CHAPTER VI
RESPONSIVE PHASE-TRANSITION OF POLY (ACRYLIC ACID) NANO FIBROUS TUBE FOR NERVE EXITATION MODEL

6.1 Abstract

PAA nanofibrous tube was successfully prepared using electrospinning process which was obtained the fine and uniform fiber. The PAA tubes were further improved their thermal stability by mean of crosslinking with Ethylene glycol. The reducing of crosslinker concentration has slightly effect on PAA fiber which showed the fused and large fiber. The phase transition of the mimic nerve was shown strongly responding when using 150 mM NaCl (which is the physiological salt concentration in the body) as an immersion medium. The imitated model was expressed the temperature dependence by mean that the Ca exchange to Na-PAA tube was favorable when temperature was increased. Moreover, the calcium influx of PAA tube was disturbed by adding some anesthetics. The mimic nerve was showed properties similar to living axons which can be suggested that this material can be use as an artificial nerve.

6.2 Introduction

It is well known that responsive living material can be constructed as Mimics of Biology. The living system was clearly showed the reaction or response when environment change which is call "irritability" [1]. One of the most compromising studies in artificial cell responding is nanofibrous cell cortex/cytoskeleton capable of phase transition. Nerve fiber (axon) is the currently accepted investigation for irritability. The origin of evolution is the study of physiological action by using electric shock [2]. The providing of electrolytes, which is used as an immersion medium for nerve fiber, clearly expressed that the membrane of axons has the cation exchange property [3, 4]. The existent of divalent cation binding bridges has effect on structure of axons as well as the polyanionic synthetic polymers [5, 6].

Electrospinning, a simple and cost-effective technique, is one of the straightforward ways utilized for fabricating fibrous materials, which was obtained large surface area to volume, flexible surface functionality, and superior mechanical performance [7].

This technique can be generated nanometer in diameter fiber which can be compared to biology size such as Cytoskeleton fiber, ECM fiber and Axon. Poly (acrylic acid), PAA is defined as a cation membrane which can be bound with positively charge ions including monovalent cations, divalent cations and ionized organic chemical [8-11]. The interested property of the PAA is their ability to swell during moist environment [12].

In this study, nanofibrous PAA tube was prepared by electrospinning process. The nanofibrous tubes were further improved thermal stabilization via crosslinking with Ethylene glycol in the acid catalyst. The PAA tube was used as an imitate nerve excitation model which can be expressed the phase transition of the Ca-influx to the surface of the tube. The several effects including concentration of surrounding salt, phase transition in the anesthetized region and temperature effect on the ion exchange reaction of the mimic axon were investigated which can be measured phase transition from the contraction percentage of the tube after providing cations. In addition, the model of mimic nerve was evaluated for their potential use as artificial axons.

6.3 Experimental

6.3.1 Materials

Poly (acrylic acid) (PAA; M_w = 450,000 $gmol^{-1}$) and Sodium Citrate were purchased from Sigma-Aldrich, USA. Poly (vinyl pyrrolidone) (PVP; M_w = 360,000) was purchased from Scientific Polymer Products, USA. Calcium chloride anhydrous, sodium chloride, chloroform, sulfuric acid and diethyl ether were purchased from Fisher Scientific, USA. All of the chemical were the analytical grade and used without further purification.

6.3.2 Electrospinning

Poly (acrylic acid), 0.6 g was dissolved in ethanol, 18.25 mL (4 % w/v). Next, ethylene glycol, 0.09 mL (16 % ethylene glycol which was related to polymer weight) was added into the PAA solution and stir over night. The resulting solution is colorless and clear. A few drops of sulfuric acid which acts as a catalyst was added just before the electrospinning process.

The experiment set up consisted of a syringe and needle, power supply (model CZE1000R, Spellman), syringe pump (KD Scientific), and a variable speed, rotating, stainless steel grounded counter-electrode drum. In order to facilitate removal of PAA tube from the mandrel, the designed experiments was using spin coating of 10% PVP in ethanol (1 mL) as the first layer of the electrospun fiber and further spin PAA on as the upper layer. Briefly, the solutions were loaded into a 5 mL syringe with a blunt-tip 18-guage needle (0.8 mm OD) and placed 20 cm away from the leading face of the rotating collection target. The solutions were electrified by applying a positive voltage (15 kV) to the syringe needle by means of an alligator clamp. The solutions were transferred through syringe pumps with the mass flow rate of 0.8 mL/h. All experiments were carried out at room temperature and relative humidity of 20%. Then, the PAA tube was further cross-linked using oven at 135 ^{o} C for 30 minutes. The final step was dipping of the processed mandrel in ethanol for 2 h in order to dissolve the excess of PAA which was not cross-linked and dissolved the PVP layer out leading to remove nanofibrous tube easily. The obtained PAA tube needed to neutralize using the equally mixed between 1 M NaCl and 1 M NaOH solution before using.

6.3.3 Effect of Salt Concentrations on Contraction Transition of Na- PAA Nanofibrous Tube

The neutralized PAA tube was used to study on their contraction behavior after binding with 1 M $CaCl_2$. First step, the tubes were cut into a 2 cm length long and further immersed into the different salt solution flasks (i.e. 0, 20, 40, 80 and 150 mM NaCl). Afterward, the $CaCl_2$ was added to the solution flask and then shakes for 15 minute. The certain amount of $CaCl_2$ was kept adding until the titration curve reached the equilibrium which meant to be the fully contraction of the tube.

6.3.4 Preparation of Ca^{2+} Rich PAA Tube (Ca-PAA tube)

The PAA tube was cut into a 2 cm length for each. Then, the tubes were put in the 150 mM NaCl solution flask that being as a Na^{+} rich medium and measured the length again because there was some swellings occur during immersion. The

specified concentration of calcium chloride was added to the flask in order to provide crosslinking bridge between the chains that led to tube contraction.

$$Length\ Contraction\ (\%) = \frac{Ls}{Lo}\ x\ 100 \qquad (1)$$

Where *Ls* is the length of the tube after shrinkage and *Lo* is the original length of the tube

The dried PAA tube, the Na-PAA tube and the obtained Ca-PAA tube were further measured on diameter as well as length change both before and after titration using microscope in order to study on anisotropic property.

6.3.5 Effect of Temperature on Ca-PAA Tube

The temperature dependence experiment was investigated the contraction behavior under the temperature change of the tube using Max Q mini 4450 incubating and shaking machine as a temperature controller. The procedure almost the same as preparing the ca^{2+} rich PAA tube with using 2, 3, 10 and 30 mM of $CaCl_2$ at given temperature including 0, 25, 37, 45 and 55 $^{\circ}$C (in 150 mM NaCl) and measure the contraction percentage under temperature change.

6.3.6 Surface Morphology of Electrospun Fibers

Firstly, the electrospun fibers with different crosslinker concentrations (i.e. 10 % and 16% ethylene glycol) were coated using gold sputtering thickness 10 nm. The morphology of the PAA electrospun tube was observed by scanning electron microscope (SEM; JSM-6510, JEOL) with 10 kv voltage operation.

6.3.7 Effect of Temperature on Lightly Crosslink Nanofibrous PAA Tube

The lightly crosslink of nanofibrous PAA tube was prepared with the same procedure of PAA tube but using 10 % ethylene glycol related to polymer weight instead. The temperature effect was carried out at various temperatures including 0, 25, 37, 45 and 55 $^{\circ}$C (in 150 mM NaCl) and investigates the percentage of contraction change with temperature.

6.3.8 PAA Nanofibrous Contraction Behaviors in the Anesthetized Region

This experiment was set to imitate the influx of calcium to responsive nerve in anesthetized region (organic compounds environment). Hydrophobic solvents including chloroform and diethyl ether were interested in their effect on swelling behavior of Ca-PAA tube. The 1 %v/v chloroform and 2 %v/v diethyl ether were added (keep adding, 0.5 mL at each time point) to the 150 mM NaCl solution flask which was used as immersion medium for Ca-PAA tube. All experiments were set at 37 ^{o} C using Max Q mini 4450 incubating and sharking machine. The contraction percentage was measured at each time points including 5, 10, 15, 30, 60, 90 and 120 minutes, respectively. The different of diethyl ether concentration were used to study on anesthetic concentration dependence on Ca-PAA tube.

6.4 Results and Discussions

6.4.1 Effect of salt concentrations on contraction transition of Na- PAA nanofibrous tube

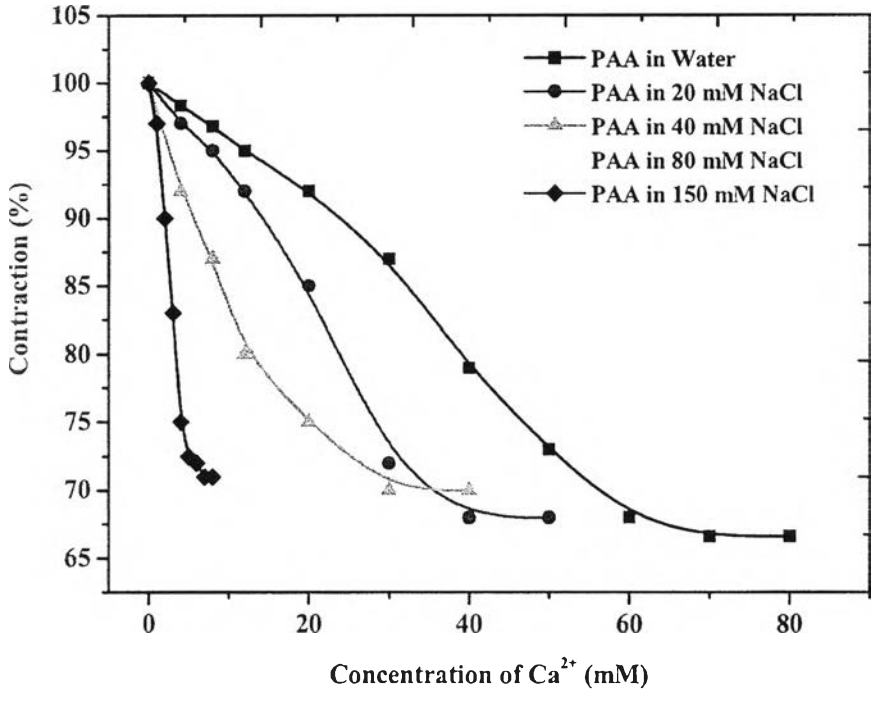

Figure 6.1 The effect of increasing surrounding salt concentrations on contraction transition of Na-PAA tubes (titration with 1 M $CaCl_2$).

After titration the Na-PAA tubes immersed in salt solutions (NaCl) with $CaCl_2$, the replacement of Ca^{2+} from $CaCl_2$ to Na-PAA tubes was occurred leading to shrinkage of the tubes which can be observed by the reduction in length of the tubes. The expected mechanism was resulting from the loosely bound of Na^+ on polyacrylate, which led to reduce repulsive force between polymer chains, was facilitated Ca^{2+} replacement [13]. All of the Na-PAA tubes in different salt concentrations including 0, 20, 40, 80 and 150 mM NaCl were clearly showed the fully contraction around 65-70 % after $CaCl_2$ titration.

As increasing salt concentrations, the contraction transition become sharp transition (Fig 6.1) (the rapid shrinkage of the tube after added even small amount of Ca^{2+}) which meant to be an increasing of cooperative calcium ions with increasing surrounding sodium ions due to ionic screening. The highest salt concentration of this experiment was equal to physiological salt concentration of the human body which is 150 mM NaCl, which possibly simplified as Na/Ca exchange of an axon.

6.4.2 Diameter and longitudinal change of Dried PAA tube, Na-PAA tube and Ca-PAA tube

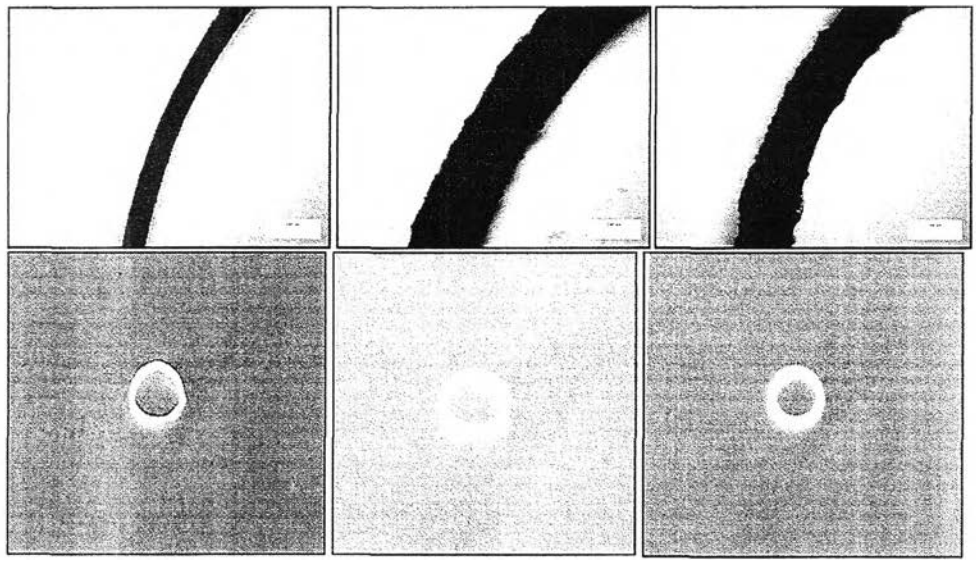

Figure 6.2 Representative of thickness and diameter of Dried PAA, Na-PAA tube and Ca-PAA tube using microscope.

Table 6. 1 Representative of thickness (μm) and diameter (mm) of Dried PAA, Na-PAA tube and Ca-PAA tube

Sample	Thickness (μm)	Diameter (mm)	Length (mm)
Dried PAA	82.03±2.63	4.22±0.01	20.23±0.01
Na-PAA tube	312.98±2.44	5.68±0.01	23.54±0.01
Ca-PAA tube	265.65±1.08	3.24±0.01	15.46±0.01

From Figure 6.2 represented of thickness, diameter and length of Dried PAA, Na-PAA tube and Ca-PAA tube. The Na-PAA nanofibrous tube showed the highest thickness, diameter and length compare to Dried PAA and Ca-PAA resulting from swelling of the tube while Na^- /H^+ exchange from carboxylic proton in the chain and Na^+ in the solution, table 1 [14].

The diameter and length change of PAA tube (after Na^+/Ca^{2+} exchange) can be calculated using equation (1). From calculation showed the significantly different in diameter and in longitudinal length of the tube which were 84.87 % diameter change and 65.67 % longitudinal change. From the results showed the responsive contraction of the PAA tube in longitudinal direction over other directions. Therefore, the PAA nanofibrous tube clearly showed an anisotropic property in comparable to nerve fiber which mainly provide oriented in longitudinal direction over other directions [15][16].

6.4.3 Effect of Temperature on Ca-PAA Tube

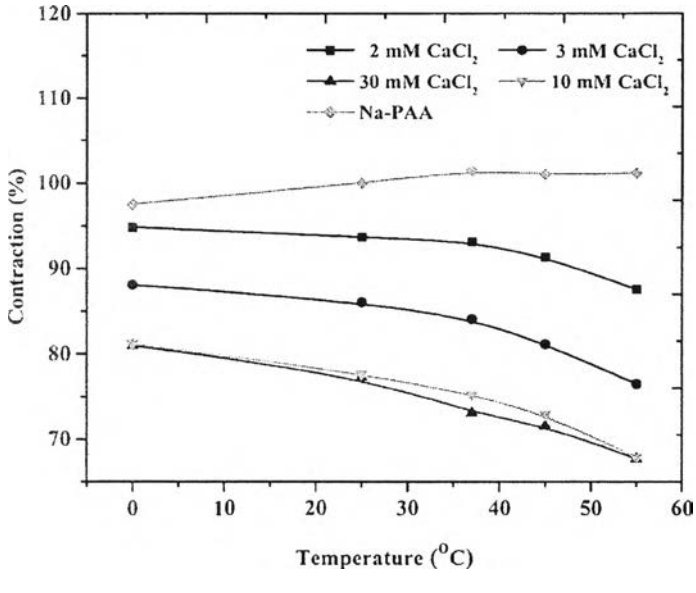

Figure 6.3 The effect of temperature change on Ca-PAA tubes with different $CaCl_2$ titration concentrations i.e. 2, 3, 10 and 30 mM respectively. The swelling behaviors of Ca-PAA tubes were compared to swelling behavior of Na-PAA tube while the temperature change.

The Na^+ from the Na-PAA tube was exchanged with Ca^{2+} during temperature change (the temperature were increased and decreased as a cycle and passed to the particular temperature point i.e. 0, 25, 37, 45 and 55 °C) with keep concentration of added Ca^{2+} ions constant, the changing in temperature had significant effect on Na/Ca-PAA exchange (Fig 6.3). The results showed that increased in temperature leading to higher contraction which meant to be higher binding site of polyacrylate to Ca^{2+}. The result showed the highest contraction was around 65 % at 55 °C.

The different added $CaCl_2$ concentrations (2, 3, 10 and 30 mM $CaCl_2$) was the another parallel factor which was interested to satisfy of this temperature dependence contraction behavior of the Na/Ca exchange of PAA tube. From Figure 3, the contraction directly depend on added Ca^{2+} concentrations which expressed higher shrinkage when increased Ca^{2+} concentration. There were significantly different contraction percentage after adding 2 and 3 mM $CaCl_2$ that around 89% and 79%, respectively. Both 10 and 30 mM of added $CaCl_2$ were not showed much different because of reaching the maximum contraction of the tubes.

$$PAA\text{-}2Na^+ + Ca^{2+} \rightleftharpoons PAA\text{-}Ca^{2+} + 2Na^+ \qquad (*)$$

*Ca^{2+}/PAA-Na^+ replacement reaction, ΔH (+)

The Ca exchange to Na-PAA chains was favorable when temperature was increased resulting from the replacement reaction of Na-ion in the exchanger with Ca-ion is endothermic reaction (*) according to Le Chatelier-Braun's law [17]. From the obtained temperature dependence result can be explained in molecular level that the increasing in temperature can be increased in entropy leading to disordered layer of water molecule around the polymer chains and providing the negatively charges binding site [18] which facilitated positively charge binding of Ca^{2+}.

6.4.4 Surface morphology of Electrospun fibers

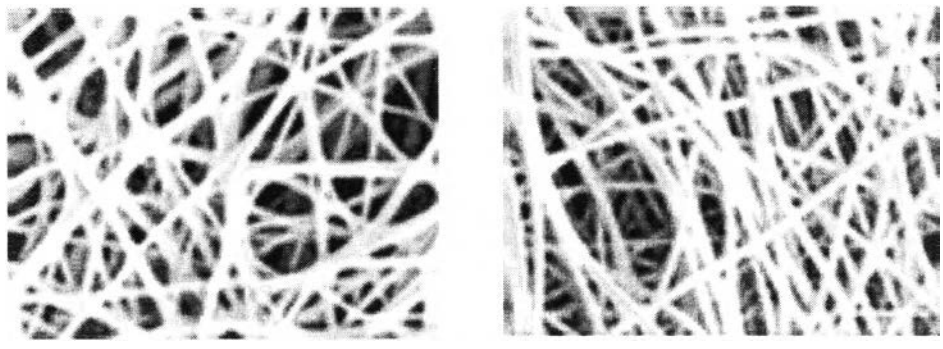

Figure 6.4 The SEM images of electrospun fiber of PAA with different crosslinker concentration i.e. 10% (a) and 16% (b) ethylene glycol related to polymer weight.

The 16 % ethylene glycol cross-linked PAA fiber showed uniform and fine fiber which had an average of the fiber diameter around 865±68 nm (Fig 6.4b). Unlike, the PAA fiber using 10 % ethylene glycol as a crosslinker which showed fused fiber as well as droplet with slightly larger fiber diameter than when using higher crosslinker concentration. There were many parameters that have effect on electrospun fiber including polymer concentration, flow rate, voltage and cross-linker concentration. By lowering concentration of the crosslinker directly led to lower viscosity and higher the surface tension resulting in the obtained fibers were not uniform as shown in Fig 6.4a. [19][20][21].

6.4.5 Effect of Temperature on Lightly Crosslink Nanofibrous PAA Tube

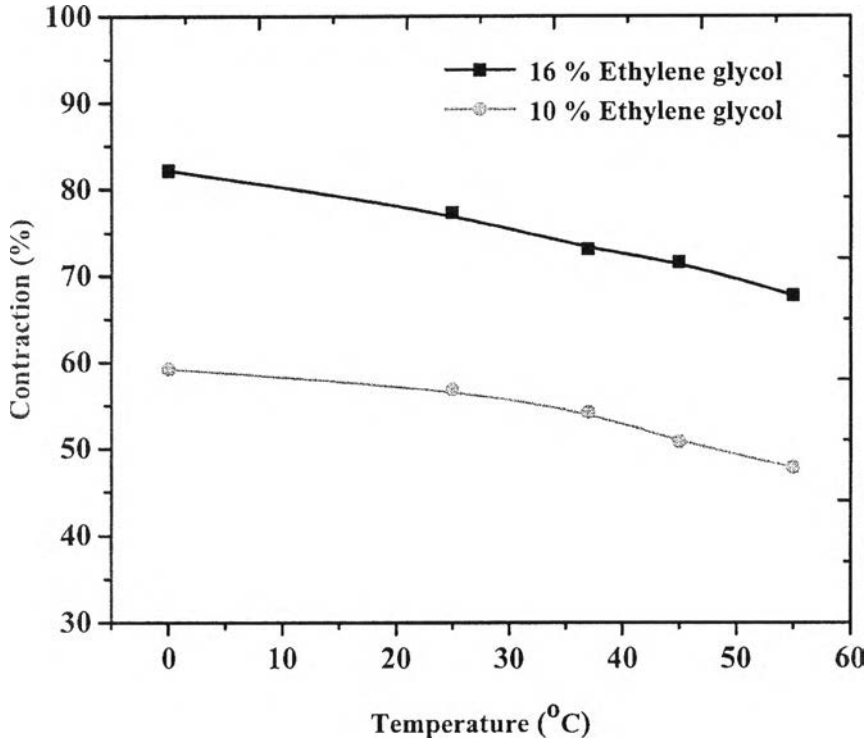

Figure 6.5 The effect of temperature on Ca-PAA tubes with different crosslink density i.e. 10% and 16% ethylene glycol related to polymer weight. The swelling behavior of Ca-PAA tube was compared to swelling behavior of Na-PAA tube whiles the temperature changes.

The Na-PAA tubes with different crosslinking conditions, which were used 10% and 16% ethylene glycol as crosslinker, showed similar temperature dependence by replacing Na^{+} from Na-PAA tube to Ca^{2+}. From Figure 6.5, lightly crosslinked PAA fiber provided strongly dependence with temperature which expressed in form of much lower contraction percentage. This result can be explained that reducing in crosslinker concentration leading to less crosslink density as well as crossliking points which provided freely movement of the chains and easily bound with other ions.

It can be expressed in term of large active surface on the fiber due to large fiber diameter leading to more binding sites on the fiber chains and increased in contraction of the tube.

The responsive property of lightly crosslink PAA fiber which had slightly large fiber diameter was similar to previous study on vertebrate nerve fibers [22]. It was reported that the responsive of the activation process was increased with increased nerve fiber diameter. Likewise, the responsive process for invertebrate nerves fiber which provided much different in responsive by using giant nerves (large diameter of the nerve fiber) of the squid [23].

6.4.6 PAA Nanofibrous Contraction Behaviors in the Anesthetized Region

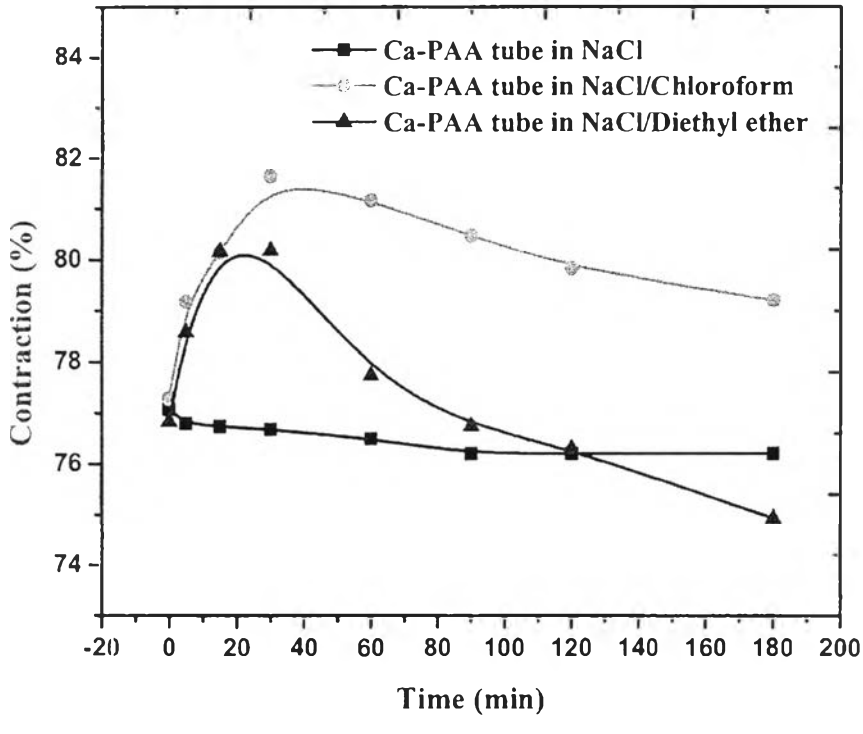

Figure 6.6 The effect of organic solvents on Ca-PAA tube in 150 mM NaCl including Chloroform and Diethyl ether. The transitions were compared to swelling behavior of Ca-PAA tube without any solvents.

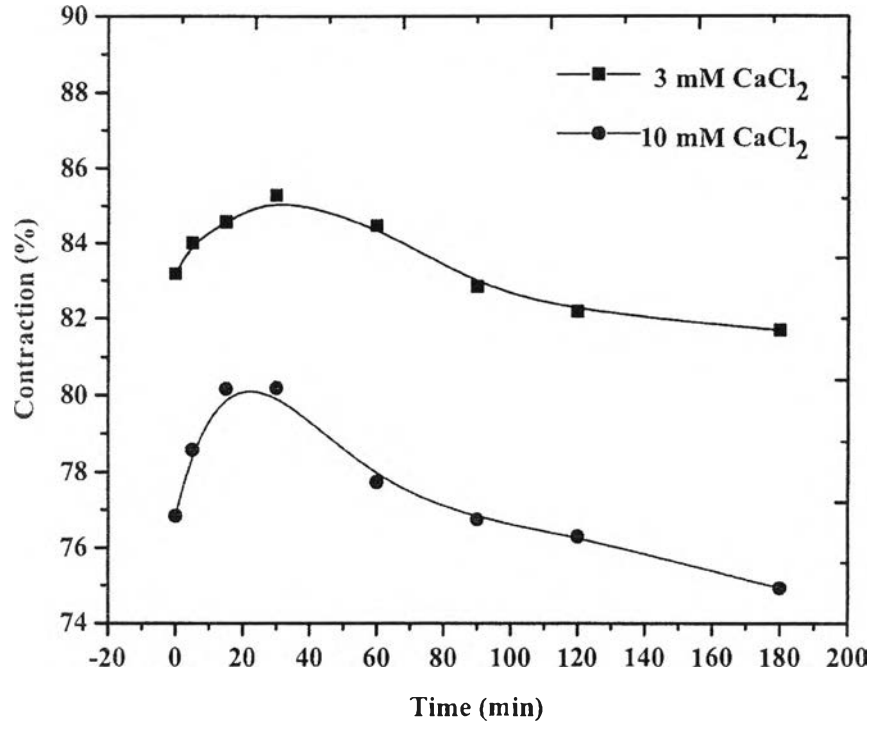

Figure 6.7 The effect of organic solvents on Ca-PAA tube in 150 mM NaCl adding Diethyl ether. The transitions were compared to different added $CaCl_2$ concentrations (3 mM and 10 mM $CaCl_2$).

Both diethyl ether and chloroform were assumed to be the anesthetics for the plastic nerve (Fig. 6.6). The results showed the time dependence of contracted plastic nerve after adding organic solvents. The effect of anesthetics showed the same transition curve even using different amount of calcium binding PAA fibers. The PAA-Ca used 3 mM and 10 mM $CaCl_2$ to prepare Ca^{2+} rich PAA tube (Fig.6.7). The Ca-PAA tube was initially expanded and then started to contract after 30 min for diethyl ether and 40 min for chloroform (Fig.6.6). There were 2 possibilities to explain this phenomenon (Fig. 6.8). First possibility is the sorption organic solvent in to hydrophobic chains in the nanofibrous tube leading to expansion.

Afterword, if the concentration of organic solvent is high enough, the Ca-PAA tube was started to shrink because of polymer collapse in poor solvent. Second possibility, organic solvent might weak calcium binding due to carboxylate interactions encouraging exchange with excess Na^+ led to expansion because of reducing in calcium bridging between the chains. However, the exchange of Na^+ to Ca-PAA tube also raised calcium free ions in the solution, $[ca^{2+}]_{ex}$ which can be bound in to polymer chains resulting in chain shrinkage.

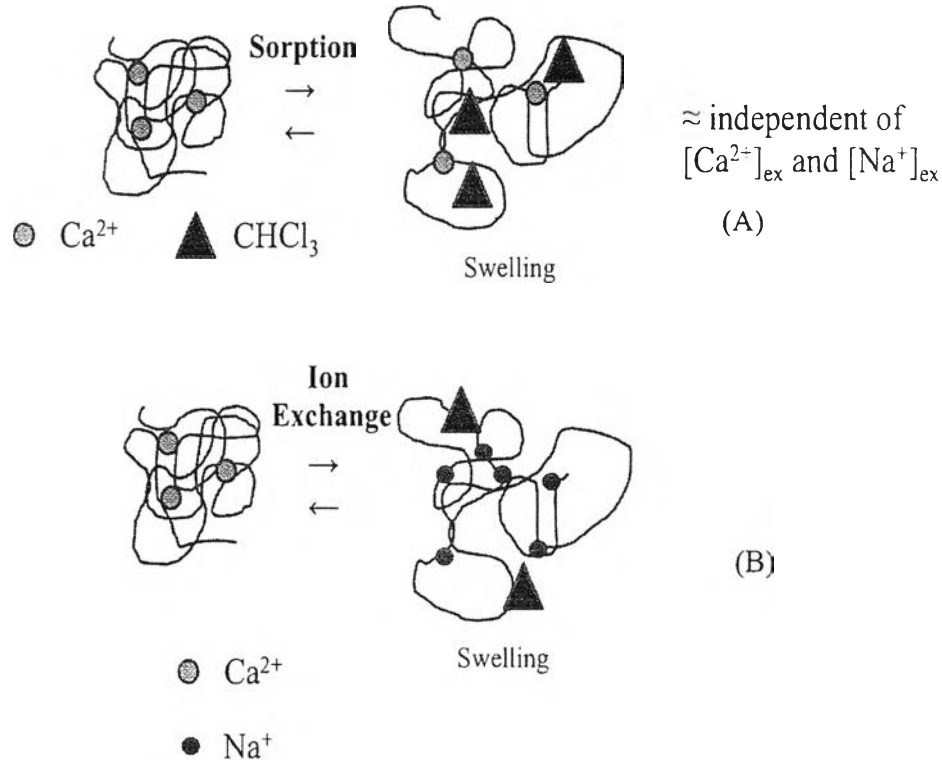

Figure 6.8 Schematic of two possibilities expression plastic nerve behavior in the anesthetic region: (a) Sorption of Chloroform, (b) Ion Exchange of excess Na^+

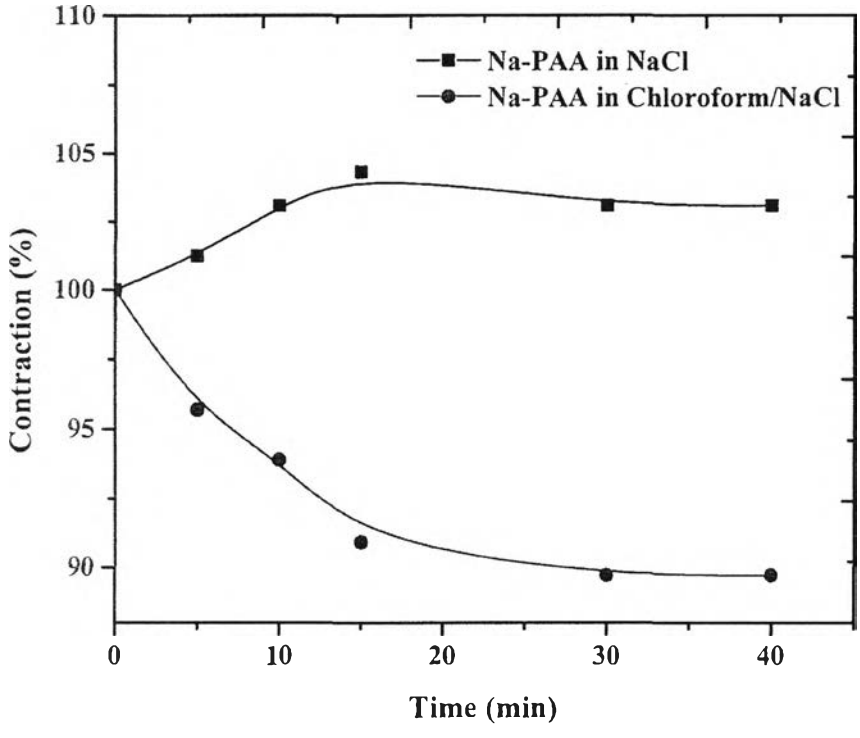

Figure 6.9 Dimensional changes of PAA-Na^+ Electrospun Tubes (in 150 mM NaCl) upon addition of chloroform

The anesthetics also have the effect on PAA-Na tube which preferred to shrink after adding chloroform instead of swelling as usual (Fig.6.9). The behavior of plastic nerves in the anesthetized region was comparable to isolated single nerve fibers from previous studies. The experiment was set by applying anesthetics to nerves fiber, the physical properties of the nerve including threshold, action current amplitude, etc. was effected immediately after adding anesthetics and gradually constant[24][25]. Moreover, it was reported that the effect of anesthetic on nerve fiber showed not only concentration dependence but also time dependence [26].

6.5 Conclusion

The PAA nanofibrous tube was successfully prepared using electrospinning process and further modified for their thermal stability by mean of crosslinking with ethylene glycol at high temperature. The obtained PAA tube showed the responsive to ions change in the medium which was clearly showed the cycle of shrinkage and expansion during ions exchange occurred. Moreover, the rapid responsive of the Na/Ca exchange of the PAA tube was occurred when using physiological salt concentration of the human body (150 mM NaCl) as an immersion medium. The transition of the PAA tube showed the dependence on temperature change of the system which was directly depend on contraction behavior (the contraction increased when temperature increased). Likewise, the PAA nanofibrous model was clearly showed the dependence on added organic solvent. The decreased in crosslinker concentration had effect on fiber shape and diameter which showed more effect on contraction when increased temperature than that when using higher crosslinker concentration. All of the model experiment can be related to ions influx of an living axon which can be concluded that PAA nanofibrous tube can be used as an artificial nerve.

6.6 Acknowledgement

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6.7 References

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