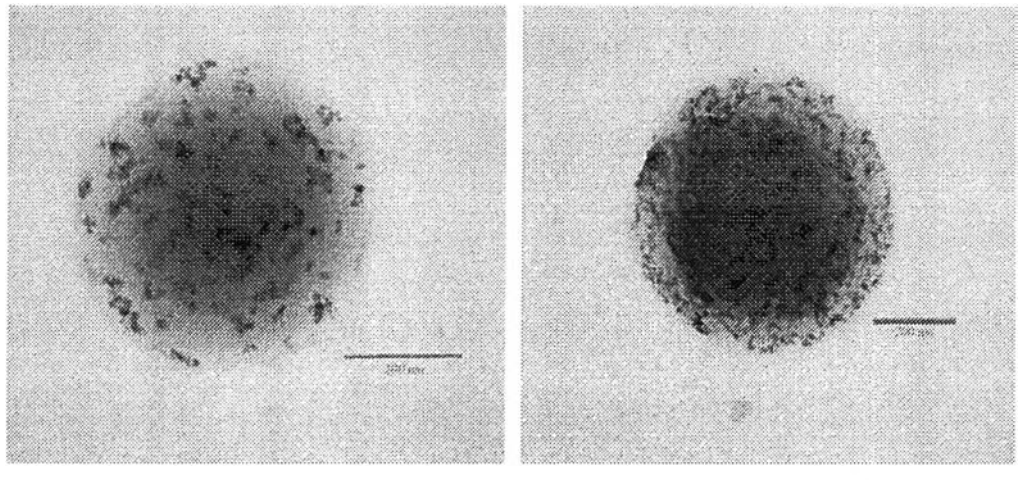

Figure. 4.4 TEM images of synthesized PLGA nanoparticles loaded with SPION coated oleic acid.

Table 1 Calculation of the percentage of encapsulation efficiency of four initial mass of dexamethasone loaded low and high molecular weight PLGA nanoparticles

Initial Mass of Dexamethasone, mg/ml	1		2		3		4	
Ratio of Drug/ PLGA, % w/w	1/27		2/27		3/27		4/27	
Molecular Weight of PLGA	Low	High	Low	High	Low	High	Low	High
Actual Loading, mg/ml	0.22	0.31	0.28	0.40	0.31	0.42	0.39	0.59
Encapsulation efficiency, %	22.49	31.23	15.70	20.02	9.49	14.05	9.82	12.83

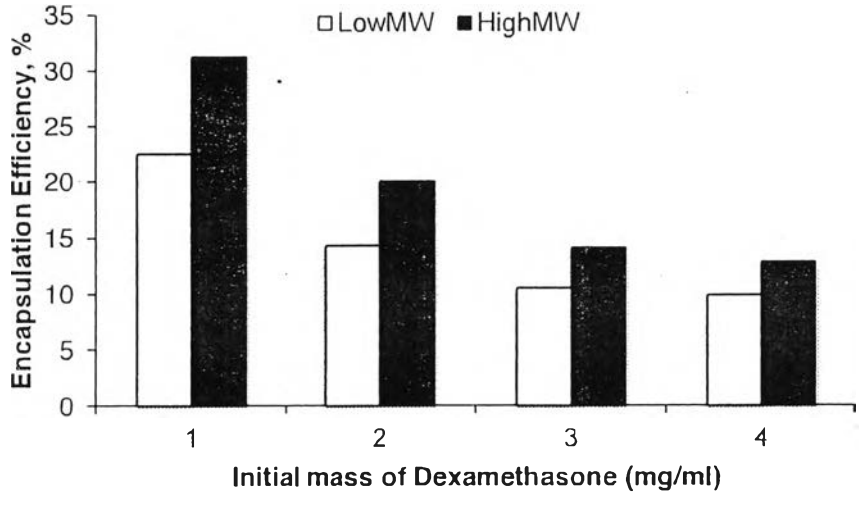

Figure. 4.5 The percentages of encapsulation efficiency on low and high molecular weight PLGA with varying the initial mass of dexamethasone.

4.2 Encapsulation Efficiency

The initial masses of Dexamethasone were varied from 1 to 4 mg. From table 1, the percentages of encapsulation efficiency of DEX loaded low molecular weight PLGA nanoparticles were approximately 22.49, 14.24, 10.47 and 9.82 %. Whereas, the percentages of encapsulation efficiency of DEX loaded high molecular weight of PLGA nanoparticles were roughly 31.23, 20.02, 14.05 and 12.83 % for the initial loading amount 1, 2, 3 and 4 mg of DEX, respectively. From the observation, it was able to conclude that high molecular weight of PLGA nanoparticles had an ability to encapsulate dexamethasone much more than low molecular weight of PLGA nanoparticles. Since high molecular weight of PLGA nanoparticles brought the organic phase much more viscous than low molecular weight of PLGA nanoparticles, the drug was harder to leak from the inner aqueous phase into the outer aqueous phase (Zhu *et al*, 2001). Moreover, all of concentrations on low and high molecular weight of PLGA nanoparticles were lower than a half of the initial mass of Dexamethasone resulting from using a hydrophilic drug. Therefore, dexamethasone on the surface diffused into the external aqueous phase causing the decrease of the percentages of encapsulation efficiency during the second emulsification (Zhu *et al*, 2001), (Lin *et al*, 2000), (Wang *et al*, 2011).

The important point needed to be extended. An increase of the initial mass of dexamethasone minimized the encapsulation efficiency of drug. Similarly, Yi-Yan Yang and her co-worker in 2001, the increase in the concentration gradient of BSA between an internal and external aqueous phase brought about the amount of BSA diffusing into an external aqueous phase. The percentages of encapsulation efficiency were 79.1, 59.8 and 55.0 % within 0.57, 1.10 and 4.80 of theoretical loading of BSA respectively (Yang et al, 2001). This phenomenon could be interpreted by an increase in concentration gradient of dexamethasone between inner and outer aqueous phase, that is, the concentration gradient of Dexamethasone was the driving force causing the diffusion of drug into external aqueous phase. The higher amount of dexamethasone was in internal aqueous phase, the more drug diffused to external aqueous phase.

4.3 In Vitro Drug Release Studies

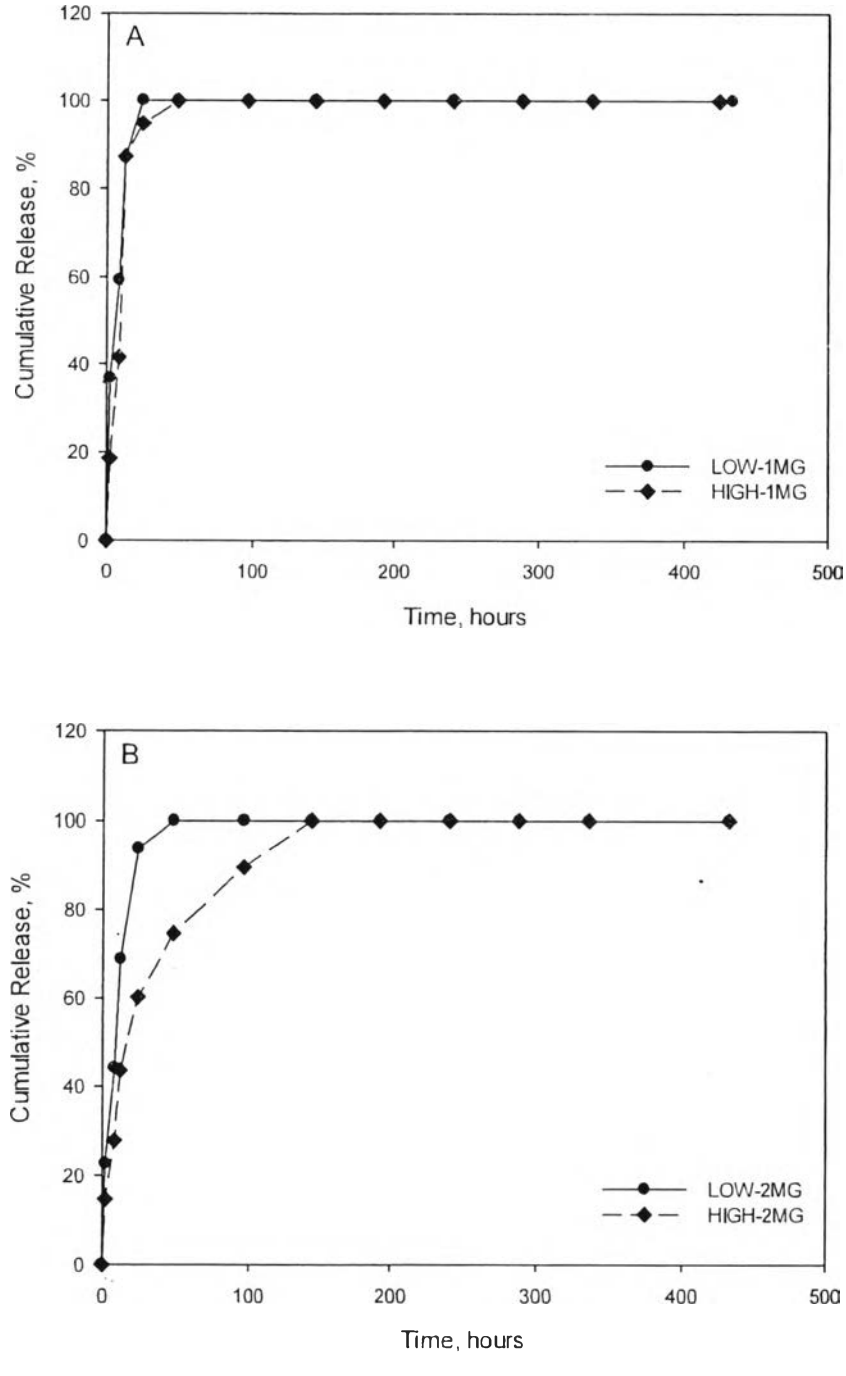

Figure. 4.6 In vitro release profiles of dexamethasone loaded low and high molecular weight of PLGA sub-micro particles; (A) 1 mg of an initial mass, (B) 2 mg of an initial mass.

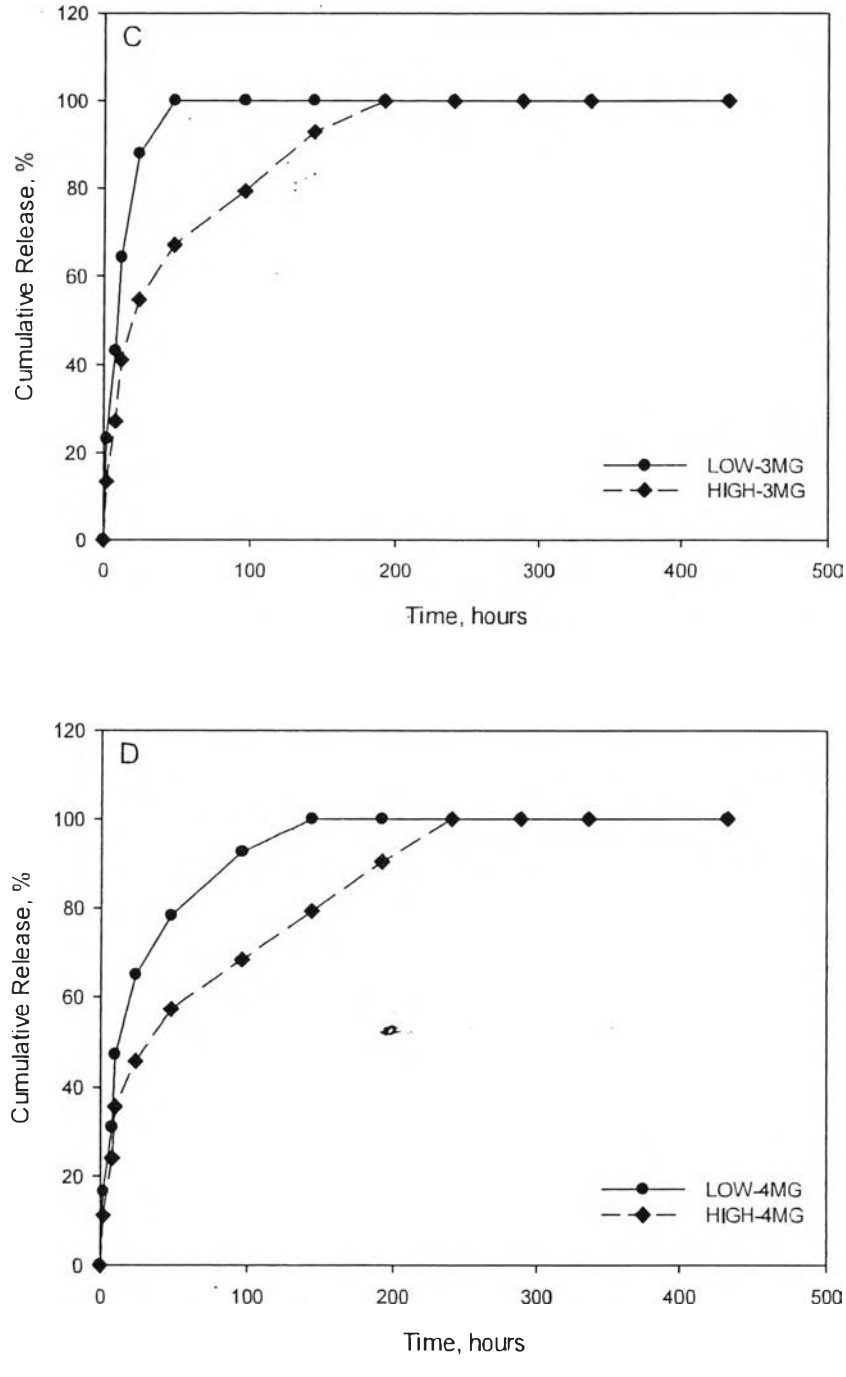

Figure. 4.7 In vitro release profiles of dexamethasone loaded low and high molecular weight of PLGA sub-micro particles; (C) 3 mg of an initial mass, and (D) 4 mg of an initial mass.

4.3.1 Effect of Molecular Weight of PLGA on Drug Release Profile

Polymer molecular weight played a critical role in the rate of polymer degradation and release kinetics of drug. The molecular weight was referred to the hydrophilicity/lipophilicity of each polymer; in other words, when the molecular weight increased, the lipophilicity of polymer increased alike. Furthermore, the molecular weight was directly related to hydrolysis reaction (Bae, Son et al. 2009). The hydrolysis reaction tended to affect water absorption and erosion, which created drug diffusion through polymer. The more hydrolysis reaction occurred, the faster rate of drug diffusion was. Besides, the high molecular weight having dense structure took longer time than the low molecular weight having porous structure in terms of the drug diffusion (Berkland *et al*, 2002). Moreover, polymer molecular weight was concerned with the rate of polymer degradation. Thus, the drug diffusion and the rate of polymer degradation could be controlled by varying the molecular weight of polymer.

The data from our experiment as shown in Fig 5, the rate of drug release significantly reduced when the molecular weight of PLGA nanoparticles increased. The burst release was analyzed by monitoring the initial drug release within 24 hours. Low molecular weight of PLGA nanoparticles had higher the percentage of drug accumulative release at initial burst release comparing to high molecular weight of PLGA nanoparticles in each concentration. The percentages of drug accumulative release at initial burst of low molecular weight were 100.00, 93.77, 87.86 and 64.99 %. As for high molecular weight, the percentages of drug accumulative release were 94.81, 60.17, 54.63 and 45.80 % in 1, 2, 3 and 4 mg of drug, respectively.

In case of the release profile during 18 days, high molecular weight of PLGA nanoparticles were much more sustained release as compared to low molecular weight of PLGA nanoparticles in each concentration. High molecular weight extended the sustained drug releases until 2, 6, 8, and 10 days. As for low molecular weight, the sustained drug releases were 1, 2, 2 and 6 days in 1, 2, 3 and 4 mg of initial drug, respectively. The interesting difference of 1 and 2 mg of Dexamethasone encapsulated in between high and low molecular weight of PLGA nanoparticles was worth discussing. The release profiles of 1 and 2 mg of

Dexamethasone encapsulated in low molecular weight of PLGA nanoparticles were controlled by a considerable burst release, which was the rapid diffusion of drug accumulated on the surface of PLGA, and occurred within 24 hours (Maulding *et al*, 1987) and (Berkland *et al*, 2002). On the other hand, the release profile of 2 mg of Dexamethasone encapsulated in high molecular weight PLGA nanoparticles were dominated by both a burst release and diffusion through water-filled pores, which were an erosion controlled mechanism within 6 days. The result could be explained by low molecular weight occurred hydrolytic degradation of ester bonds much easier than high molecular weight. Thus, the sustained drug release of high molecular weight was longer than low molecular weight in each concentration.

4.3.2 The Effect of the Mass of Dexamethasone on Drug Release Profile

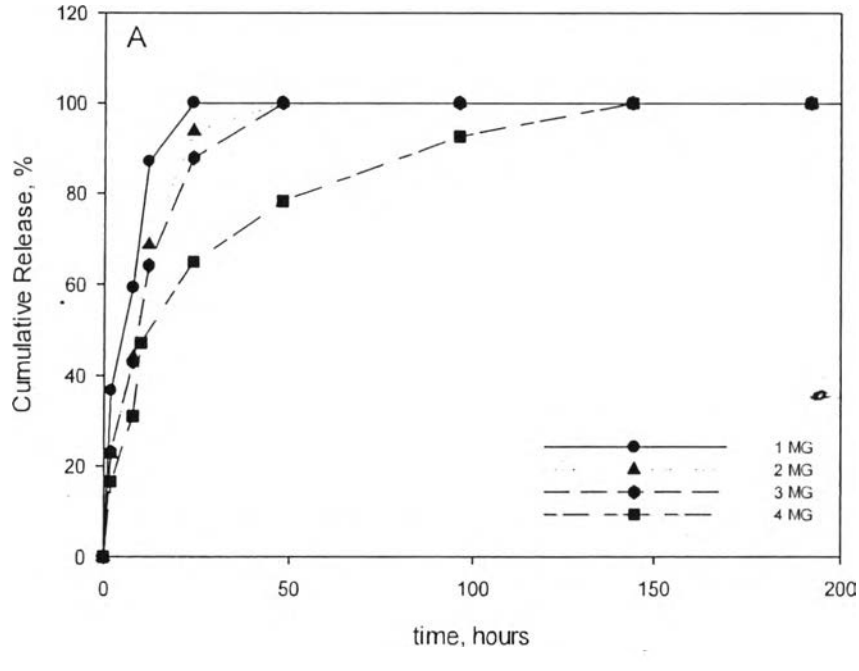

Figure. 4.8 In vitro release profiles of 1, 2, 3 and 4 mg of dexamethasone loaded PLGA nanoparticles; (A) loaded low molecular weight, (B) loaded high molecular weight.

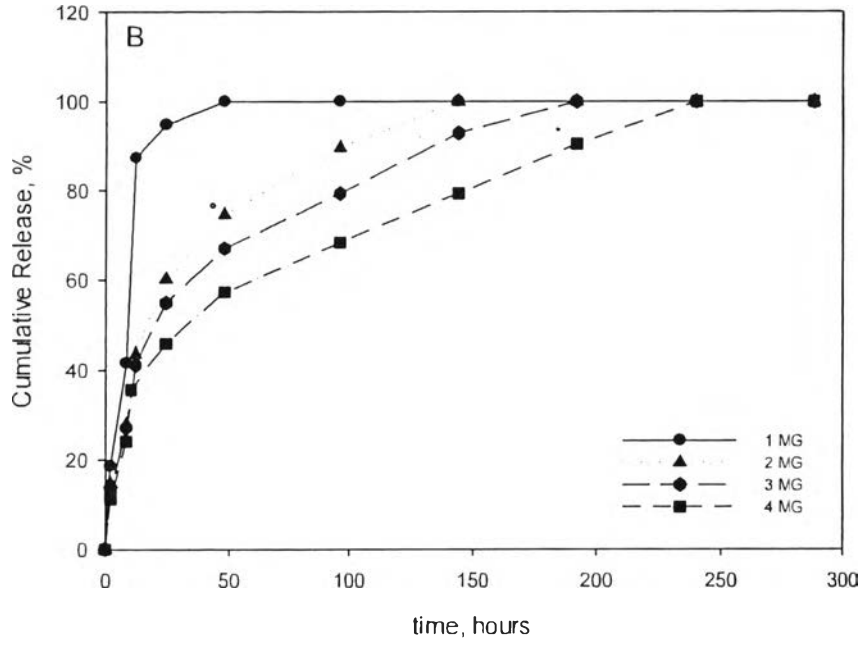

Figure. 4.8 In vitro release profiles of 1, 2, 3 and 4 mg of dexamethasone loaded PLGA nanoparticles; (A) loaded low molecular weight, (B) loaded high molecular weight. (count)

The initial mass of Dexamethasone is one of the important factors controlling the sustained release of drug in PLGA nanoparticles. The sustained releases of both high and low molecular weight of PLGA nanoparticles took longer time with increasing the initial mass of Dexamethasone (see Fig. 6.) This phenomenon due to the increase in concentration of drug resulting in improving drug encapsulated inside water-filled pores of PLGA sub-micro particles (Bae *et al*. 2009).

In this research as shown in Fig 6, the drug accumulative releases of high molecular weight of PLGA nanoparticles extended the maximum percent of total entrapped dexamethasone within 2, 6, 8 and 10 days. In comparing with low molecular weight of PLGA nanoparticles, the drug accumulative releases reached the maximum percent of total entrapped dexamethasone within 1, 2, 2 and 6 days at 1, 2, 3 and 4 mg of drug, respectively. This phenomenon could be identified. When adding much more a quantity of initial mass of drug, the polymer was better to encapsulate Dexamethasone, resulting in acquiring the longer time of drug release.

The drug loading increased as the initial mass of drug increased, until the matrix was saturated with the drug (Gómez-Gaete *et al*, 2007). For this reason, the more initial mass of Dexamethasone increased, the longer time of drug release took (Ali *et al*, 2013).