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EFFECT OF CALCIUM CHLORIDE ON ELECTROSPINNING OF SILK FIBROIN NANOFIBRES

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Abstract

Silk fibroin is one of the candidate materials for biomedical application because it has good biocompatibility and minimal inflammatory reaction. Electrospinning is a simple method capable of producing nanofibres for biomedical applications. This study was focused on the production of a nonwoven sheet from silk fibroin using a needleless electrospinning method and concentrated on an alternative way of spinning solution preparation by using a mixture of formic acid and calcium chloride as a solvent. The effects of salt concentrations in formic acid and the voltage of electric field on fibre morphology were studied. It was observed that nanofibre has a good uniform fibre distribution on the nonwoven sheet by increasing the applied voltage. Calcium chloride increases the solubility of silk fibre in formic acid with 2 wt% calcium chloride being preferred. The silk nanofibres had diameters ranging from 200 to 2300 nm.

Keywords: silk fibroin, needleless electrospinning, calcium chloride

1. Introduction

In recent years polymer nanofibres have gained considerable attention as promising materials with many possible areas of application due to their unique properties such as a high specific surface area, small pore diameters, high surface to weight ratio, and good barrier characteristics against microorganisms [1,2]. They can be used as filters, wound dressings, tissue engineering scaffolds etc. There are several methods to produce fibres at a nanoscale, electrospinning is one of these methods that has gained much attention because it is an effective method for the manufacture of ultrafine fibres or fibrous structures from both synthetic and natural polymers with diameters ranging from several micrometers down to tens of nanometres [3]. In the spinning process, a high voltage is used to create an electrically charged jet of a polymer solution or a molten polymer. This jet is collected on a target as a non-woven fabric.

Silk fibroin is the protein that forms filaments of silkworm and gives high mechanical strength, elasticity and softness. In addition to the outstanding mechanical properties, silk fibroin is a candidate material for biomedical application because it has a good biological compatibility, good oxygen and water vapour permeability, biodegradability and minimal inflammatory reaction. Silk fibroin can be used in surgery as implant material as well as for tissue engineering applications in the form of non-woven membranes and fibres or in the form of woven membranes and fibres. It has been demonstrated that silk fibroin-derived scaffolds may have a widerange of applications in the fabrication of replacement tissue. However, recent studies demonstrated that thesericin glue-like proteins are the major cause of adverse problems and the biological responses to the fibroin fibres appear to be improved if sericin is removed [4].

In order to regenerate silk fibre into a membrane, nonwoven mat or nanofibre sheet, the preliminary dissolution of silk fibreis required to prepare the spinning solution. Natural silk fibres dissolve only in a limited number of solvents; close packing of the polypeptide chains in the protein is possible due to their presence in fibroin which has a large quantity of glycine and amino acids with hydrocarbon side chains. Consequently, hydrogen bonds are an important bond in the conformation and structure of the fibroin. The influence of hydrogen bonding on the stability of fibroin molecules can be shown by the ease of dissolution of the protein in known hydrogen bond breaking solvents [5, 6].

Fibroin does not dissolve in water or in the majority of organic solvents but only swells to 30-40%;two thirds of the absorbed solvent is retained by the amorphous fraction of the polymer.Fibroin can be dissolved in concentrated aqueous solutions of acids (phosphoric, formic, sulphuric and hydrochloric) and in high ionic strength aqueous salt solutions such as lithium bromide (LiBr), calcium chloride ($CaCl_2$), zinc chloride ($ZnCl_2$), magnesium chloride ($MgCl_2$) [5]. The main disadvantage of a salt-containing aqueous solvent is the long preparation time due to the fact that aqueous solutions of fibroin should be dialyzed for several days to remove the salts and to recoverthe polymer as a film from the aqueous solution by dry forming. In some component organic solvents e.g. hexafluoroisopropanoland hexafluoroacetone, fibroin can be dissolved only after its preliminary activation by dissolution in aqueous salt systems [7].

This study focused on the production of a regenerated nonwoven silk from *Bombyx mori* silk. The experiment intensively concentrated on the alternative way of silk solution preparation for electrospinning by using a mixture of formic acid and calcium chloride as a solvent.

2. Experiment

2.1 Materials

The silk cocoon used in this experiment was *Bombyx mori* Linn. (Nang-Noi Srisakate 1) from Amphoe Mueang Chan, Si Sa Ket Province, Thailand. ECE Phosphate Reference Detergent FBA free (Union TSL Co., Ltd.) was used as soaping agent for degumming. Calcium chloride (Fluka AG, Switzerland) and98% formic acid (Penta, Czech Republic) were used as the solvent for spinning solution preparation.

2.2Preparation of spinningsolution

- Degumming process

Raw silk cocoons were degummed twice with 1% sodium carbonate and 0.5% soaping agent at 100 ^{O}C for 30 minutes (the ratio of raw silk cocoon over degumming solution was 1:30), then rinsed with warm water in order to remove the sericin (silk gum) from the surface of the fibre and then dried at room temperature.

- Silk fibroin solution preparation

silk fibre was dissolved in 98% formic acid. The formic acid solution used in the process contains 1-8 wt% calcium chloride. The silk fibroin concentration was fixed at 8 wt%. The solutions were mixed by magnetic stirring at room temperature for 6 hours. Viscosity of the solution was measured by HAKKE RotoVisco RV1.

2.3 Electrospinning

A schematic representation of the equipment used in the experiment is illustrated in Figure 1. A high voltage in the range of 20 kV to 50 kV was applied to the spinning solution. Electrospinning was carried out at a distance of 10 cm, the temperature was 20±1 ^{O}C and air humidity was 38±2%. The morphology of the electrospun fibreswas examined by a scanning electron microscope (SEM, Phenom FEI). Fibre diameter was determined by using NIS-Elements AR software. About 100 randomly selected fibres were used to determine the average fibre diameter and their distribution.

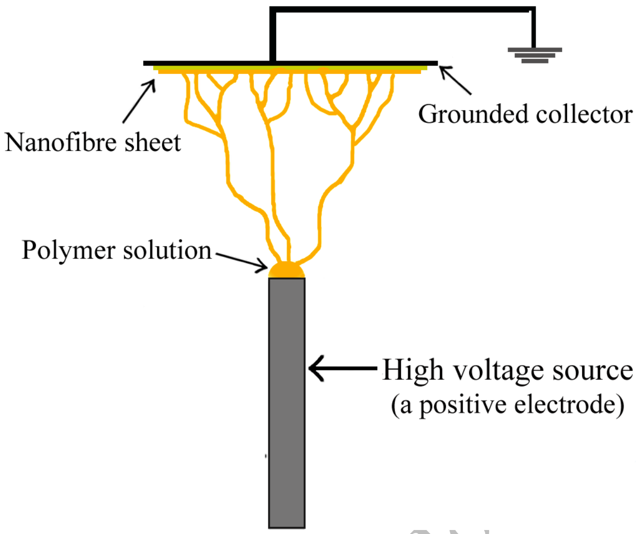

Figure 1: *Schematic of a simple electrospinning experiment*

3. Results and Discussion

- Effect of salt concentration on solubility of silk fibroin

Silk fibroin cannot dissolve in formic acid because of large quantities of glycine and amino acids with hydrocarbon side chains as well as close packing of the polypeptide chains. In this work calcium chloride was used to increase solubility of silk fibroin in formic acid; spinning solutions with 2 - 5 wt% calcium chloride could be used but 2 wt% calcium chloride was preferred. It is suggested that calcium chloride has a chaotropic property that disrupts stabilizing intra-molecular forces such as hydrogen bondsin silk fibroin structures by shielding charges and preventing the stabilization of salt bridges. Hydrogen bonds are stronger in nonpolar media. Salts which increase the chemical polarity of the solvent can also destabilize hydrogen bonding. They will make hydrophobic proteins more soluble [5, 8].

In this experiment, spinning solutions with 6-8 wt% calcium chloride were not successful in electrospinning; besides, silk solutions tend to transform into a gel after being left at room temperature. It is possible that calcium cations (Ca^{++}) can also enhance rates of the gelation of *Bombyx mori* silk with an increase in calcium chloride concentration. Gelation can occur at room temperature and the time of silk fibroin gelation decreases [6, 10].

- Effect of salt concentration on fiber diameter

Scanning electron micrographs at various salt concentrations and the average fibre diameter of obtained fibresare shown in Figure 2 and Table 1, respectively. The results show that there is a significant increase in the mean fibre diameter with an increase in the calcium chloride concentration. It is possible that the addition of ionic salt may cause an increase in the viscosity of the solution (results are shown in Figure 3). Thus, although the conductivity of the solution is improved, the viscoelastic force is stronger than the columbic force resulting instead in an increasein the fibre diameter [11, 12].

Table 1: *Effect of concentrations of $CaCl_2$ on average fibre diameter*

$CaCl_2$ (%wt)	Solubility	Average fiber diameter (nm)	S.D.	Minimum (nm)	Maximum (nm)
0	Swell able	-	-	-	-
1	Partially soluble	-	-	-	-
2	Soluble	380	115	205	718
3	Soluble	505	109	301	966
4	Soluble	824	268	403	1533
5	Soluble	1042	400	436	2326

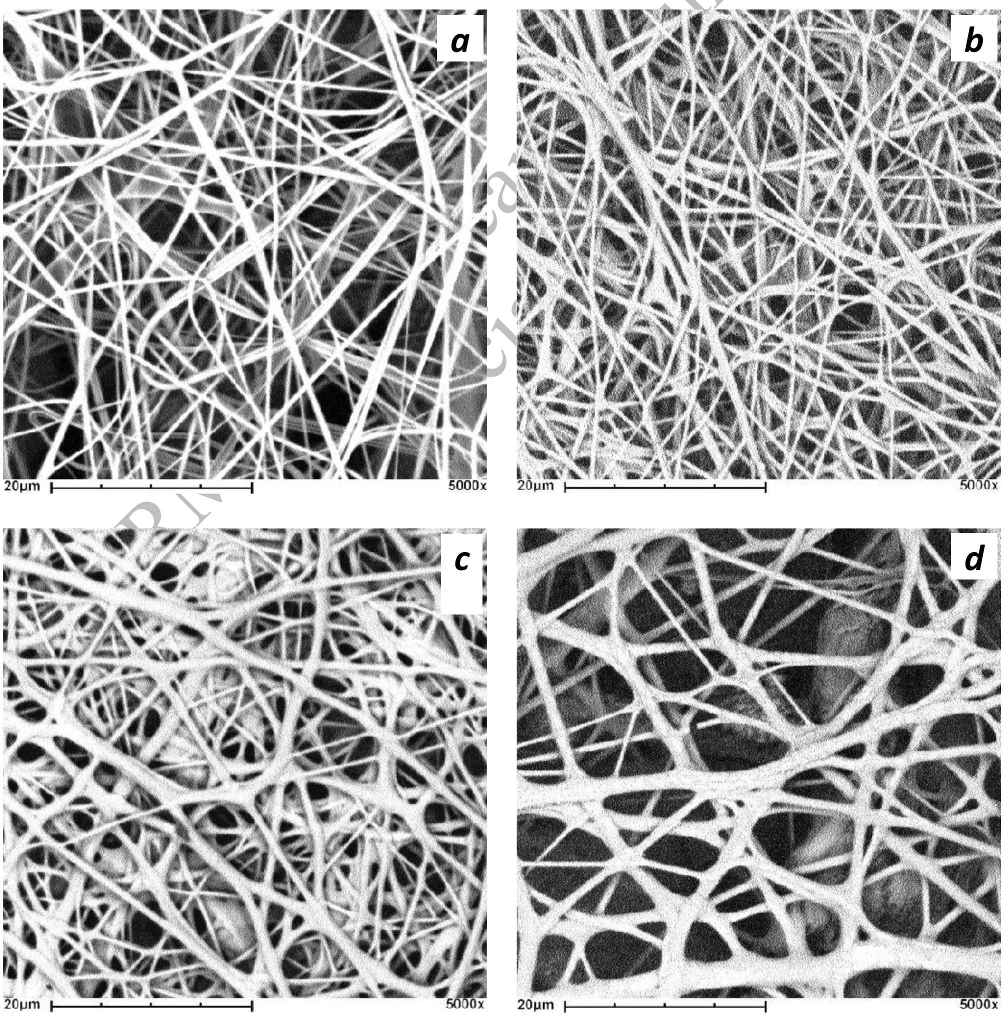

***Figure 2**: SEM micrographs of silk nanofibres prepared from 8 wt% silk fibroinat various concentrations of $CaCl_2$ by a) 2 wt%; b) 3 wt%; c) 4 wt%; d) 5 wt%*

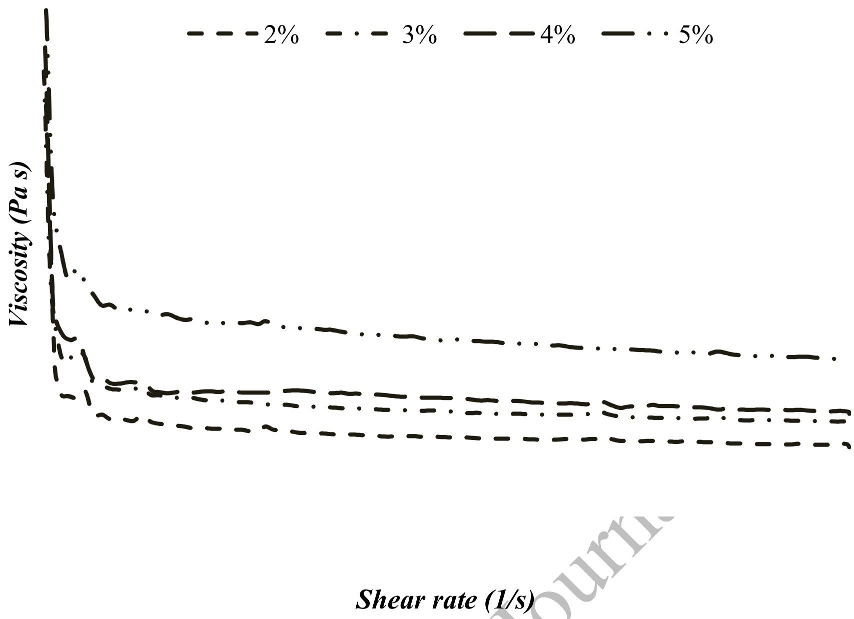

Figure 3: *Rheological behaviour of silk solutions prepared from different salt concentrations*

- Effect of applied voltage on fibre diameters

In order to study the effect of the electric field on fibre morphology, a silk solution with calcium chloride 2 wt% was electrospun with a voltage between 20 kV and 50 kV. Scanning electron micrograph at different applied voltages is shown in Figure 4. The results show that the variation of applied electric fields had a less significant effect on the average fibre diameter but produces an influence on the uniform fibre distribution of the obtained nonwoven sheet. It is suggested that a higher voltage will lead to greater stretching of the solution due to the greater columbic forces in the jet as well as the stronger electric field. These have the effect of reducing the diameter of the fibres. Higher applied voltage causes multiple jet formations which would provide uniform fibre distribution and broad fibre diameter distribution.

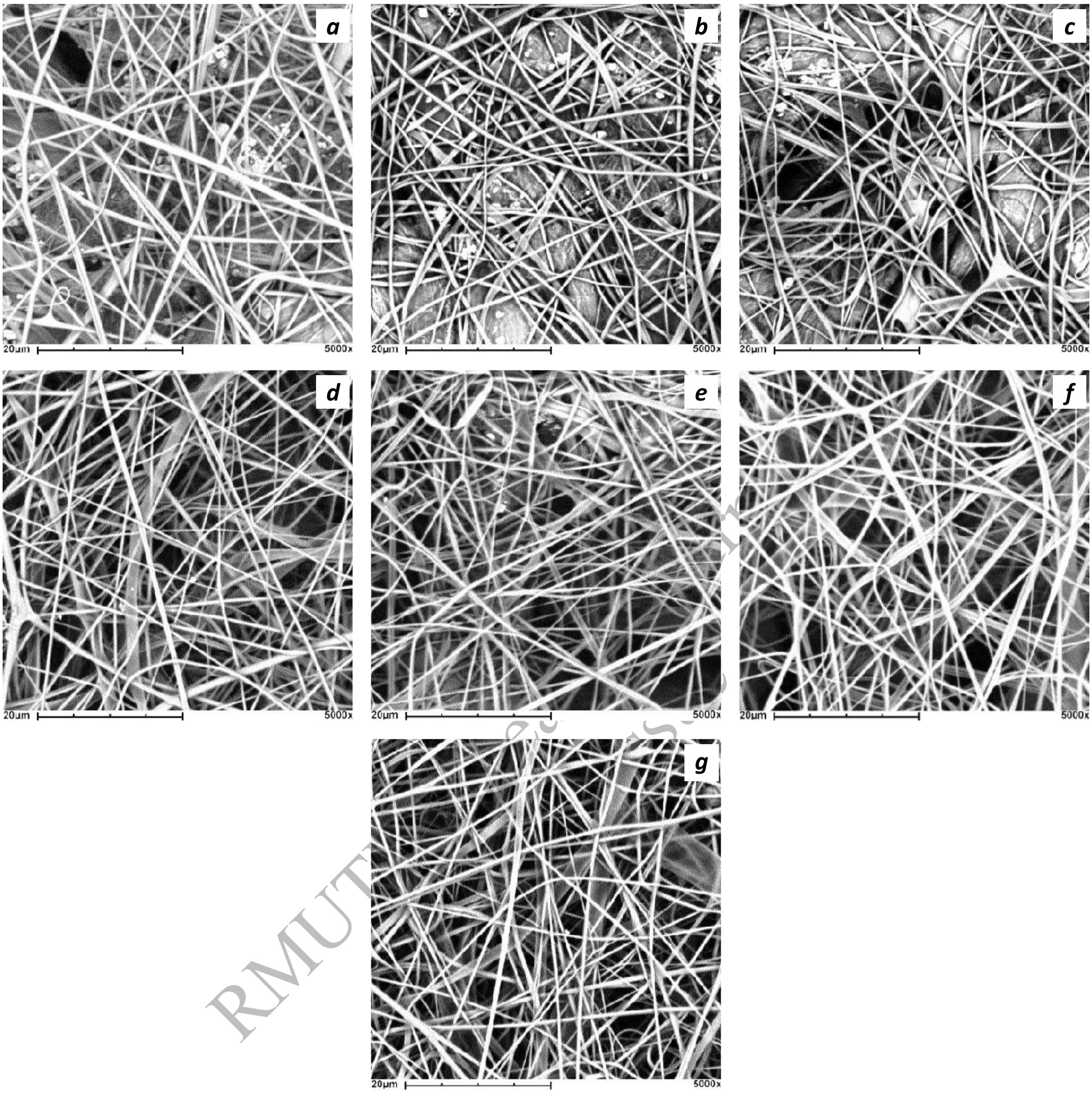

***Figure 4**: SEM micrograph of silk nanofibres prepared from the mixture of silk fibroin8 wt% and 2 wt% $CaCl_2$ by a) 20 kV; b) 25 kV; c) 30 kV; d) 35 kV; e) 40 kV f) 45 kV; g)50kV*

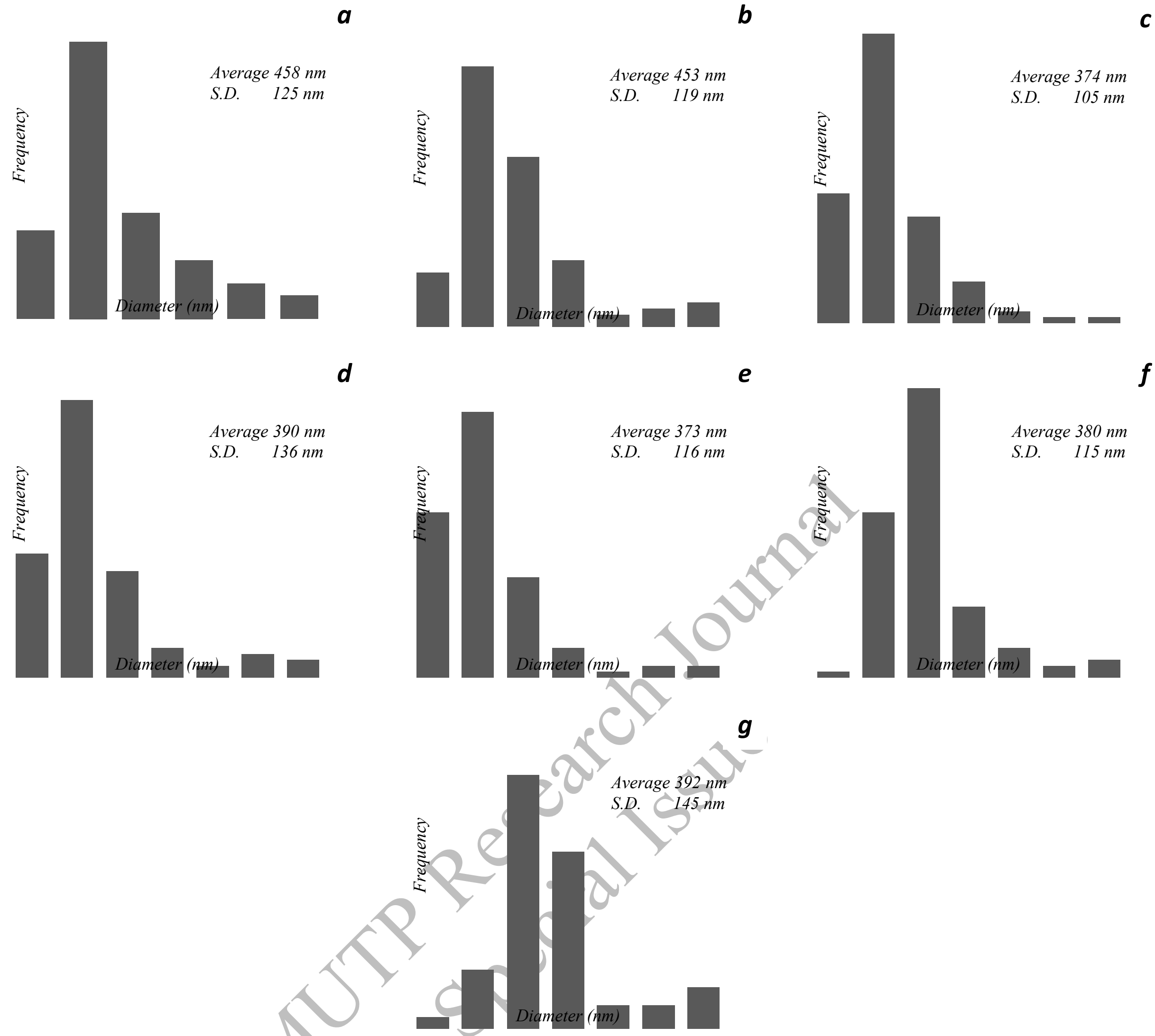

Figure 5*: Histogram of silk nanofibres prepared from the mixture of silk fibroin8 wt% and 2 wt% $CaCl_2$ by a) 20 kV; b) 25 kV; c) 30 kV; d) 35 kV; e) 40 kV f) 45 kV; g) 50 kV*

4. Conclusion

The solutions of silk fibroin in a mixture of formic acid and calcium chloride were electrospun into nanofibres bya needleless electrospinning method. Effects of salt concentrations in formic acid and the voltage of electric field on fibre morphology were examined.Concentration of calcium chloride played an important role in silk solution spinability and the diameter of the obtained fibres. An increase of salt concentration leads to an increase in fibre diameter ranging from 200 to 2300 nm. Concentrations of 2-3 wt% calcium chloride seem to be a suitable amount for improved solubility of silk fibroin in formic acid. The resultsshow that this could be a new direction in the preparation of solutions for electrospinning from silk fibroin.

5. Acknowledgements

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6. References

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