CHAPTER IV
RESULTS AND DISCUSSION

4.1 Parameter Controlling the Loading into PEM

In order to load the hydrophobic molecules such as curcumin as a therapeutic compound directly on the surface of materials, PEM are used here as a primer for loading. Because of their hydrophilic property, the polar group of hydrophobic molecules can interact with PEM and help these molecules penetrate in between the PEM layers. The driving forces behind PEM formation can study by various the number of PEM film layers, polymer type that used to fabricate the PEM thin film, and solvent type that used in loading mediated.

4.1.1 Effect of the Number of Layers on the Thickness of PEM

The PEM fabricated by increasing the layers of 10mM PDADMAC/PSS containing 1M NaCl on quartz slide. The deposition time of each layer is 1 min and then, rinses the PEM in water 1 min for 1 time. The growth of PEM thin films were characterized by UV-Vis spectrophotometer to confirm that the film growth with increasing the number of PEM layers. The absorbance at 230 nm under the ultraviolet range shown in Figure 4.1.

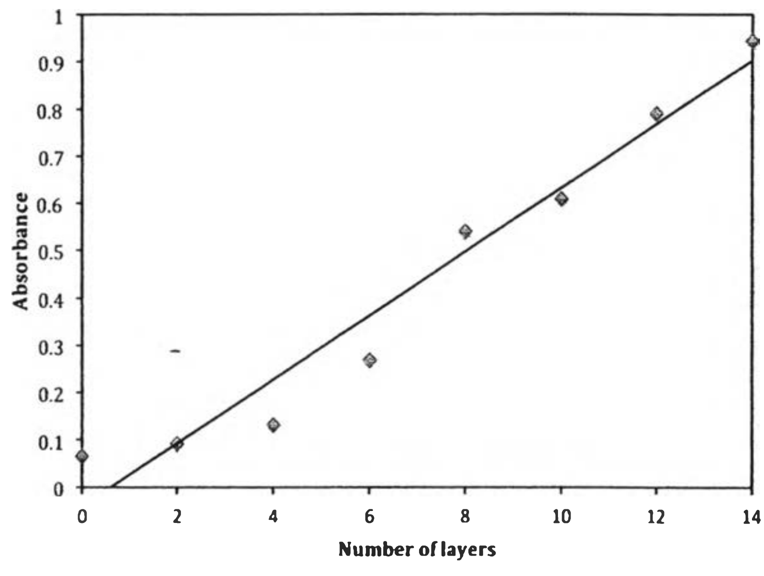


Figure 4.1 The absorbance at 230 nm of PDADMAC/PSS film on quartz slide with increasing the number of layers.

The absorbance increase with increasing the number of PEM layers that are the PSS on the top of layer. There were found the increase of sulfonate ($-SO_4^{2-}$) group peak in the absorbance graph. It can be state that increase PEM layers lead to increase the stickness of PEM films.

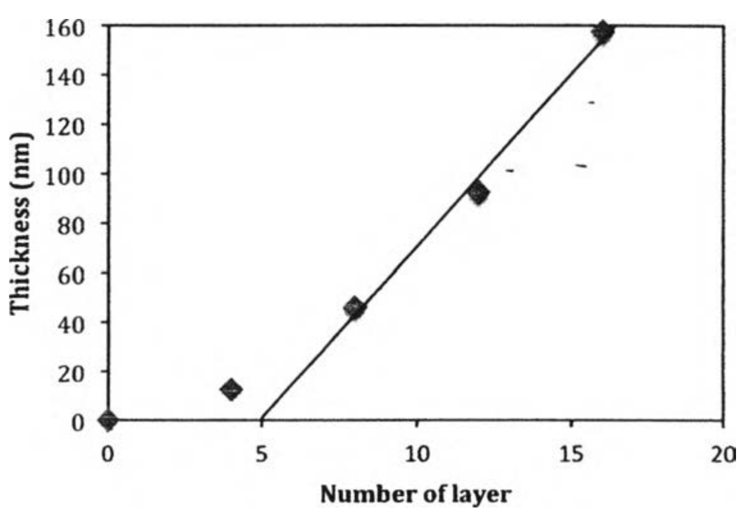


Figure 4.2 The thickness of 10mM PDADMAC/PSS film on silicon wafer with increasing the number of layers measured by ellipsometry.

The thickness of PEM film fabricated by 10mM PDADMAC/PSS contain 1M NaCl deposite by dip-coating on silicon wafer after pre-treatment by piranha and hot ammonia solution was measured by ellipsometry at angle of incidence 60 degrees (Delta: 174-178 and Psi: ~23 for bare silicon wafer) and air-dried every 4 layers. Multilayer thickness as a function of the number of layers show in Figure 4.2, the thickness increase with incrasing the number of layer in a linear relationship after 8 layers ofPEM as a equation, that

$$y = 13.972x - 68.95 \tag{1}$$

The result was superimposed to the thickness of the number of layers for a 1mM PSS/PDADMAC multilayer with 1.0M NaCl measure by ellipsometer by Dubas *et al.*, 1999. From (1) equation and calculated the thickness of 13-layer PEM equal to 111.146 nm which can state that the coating of PEM on surface is in nanoscale.

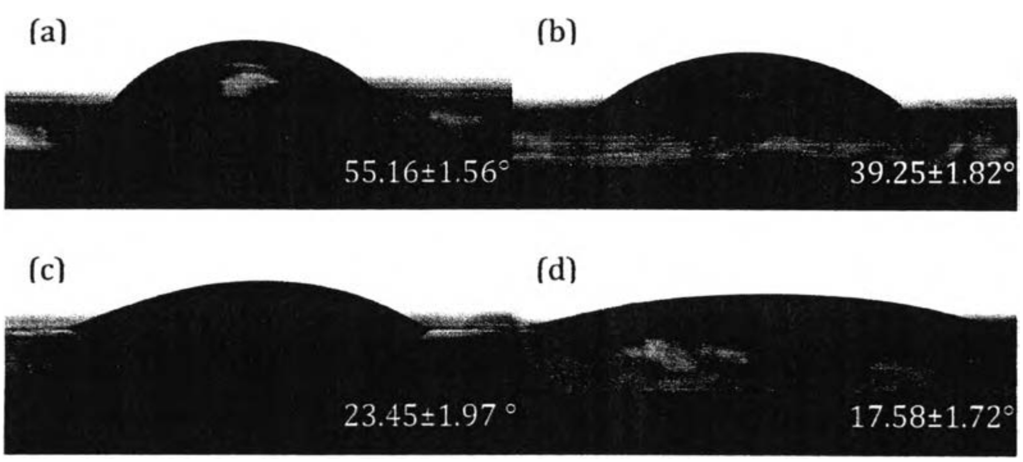


Figure 4.3 Contact angle of 10mM PDADMAC/PSS with 1M NaCl on glass slide with increasing the number of layers; (a) 10-layer (b) 20-layer (c) 30-layer (d) 40-layer.

Figure 4.3 show the contact angle measurement of PEM thin film when increase the number of layers. The decreasing of contact angle with more deposited layer of PEM can state to the increasing of hydrophilicity of films surface as show in figure 4.4. The PEM can provide more hydrophilic on glass slide which can used as a matrix for incoparated with water-soluble molecules directly on the surface of substrate.

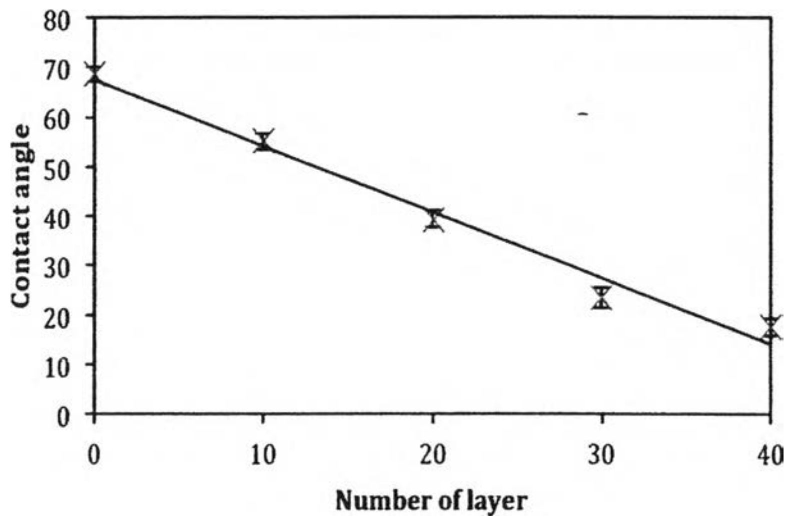


Figure 4.4 The decreasing of contact angle when increase the number of PEM layer.

4.1.2 <u>Effect of Polymer Type on the Final Dipped on PEM</u>

In this study, two types of PEM thin film, PDADMAC/PSS and chitosan/alginate, were used to compare the ability of dipped molecule. The selected of these two types of PEM was focused on that the PDADMAC/PSS pair is a synthetic polymer commonly used in PEM studies because of their pH-independent property while the chitosan/alginate pair is natural polymer use in a pH value 5.5 which is condition that pH near their pKa and both polyelectrolytes are well ionized.

The synthetic PEM fabricated from 5mM PDADMAC/PSS containing 1M NaCl and the natural PEM by 5mM chitosan/alginate with 1M NaCl in acetic-acetate buffer pH 5.5 were deposited on glass slide with deposition time of each layer 1 min and rinse with the solvent of each PEM 1 min for 1 time by increased the number of layers to 4 and 8 layers. Then, dipped the PEM in 2% w/v chitosan (low molecular weight) with purple dye (Lannaset Bordean B.) 5 mins to found the ability of coating chitosan on PEM. The PEM after coated with 2% chitosan shown in Figure 4.5.

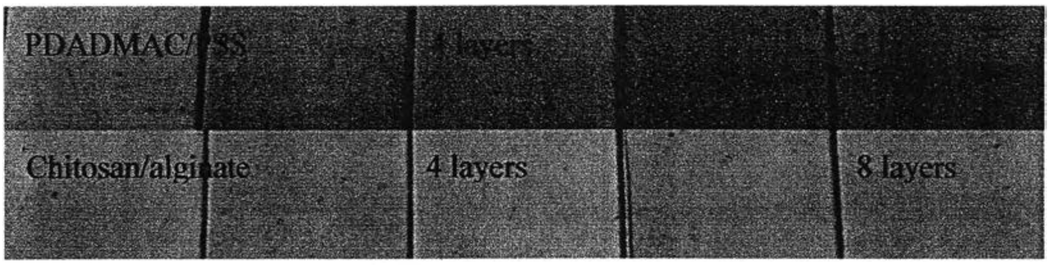


Figure 4.5 The 5mM PEM containing 1M NaCl on glass slide dipped with 2% w/v chitosan contain purple dye.

The coated chitosan on PEM were dry in air and measure the weight of chitosan in gram unit that show in Table 4.1.

Table 4.1 Weight (g) of 2% w/v chitosan dipped on PEM

Number of layers	Average weight (g) of 2% chitosan	
	PDADMAC/PSS	Chitosan/alginate
0	0.0366	0.0366
4	0.0480	0.0702
8	0.0677	0.0854

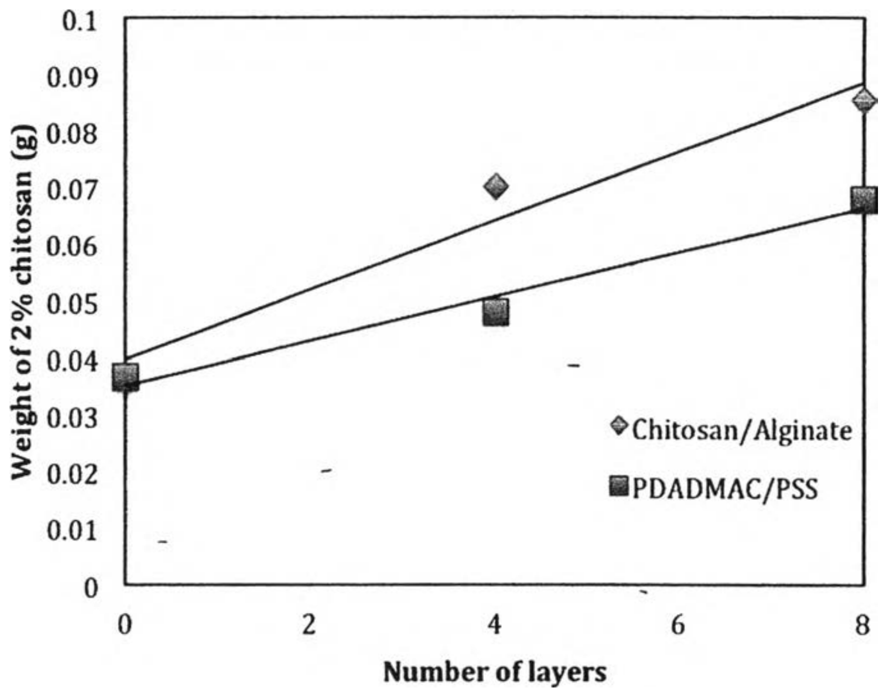


Figure 4.6 The weigth of 2% w/v chitosan (g) dipped on PEM in a function of number of layers compare between PDADMAC/PSS and chitosan/alginate.

The weight of 2% chitosan on both of PEM increase with increasing the number of layers by linear relationship that shown in Figure 4.6. The coated chitosan on chitosan/alginate PEM was higher than PDADMAC/PSS PEM because natural PEM have a large amount of hydroxyl groups along the polymer repeat units which provide very stronger interaction with dipped chitosan so, it can penetrate in between the layer of each chitosan/alginate layer. Deep purple color in PDADMAC/PSS state that the purple dyes (Lannaset Bordean B.), which have sulfate ($-SO_4^{2-}$) group same as the functional group along PSS polyelectrolyte chain, prefer to diffuse in synthetic PDADMAC/PSS than natural chitosan/alginate (Figure 4.5). Moreover, more purple when increase the number of layers confirm the increase of PEM thickness.

The hydrophilic character of PDADMAC/PSS and chitosan/alginate thin film can caused a increasing of 2% w/v chitosan and purple dye penetrate into the film which increase the purple color of film.

4.1.3 Effect of Solvent Type on the Loaded of Curcumin into PEM

The 13-layer of 10mM PDADMAC/PSS containing 1M NaCl on glass slide loaded with 0.01% w/v curcumin in difference solvent composition 80:15, 90:10 and 95:5 water:solvent which using EtOH, MeOH and acetone as solvent with loading time 3 hours. The final amount of curcumin in PEM was characterized by UV-Vis spectrophotometer. The high loaded lead to the dark yellow color in PEM thin films and final loaded shown in Figure 4.7.

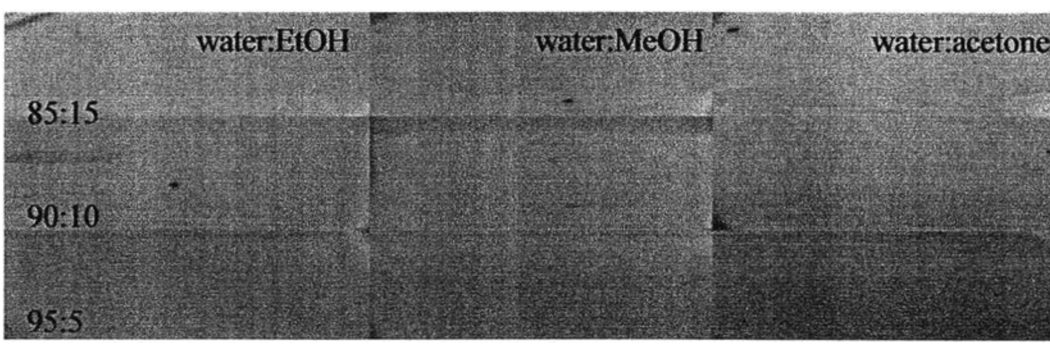


Figure 4.7 The final curcumin loaded into 13-layer of 10mM PDADMAC/PSS containing 1M NaCl by various the composition of solvent and solvent type.

The absorbance of final loaded curcumin in PEM has λ_{max} at 435 nm states to the amount of curcumin in Table 4.2 and then, plot graph the relation between the absorbance when increasing % water in three types of solvent shown in Figure 4.8.

Table 4.2 Absorbance at λ_{max} (435 nm) of the final curcumin loaded into 13-layer of PDADMAC/PSS containing 1M NaCl

Solvent composition	%Water content		
	85	90	95
Water/EtOH	0.0938	0.1275	0.1625
Water/MeOH	0.1350	0.1688	0.1600
Water/Acetone	0.0450	0.0850	0.1562

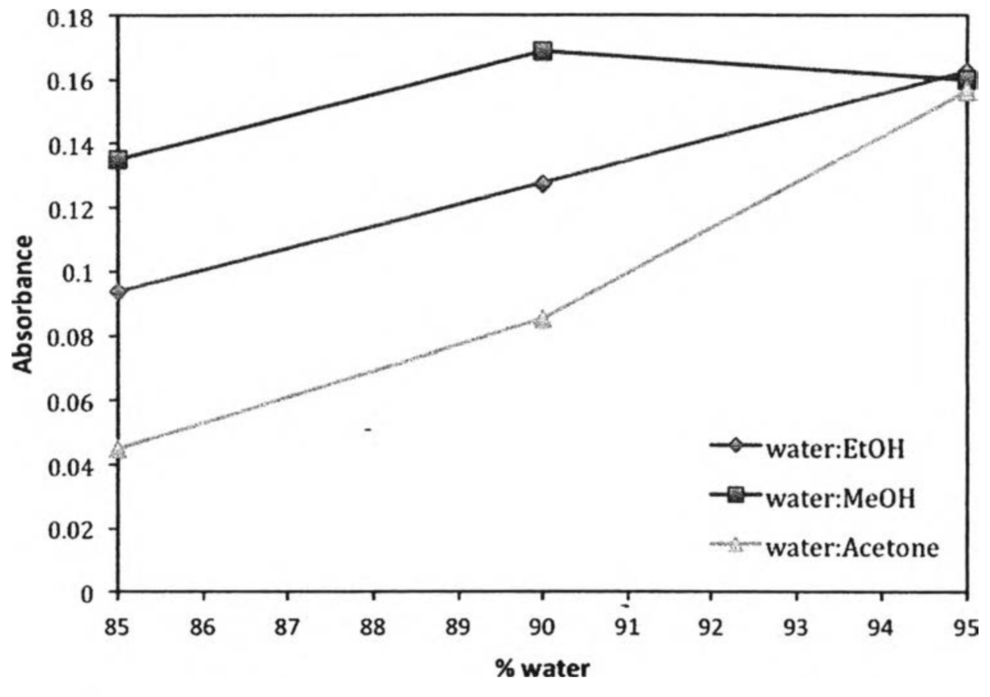


Figure 4.8 The absorbance of the final curcumin loaded into 13-layer of PDADMAC/PSS contain 1M NaCl by various %water in solvent and solvent type.

The absorbance at λ_{max} 435nm relate to the final amount of curcumin loaded into PEM, high absorbance mean high amount of curcumin. After loading curcumin for 3 hours, the water:EtOH and water/acetone solvent show increase of curcumin in PEM with increasing %water in solvent. The polarity of solvents are increase from acetone < EtOH < MeOH, MeOH is the highest polar solvent in three of these solvent, so, the hydrophobic CUR molecule hardly to dissolve in MeOH because of their hydrophobic property and CUR prefer diffuse into PEM which show higher hydrophobicity than MeOH solvent results to increase the amount of CUR in the PEM. Althrough, the water:MeOH solvent show the highest amount of curcumin in all of solvent but MeOH has a high toxicity in humans. So, we prefer use water:EtOH as a loading solvent to reach the high final loading curcumin into PEM.

4.2 Temperature Mediated Loading of Curcumin in PEM Thin Films

The PEM were fabricated by 13-layer of 10mM PDADMAC/PSS containing 1M NaCl on glass slide with deposition time 1 min and rinse in water 1 min. To study the loading of 0.01% curcumin in water:EtOH solvent by various the solvent composition to 50:50, 75:25, 80:20, 85:15, 90:10 and 95:5. The PEM films were dipped in each solution at various temperature 4, 15, 28 and 50°C. Finally, measure the absorbance at 440 nm at various loading time begin with 5, 30 mins, 1, 2 and 3 hrs. The final loaded curcumin in PEM shown the yellowness in Figure 4.9.

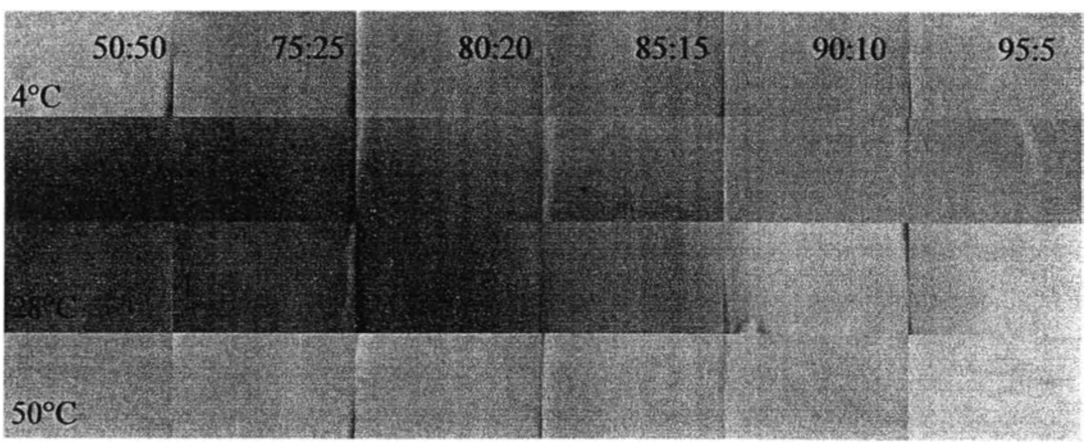


Figure 4.9 The final loaded curcumin in PEM at loading time 3 hours by various water:EtOH composition and temperature.

4.2.1 <u>Effect of Water:ethanol Solvent Composition at Increasing Temperature</u>

The absorbance at 440 nm of the water:EtOH solvent by increase %water content as 50:50, 75:25, 80:20, 85:15, 90:10 and 95:5 at temperature mediated 4, 15, 28 and 50°C are shown in Figure 4.10.

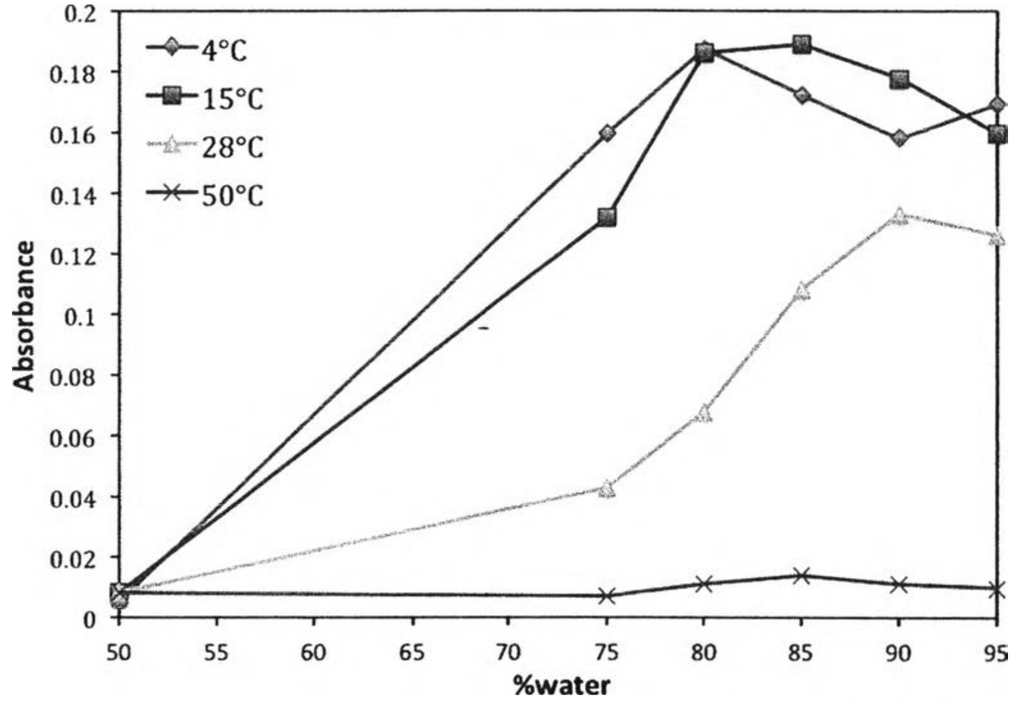


Figure 4.10 Absorbance at 440 nm of a PEM at increasing temperature for various solvent composition.

The results show that the absorbance at 440 nm is proportional to the amount of curcumin in the PEM thin films. In Figure 4.10, the loading of curcumin into PEM can be control by solvent composition force by solid-liquid partitioning mechanism. When PEM was dipped into curcumin solution, PEM show more hydrophobic than water:EtOH solvent then curcumin prefer diffused into PEM. Increasing hydrophilic property of solvent by increase %water lead to high loading of curcumin into PEM films. In addition, the loading decrease with increasing temperature driven by the solubility of curcumin in each temperature mediated. At low temperature, curcumin have less solubility in solvent, so, it prefer diffused into PEM lead to high absorbance.

Curcumin is dispersed in water at high temperature because of the breakage of intramolecular H-bonding and exposes the polar hydroxyl (-OH) and keto (>C=O) group that leads to enhance the availability of the CUR molecules dissolve in solution (Jagannathan *et al.*, 2012). The absorbance show rising trend when increase volume of water in solvent because of the partitioning of CUR, PEM show more hydrophobic than solvent so CUR prefer to diffuse into PEM (Kittitheeranun *et al.*, 2007).

4.2.2 Effect of Loading Time at Increasing Temperature

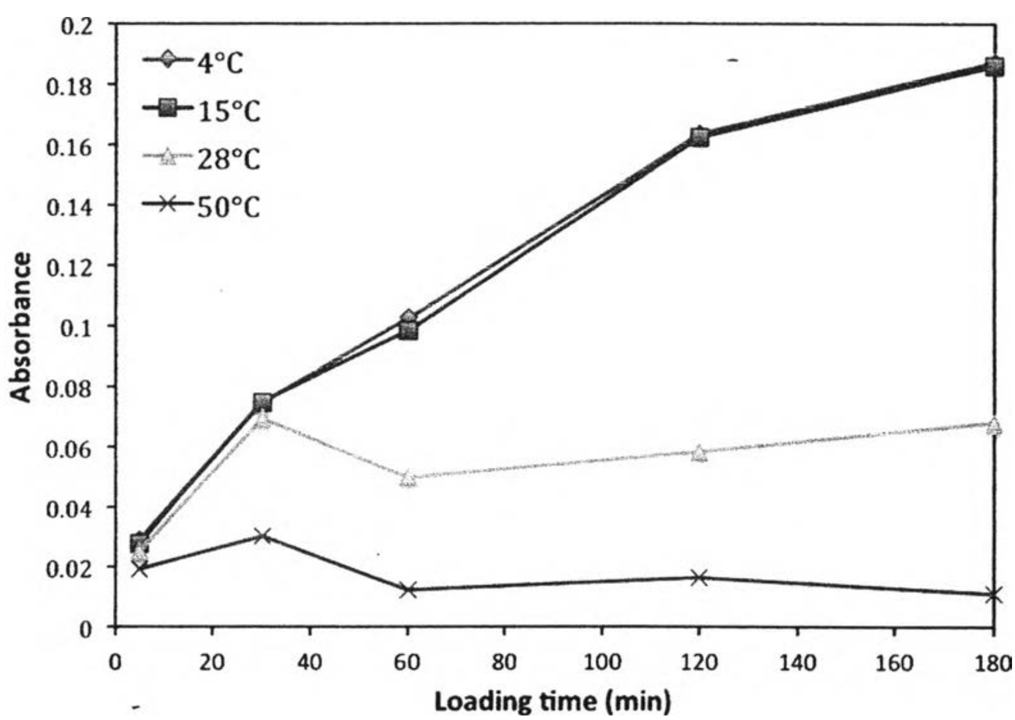


Figure 4.11 Kinetic property of loaded curcumin in PEM at 80:20 water:EtOH.

The 80% water solvent at low temperature (4 and 15°C) have the highest absorbance as show in Figure 4.11. The loading of curcumin into PEM was found to saturated in each condition after 3 hours. The kinetic of loading shown a sharp increase at 4 and 15°C, and more stable at 28°C after 60 minutes to maintain the amount of curcumin in PEM at room temperature.

These results can conclude that PEM fabricated form synthetic PDADMAC/PSS or natural chitosan/alginate can be used as a coating for loading EOs or hydrophobic molecules such as curcumin directly on a surface of material.

4.3 Surfactant Modification of PEM

The PEM fabricated by 10mM PDADMAC/PSS containing 1M NaCl on glass slide. Then, dipped the PEM in surfactant solution for 1 hour and loaded 0.01% w/v curcumin in water:EtOH (90:10) for 3 hours. The loading of curcumin into PEM was characterized by UV-Vis spectrophotometer.

4.3.1 Sodium Dodecyl Sulfate (SDS)

The 9-layer PEM were dipped into SDS surfactants in water at concentration of 0.725, 1.5, 3, 6, 10, 14, 18 and 22mM for 1 hour. Then, loading with 0.01% w/v curcumin at 90:10 water:EtOH solvent for 3 hours. The final loading of curcumin in PEM with SDS show in Figure 4.12.

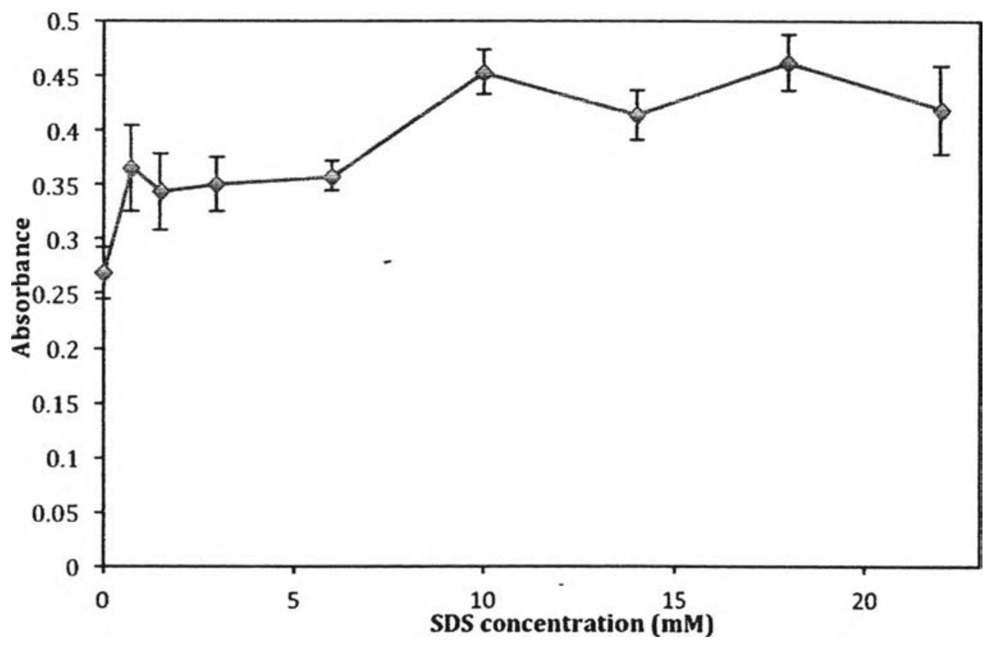


Figure 4.12 The absorbance of final loaded curcumin in PEM by increasing SDS concentration.

The absorbance at λ_{max} was increased with increasing the SDS concentration and it was found to rapidly increased when reach 6mM, which is the critical micelle concentration (CMC) of SDS at 8.0 – 9.4mM, and SDS molecules orient themselves in monolayer formed later the absorb at the surface of PEM (Khan *et at.*, 2008). When immersed the PEM in SDS solution, the hydrophillic head of SDS with sulfate ($-SO_4^-$) group create a relative wesk attractive interaction with cationic PDADMAC on PEM surface after loading with curcumin, the hydrophobic tail of SDS attract curcumin into PEM lead to increasing amount of curcumin in the films thus increasing surfactant concentration leads to strong increase amount of surfactant adsorbed on hydrophillic surface of PEM (Wijmans *et al.,* 1996). There are SDS surfactant can form different aggregates above CMC, in 2007, Dominquez and coworkers studied the self-aggregated of SDS of solid-liquid interface and was found that at high concentration of SDS, they can form half-cylinder micelle, that show hydrophobicity out of PEM surface. It can state that the final amount of curcumin in PEM can be increased by modified the PEM surface with high concentration of SDS surfactants as shown in Figure 4.13.

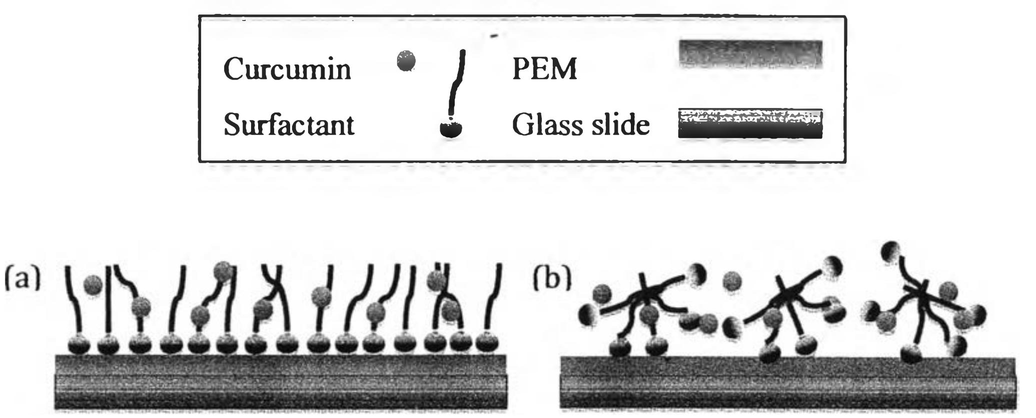


Figure 4.13 The loading of curcumin into PEM modified by SDS surfactant; (a) SDS monolayer form at below CMC (b) SDS half-cylinder form at above CMC.

4.3.2 Hexadecyltrimethylammonium Bromide (CTAB)

The 8-layer PEM were dipped into CTAB surfactants in water at concentration of 0.125, 0.25, 0.5, 1, 2 and 4mM for 1 hour. Then, loading with 0.01% w/v curcumin at 90:10 water:EtOH solvent for 3 hours. The final loading of curcumin in PEM with CTAB show in Figure 4.14.

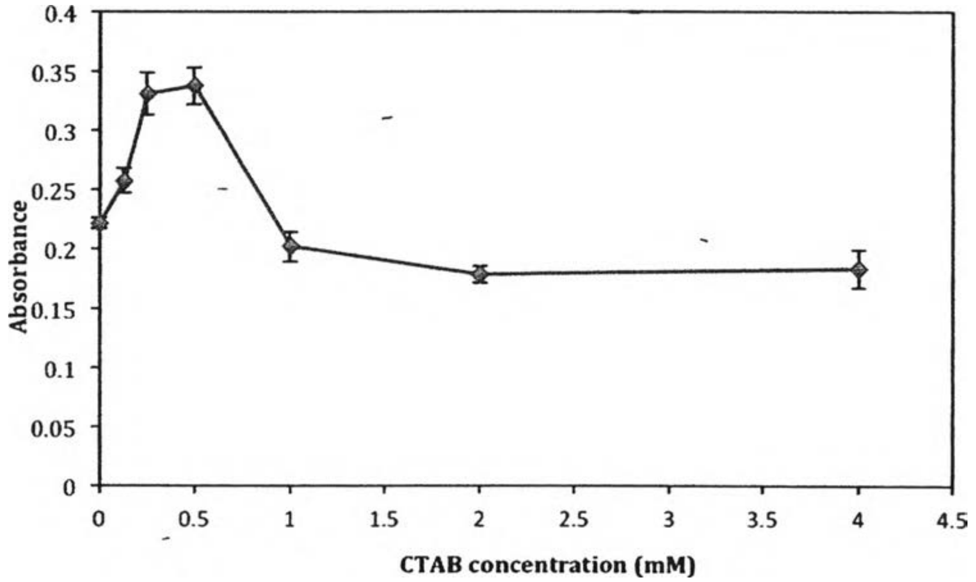


Figure 4.14 The absorbance of final loaded curcumin in PEM by increasing CTAB concentration.

At CTAB concentration below CMC (about 1mM), the absorbance at λ_{max} shows that the final amount of curcumin in PEM has increased from the PEM with no surfactant. In the other hand, the absorbance decreased with increasing CTAB concentration and has found to constant at concentration above CMC. Further, when dipped the PEM in CTAB solution above CMC (about 1mM), the CTAB in form of micelle stick on the anionic PSS surfacce on PEM films and after that when dipped in curcumin solution, the curcumin can not diffuse into PEM because of the hydrophilicity of CTAB micelle lead to decreasing amount of curcumin in films. It can state that the final amount of curcumin in PEM can be increased by modified the PEM surface with CTAB surfactants which have concentration below CMC as shown in Figure 4.15.

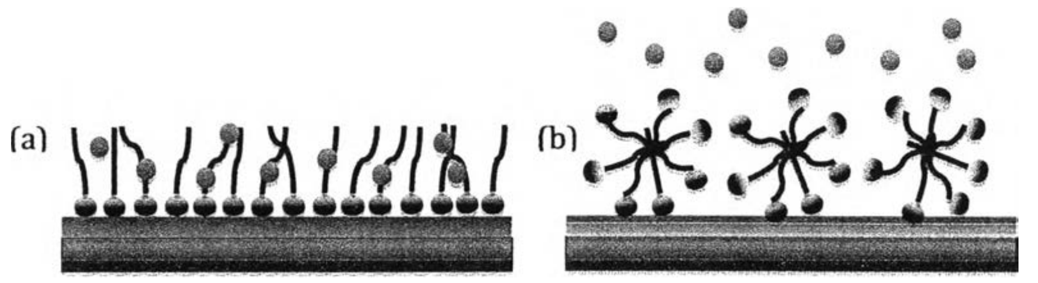


Figure 4.15 The loading of curcumin into PEM modified by CTAB surfactant; (a) CTAB monolayer form at below CMC (b) CTAB micelle form at above CMC.

4.3.3 Characterization of SDS Modification PEM Thin Film

The final loading of curcumin in PEM with 10mM SDS (Figure 4.12) showed the highest amount of curcumin (absorbance 0.45) with minimum SDS concentration. The 13-layer PEM fabricated by 10mM PDADMAC/PSS contain 1M NaCl on glass slide were dipped into 10mM SDS surfactants in water for 1 hour. Then, loading with 0.01% w/v curcumin at 90:10 water:EtOH solvent for 3 hours.

The PEM films change from yellow to green under UV light (Figure 4.16) show strong absorbance in the visible range at 440 nm (Kittitheeranun *et al.*, 2010) which can state that the amount of curcumin in the films.

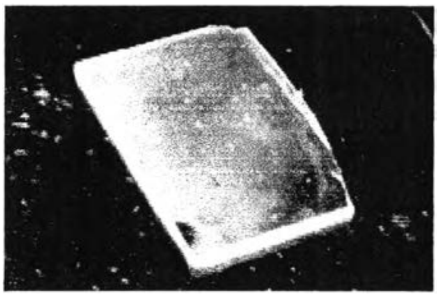


Figure 4.16 The 13-layer PEM film loaded with 0.01%w/v curcumin under UV light.

Moreover, curcumin itself is a fluorescent compound, curcumin films have emission fluorescence spectrum at 540 nm. The fluorescence intensity of 13-layer PEM loaded with curcumin showed in figure 4.17.

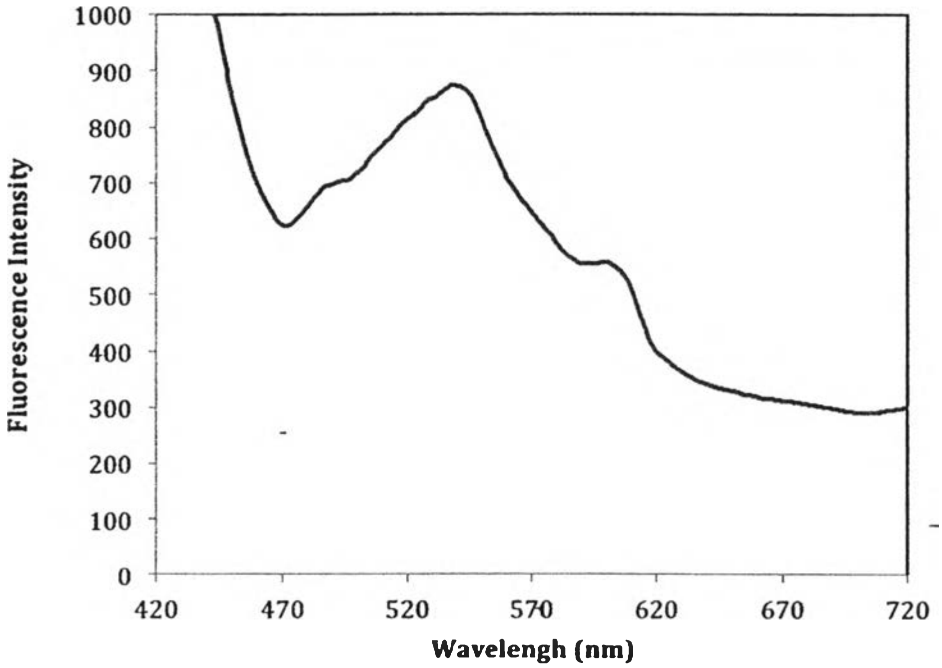


Figure 4.17 The fluorescence intensity spectra of 13-layer PEM film loaded with 0.01%w/v curcumin.

The hydrophobicity of PEM surface before and after modified with 10mM SDS surfactant was investigated by contact angle measurement. The optical images of water droplet on the PEM surface showed in Figure 4.18. The contact angle of PEM increase from 63.16±1.43° to 75.98±0.78° after loaded with curcumin because curcumin diffuse into the film, the strong hydrophobicity of the conjugated alkene chain of curcumin lead to more hydrophobic of surface. After surfactant treatment, the hydrophilic head of SDS create monolayer forming by the electrostatic interaction with PEM surface and the other side that are hydrophobic tail increase the contact angle of water droplet to 90.41±0.31°. When loaded curcumin into surfactant modified surface PEM, the contact angle was slightly decreased to 86.69±0.58° because of the polar group of curcumin in the films.

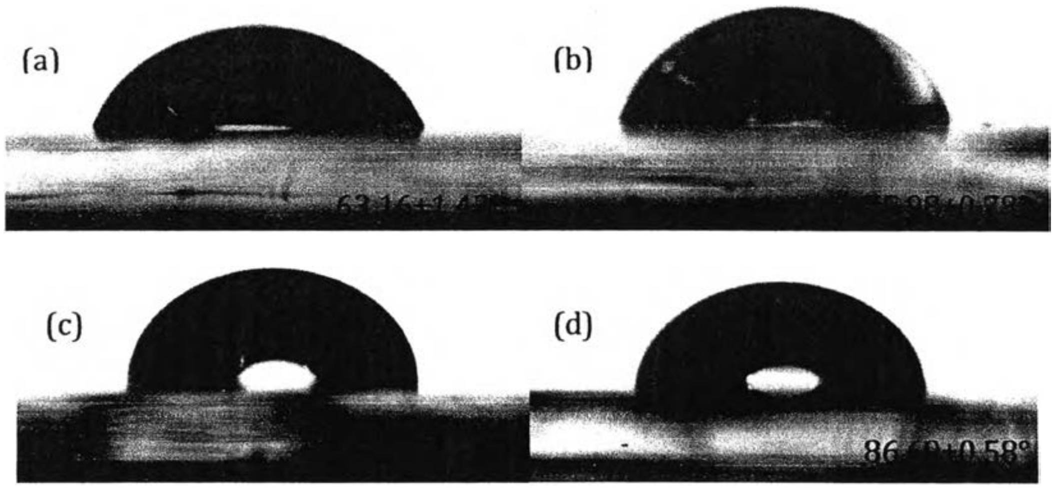
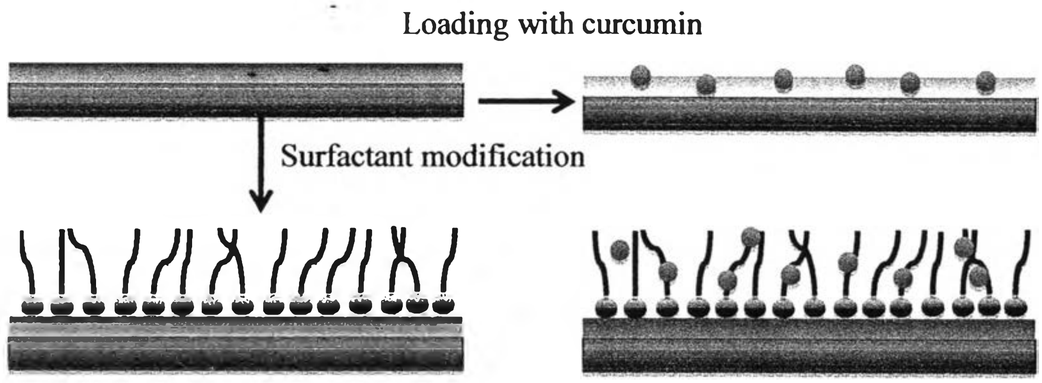


Figure 4.18 Contact angle of 13-layer 10mM PDADMAC/PSS contain 1M NaCl; (a) PEM (b) PEM after loading with 0.01%w/v curcumin for 3 hours (c) PEM immersed in 10mM SDS for 1 hour (d) PEM immersed in SDS and loading with curcumin.

The strong hydrophobicity of surfactant monolayer on the PEM surface improved the loading of curcumin which is hydrophobic molecule by increase the diffusion of curcumin particles into the films more than two times from absorbance 0.18 to 0.45 before and after treatment, respectively. The behavior of the increasing of curcumin in PEM with surfactant modification as showed in Scheme 4.1.



Scheme 4.1 Increasing the amount of curcumin in PEM with surfactant modification.

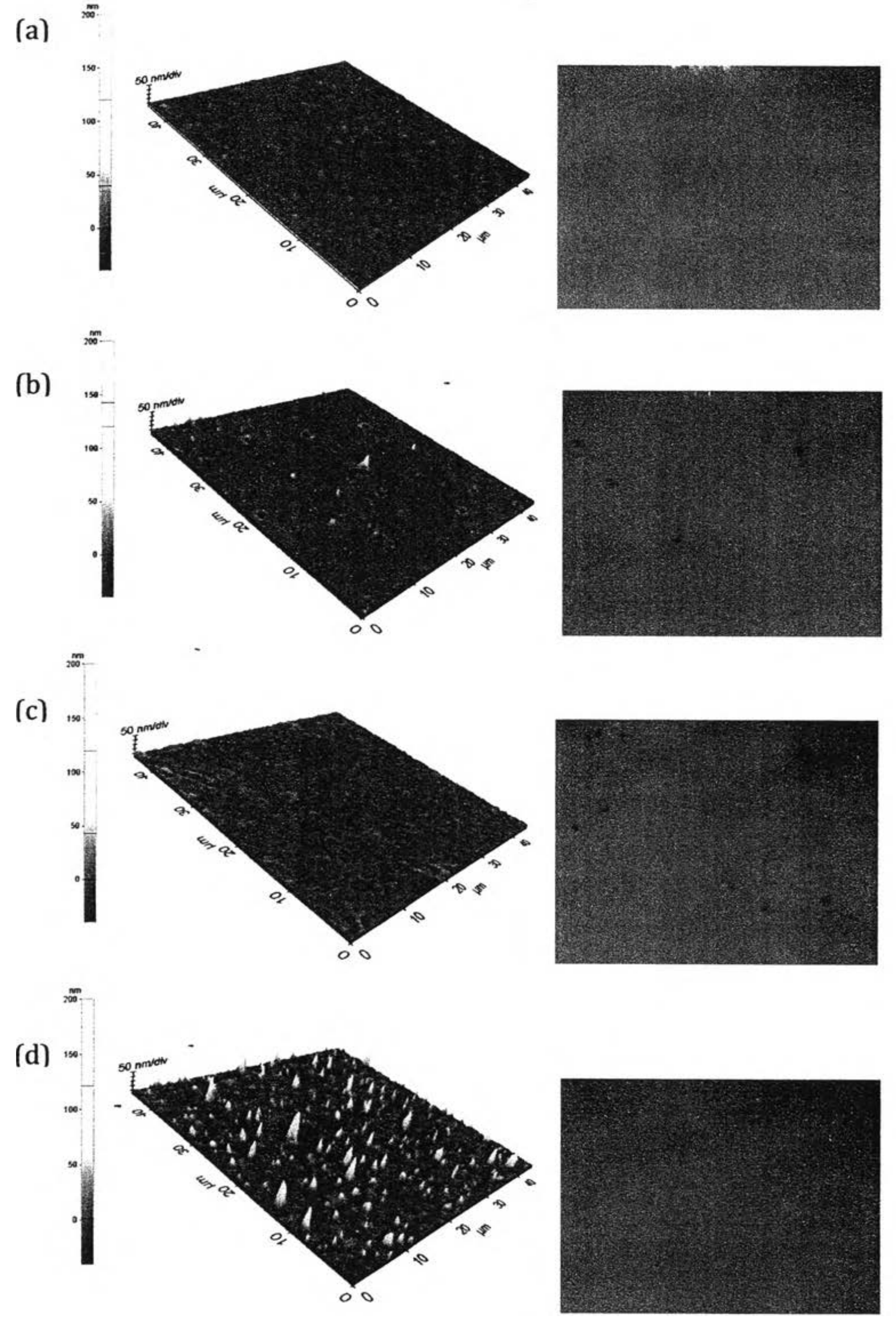


Figure 4.19 3D-AFM images and Optical images of 13-layer 10mM PDADMAC/PSS contain 1M NaCl; (a) PEM (b) PEM after loading with 0.01%w/v curcumin for 3 hours (c) PEM immersed in 10mM SDS for 1 hour (d) PEM immersed in SDS and loading with curcumin.

Figure 4.19 showed the 3-D AFM images of PEM and surfactant modified PEM film before and after loading with 0.01%w/v curcumin in water:EtOH (90:10) was found that the height of PEM film was found to increase twice a time from 142.096 to 302.569 nm when loading curcumin after surfactant treatment. As same as in Figure 4.20, the increasing of root mean square roughness (R_{rms}) of PEM film surface from 4.354 to 6.189 after loading that mean the curucmin diffuse into PEM film and modified film surface by immerse in SDS surfactant the R_{rms} is 7.163 and highly increase to 21.018 after loading because hydrophobic tail of surfactant can help curcumin to diffuse into PEM.

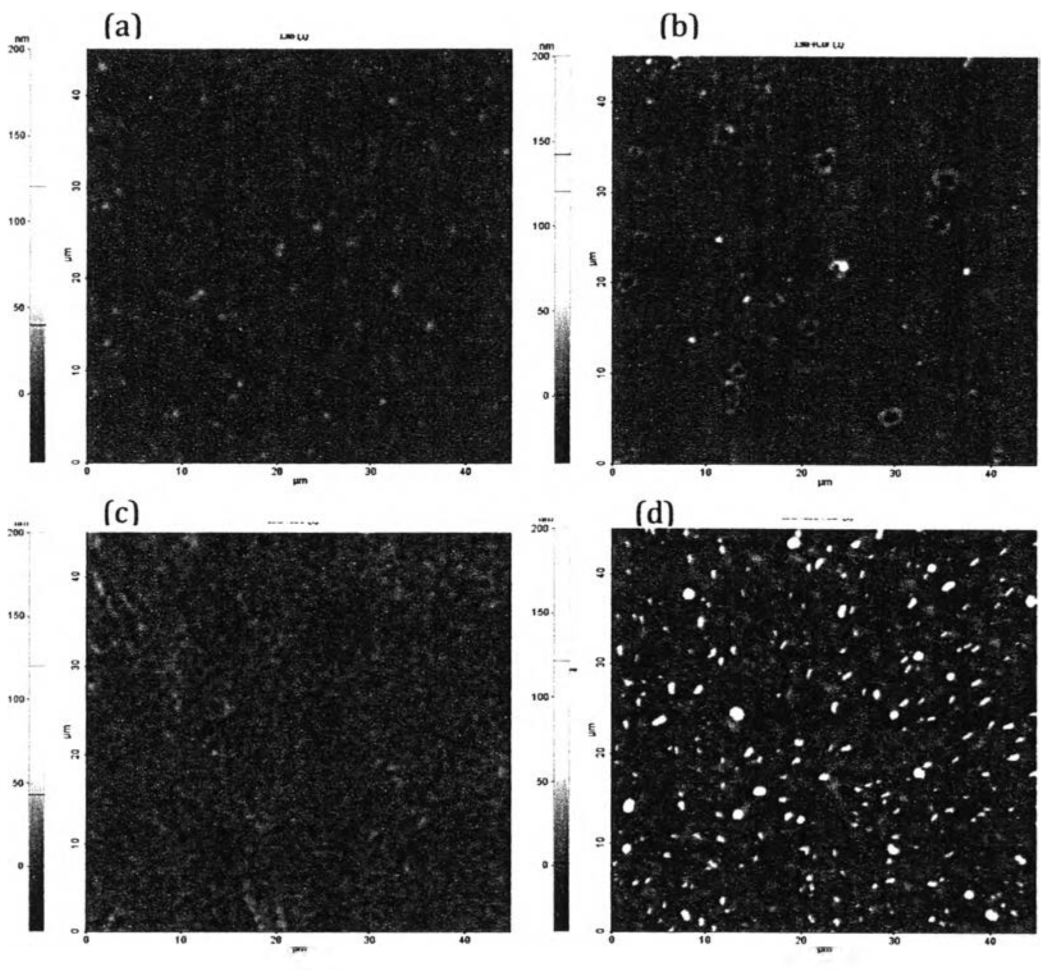


Figure 4.20 Morphology of 13-layer 10mM PDADMAC/PSS contain 1M NaCl; (a) PEM (b) PEM after loading with 0.01%w/v curcumin for 3 hours (c) PEM immersed in 10mM SDS for 1 hour (d) PEM immersed in SDS and loading with curcumin.