



## CHAPTER IV

### RESULTS AND DISCUSSION

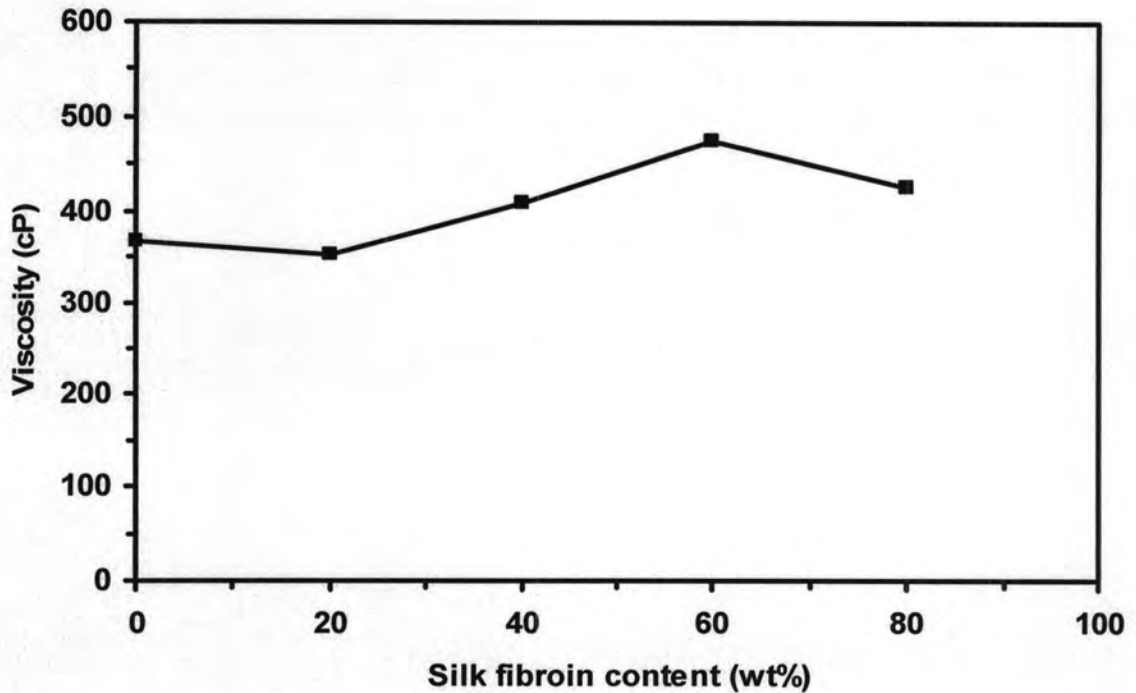
#### 4.1 Physical characteristics of the blended Thai silk fibroin/type B gelatin solution and electrospun fiber mats

##### 4.1.1 Viscosity and conductivity of Thai silk fibroin/type B gelatin blended solution

In this study, the viscosity and conductivity of Thai silk fibroin/type B gelatin (SF/GB) solutions in formic acid at different weight blending ratios was measured to examine the relationship between these parameters and a morphology of the obtained electrospun fibers.

As seen in Figure 4.1, the viscosity of solution increased from 351 centipoises in a neat gelatin solution to 475 centipoises for SF/GB with 80 wt% of silk fibroin content. Solution with silk fibroin content above 80 wt% became gel, and the viscosity of the solution was out of measurable range of the instrument.

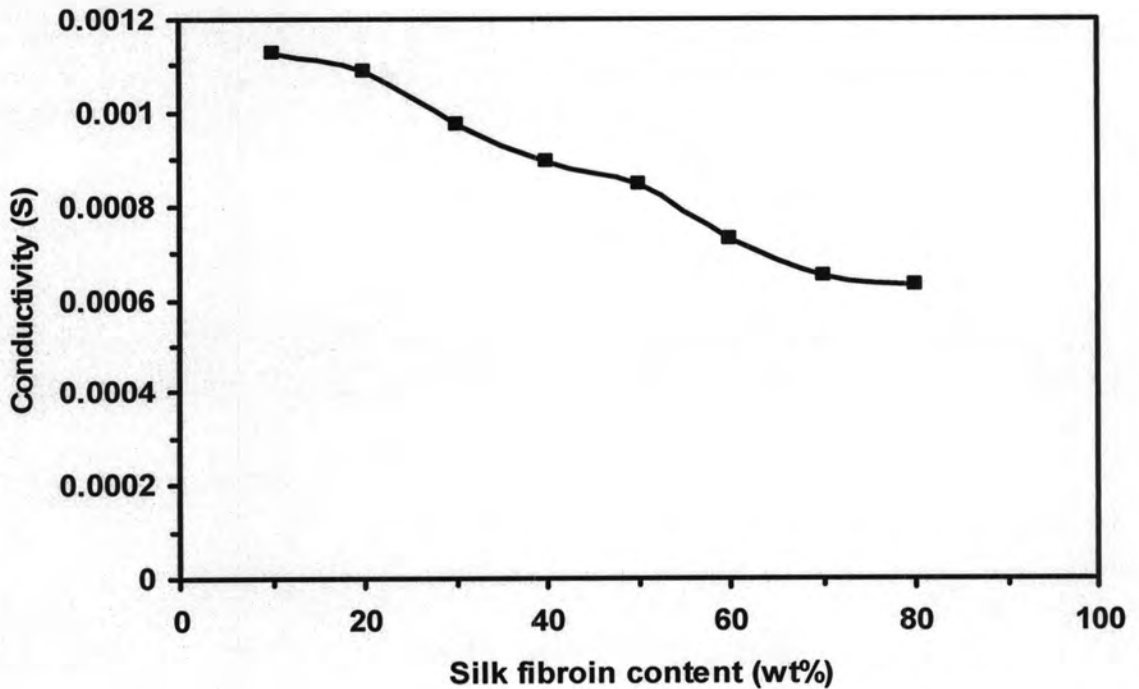
From visual observation during spinning process, the SF/GB solution with silk fibroin content lower than 50wt% of silk fibroin content, were easier to spin with large coverage area during fiber deposition with low irregularity such as beaded fiber or large droplet.



**Figure 4.1** Viscosity of Thai silk fibroin/type B gelatin (SF/GB) blended solution at various weight blending ratios (solution concentration of 20 %w/v)

The conductivity of the blended solution was showed in Figure 4.2. As the silk fibroin content increased from 10 to 80 wt%, the conductivity of the solution lowered from  $11.25 \times 10^{-4}$  to  $6.3 \times 10^{-4}$  S. Since conductivity of the fluid is an indication of number of charges available in the solution or charge mobility through the media or both, the lower conductivity at high SF content could be due to lower charge mobility in a more viscous fluid. In addition, silk fibroin consisted of charged amino acids less than gelatin, the conductivity of blended solution with higher silk fibroin content was lower than that of blended solution with lower silk fibroin content and lower number of excess charge in the blend solution.

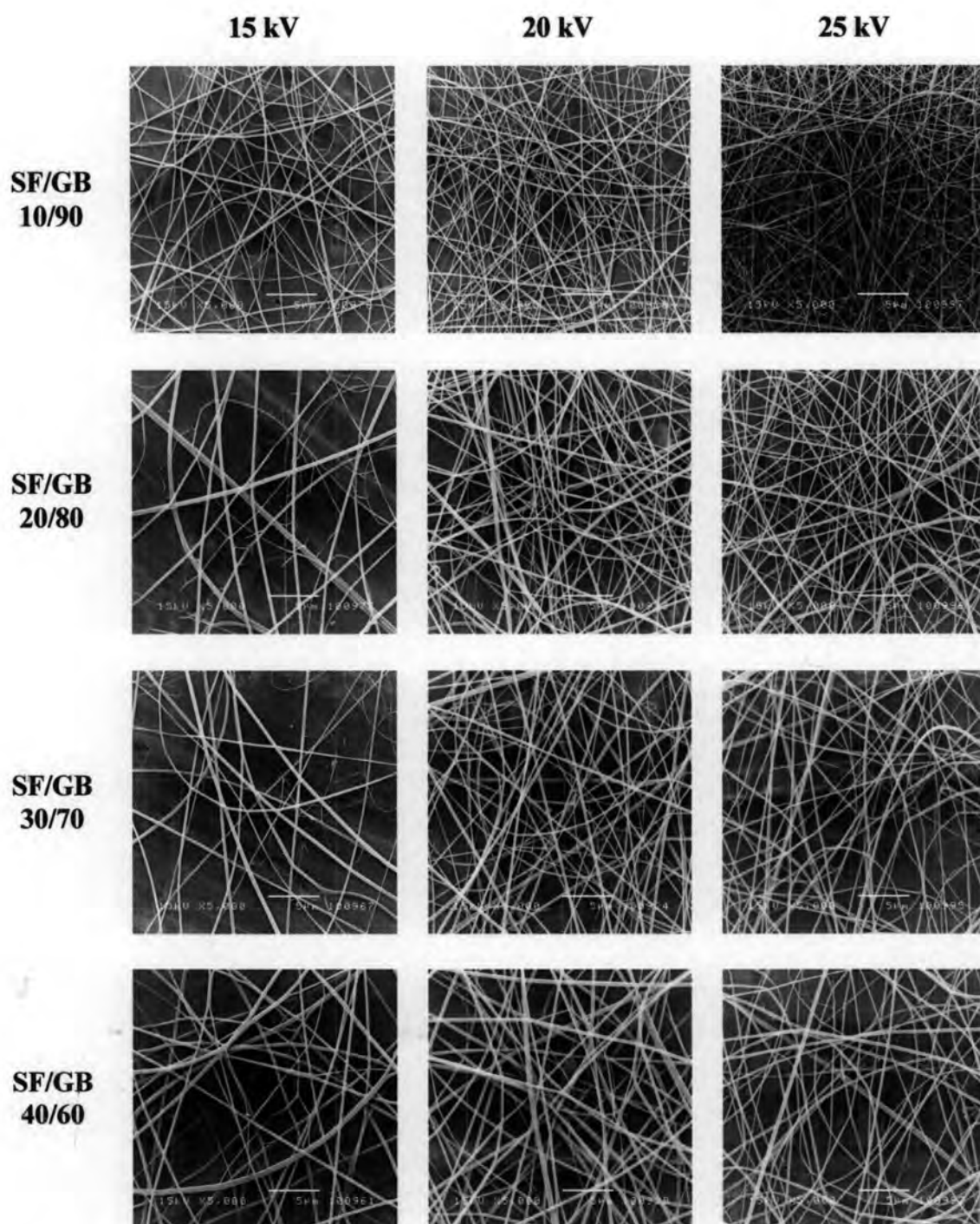
The effect of both viscosity and conductivity of the solution was apparent when compared sizes of fiber electrospun at the same processing parameter as shown in section 4.1.3.



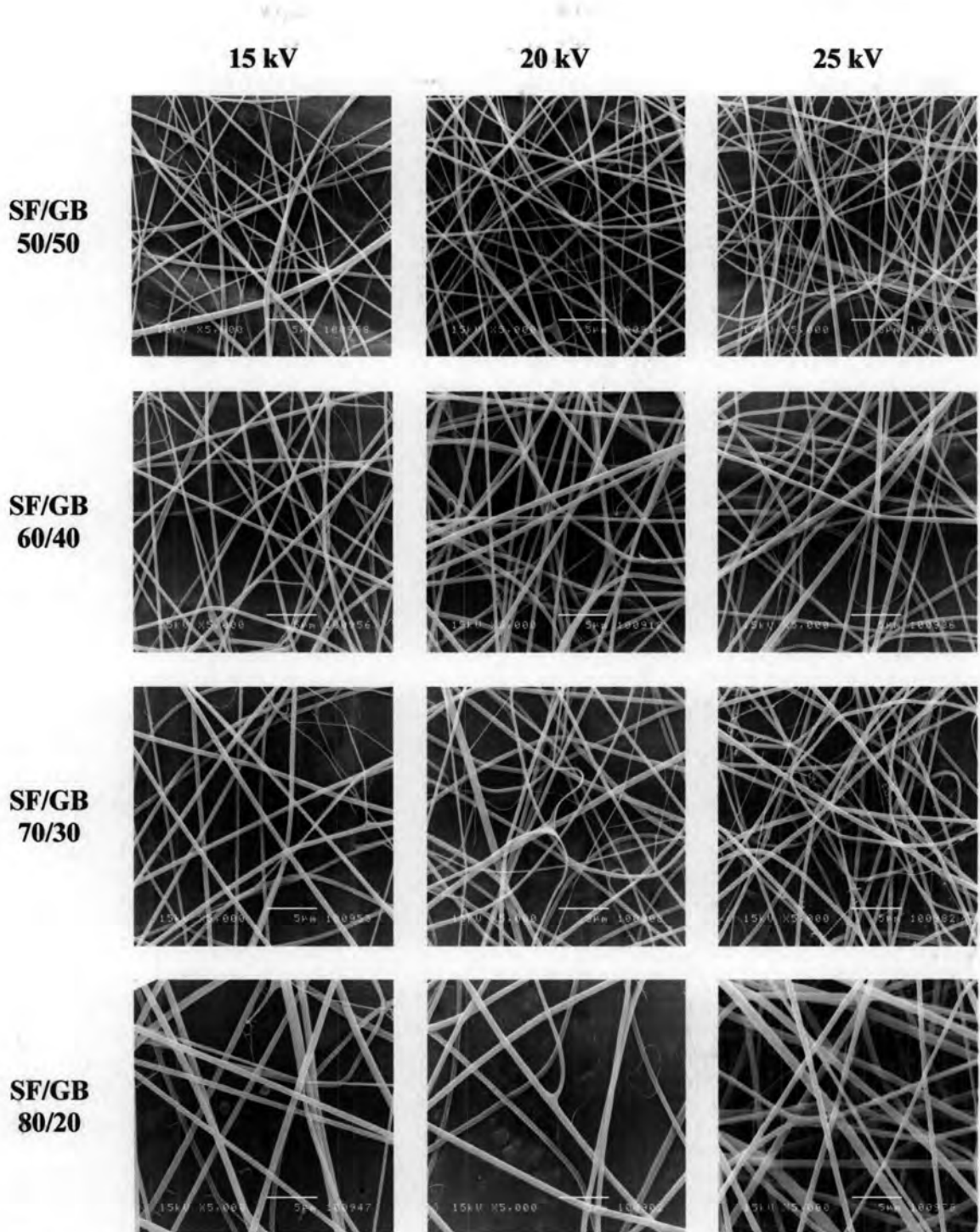
**Figure 4.2** Conductivity of Thai silk fibroin/type B gelatin (SF/GB) blended solution at various weight blending ratios (solution concentration of 20 %w/v)

#### **4.1.2 The effect of applied voltage on morphology and distribution of Thai silk fibroin/type B gelatin fiber diameter**

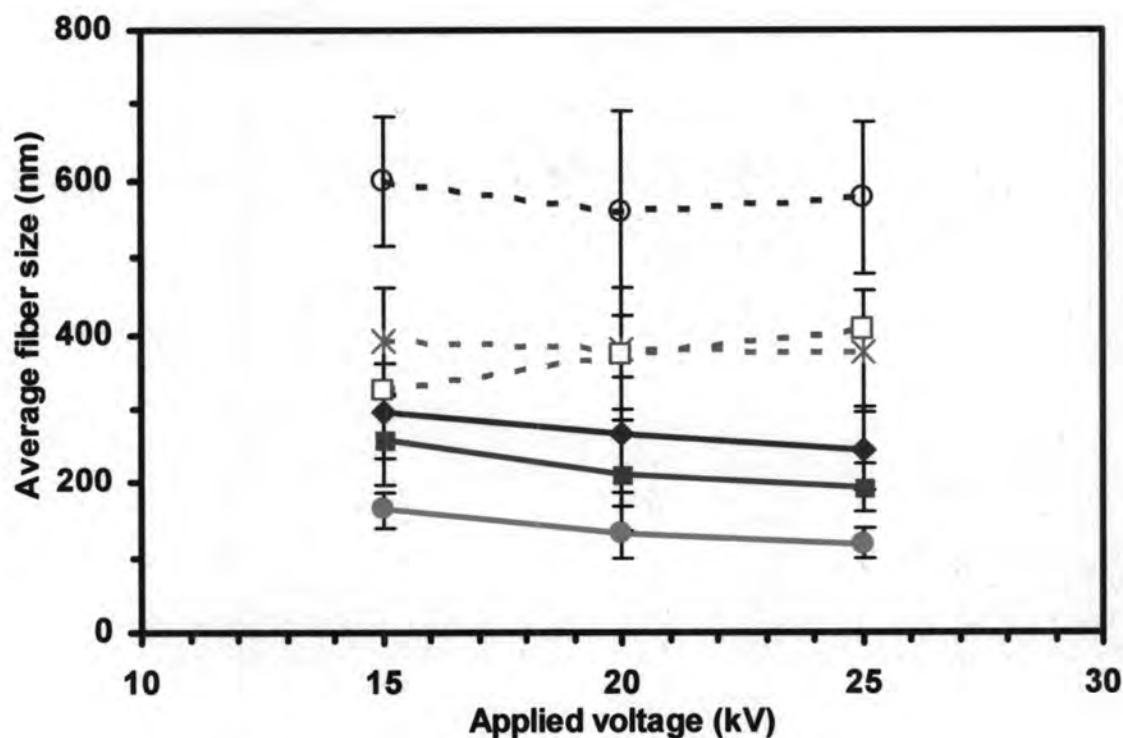
The blended solution of SF/GB was first prepared for the fabrication of electrospun fiber mats. Because the incomplete dissolution of silk fibroin content in formic acid was occurred when silk fibroin content was higher than 80 wt%, the homogeneous Thai silk fibroin/type B gelatin blended solutions with silk fibroin content of 10 - 80 wt% were prepared. Morphology of the SF/GB electrospun fiber mats at various weight blending ratios, which produced from the applied voltage of 15, 20, and 25 kV was demonstrated in Figure 4.3.



**Figure 4.3** SEM micrographs of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats at various weight blending ratios : applied voltage of 15, 20, and 25 kV



**Figure 4.3** SEM micrographs of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats at various weight blending ratios : applied voltage of 15, 20, and 25 kV (continued)



**Figure 4.4** Average fiber size of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats (—●— SF/GB 10/90, —■— SF/GB 30/70, —◆— SF/GB 50/50, - -□- - SF/GB 60/40, - -x - - SF/GB 70/30, - -○- - SF/GB 80/20)

Evidently, non-woven fiber mat arranged randomly with nanometer-scaled fiber diameter. The smooth and continuous fiber with no bead could be produced from all blended solution at three different applied voltages. The uniformity of fiber tended to decrease with an increasing in silk fibroin content. The flat-like fiber was found when silk fibroin content was higher than 50 wt%. Figure 4.4 presented the relationship between fiber size distribution and average fiber size of fiber mats produced from different applied voltage and Table 4.1 summarized the range of fiber size and their average fiber size from SF/GB electrospun fiber at various weight blending ratios and applied voltages. These results clearly showed that higher applied voltage tended to produced smaller fiber size for the solution with SF content of 50 wt% or less. For solutions with higher SF content, the effect of applied voltage was ambiguous. This may be attributed a change in fiber morphology with more ribbon like fiber formation

at these SF content ratio making it more difficult to compare fiber size with different morphology in nature.

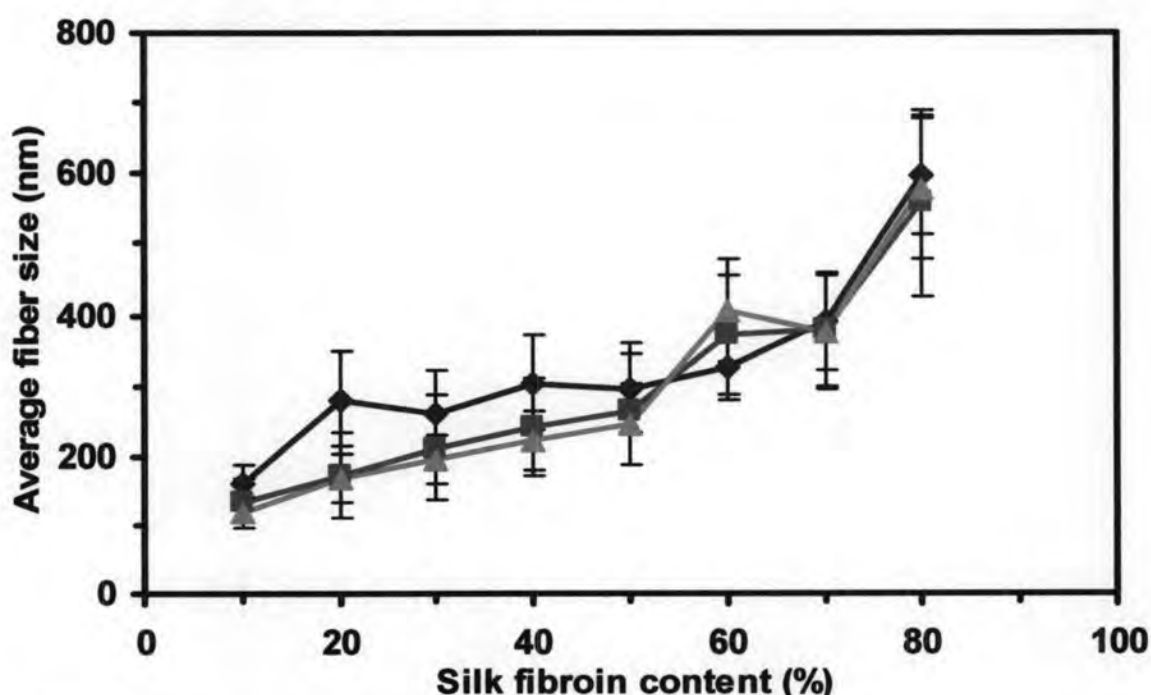
**Table 4.1** SF/GB fiber size at various weight blending ratios and applied voltage

SF/GB blended ratios	The size of SF/GB fiber (nm) at various applied voltage					
	15 kV		20 kV		25 kV	
	Min - Max	Average	Min - Max	Average	Min -Max	Average
<b>10/90</b>	117 - 206	162±24	58 - 248	133±36	79 - 174	118±21
<b>20/80</b>	157 - 404	280±66	64 - 343	172±61	109 - 238	167±35
<b>30/70</b>	153 - 373	258±60	56 - 456	211±75	140 - 267	195±32
<b>40/60</b>	194 - 437	304±65	57 - 444	240±67	137 - 322	221±43
<b>50/50</b>	195 - 427	296±63	58 - 414	265±78	148 - 373	245±56
<b>60/40</b>	210 - 408	325±44	136 - 526	371±82	277 - 529	406±71
<b>70/30</b>	245 - 505	389±68	181 - 512	380±80	220 - 572	376±81
<b>80/20</b>	395 - 735	598±83	102 - 891	557±133	391 - 765	577±98

#### 4.1.3 The effect of silk fibroin content on morphology and distribution of Thai silk fibroin/type B gelatin fiber size

As seen in Figure 4.5, lower silk fibroin content in blended solution resulted in narrow fiber size distribution and low average fiber size. This differed from higher silk fibroin content, which showed broad fiber size distribution and higher average fiber size. It might be affect by the un-uniformity of fiber mats when silk fibroin content was higher than 50 wt%. The result clearly showed that as the silk fibroin content in the solution increased (the viscosity of the solution increased and the conductivity of the solution decreased), the average fiber size decreased. This is because the solution viscosity was a restraint for stretching a charged jet during spinning process, higher viscosity solution requires more force to stretch or elongate the jet segment apart, while

the conductivity can be viewed as a parameter that favors the elongation of the jet. For a given solution dielectric constant and charge mobility, a solution with higher conductivity could have a higher surface charge density, thus, higher columbic repulsion between jet segment which leads to more elongation and a smaller fiber size. Jeeratawatchai (2008) has investigated the morphology of electrospun fiber mats obtained from type B gelatin in formic acid at a solid concentration of 20 wt%. She reported that the fiber diameter of pure type B gelatin was about 150 nm. In addition, Ayutsede *et.al.* (2005) have reported that the diameter of pure silk fibroin electrospun fibers was 397 nm. It can be compared that most fiber diameters of our obtained SF/GB electrospun fiber were in the range of the diameter of two pure components.



**Figure 4.5** Average fiber size of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats obtained from various applied voltage (—◆— 15 kV, —■— 20 kV, —▲— 25 kV)

The results from morphology and fiber size distribution, fiber mats with lower silk fibroin content and obtained from high applied voltage showed more uniformity compared to those of higher silk fibroin content and obtained low applied voltage. Therefore, SF/GB solution at the weight blending ratios of 10/90, 30/70, and



50/50 were chosen to fabricate into fiber mats at applied voltage of 20 kV for further investigation.

## **4.2 The effect of treatment methods on weight loss and morphology of Thai silk fibroin/type B gelatin electrospun fiber mats**

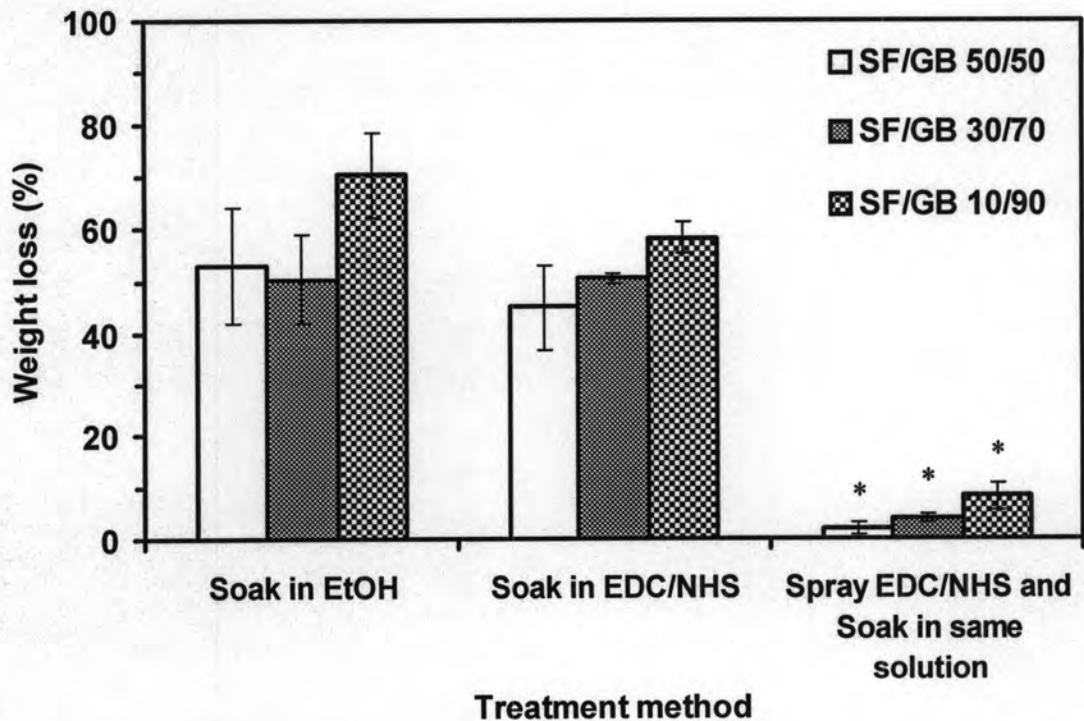
It is known that the structure of regenerated silk fibroin and both types of gelatin are random coil and helix conformation, respectively, which are water soluble and mechanically weak (Weiwei B. *et.al.*, 2008; Guibo Y. *et.al.*, 2008; Songchotikunpun P. *et.al.*, 2008). This can limit their applications. The aim of this part was to prevent the solubility of SF/GB electrospun fiber mats for the controlled release applications.

The experiment consisted of two main parts. First part was the investigation on the alcohol treatment on weight loss and morphology of SF/GB electrospun fiber mats. There were many reports induced the conformational change of silk fibroin from random coil to beta-sheet (crystallization) using methanol (Um I.C., 2001; Gil E., 2006; Ueberax L., 2007). However, the toxicity of methanol has been concerned. Ethanol has become an alternative alcohol, which was also able to induce the conformational change of silk fibroin without the toxicity (Zhang X., 2008).

Second part was the investigation on the effect of crosslinking agent on the weight loss and morphology of SF/GB electrospun fiber mats. Several physical methods including dehydrothermal and ultraviolet irradiation are widely used for the crosslinking treatment. However, these methods are less effective. Some chemical crosslinking agents such as glutaraldehyde, formaldehyde and carbodiimide have been employed to crosslink gelatin molecules (Songchotikunpun P., 2008; Zhang Y.Z., 2006; Yang D., 2007; Lee J., 2008). Although glutaraldehyde is a very effective agent, many researches avoid using this agent due to its cytotoxicity (Liu X., 2009). The 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) with N-hydroxysuccinimide (NHS) was introduced as an alternative crosslinking agent.

#### 4.2.1 Percentage of weight loss of Thai silk fibroin/type B gelatin electrospun fiber mats after treatment

As seen in Figure 4.6, weight loss (%) of the blended SF/GB electrospun fiber mats at 50/50, 30/70, and 10/90 after treated with absolute ethanol for 15 minutes were 53.00, 50.27, and 70.25%, respectively. Gil E. *et.al.* (2006) and Guibo Y. *et.al.* (2008) have reported the conformational change of silk fibroin, the blended membrane with gelatin, from random coil to  $\beta$  sheet using ethanol treatment. Meanwhile, gelatin was not alcohol soluble and not affected by ethanol treatment. Therefore, most of weight loss (%) was expected to come from gelatin content rather than silk fibroin content which might occur during rinsing the electrospun fiber mats with water to removal ethanol residue.



**Figure 4.6** Percentage of weight loss of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats after various treatment methods (\*  $p < 0.05$  compared with other treatment methods at the same weight blending ratios)

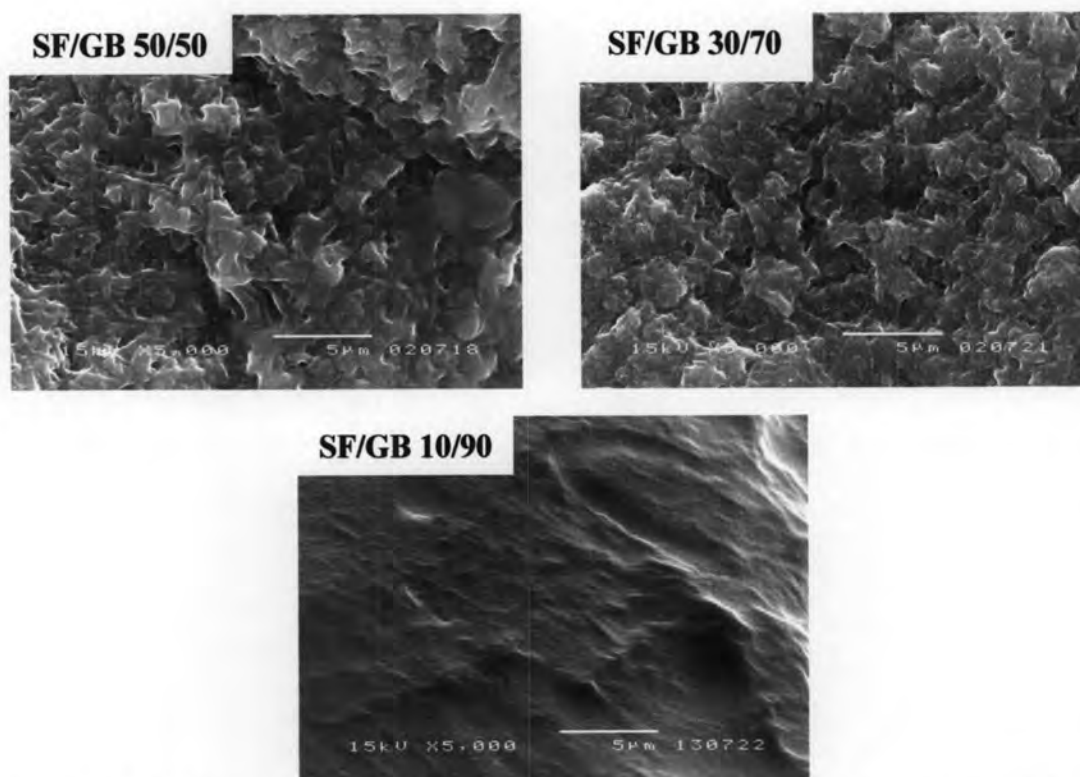
Then the crosslinking of SF/GB electrospun fiber mats by soaking in EDC/NHS dissolved in ethanol solution was introduced to reduce the solubility of blended fiber mats. Considering the blended SF/GB electrospun fiber mats at 50/50, 30/70, and 10/90 after treatment, the weight loss was 44.94, 50.59, and 58.23%, respectively. Therefore, the direct contact of electrospun fiber mats to the EDC/NHS in ethanol solution may cause gelatin loss before the crosslinking reaction could completely occurred. Therefore, the weight loss of fiber mats after this treatment was still high and not suitable for further applications.

To avoid the direct contact with EDC/NHS in ethanol solution, the spraying and soaking in EDC/NHS in ethanol solution (Jeeratawatchai, 2008) was introduced in this blended system. In this case, the weight loss (%) of SF/GB electrospun fiber mats at 50/50, 30/70, and 10/90 after treatment were only 2.02, 4.04, and 8.17%, respectively. The results indicated that the increasing in gelatin content resulted in higher percentage of weight loss. When compared to the system of pure type B gelatin electrospun fiber mats after treatment with the same method, it can be seen that weight loss of blended fiber mats was less than that of pure type B gelatin fiber mats. Therefore, it could be implied that the addition of silk fibroin hindered the dissolution of the blended electrospun fiber mats.

When compared to the other two treatment methods in this study, the spraying and soaking in EDC/NHS in ethanol solution method resulted in much lower weight loss (%). The first spraying EDC/NHS in ethanol solution onto fiber mats allowed the partially crosslinked gelatin molecules and then the treatment by soaking fiber mats into EDC solution could directly crosslink the residue gelatin molecules. In addition, it was suspected that the presence of EDC/NHS did not obstruct the conformational change of silk fibroin (from random coil to  $\beta$ -sheet) by alcohol. On the other words, if no conformational change, silk fibroin would be soluble during washing the fiber mats to remove the residue EDC/NHS. This could be concluded that the treatment by spraying and soaking in EDC/NHS in ethanol solution was an effective method to enhance the dissolubility of the blended SF/GB electrospun fiber mats.

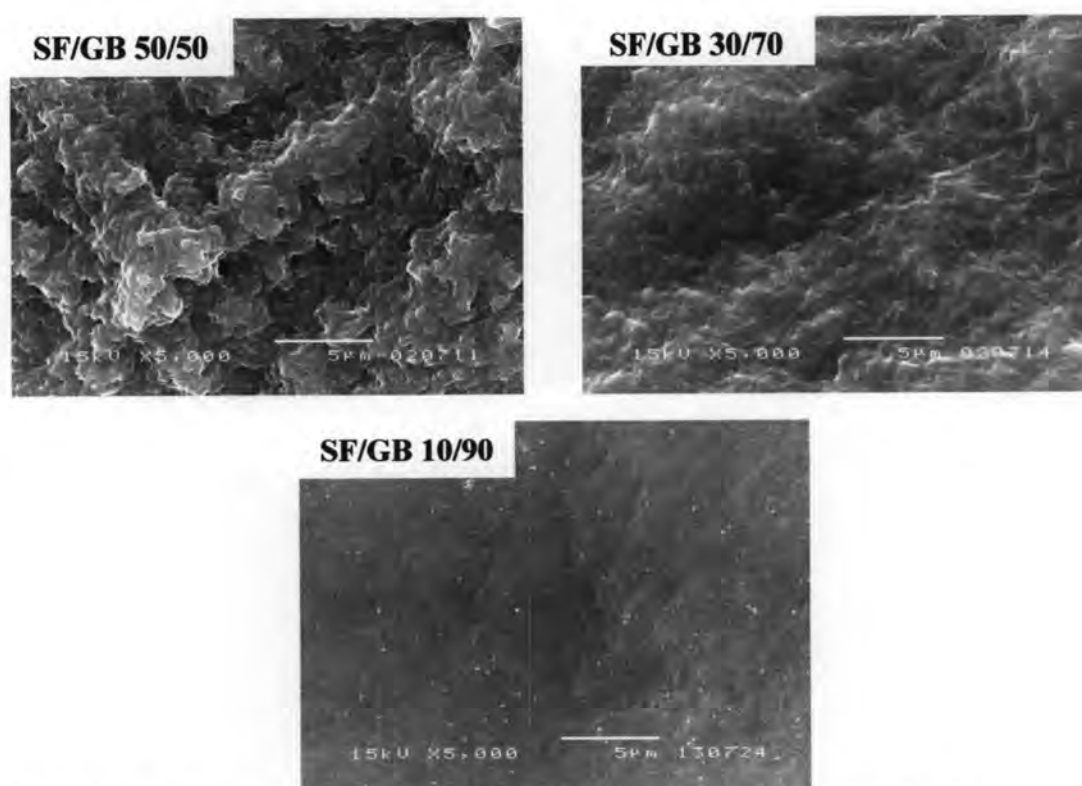
#### 4.2.2 Morphology of Thai silk fibroin/type B gelatin electrospun fiber mats after treatment

Figure 4.7 and Figure 4.8 presented SEM micrographs of SF/GB electrospun fiber mats after soaking in ethanol and EDC/NHS solution. The morphology of blended electrospun fiber mats was similar. It could be seen that both treatment methods induced change in morphology of the blended fiber mats, comparing to the original morphology. Swollen fiber mats in the treated SF/GB at 50/50, and 30/70 were observed. The fiber mats at both contents seemed to fuse together and nearly lost its fibrous structure. On the contrary, the treated SF/GB at 10/90 was fused into film and completely lost its fibrous structure. The much swollen of post-treatment fiber mats corresponded to the high weight loss of fiber mats after soaking in ethanol and EDC/NHS solution.



**Figure 4.7** SEM micrographs of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats at various weight blending ratios after soaking in ethanol for 30 min

The morphology of the blended SF/GB fiber mats after spraying and soaking in EDC/NHS in ethanol was illustrated in Figure 4.9. It could be seen that the morphology of post-treatment electrospun fiber mats differed from the first two treatment methods. Comparing among three blended ratios, the SF/GB fiber mats at 50/50 was less swollen than the others. Its fibrous structure still remained. Whereas, the SF/GB fiber mats at 10/90 was much swollen than the others but some fibers structure could be observed, which different from first two treatment methods (The fiber mats at same blending ratios completely lost porous structure and became film-like). The partially crosslinked by spraying EDC/NHS solution and further soaking in the same solution was shown to be able to maintain fibrous structure of SF/GB fiber mats at 50/50, and 30/70, comparing to the direct soaking in EDC/NHS in ethanol.

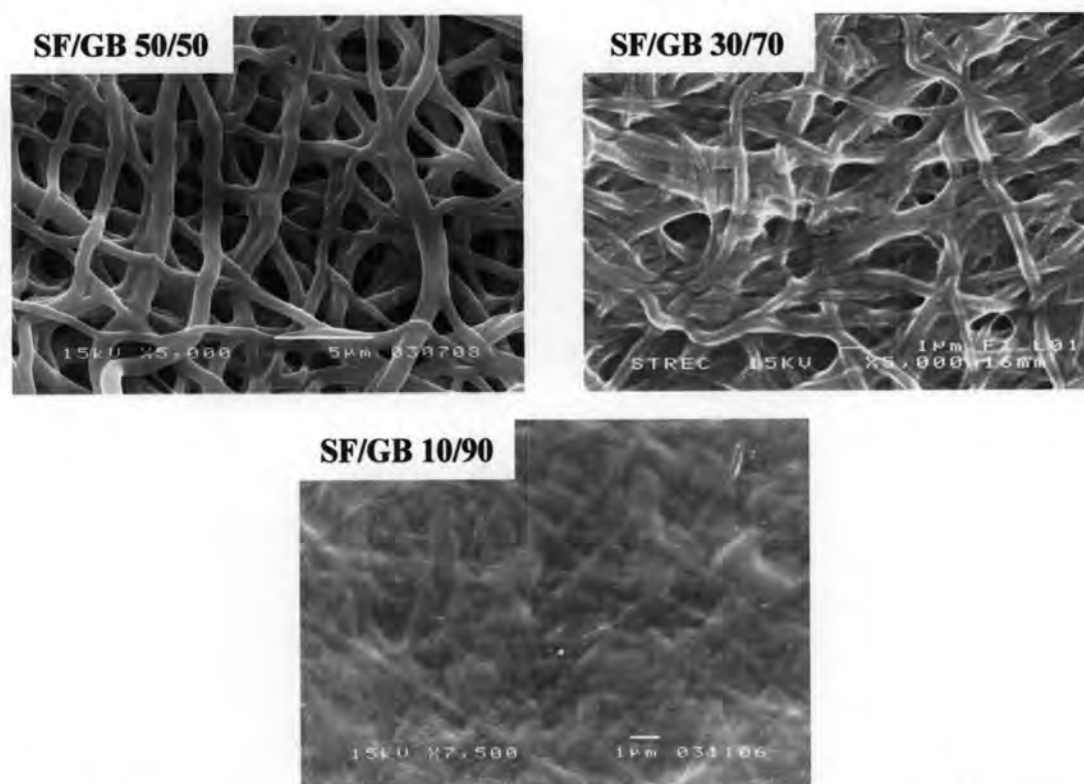


**Figure 4.8** SEM micrographs of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats at various weight blending ratios after soaking in EDC/NHS dissolved in ethanol for 2 h.

The morphology of post-treatment fiber mats also agreed with weight loss of electrospun fiber mats. The blended fiber mats with less lost of weight still remained

the fibrous structure. Comparing among three treatment methods, it could be seen that the fibrous morphology of fiber mats after treatment by spraying and soaking in EDC/NHS in ethanol could still be noticed, similar to the original morphology. In addition, the increasing in gelatin content resulted in fused fiber and less porous structure for all treatment methods.

Based on the results of weight loss and morphology of post-treatment fiber mats, spraying and soaking in EDC/NHS in ethanol solution method was chosen to treat Thai silk fibroin/type B gelatin electrospun fiber mats for further investigation.



**Figure 4.9** SEM micrographs of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats at various weight blending ratios after spraying and soaking in EDC/NHS dissolved in ethanol for 2 h.

#### 4.2.3 Mechanical properties of SF/GB electrospun fiber mats

The electrospun fiber mats should have appropriate mechanical properties in order to be handled in controlled release applications. The tensile strength, Young's modulus and strain at break of SF/GB electrospun fiber mats after treatment by

spraying and soaking in EDC/NHS solution were summarized in Table 4.2. The Young's modulus of SF/GB fiber mats at 50/50, and 30/70 were 2461.20 MPa, and 308.32 MPa, respectively. The fiber mats with high gelatin content had low Young's modulus, representing a soft characteristic. While the fiber mats with high silk fibroin content was more rigid. Furthermore, the tensile strength of both fiber mats exhibited correlated behavior as same as Young's modulus. The tensile strength of SF/GB electrospun fiber mats at 50/50, and 30/70 were  $653.77 \pm 129.43$  kPa, and  $406.75 \pm 81.66$  kPa, respectively. The results indicated that the weight blending ratio affected the tensile strength of the electrospun fiber mats. The tensile strength of the electrospun fiber mats with high silk fibroin content was higher than that of fiber mats with low silk fibroin content. Therefore, the addition of silk fibroin could enhance mechanical properties of blended fiber mats. In this study, we found that the wet SF/GB fiber mats at 10/90 was too weak to be tested for its mechanical properties. It was damaged during the test.

**Table 4.2** Mechanical properties of wet Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats

SF/GB blending ratios (wt%)	Tensile strength (kPa)	Young's modulus (MPa)	Strain at break (%)
50/50	$653.77 \pm 129.43$	2426.10	$21.30 \pm 11.53$
30/70	$406.75 \pm 81.66$	308.32	$41.60 \pm 19.59$
10/90	N/A	N/A	N/A

Yang D. *et.al.* (2007) have investigated poly vinyl alcohol (PVA)/gelatin electrospun fiber mats for the controlled release of raspberry ketone. They reported that the addition of PVA could improve tensile strength of gelatin nanofibers. At 10-50 wt% of PVA content, the tensile strength increased from 2.4 MPa to 6.3 MPa. Similarly, Mobarakeh G.L. *et.al.* (2008) reported that the blending of gelatin with polycaprolactone (PCL) caused a reduction in mechanical strength of the blended electrospun fiber mats. Both reports corresponded to the weak mechanical properties of fiber mats with high gelatin content found in this work.

Chen C. *et.al.* (2006) have been studied the electrospinning of all-aqueous silk fibroin and the mechanical properties of as-spun fiber before and after methanol treatment. The break stress of as-spun fiber (0.82 MPa) was found to increase after methanol treatment (1.49 MPa). Compared between the blended SF/GB fiber mats with the as-spun silk fibroin fiber after methanol treatment, the addition of gelatin content reduced the strength of the blended fiber mat. The less in tensile strength of the blended SF/GB fiber mats compare to the literatures caused by wet condition in tensile processing.

Therefore, the mechanical properties of the blended SF/GB fiber mats depended on the blending ratios of silk fibroin and gelatin. Higher amount of silk resulted in stronger Thai silk fibroin/type B gelatin electrospun fiber mats.

### **4.3 *In vitro* biodegradation of Thai silk fibroin/type B gelatin electrospun fiber mats**

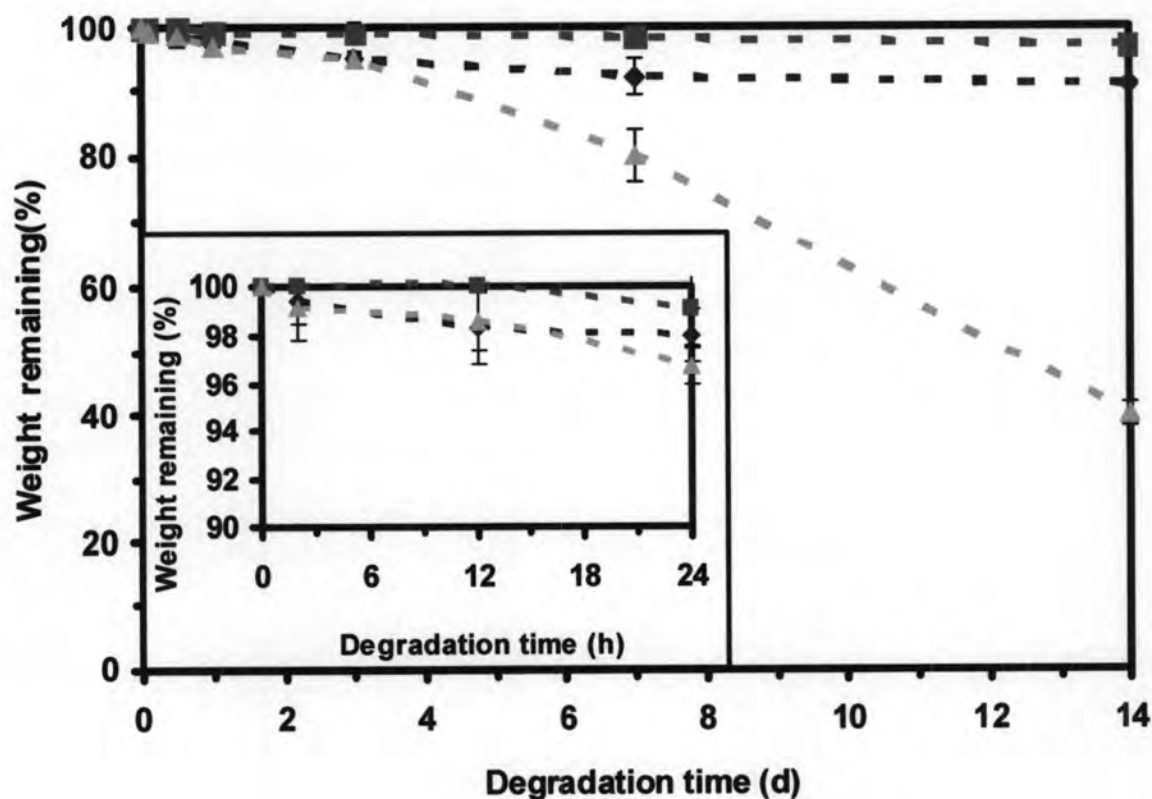
The biodegradation behavior of three blended electrospun fiber mats was first examined in phosphate buffer saline (PBS at pH 7.4) at 37°C. To simulate the degradation behavior in human body, the degradation of three blended fiber mats was further investigated in collagenase solution (pH 7.4) at 37°C. The remaining weight of electrospun fiber mats after degraded at various degradation times was reported.

#### **4.3.1 *In vitro* biodegradation in phosphate buffer saline**

The degradation of Thai silk fibroin/type B gelatin electrospun fiber mats were investigated in PBS solution over 14-day period. As seen in Figure 4.10, the difference in weight loss of the blended fiber mats can be observed along incubation time. The weight of SF/GB electrospun fiber mats gradually decreased when incubated in PBS for a longer time, except the fiber mats at 10/90. SF/GB fiber mats at 10/90 degraded more rapidly than the other two. After 14-day of incubation, the remaining weights of SF/GB fiber mats at 50/50, 30/70, and 10/90 were 90.87, 96.97, and 40.16 %, respectively. Comparing three blending ratios, the higher silk fibroin content in the blended fiber



mats resulted in less loss of weight. Therefore, the addition of silk fibroin could enhance hydrolytic degradation of silk fibroin/gelatin electrospun fiber mats. Owing to silk fibroin after exposed in alcohol is  $\beta$ -sheet conformation and hydrophobic, the weight loss of the blended fiber mats was considered to be the weight loss of gelatin content. Mandal B. *et.al.* (2009) reported that the presence of crystalline silk fibroin act as water insoluble backbone hindering the complete degradation of the blended silk fibroin/gelatin fiber mats. Furthermore, Ulubayram K *et.al.* (2001) also stated that both bulk and surface degradation of gelatin rapidly occurred because gelatin is highly hydrophilic, which corresponded to this present work. The weight of fiber mats with 90wt% gelatin lost very rapidly.



**Figure 4.10** Remaining weight of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats after degraded in phosphate buffer saline solution at 37°C over 14-day period (- -◆- -SF/GB 50/50, - -■- - SF/GB 30/70, - -▲- - SF/GB 10/90)

#### 4.3.2 *In vitro* biodegradation in collagenase solution

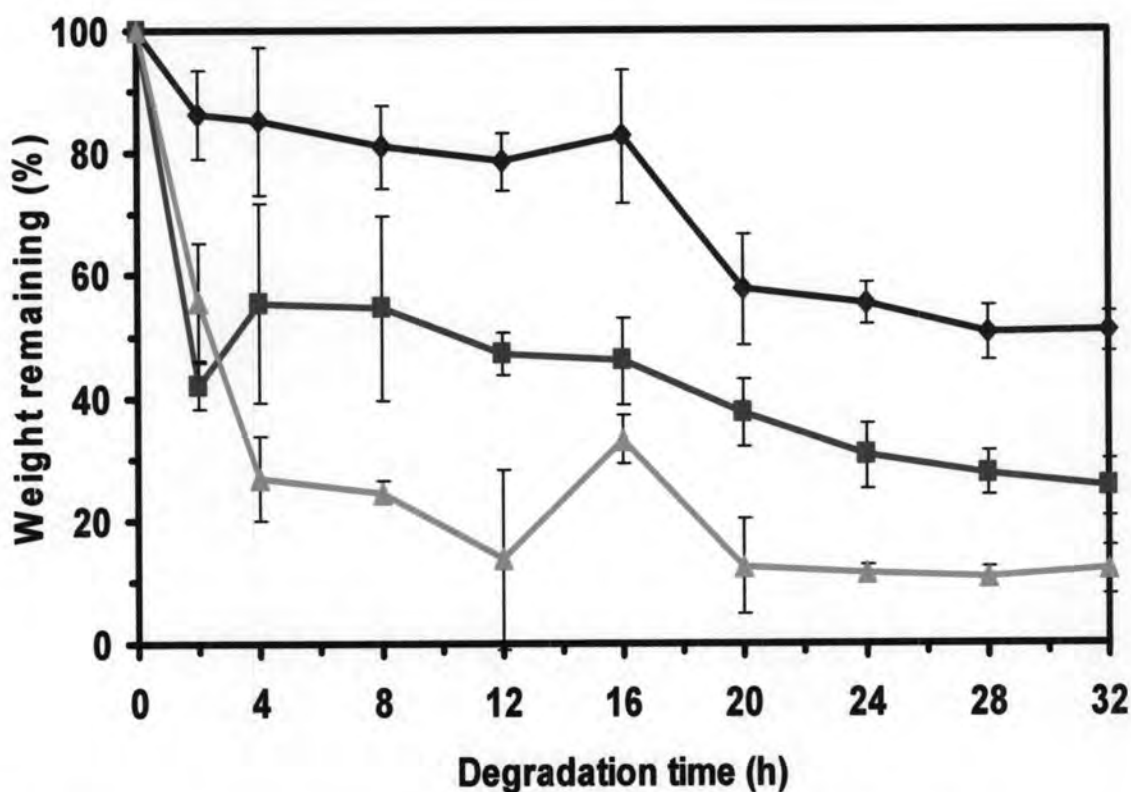
As collagenase naturally presented in body fluid, *in vitro* biodegradation behavior of Thai silk fibroin/type B gelatin electrospun fiber mats in collagenase solution at 37°C was investigated. Figure 4.11 illustrated the remaining weight of SF/GB electrospun fiber mats at various weight blending ratios after degraded in collagenase solution at 37°C for 32-h period. After 2 h of incubation, the weight of SF/GB electrospun fiber mats at 50/50 was decreased by 10% while that of the other two fiber mats at the blending ratios of 30/70 and 10/90 was decreased rapidly by 40% and 50%, respectively. Considering the time needed to achieve 50wt% weight loss as the half-life of SF/GB fiber mats, it was found that the half-life of SF/GB fiber mats at 50/50, 30/70, and 10/90 weight blending ratios was 32, 10, and 2 h, respectively (Table 4.3). The results revealed that SF/GB fiber mats with higher gelatin content degraded faster than the ones with lower gelatin content. After 32 h of incubation, the weight of SF/GB fiber mats at 50/50, 30/70, and 10/90 decreased to 50.71%, 25.30%, and 11.90% of their initial weight. Also, it can be obviously observed that the weight loss of fiber mats after exposed to collagenase for 32 h was approximately the amount of gelatin contained in blended fiber mats. This implied that gelatin component was directly hydrolysed by collagenase, while silk fibroin component might resist to collagenase hydrolysis, due to its  $\beta$ -sheet conformation and hydrophobicity.

**Table 4.3 Half life of the blended electrospun fiber mats**

SF/GB electrospun fiber mats	Half life (h)
50/50	32
30/70	10
10/90	2

This result corresponded to the research of Jeeratawachai (2008). She has investigated the *in vitro* biodegradation of type B gelatin electrospun fiber mats in 0.01 U/ml collagenase solution. The fiber mats was found to remain about 20% of its initial weight after 4 h of degradation. Comparing to the degradation behavior of SF/GB fiber mats in this study, the remaining weight of the SF/GB fiber mats at 10/90 was 26.88%

after 4 h at degradation and about 11.89% after 32 h of biodegradation. It could be concluded that the blended SF/GB fiber mats degraded slower than the pure gelatin fiber mats. The addition of silk fibroin could prolong the biodegradation of SF/GB blended fiber mats in collagenase solution.



**Figure 4.11** Remaining weight of Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats after exposed in collagenase solution at 37°C (—◆— SF/GB 50/50, —■— SF/GB 30/70, —▲— SF/GB 10/90)

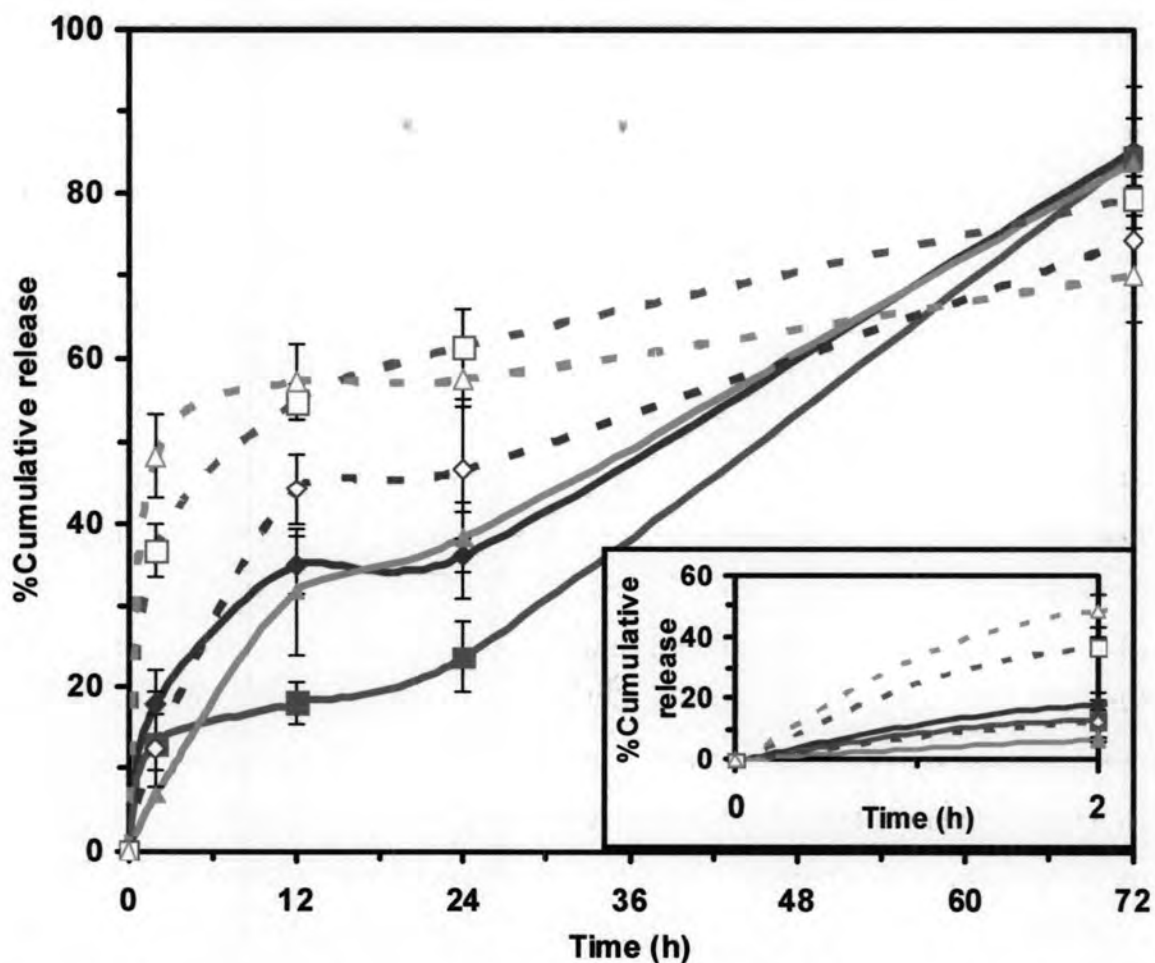
#### 4.4 *In vitro* release of model compounds from Thai silk fibroin/type B gelatin electrospun fiber mats

An advantage of nanofibers favorable for controlled release applications is their high surface to volume ratio. Active agents can be loaded at higher doses compared to materials prepared from other techniques. Various techniques used to load active agents onto fiber mats including adsorption, embedding and encapsulation (Sill T. *et.al.*, 2008). In this study, adsorption onto fiber mats was selected in order to avoid dissolution of

fiber mats. Some volume of active agents was used to ensure all active agents absorbed onto fiber mats. Three model active agents including methylene blue, azo-casein and nerve growth factor were investigated. Estimated electrostatic charges of materials and three model active agents are shown in Table 4.4.

#### 4.4.1 Release behavior of azo-casein

The release profile of azo-casein from SF/GB electrospun fiber mats was illustrated in Figure 4.12. Rapid burst of azo-casein was observed in all samples in the first 2-12h indicating its excess amount which not absorbed tightly on the fiber mats. The release was plateau at 2-12 h before the continuous increases. At the beginning (0 h), no release of azo-casein was observed from all weight blending ratios of the blended fiber mats containing 5 and 10 mg of azo-casein per g of fiber mats. Considering the release of azo-casein from fiber mats with loading amount of 5 mg of azo-casein per g of fiber mats, 17.90%, 13.19%, and 6.86% of azo-casein were released from SF/GB 50/50, 30/70, and 10/90 fiber mats, 2-h of incubation, respectively. The release of azo-casein from SF/GB 50/50, 30/70, and 10/90 blended fiber mats was found to increase to 36.07%, 23.70%, and 38.28%, respectively at 24-h of incubation. After that, it was continuously released from three blended fiber mats, to approximately 85% at 72-h of incubation. Considering 10 mg of azo-casein loading per g of fiber mats, SF/GB at 50/50, 30/70, and 10/90 released azo-casein about 46.74%, 64.44%, and 57.48% within 24-h. Then the cumulative release of NGF from SF/GB at 50/50, 30/70, and 10/90 fiber mats was 74.24%, 79.36%, and 70.09% at 72-h, respectively. Comparing between two loading amount, the release behavior was similar. The release behavior of azo-casein from SF/GB electrospun fiber mats could be explained based on electrostatic charge as shown in Table 4.3. In PBS (pH 7.4), azo-casein with an isoelectric point of 6 was negatively charged, same as both silk fibroin and type B gelatin with an isoelectric point of 3 and 4-5, respectively. The repulsion force between azo-casein and materials would cause the rapid release of azo-casein from electrospun fiber mats along a period of 72-h. This resulted in continuously diffusion of azo-casein from SF/GB electrospun fiber mats.



**Figure 4.12** *In vitro* release of azo-casein from Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats in phosphate buffer saline over 72-h period (—◆—SF/GB 50/50, —■—SF/GB 30/70, —▲— SF/GB 10/90; close and open marker represented 5 and 10 mg of azo-casein/g of fiber mats, respectively.)

**Table 4.4** Charges of materials and model compounds

Materials and compounds	pI	Charge at pH 7.4	References
Silk fibroin	~3	negative (-)	(Philips G.O., 2000)
Type B gelatin	~ 4-5	negative (-)	(Philips G.O., 2000)
Azo-casein	~ 6	negative (-)	(Philips G.O., 2000)
Nerve growth factor	~ 9	positive (+)	(Philips G.O., 2000)
Methylene blue	-	positive (+)	www.americanchemistry.com

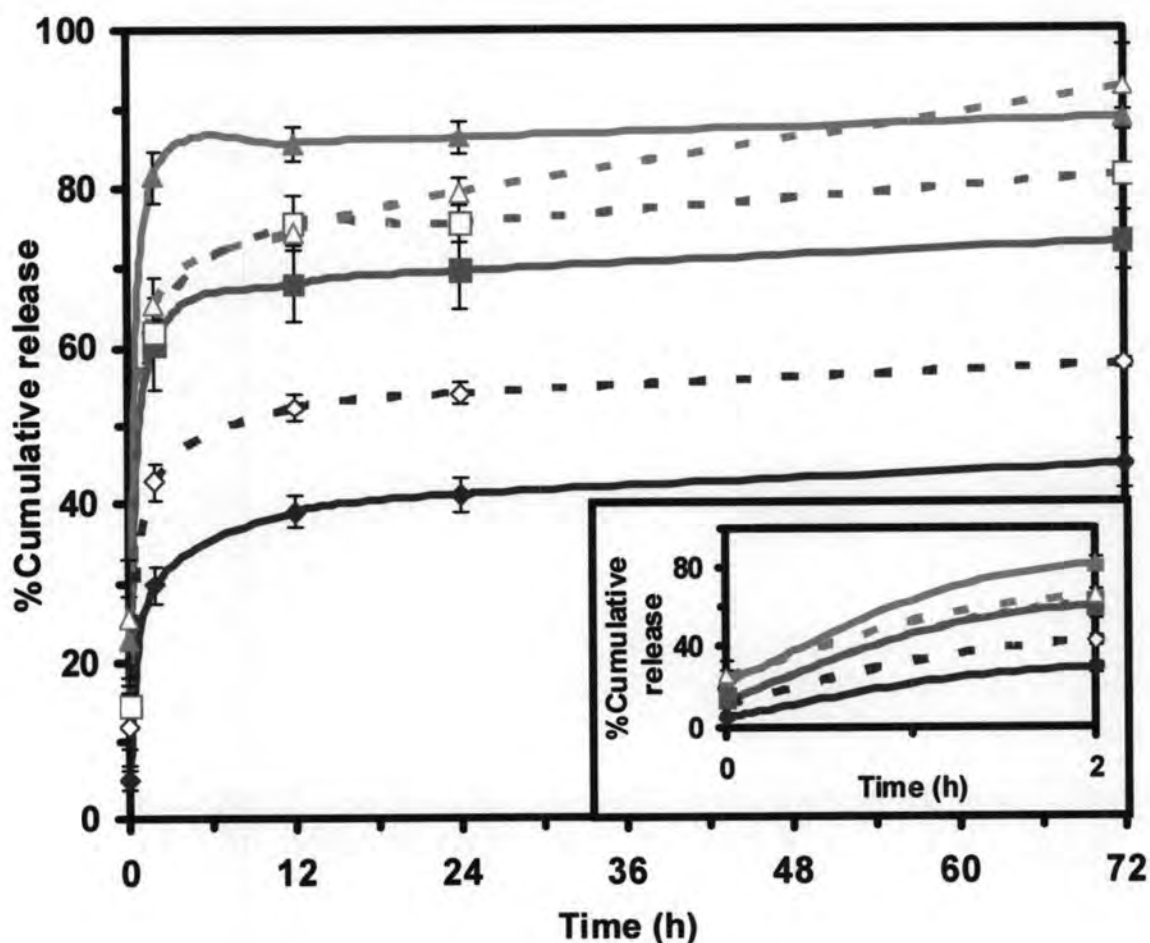
#### 4.4.2 Release behavior of methylene blue

The release profile of methylene blue in PBS (pH 7.4) at 37°C was illustrated in Figure 4.13. The influence of loading amount of methylene (5 and 10 mg of methylene blue per g of fiber mats) on release profile was examined, similar to the case of azocasein. The blended fiber mats containing high loading amount of methylene blue showed high percentage of methylene blue released. Considering the release behavior of methylene blue from SF/GB fiber mats containing low loading amount of methylene blue, at the beginning (0 h), SF/GB 50/50, 30/70, and 10/90 electrospun fiber mats released 5.14%, 14.54%, and 22.73% of methylene blue, respectively. The percentages of cumulative release were increased to 29.69%, 60.53%, and 81.51% within the first 2 h, respectively. This result implied the excess amount of methylene blue, which could not be absorbed into fiber mats and could be released rapidly. At 24h, the release of methylene blue from SF/GB fiber mats at 50/50, 30/70, and 10/90 increased slightly to 38.90%, 68.05%, and 85.68% and sustained to 44.67%, 73.11%, and 88.71% at 72 h of incubation, respectively. From the result, it could be stated that the blended fiber mats was able to control the slow release of methylene blue.

The charges of methylene blue and blended fiber mats would be considered to comprehend the release behavior of methylene blue. As shown in Table 4.4, silk fibroin and type B gelatin carried negative charges, while methylene carried positive charge, resulting in the formation of electrostatic attraction force between methylene blue and fiber mats. Furthermore, it was observed that, at  $t=0$ , the amount of methylene blue released from the fiber mats with higher silk fibroin content was less than that released from the fiber mats with lower silk fibroin content. This result indicated that three SF/GB blended fiber mats could absorb different amount of methylene blue. As shown in Table 4.4, IEP of silk fibroin was lower than that of type B gelatin (IEP of silk fibroin and type B gelatin was 3 and 4-5, respectively). An attraction force between silk fibroin and methylene blue was then stronger than that of type B gelatin and methylene blue, resulting in less methylene blue released from fiber mats with higher silk fibroin content at the beginning (0 h).

The release profiles which plateaued after 12 h in all samples showed that the SF/GB blended fiber mats could sustain release of methylene blue along the period of

72 h, the high percentage of cumulative release was shown (i.e. SF/GB fiber mats at 30/70, and 10/90 released 73.11% and 88.71% of methylene blue, respectively). Each release profile showed amount of methylene blue absorbed on each type of fiber mats.



**Figure 4.13** *In vitro* release of methylene blue from Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber in phosphate buffer saline over 72-h period (—◆—SF/GB 50/50, —■—SF/GB 30/70, —▲—SF/GB 10/90 close and open marker represented 5 and 10 mg of methylene blue/g of fiber mats, respectively.)

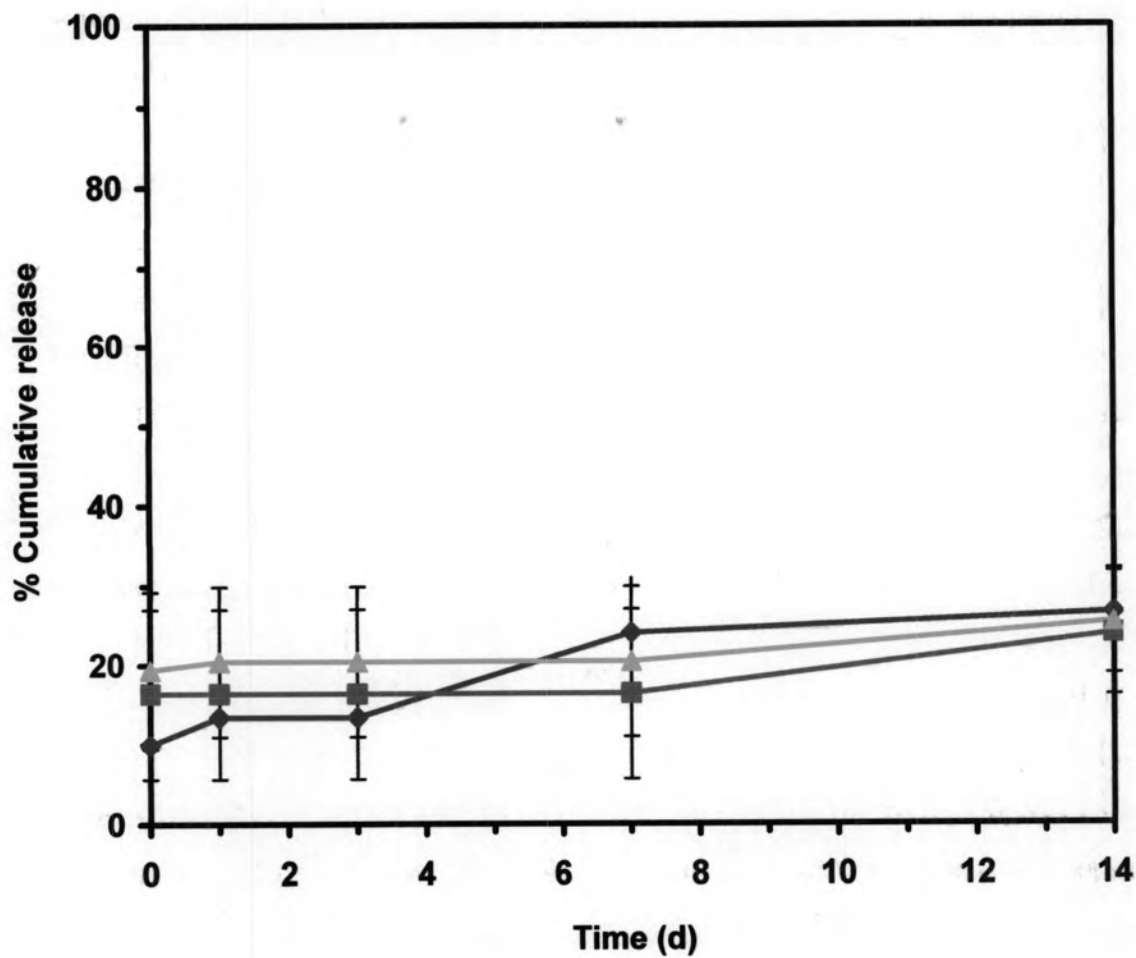
From these results, electrostatic interaction was an important parameter which affected the release profiles of two model compounds at 72-h. Repulsion force between fiber mats and azo-casein resulted in a rapid diffusion of compound throughout fiber matrix, while attraction interaction between fiber mats and methylene blue resulted in a

low diffusion of compound at the beginning. Then, the release of compound was controlled by biodegradation of fiber mats. More biodegradation of fiber mats could result in higher amount of released compounds.

#### 4.4.3 Release behavior of nerve growth factor

Figure 4.14 demonstrated *in vitro* release behavior of NGF from SF/GB blended fiber mats in phosphate buffer saline. The burst release of NGF from SF/GB fiber mats at 50/50, 30/70, and 10/90 was obviously observed at 9.90%, 16.36%, and 19.32%, respectively ( $t=0$  h), indicated amount of nerve growth factor absorbed on the fiber mats. The amount of released NGF indicated excess NGF that was not absorbed into the blended fiber mats. It could be seen that the blended fiber mats with higher silk fibroin content released less NGF than the ones with lower silk fibroin content. This implied that silk fibroin content might be able to absorb NGF higher than gelatin content because an attraction interaction between silk fibroin and NGF was greater than that of type B gelatin and NGF. After 14 days of incubation, NGF released from three SF/GB blended fiber mats at 50/50, 30/70, and 10/90 was slightly increased to 26.58%, 23.88%, and 25.40%, respectively. This result suggested that three SF/GB blended fiber mats were able to sustain the release of NGF by an attraction force. After 14 days of incubation, the fiber mats were then completely digested by excess collagenase, in order to release all residual NGF from fiber mats. 71.46%, 74.26%, and 71.85% of NGF was detected from SF/GB fiber mats at 50/50, 30/70, and 10/90, respectively. This result could ensure that the biodegradation of SF/GB fiber mats was main mechanism of the NGF release. However, the effect of SF/GB blending ratios on release behavior of NGF in phosphate buffer saline was not clearly observed. Further investigation on the controlled release test of this system in collagenase solution should be performed to confirm the influence of three SF/GB fiber mats with different blending ratios.





**Figure 4.14** *In vitro* release of nerve growth factor from Thai silk fibroin/type B gelatin (SF/GB) electrospun fiber mats in phosphate buffer saline over 14-day period (—◆—SF/GB 50/50, —■—SF/GB 30/70, —▲—SF/GB 10/90)