CHAPTER IV
RESULTS AND DISCUSSION

4.1. Part A: Loading Curcumin onto PEMU

Curcumin-based sensors can be fabricated in various techniques. One of those techniques is the LbL deposition. LbL is a surface modification technique where the surface of the substrate is modified with PEMU to enhance its property using other loaded material. In this part, curcumin is loaded onto two types of PEMU i.e. PDADMAC/PSS and CHI/ALG thin films. PDADMAC and PSS are synthetic polymers which produce efficient PEMUs; however, CHI and ALG are natural polymers which are more suitable to be applied in food packaging applications.

4.1.1. Effect of Solvent Composition

Curcumin could be loaded onto PEMU by adding curcumin stock into a mixed solution of water and ethanol. Curcumin is hydrophobic and dissolves in ethanol (1mg/1ml) (Curcumin, Sigma Aldrich). Therefore, to avoid being in contact with water, curcumin would prefer to deposit in the PEMU i.e. solvent partitioning. Here, 0.2% curcumin was mixed into the mixed solution with various ratios of water and ethanol. Then, 11 layers of PDADMAC/PSS and CHI/ALG thin films were prepared and dipped into the curcumin solution for 2 hours. UV-spectroscopy was measured to observe the absorbance of the yellow color at 440 nm.

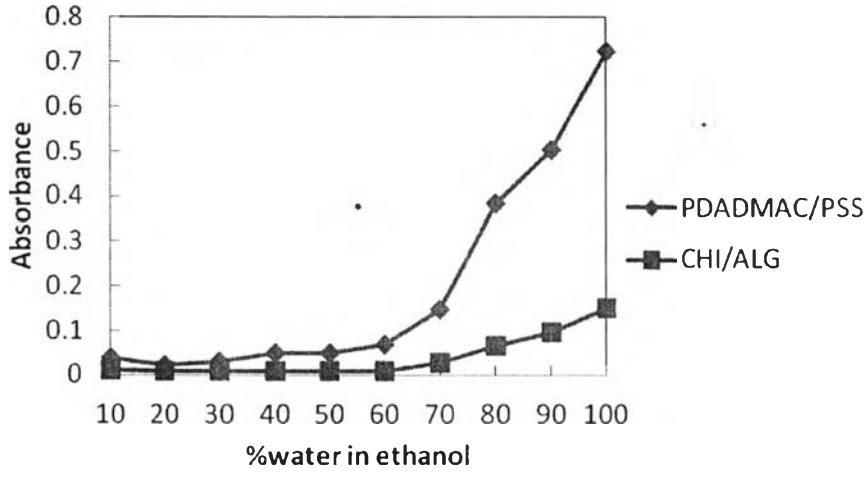

Figure 4.1 Absorbance at 440 nm of curcumin loaded using different solvent compositions on 11 layers PDADMAC/PSS and CHI/ALG PEM.

As mentioned previously, more %water in ethanol induces the loading of curcumin into the PEM thin films. According to Kittitheeranun and coworkers, since curcumin is hydrophobic and it will prefer the presence of a hydrophobic environment which is the PEMU. Even though the top layer of the PEMUs is charged and is hydrophilic, within the bulk is nonpolar and more hydrophobic compared to the mixed solution; as a result, it is more favorable for curcumin to diffuse into the PEMU bulk (Kittitheeranun, Sanchavanakit, Sajomsang and Dubas, 2010). Figure 4.1 describes the loading effect on two types of PEMU thin films which are PDADMAC/PSS and CHI/ALG thin films. From 10% to 60% water in ethanol, is very low and no curcumin was loaded on the either thin films due to high ethanol content. After 60% the absorbance of curcumin at 440 nm increases exponentially for both types of PEMU because there is more water content which made the solvent more hydrophilic and is less favorable to curcumin; resulting with curcumin diffusing into the PEMU bulk. Additionally, the curcumin loaded on CHI/ALG thin films have lower absorbance compared to PDADMAC/PSS thin films due to the thicknesses of thin films.

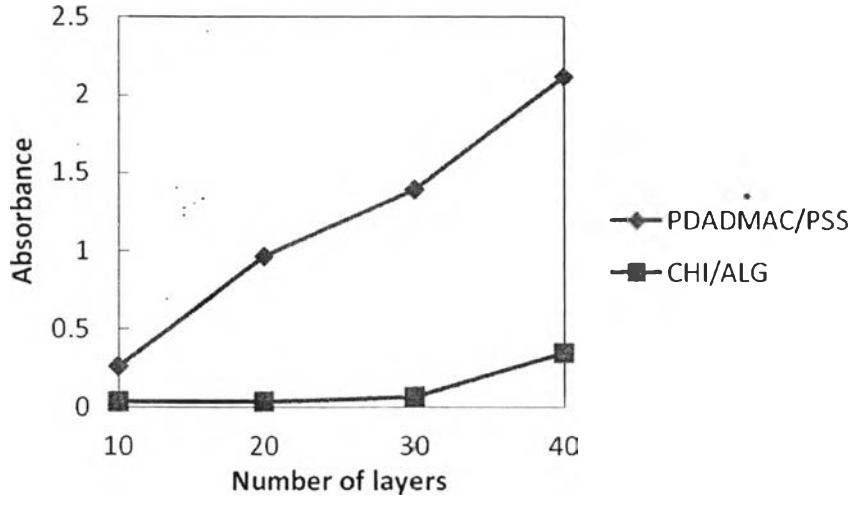

Figure 4.2 Absorbance at 440 nm of curcumin loaded on PDADMAC/PSS and CHI/ALG PEM thin films versus number of layers.

The effect of PEMU thickness towards loading of curcumin was described in Figure 4.2. PDADMAC/PSS thin films showed constant linear increment with increasing number of layers but CHI/ALG showed less absorbance. The CHI/ALG thin film was able to load more curcumin at 40 layers which has the absorbance equivalent to 10 layers of PDADMAC/PSS thin films. By comparing the thicknesses at 10 layers of PDADMAC/PSS and CHI/ALG from Figure 2.8 and Figure 2.12, showed that PDADMAC/PSS thin films are 5 times thicker than CHI/ALG thin films (Dubas and Schlenoff, 1999, and Fujie and Takeoka). This is because chitosan and alginate has ringed molecular structure which made the polymer chains difficult to bend even with high salt concentration; therefore, the CHI/ALG thin films are tightly packed compared to PDADMAC/PSS thin films. Resulting with lower curcumin loaded on the CHI/ALG thin films.

4.1.2. Loading of Curcumin on Various Number of Layers

Films prepared by layer by layer (LbL) can be loaded with many materials such as poly(aniline), graphite oxide, silver nanoparticles, and specifically with curcumin. This part studied the effect of the top layer of PDADMAC/PSS thin films. PDADMAC/PSS thin films composed of layers 5 to 20 were prepared from

10mM PDADMAC/PSS in 1M NaCl; then the films were immersed in 0.2% curcumin solution for 2 hours which was mixed in a water-ethanol mixed solution (90:10).

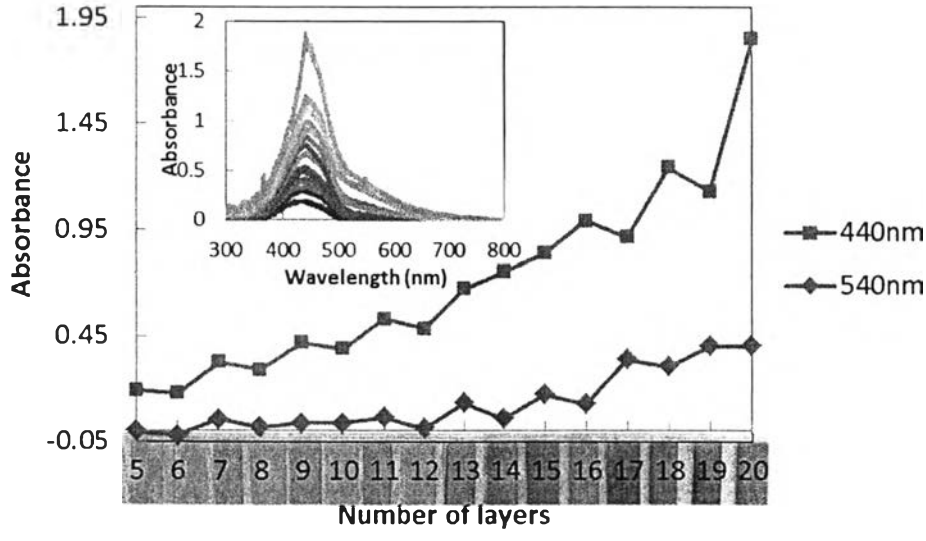

Figure 4.3 The UV spectra (inserted graph) and absorbance at 440 nm and 540 nm of curcumin loaded on PDADMAC/PSS thin films (large graph) and the actual thin films (images on x-axis).

Figure 4.3 (large graph) shows the absorbance of the films at different layers where the absorbance at 440 nm and 540 nm corresponds to the yellow and orange color, respectively. The more layers PEMU, the darker the yellow. The increasing amount of layers produces more space within the films which enhances curcumin diffusion into the bulk. Figure 4.3 (images on x-axis) shows alternating curcumin intensity where the layers with PDADMAC on top (odd layers) have darker tones and layers with PSS on top (even layers) have lighter tones. This is due to the opposite interactions of the positive PDADMAC with the negative curcumin which attracts more curcumin into the PEMU bulk. PSS is negatively charge, thus repels curcumin; however, due to the solvent polarity, the curcumin will still diffuse into the PEMU.

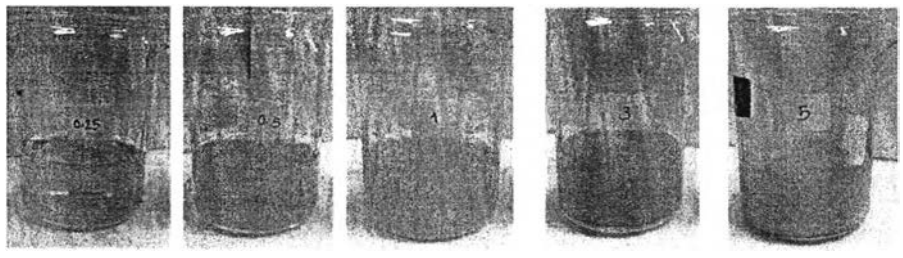

Figure 4.4 Various curcumin/PDADMAC ratio %v/v.

Figure 4.4 shows that with increasing curcumin content, complexation occurs due to the electrostatic interaction between the PDADMAC and curcumin. Thus, as a film, PDADMAC odd layers appear thicker and the color is more orange compared to PSS even layers. However, similar tests between curcumin and PSS did not show any precipitation which suggests that the interactions between curcumin and PSS are little to none.

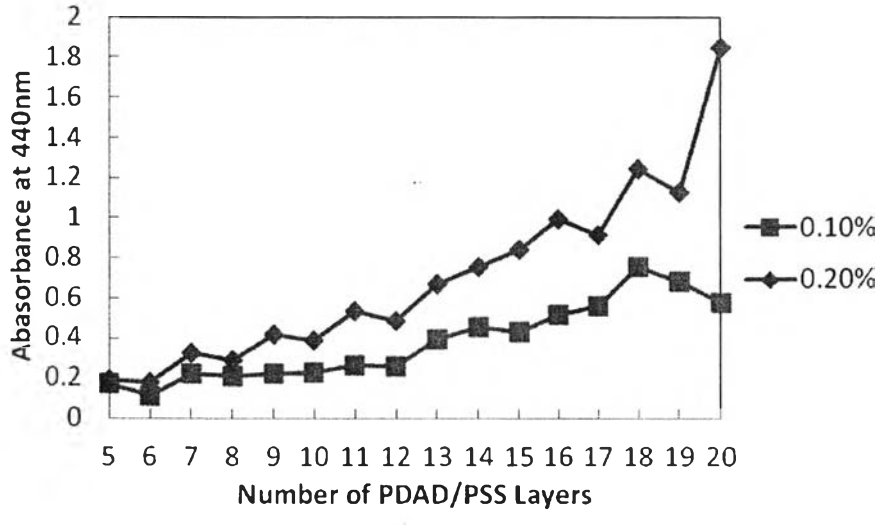

Figure 4.5 Absorbance at 440 nm of curcumin at different layers with 0.1% and 0.2% curcumin.

Figure 4.5 compares 0.1% and 0.2% curcumin loaded on PDADMAC/PSS with increasing layers. Thin films with 0.1% curcumin has lower absorbance; however, did not show variation between odd and even layers as much as 0.2% curcumin because it has lower concentrations.

4.1.3. Effect of Loading Time

The amount of curcumin loaded on the thin films does not only depend on the number of film layers and concentration but also varies with time. 0.2% curcumin in 90:10 water and ethanol mixed solution was prepared and loaded on 11 and 12 layers PDADMAC/PSS thin films from 0 to 4 hours to observe the effect of loading time and also to compare the differences between odd and even layers.

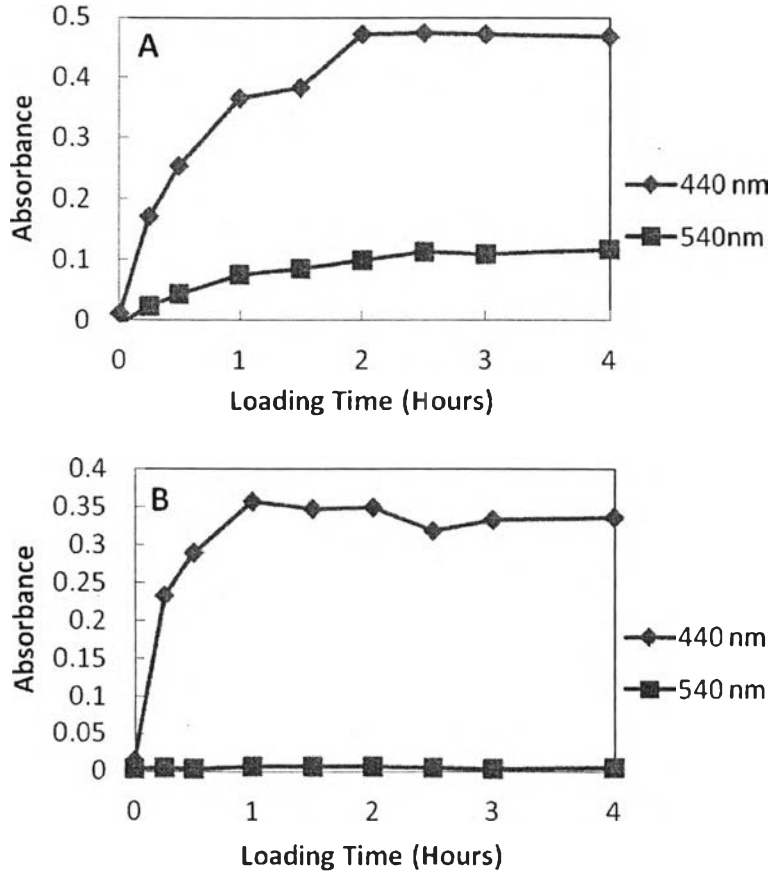

Figure 4.6 Absorbance of curcumin at 440 nm and 540 nm on (A) 11 layers and (B) 12 layers PDADMAC/PSS with varying loading time.

By observing the absorbance at 440 nm and 540 nm, Figure 4.6 has shown that the curcumin loading starts to saturate at 2 hours for 11 layers. This is due to the electrostatic interaction between PDADMAC and curcumin; additionally, the 11 layered PEMU has higher absorbance. However, at 12 layers, the saturation starts at 1 hour, because PSS is negative; therefore repels curcumin, resulting with less curcumin loaded on the film.

4.1.4. Effect of Curcumin Concentration

To study the maximum curcumin content which could be loaded on the PEMU, various stocks of curcumin with different concentrations were prepared. Curcumin is not as soluble in water as in ethanol, so curcumin was dissolved in pure ethanol to produce curcumin stock. The maximum amount of curcumin soluble in ethanol is 0.2%.

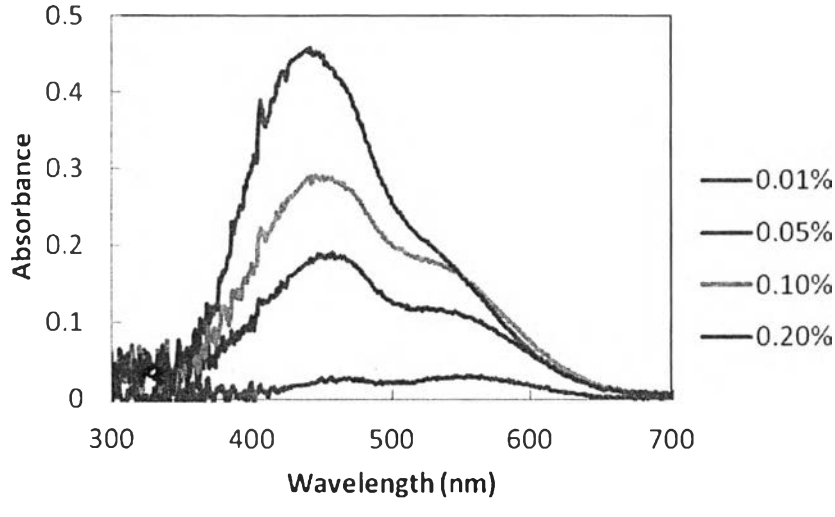

Figure 4.7 The UV spectra showing the absorbance versus the wavelength comparing curcumin loaded on 11 layered PDADMAC/PSS thin films using various curcumin concentrations.

Figure 4.7 shows the UV spectra of curcumin loaded on 11 layered PDADMAC/PSS thin films with varying curcumin concentrations. The peaks at 440 nm and 540 nm represent yellow and orange color, increases with increasing

curcumin concentration due to the increasing complexation between curcumin and PDADMAC.

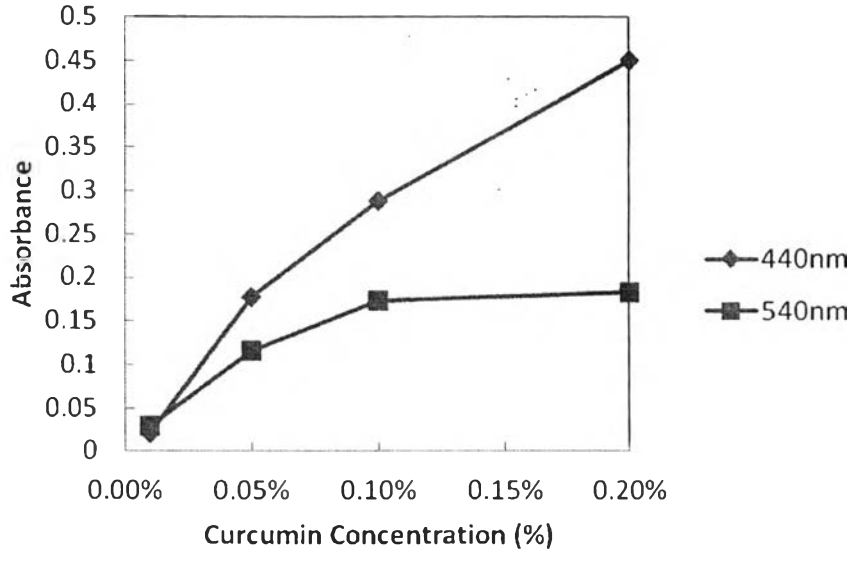

Figure 4.8 Absorbance of curcumin at 440 nm and 540 nm on 11 layered PDADMAC/PSS with varying curcumin concentration.

Figure 4.8 shows the absorbance of curcumin loaded on 11 PDADMAC/PSS with varying curcumin concentration correlating to Figure 4.7. As expected, the intensity of curcumin increases with stronger curcumin concentration. The peak at 540 nm which represents orange color also increases in terms of curcumin concentration as well. Furthermore, the absorbance or the maximum amount of curcumin starts to saturate at 0.15 %wt./v curcumin.

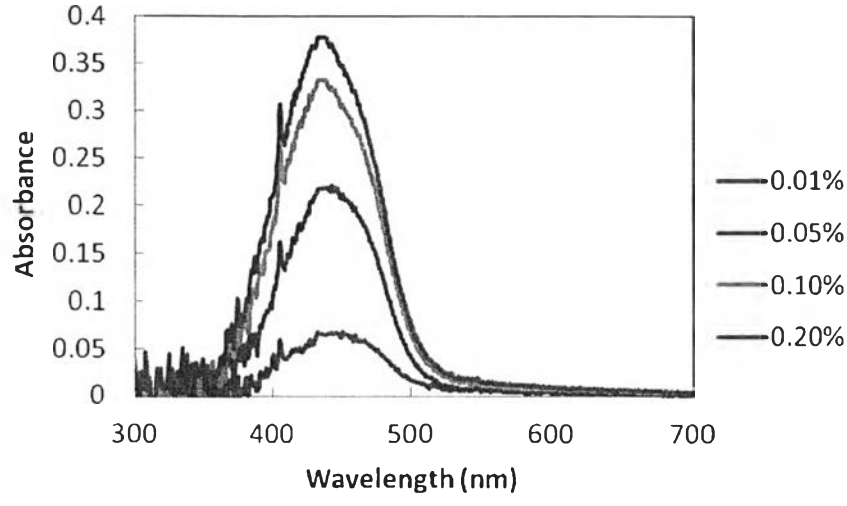

Figure 4.9 The UV spectra of curcumin loaded on 12 layered PDADMAC/PSS at 440 nm and 540 nm on 12 layers with varying curcumin concentration.

Figure 4.9 is the UV spectra of curcumin loaded on 12 layered PDADMAC/PSS thin films with various curcumin concentrations. Unlike 11 layers, curcumin loaded on 12 layered PDADMAC/PSS does not show peaks at 540 nm due to the charge repulsion between PSS and curcumin which causes less curcumin to be loaded on the film surface and there was no complexation between curcumin and PSS. Nevertheless, the "yellowness" of curcumin still increases with curcumin concentration.

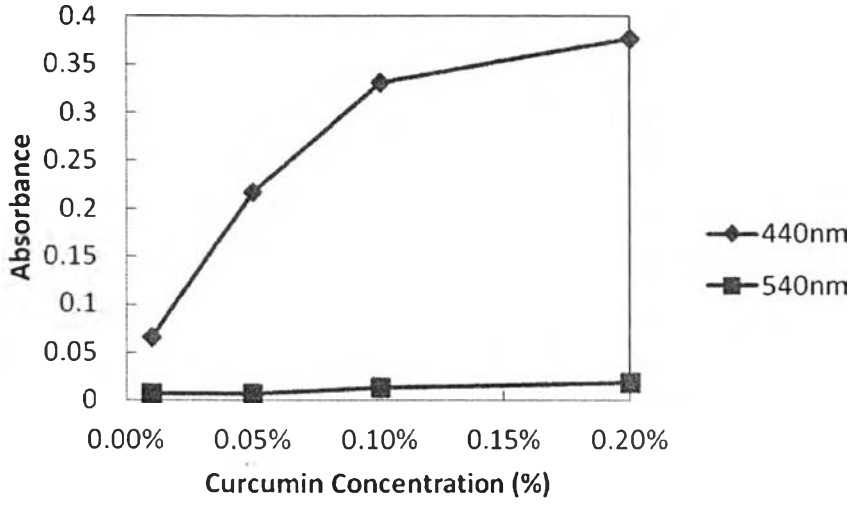

Figure 4.10 Absorbance of curcumin at 440 nm and 540 nm on 12 layers with varying curcumin concentration.

Figure 4.10 shows the absorbance of curcumin at 440 nm and 540 nm which correlates to the UV spectra (Figure 4.9). The absorbance at 550 nm did not increase with increasing curcumin concentration like curcumin loaded on odd layers. However, the peak at 440 nm still increases.

4.1.5. pH Sensitivity of Curcumin

Curcumin is known for being sensitive to pH change. It could change color from yellow to orange at approximately pH 8-9; therefore, it could be used as an optical sensor. 0.2% curcumin was loaded on 11 and 12 layers of PDADMAC/PSS and CHI/ALG thin films. Then, they were dipped in 10 mM phosphate buffer at pH 5-10 to observe the color change at each pH using the UV-Vis spectrometer.

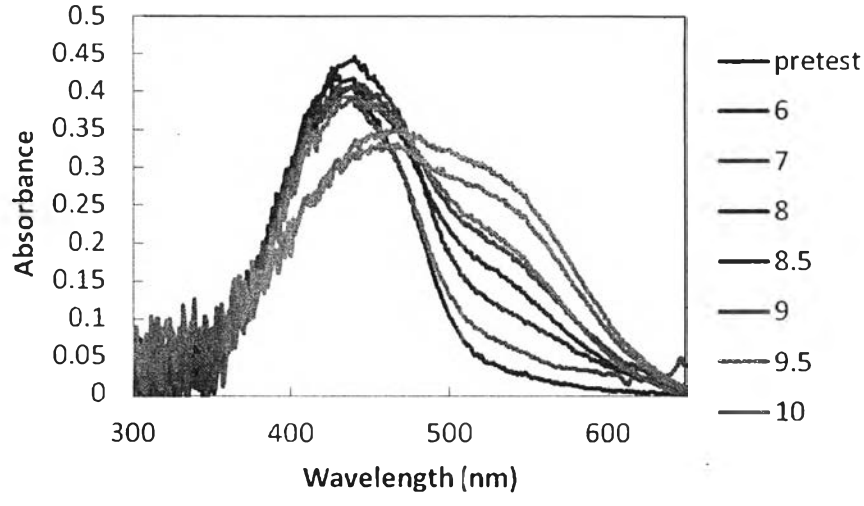

Figure 4.11 UV spectra of curcumin loaded on 11 layered PDADMAC/PSS thin films dipped in various phosphate buffer pH in terms of absorbance versus wavelength.

Figure 4.11 is an example of the UV spectra which could be seen with curcumin loaded on odd layered PDADMAC/PSS. There are two main peaks which are at 440 nm and 540 nm which represents yellow and orange, respectively. The increasing pH did not affect the peaks at 440 nm until pH 9.5 which showed slight shift to 450 nm. This indicates color change. However, obvious absorbance changes can be observed at 550 nm.

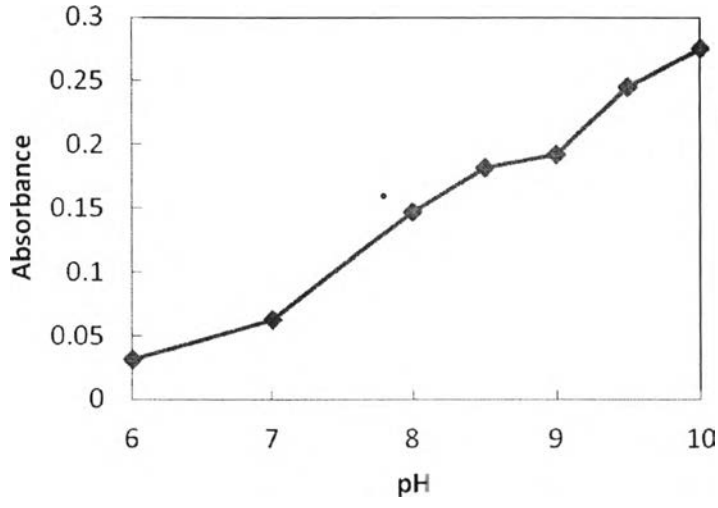

Figure 4.12 The absorbance at 540 nm of curcumin loaded on 11 layered PDADMAC/PSS thin films at various phosphate buffer pH.

The absorbance at 550 nm of curcumin loaded on odd layered PDADMAC/PSS thin films increases linearly with increasing pH (Figure 4.12). Figure 4.13 is the actually image of the thin film dipped in phosphate buffer at various pH.

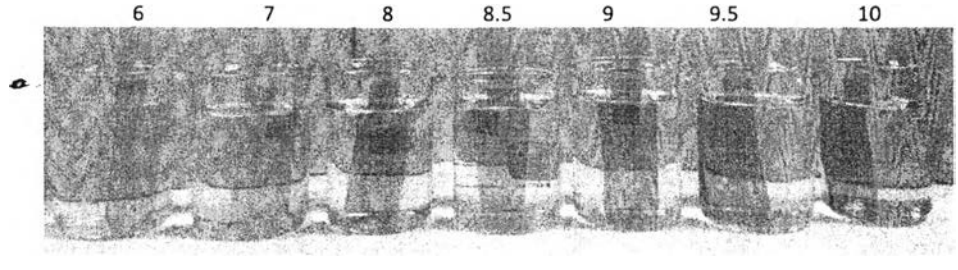

Figure 4.13 Actual image of curcumin loaded on 11 layers thin film dipped at different phosphate buffer pH.

From this image, at pH 6 and pH 7 the color of the film is yellow. When the pH reaches 8, the color changed to orange which explained the increase in absorbance at 550 nm. The color change is due to curcumin's protonation form at

different pH where the structure of curcumin dissociates three acidic protons when reaches pH 9; therefore, changes color to red. This shows that curcumin is sensitive to pH 8, which could later be used to detect ammonia gas.

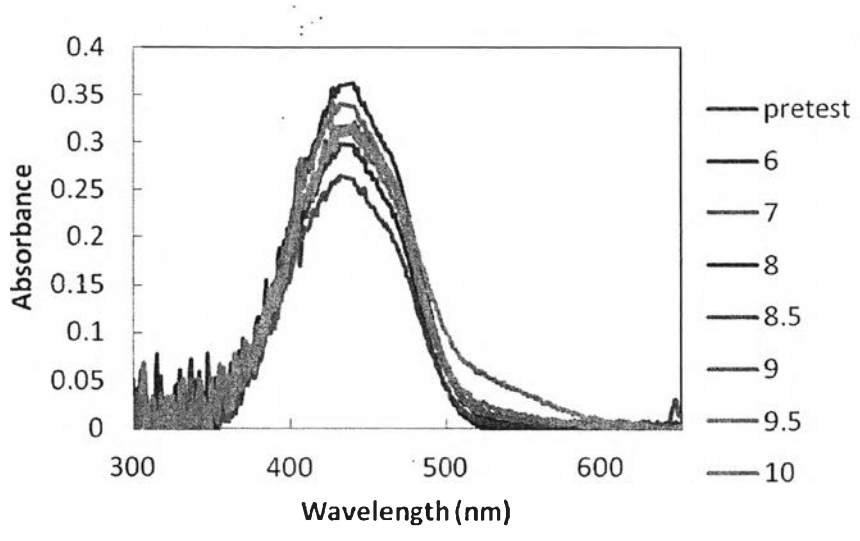

Figure 4.14 UV spectra of curcumin loaded on 12 layered PDADMAC/PSS thin films dipped in various phosphate buffer pH in terms of absorbance versus wavelength.

Unlike odd layers, curcumin loaded on even layered thin films did not show peaks at 550 nm until the pH_b is 10 (Figure 4.14). However, when the absorbance at 550nm was plotted with increasing pH, the change can be observed.

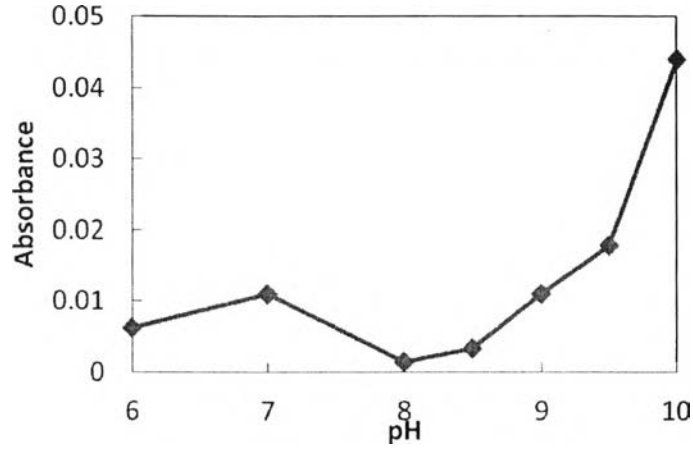

Figure 4.15 The absorbance at 540 nm of curcumin loaded on 12 layered PDADMAC/PSS thin films at various phosphate buffer pH.

Figure 4.15 is the graph of absorbance at 540 nm plotted against pH of curcumin loaded on even layered thin films. The absorbance increases exponentially from pH 8 to 10. However, the absorbance showed a sharp increment at pH 10.

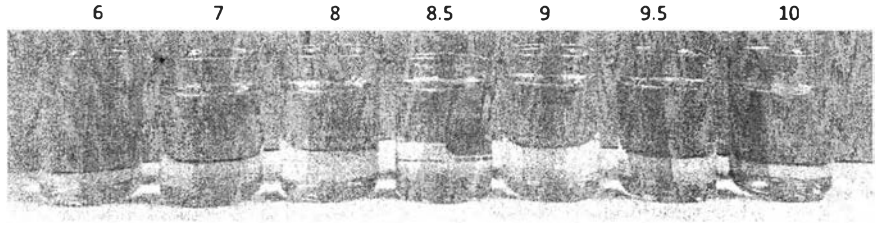

Figure 4.16 Actual image of curcumin loaded on 12 layers thin film dipped at different phosphate buffer pH.

As shown in the image (Figure 4.16), curcumin loaded on even layered films showed that the color change occurred after pH 9.5 which was delayed compared to odd layers in Figure 4.13. These results suggest that the pH sensitivity or pKa of curcumin can be tuned by the top layer of the PEMU.

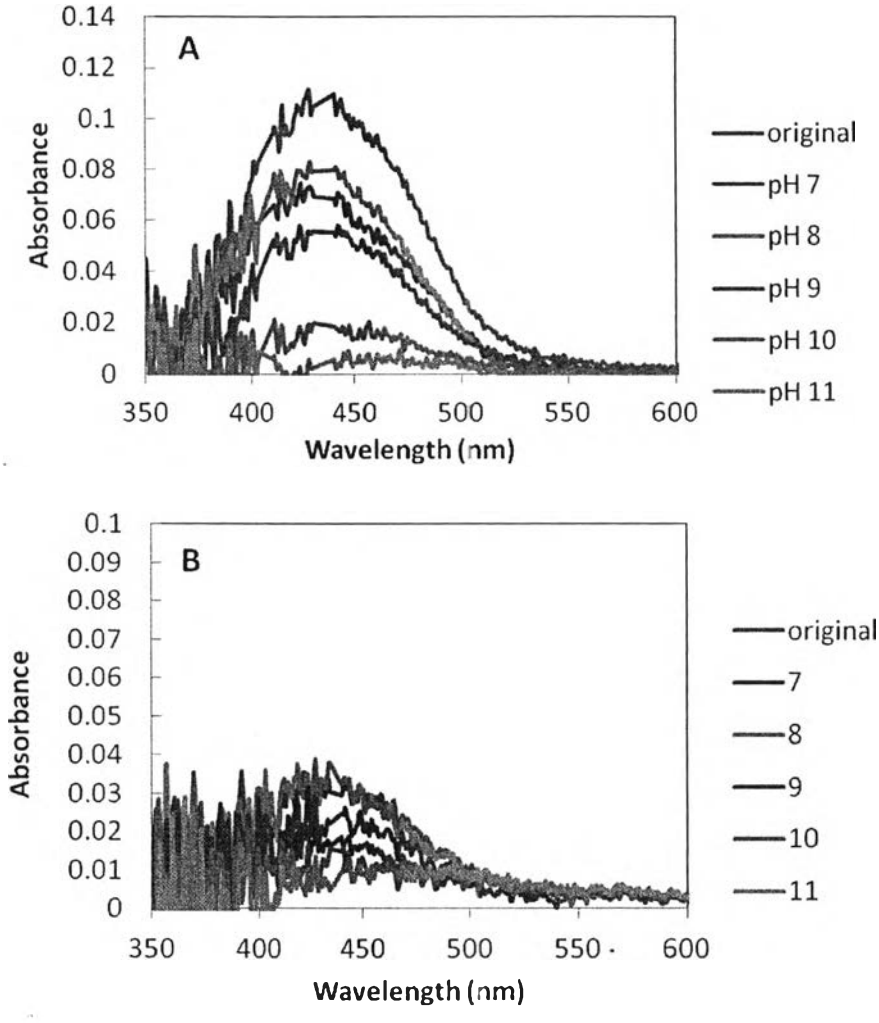

Figure 4.17 UV spectra of curcumin loaded on (A) 11 and (B) 12 layered CHI/ALG showing the effects of pH.

Unlike PDADMAC/PSS PEM, curcumin loaded on CHI/ALG shows different pH sensitivity since CHI/ALG has slow thin film growth and is very thin compared to PDADMAC/PSS; therefore, the absorbance at 440 nm which is the amount of curcumin loaded on CHI/ALG thin films is less than that of PDADMAC/PSS thin films (Figure 4.17). In addition, when these films were immersed in phosphate buffer, the thin films did not appear orange, instead it became colorless. As a result, there are no peaks at 540 nm.

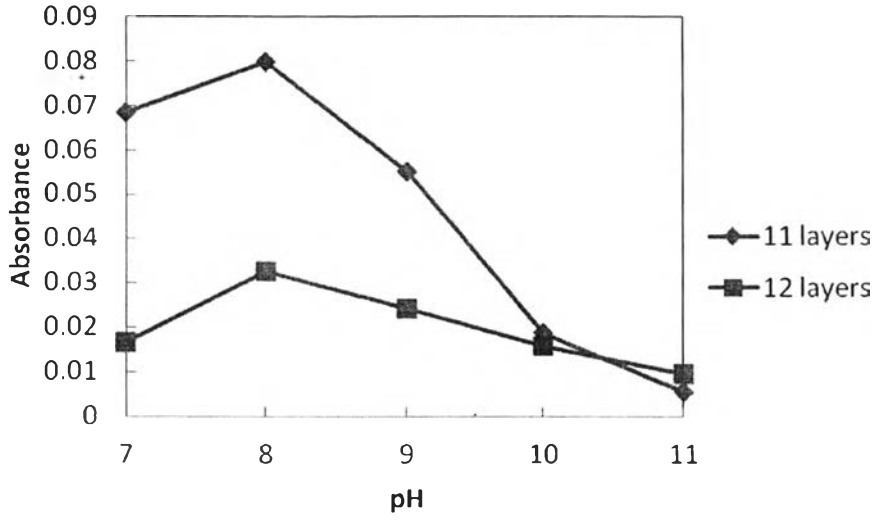

Figure 4.18 Absorbance at 440 nm of curcumin loaded on 11 and 12 layered CHI/ALG showing the effects of pH.

Figure 4.18 shows the absorbance reduction of curcumin with increasing pH. Curcumin loaded on both odd and even layers turns from yellow to colorless. However, odd layers have higher intensity than even layered CHI/ALG which indicated that there was more curcumin loaded on the PEMU due to the electrostatic interactions between the positive CHI and the negative curcumin.

An actual shrimp test was applied to observe the sensitivity of curcumin on films. Since curcumin loaded on PDADMAC/PSS thin films showed stronger color and clearer color change than CHI/ALG thin films, PDADMAC/PSS were chosen for this experiment. The shrimps and curcumin loaded PDADMAC/PSS thin films was stored in a closed container at room temperature. However, the films did not change color. Alternatively, alginate with curcumin gels and curcumin solution were used for the shrimp test (Figure 4.19).

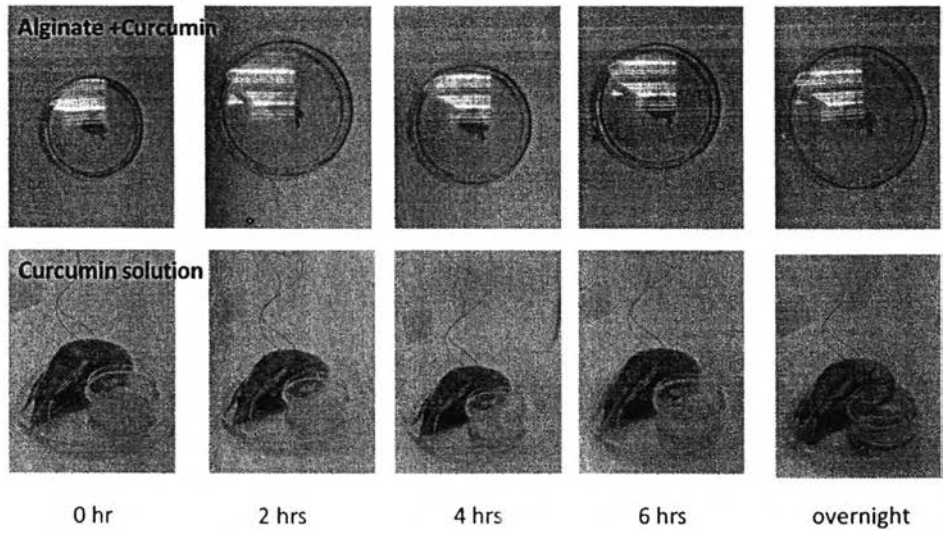

Figure 4.19 (Top) Alginate with curcumin gel films coated on top and bottom of the petri-dish and (Bottom) curcumin solution. Both were monitored and recorded every 2 hours.

Unfortunately, both the types of mediums were not affected by the ammonia released from the spoilt shrimp even overnight at room temperature. As a result, ammonia solution was used to observe the limits of detection.

From the previous graphs, we can conclude that PDADMAC/PSS thin films produced better curcumin sensors compared to the biocompatible CHI/ALG thin films. The PDADMAC/PSS films were then put into a closed container with various ammonia solutions without being in contact to the solution. The curcumin thin films did not change color at low concentrations of ammonia; however, it changes when interacted with pure ammonia vapor. This is due to the limit of detection of curcumin since the concentration of ammonia vapor produced from shrimps was most likely insufficient for detection. Also, the possibility of the samples being too dried for the curcumin to be active was also considered.

4.2. Part B: Curcumin Loaded Electro-spun Fibers

Another technique to produce curcumin sensors is by combining it with a polymer and spins it into fibers known as electrospinning. By changing the morphology from film to fiber increases its surface area to detect ammonia vapor. Additionally, the polymer used was PVA which has the ability to absorb water i.e. hydroscopic property. As a result, as PVA absorbs the water vapor it also absorbs ammonia vapor which increases the contact to curcumin particles.

4.2.1. Effect of Curcumin Concentration

In order to keep the curcumin hydrated, poly (vinyl alcohol) (PVA) was used as a hydroscopic polymer which could maintain moist for the curcumin. 6% PVA was mixed with various curcumin concentration. Afterwards, the mixture undergoes electrospinning for 2 hours to obtain thick bright yellow sheets. Then UV-vis spectroscopy was taken to observe the color of the fibers. SEM and optical microscope was used to observe fiber morphology.

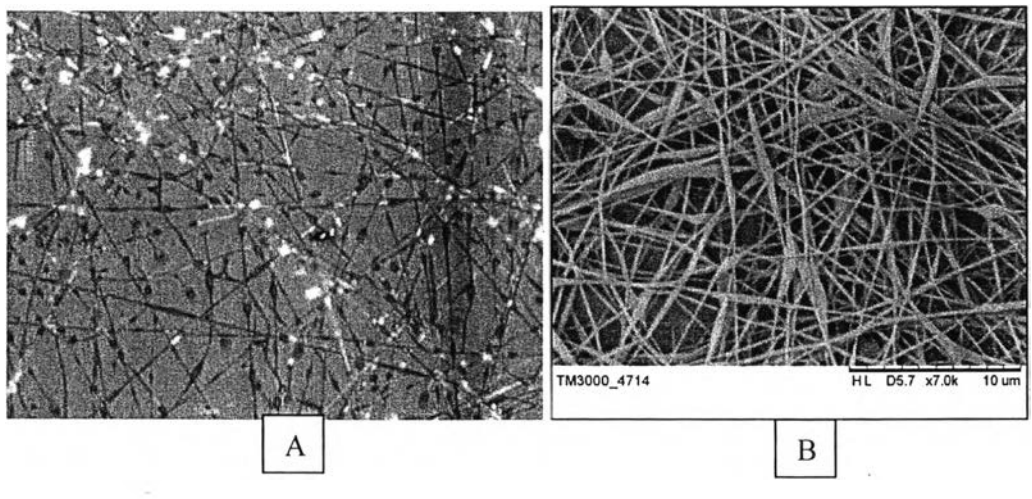

Figure 4.20 (A) Image of the PVA/curcumin electro-spun fiber from optical microscopy x500. (B) The SEM image of the PVA/curcumin electro-spun fiber.

Figure 4.20 is the image obtained from using the optical microscopy SEM. The fiber appears to be in a bead and thread form due to the curcumin particles within the PVA. The fibers contain beads but it did not affect the color of the fibers.

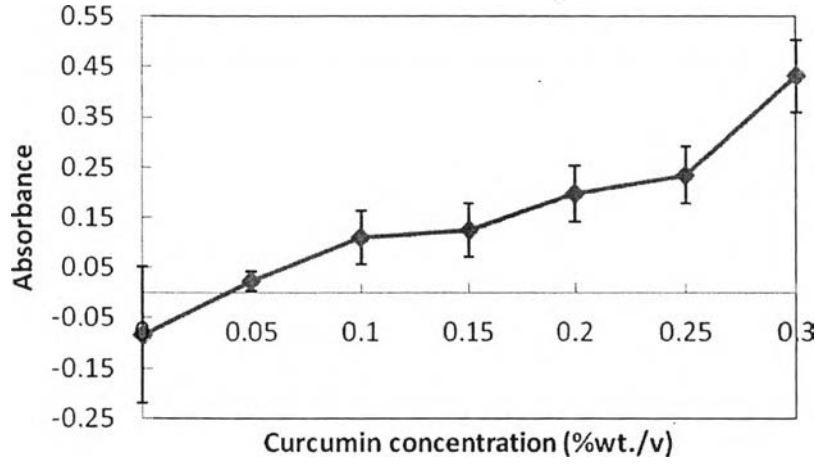

Figure 4.21 Absorbance at 440 nm of PVA/curcumin electro-spun fiber at different curcumin stock concentration.

Figure 4.21 shows the absorbance of PVA/curcumin electro-spun fiber with varying curcumin concentration. Therefore, the higher the curcumin concentration, the more color intensity because more curcumin is mixed in the fiber.

4.2.2. pH Sensitivity of Curcumin in Fibers

Ammonia vapor has pH 9 which is detectable using curcumin. PVA/curcumin electro-spun fibers were cut to small pieces and observe the color change when exposed in ammonia vapor (Figure 4.22).

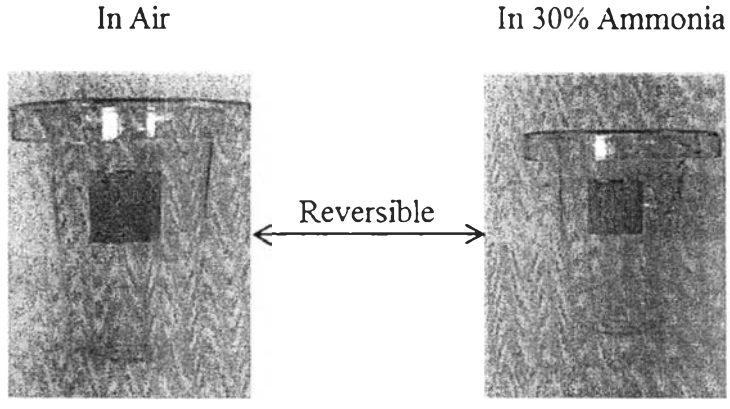

Figure 4.22 Reversible effect of PVA/curcumin electro-spun fiber when exposed in air and in pure ammonia vapor.

The color change from yellow to purple can be observed directly by the naked eye. The sensitivity of curcumin is reversible so the spectrum of the color change cannot be measured when it is in air. However, it is possible to observe kinetically.

The concentration of ammonia vapor produced from shrimps is unknown. Although, the limit of detection could be estimated by varying diluted ammonia solutions and then expose the fiber to the vapor in a closed container (Figure 4.23).

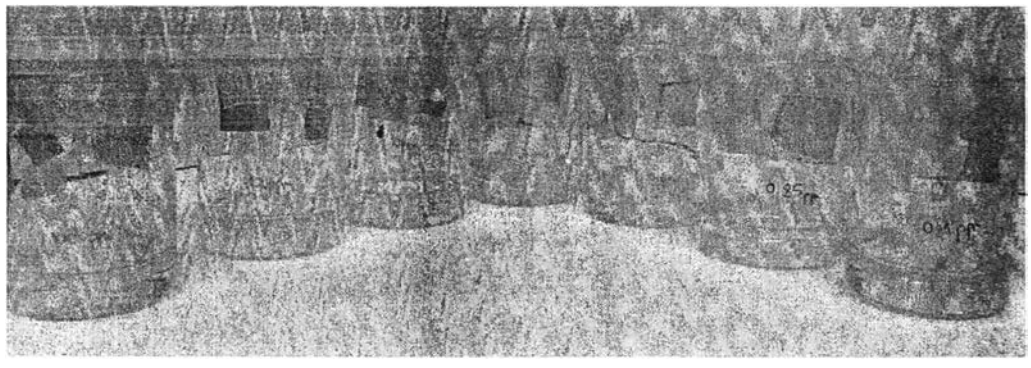

Figure 4.23 Testing the electro-spun fibers with ammonia solutions of the following concentrations: (left to right) 1000 ppm, 100 ppm, 10 ppm, 1 ppm, 0.5 ppm, 0.25 ppm, 0.1 ppm.

The sample exposed to vapor produced from 1000 ppm ammonia shows the clearest color change (yellow to purple). 100 ppm ammonia solution produces vapor that changes curcumin fiber from yellow to orange but with slower change at after 1 hour. Although, the electro-spun fibers show less sensitivity when interact with ammonia vapor concentration below 100 ppm. However, after the fibers were kept in the containers overnight, there are droplets of water on the fiber due to the hydroscopic properties of PVA and what remained on the foil was the clear, yellow color. It seemed like the fibers were deformed and molten.

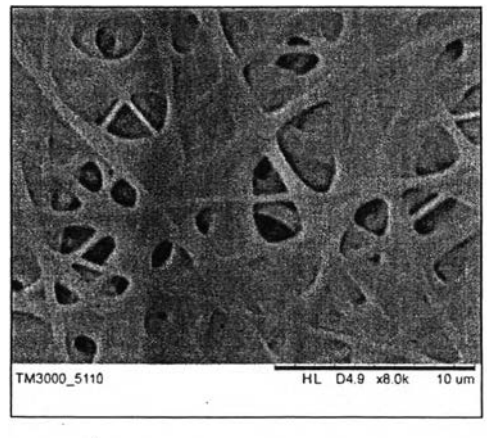

Figure 4.24 Fiber after exposed to ammonia.

Figure 4.24 shows the comparison between electro-spun PVA/curcumin fibers before and after exposure to ammonia vapor. From the naked eye the fibers were molten; however, from SEM imaging the fibers were swollen and merged together. This reduces surface area of the fibers, therefore, reduces the pH sensitivity of curcumin.

4.2.3. Crosslinking Electro-spun Fibers

In order to maintain fiber strength, the fiber should be crosslinked. Boric acid was used to crosslink PVA. The electro-spun fibers were soaked in boric acid for 5 minutes and then dried in a 60°C oven.

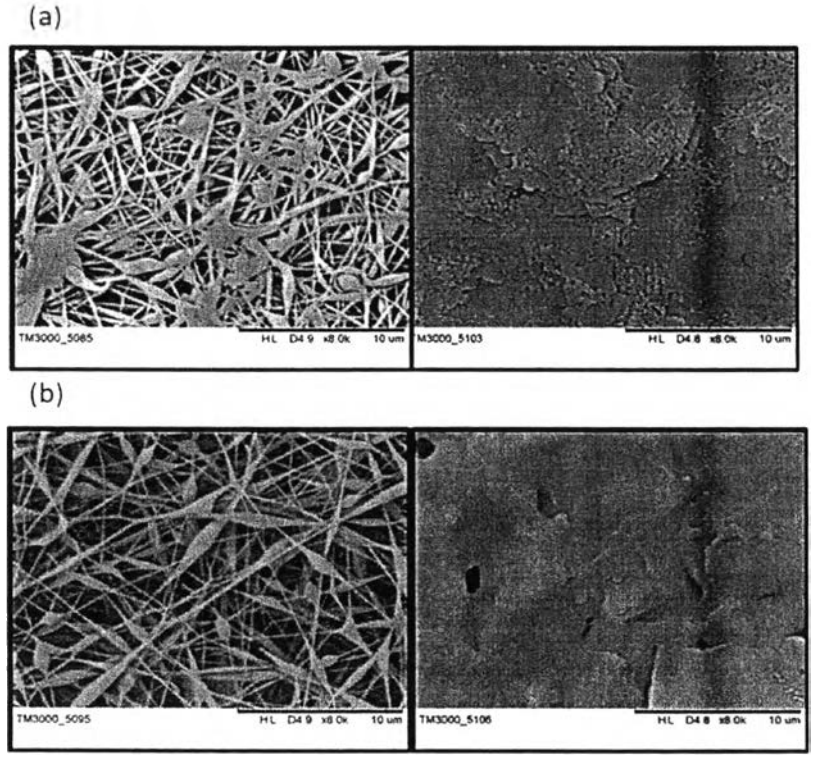

Figure 4.25 Electro-spun fiber of PVA with (a) 0% and (b) 0.2% curcumin before (left) and after (right) crosslinked with boric acid.

Figure 4.25 showed that both crosslinking agents successfully increase crosslinking of electro-spun fibers. However, boric acid caused the PVA to swell and

completely destroyed the porous structure which reduced the hydroscopic property i.e. surface area of PVA; therefore, reduces the sensitivity of curcumin. As a result, the color did not change.

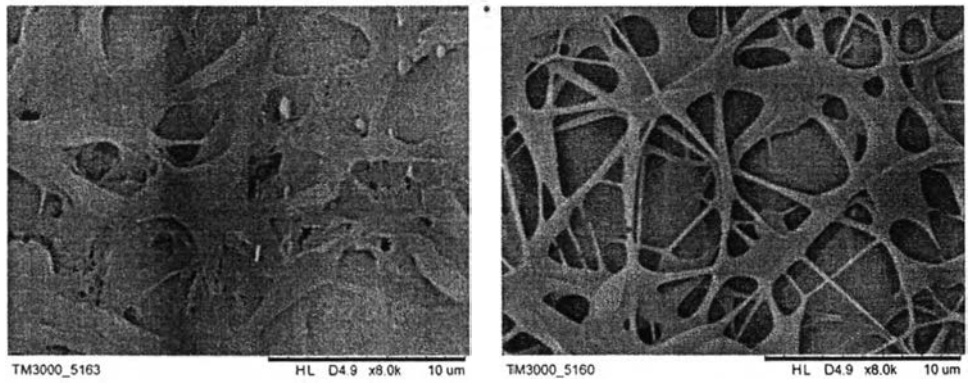

Figure 4.26 Crosslinked PVA/curcumin electro-spun fiber using (Left) 4% Boric acid and (Right) 0.5 M glutaraldehyde vapor crosslinking.

Another crosslinking method of PVA is by vapor crosslinking using glutaraldehyde. Although, vapor crosslinking with glutaraldehyde maintained fiber structure but curcumin sensitivity was reduced and there was no visible color change of the fibers (Figure 4.26).