NANOBEADS FORMATION FOR ANTICANCER DRUG RELEASE CONTROLLED BY ELECTROSPINNING

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A thesis submitted in partial fulfillment of the requirement for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

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DECLARATION OF STUDENT

I declare that this thesis is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Dedicated , in thankful appreciation for support, encouragement and understanding to my beloved supervisor, family and friends. May Allah S.W.T bless our life.

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ABSTRACT

Anticancer drug nowadays have become one of the most important industry in the world. In order to improve drug delivery, the used of nanoparticles is the main purpose for this research study. The objectives for this study are to investigate the morphology of nanoparticles produced based on different molecular weight of and concentration of Polyethylene Oxide (PEO). In this research, a very high molecular weight (MR) PEO is used as the core polymer. Two types of PEO used are with 400 000 MW and 900 000 MW. There are two types of solvents used that are distilled water and ethanol. Whey protein is used because it containing Cysteine, one of the sulphur-containing amino acid and has same properties as anticancer drug. Henceforth, whey protein is used because it is easily to purchase and cheaper. PEO acts as shell that encapsulated the drug. There are two main processes for the nanoparticles preparation that are solution preparation and electrospinning. Four types of solution are prepared; PEO with water, PEO with Ethanol, PEO with protein, and PEO with protein and ethanol. All samples are prepared till 10 mL and 20 mL and then go through the electrospinning about 5 hours. After electrospinnig the samples will be analyzed under Field Emission Scanning Electron Microscopy (FESEM) to observe the morphology of nanoparticles and Energy Dispersive X- Ray (EDX) for sulphur determination. The resulted obtained showed the lower the viscosity, the more the formation of nanobeads. Lower molecular weight also affects the nanobeads formation as lower molecular weight is more beads than higher molecular weight.

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ABSTRAK

Ubat antikanser pada masa kini telah menjadi salah satu industri yang paling penting di dunia. Dalam usaha untuk meningkatkan penyampaian ubat, penggunaan nanopartikel menjadi tujuan utama kajian ini dibuat. Objektif kajian ini adalah untuk menyiasat morfologi nanopartikel yang dihasilkan berdasarkan berat molekul yang berlainan dan kepekatan Oksida Polyethylene (PEO). Dalam kajian ini, berat molekul yang sangat tinggi (MR) PEO digunakan sebagai polimer teras. Dua jenis PEO digunakan dengan 400 000 MW dan 900 000 MW. Terdapat dua jenis pelarut yang digunakan iaitu air suling dan etanol. Whey protein digunakan kerana ia mengandungi Cysteine, salah satu daripada asid amino yang mengandungi sulfur dan mempunyai ciri-ciri yang sama sebagai ubat antikanser. Seterusnya, protein whey digunakan kerana ia adalah mudah dibeli dan murah. Terdapat dua proses utama untuk penyediaan nanopartikel iaitu penyediaan larutan dan electrospinning. Empat jenis larutan disediakan; PEO dengan air, PEO dengan Etanol, PEO dengan protein, dan PEO dengan protein dan etanol. Semua sampel disediakan antara 10 mL hingga 20 mL dan kemudian akan melalui electrospinning kira-kira 5 jam. Selepas electrospinnig, sampel akan dianalisis di bawah Pelepasan Lapang Mengimbas Mikroskopi Elektron (FESEM) untuk melihat morfologi nanopartikel dan Sebaran Tenaga X-Ray (EDX) bagi menentukan sulfur. Keputusan yang diperolehi menunjukkan lebih rendah kelikatan, lebih banyak pembentukan nanopartikel. Berat molekul yang lebih rendah juga memberi kesan kepada pembentukan nanopartikel iaitu apabila berat molekul yang rendah adalah menghasilkan lebih banyak manik daripada berat molekul yang lebih tinggi.

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LIST OF ABBREVIATIONS

C	Carbon
DC	Direct Current
DDS	Drug Delivery System
EDX	Energy Dispersion X-Ray
Etc	Et cetera
Etoh	Ethanol
FE-SEM	Field Emission Scanning Electron Microscope
K	Potassium
MW	Molecular Weight
Na	Sodium
NPs	Nanoparticles
PEO	Polyethylene
S	Sulphur
Si	Silicone

LIST OF SYMBOLS

%	Percentage
M	Micro
G	Gauge
h	hour
k	kilo
m	Meter
ml	mililiter
mm	millimeter
MPa	Mega Pascal
Nm	Nanometer
RPM	Revolution Per Minutes
s	second
S	Siemens (unit of conductivity)
V	Voltage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cancer is a disease in which the control of growth is lost in one or more cells, leading either to a solid mass of cells known as a tumour or to a liquid cancer .It is one of the leading causes of death throughout the world, in which the main treatments involve surgery, chemotherapy, and radiotherapy (Nussbaumer *et al*.,2011). Nanotechnology, which deals with features as small as a 1 billionth of a meter, began to enter into mainstream physical sciences and engineering some 20 years ago. Lamprecht (2009) describes the use of nanoparticulate drug delivery systems as one important aspect in the newly developing field of nanomedicine to allow innovative therapeutic approaches.

Nanotechnology as a delivery device has a very promising application in drug delivery as it has advantages to enhance drug transport across biological barriers and deliver the drug at selective or targeted tissue or organ. As the principle of drug targeted is to reduce the total amount of drug administration, ongoing efforts have

been made over the past decade to develop systems or drug carriers that are capable of delivering the active molecules specifically to the target organs to increase the therapeutic efficacy. The site-specific delivery systems will allow an effective drug concentration to be maintained for a longer time interval and decrease the side effects. Recent applications of nanoscience include the use of nanoscale materials in electronics, catalysis, and biomedical research. Among these applications, strong interest has been shown to biological processes such as blood coagulation control and multimodal bioimaging, which has brought about a new and exciting research field called nanobiotechnology (Suh *et al*., 2009).

Nanoparticulate delivery systems are extensively investigated as a drug delivery strategy in the pharmaceutical research. In general, nanocarriers may protect a drug from degradation, enhance drug absorption by facilitating diffusion through epithelium, modify pharmacokinetic and drug tissue distribution profile and improve intracellular penetration and distribution (Elzoghby *et al*., 2012). Nanoparticles provide a new mode of cancer drug delivery functioning as a carrier for entry through fenestrations in tumour vasculature allowing direct cell access. Different types of nano-sized carriers, such as polymeric nanoparticles, solid lipid nanoparticles, ceramic nanoparticles, magnetic nanoparticles, polymericmicelles, polymer-drug conjugates, nanotubes, nanowires, nanocages and dendrimers, are being developed for various drug-delivery applications

Polymeric nanoparticles can be fabricated from polysaccharides proteins and synthetic polymers (Elzoghby *et al*., 2012). Among the available potential colloidal drug carrier systems, protein-based nanoparticles are particularly interesting as they hold certain advantages such as greater stability during storage and in vivo, being non-toxic and non-antigenic and their ease to scale up during manufacture over other

drug delivery systems (Elzoghby *et al*., 2012). The efficiency of drug delivery to various parts of the body is directly affected by particle size. Nanostructure mediated drug delivery, a key technology for the realization of nanomedicine, has the potential to enhance drug bioavailability, improve the timed release of drug molecules, and enable precision drug targeting (Hughes, 2005).

Electrospinning is a technique that can easily fabricate nanofiber, nanobeads and microfiber meshes from different types of polymer. Due to their unique features such as high surface-to-volume ratio, morphological design flexibility and extracellular matrices structure-like, nanoparticles are used as scaffolds for drug delivery and tissue engineering. Low molecular weight drugs and biomolecules such as proteins and nucleic acids can be encapsulated into the electrospun fibers (Xu *et al*., 2008).

1.2 Problem Statement

Most of the drugs used nowadays give side effect to patient due to toxicity and lack of therapeutic ethic. Moreover, the efficiency toward cancer cell also is still in lower form. Henceforth, in order to solve this problem, the used of nanotechnology have become more important for cancer treatment. According to Kim and Pack (2006), a wide variety of new, more potent and specific therapeutics are being created in advances in biotechnology. A drug delivery system is designed to provide a therapeutic agent in the needed amount, at the right time and to the proper location in the body in a manner that optimizes the efficacy, increases compliance and minimizes side effects. Due to common problems in drug delivery

such as low solubility, high potency and poor stability, it can impact the efficiency and potential of the drug itself. Thus, there is a corresponding need for safer and more effective methods and devices for drug delivery.

Biodegradable nanoparticles are frequently used to improve the therapeutic value, improving bioavailability, solubility, and retention time. It also helps to reduces expenses for the treatment and risk of toxicity.

1.3 Objectives

1.3.1 To study the effect of PEO of different molecular weight toward the formation of nanobeads.

1.3.2 To study the effect of solution viscosity on the nanobeads formation.

1.4 Scope of Study

1.4.1 To study the effect of different molecular weight of Polyethylene Oxide (PEO) on nanobeads formation.

1.4.2 To study the effect on nanobeads formation by using different concentration of PEO that will affect the viscosity

1.5 Rationale of Significant Study

The significance of this research is to identify the formation of nanoparticles using electrospinning of different concentration and viscosity of solution. By using nanobaeads membrane encapsulated with drug as device, the drug can be released at controlled rates for a long period of time. The advantages of employing such systems are the drug release rates can be designed to the needs of a specific application. Apart from that, controlled drug delivery systems may provide drug protections especially proteins that are easily destroyed by the body. Controlled drug delivery systems can also increase patient comfort and compliance by substituting frequent doses (daily injectibles) with infrequent injection (once a month injection or less).

CHAPTER 2

LITERATURE REVIEW

2.1 Electrospinning

2.1.1 History of Electrospinning

Elcetrospinning is a technique that used very high voltage power supply to produce nanofibers. It also used to produce nanobeads using certain variation in parameter. According to Liang *et al*., (2007), the word electrospinning is derived from electrostatic spinning. This technique has been used recently since 1994. However, Liang *et al*., (2007) stated that electrospinning was first observed in 1897 by Rayleigh.

The popularity of this technique can be approved by the statistic and fact that over 200 universities and research institutes from around the world are studying electrospinning as claimed by Zussman *et al*., (2003). There are various aspects of the electrospinning process and the fiber it produces and also the number of patents for applications based on electrospinning has grown in recent years. Some companies

such as eSpin Technologies, NanoTechnics, and KATO Tech are actively engaged in reaping the benefits of the unique advantages offered by electrospinning. Meanwhile, companies such as Donaldson Company and Freudenberg have been using this process for the last two decades in their air filtration products (Ramakrishna *et al*., 2006).

This electrospinning is broadly used technology for electrostatic fiber formation which uses electrical forces to produce electropun fibers with diameters ranging from 2nm to several micrometers. It uses polymer solutions of both natural and synthetic polymers. This technique seems to be increasingly applied in research and had take the commercial attention over the past decade according to Ahn *et al*., (2006).As claimed by Zussman *et al*., (2003), this process offers unique capabilities and advantages for producing novel natural nanofibers and fabrics with controllable pore structure.

According to Reneker *et al*., (2000) this process of electrospinning has gained a lot of attention over the world. This phenomenon in the last decade not only due to its versatility in spinning a wide variety of polymeric fibers, but also its capability in producing consistent fibers and nanobeads in the submicron range consistently.This consistency is otherwise difficult to achieve by using standard mechanical fiber-spinning technologies techniques (Reneker *et al*.,2000). According to Luu *et al*., (2003), electrospun fibers have been successfully applied in various fields, such as, nanocatalysis, tissue engineering scaffolds, protective clothing, filtration, biomedical, pharmaceutical, optical electronics, healthcare, biotechnology, defense and security, and environmental engineering because of the smaller pores and higher surface area than regular beads.

Overally, this electrospinning is relatively simple and effective to produce nanobeads from a variety of polymers. Although it is more suitable to produce nanofiber, but nanobeads formation are also possible using electrospinning as claimed bt Liang *et al*., (2007). Spun nanobeads also offer some advantages such as high surface-to-volume ratio, tunable porosity, malleability to conform to a wide variety of sizes and shapes (Liang *et al*., 2007). Hence forth, Liang *et al*., (2007) also claimed that because of these advantages, electrospun nanofibers have been widely investigated in the past several years. Those advantages are probably had been applied to several process such as filtration, optical and chemical sensors, electrode materials and biological scaffolds (Liang *et al*., 2007).

2.1.2 Electrospinning Process

Electrospinning , a spinning technique that used unique approach that is by using electrostatic forces to produce fine electrospun from polymer solutions. The electrospun produced have a thinner diameter (from nanometer to micrometer) as stated by Kidoaki *et al*., (2005). Moreover, a very high voltage supply a range of several tens of kVs is necessary for the process. According to Chew *et al*., (2006a), various techniques such as electrostatic precipitators and pesticide sprayers work similarly to the electrospinning process and this process, mainly based on the principle that strong mutual electrical repulsive forces overcome weaker forces of surface tension in the charged polymer liquid. Based on the Kidoaki *et al*., (2005), there are two types of electrospinning that had been used nowadays. The two types of electrospinning are vertical and horizontal. With the expansion of this technology,

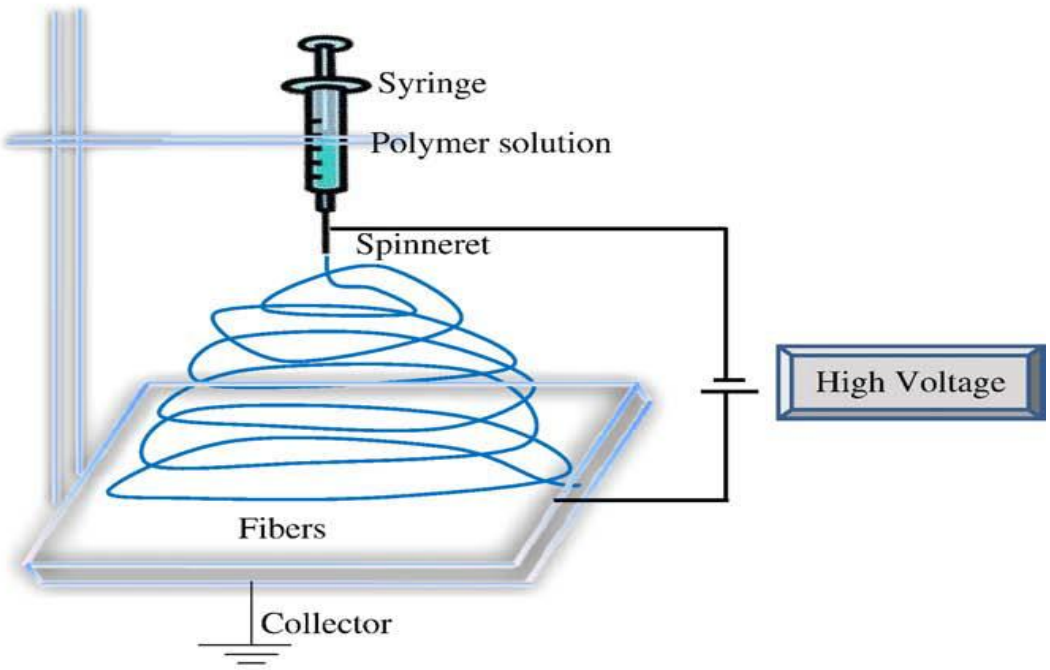
several research groups have developed more sophisticated systems that can fabricate more complex nanofibrous structures in a more controlled and efficient manner.

This process of electrospinning should be running at room temperature with standard atmosphere conditions. The standard set up of electrospinning apparatus is shown in Figure 2.1 (a and b). The standard electrospinning process consists of three major components that are a high voltage power supply, a spinneret and a grounded collecting plate and utilizes a high voltage source to inject charge of a certain polarity into a polymer solution or melt, which is then accelerated towards a collector of opposite polarity (Liang *et al*., 2007).

According to the article from Huang *et al*., (2003), they claimed that most of the polymers are dissolved in some solvents before electrospinning, and when it completely dissolves forms polymer solution. The polymer fluid is then introduced into the capillary tube for electrospinning. However, Huang *et al*., (2003) also claimed that some polymers may emit unpleasant or even harmful smells, so the processes should be conducted within chambers having a ventilation system. In the electrospinning process, a polymer solution held by its surface tension at the end of a capillary tube is subjected to an electric field and an electric charge is induced on the liquid surface due to this electric field. When the electric field applied reaches a critical value, the repulsive electrical forces overcome the surface tension forces.

Eventually, a charged jet of the solution is ejected from the tip of the Taylor cone and an unstable and a rapid whipping of the jet occurs in the space between the capillary tip and collector which leads to evaporation of the solvent, leaving a polymer behind (Milasius, 2007). The jet is only stable at the tip of the spinneret and after that instability starts. Thus, the electrospinning process offers a simplified technique for fiber formation.

a)



b)

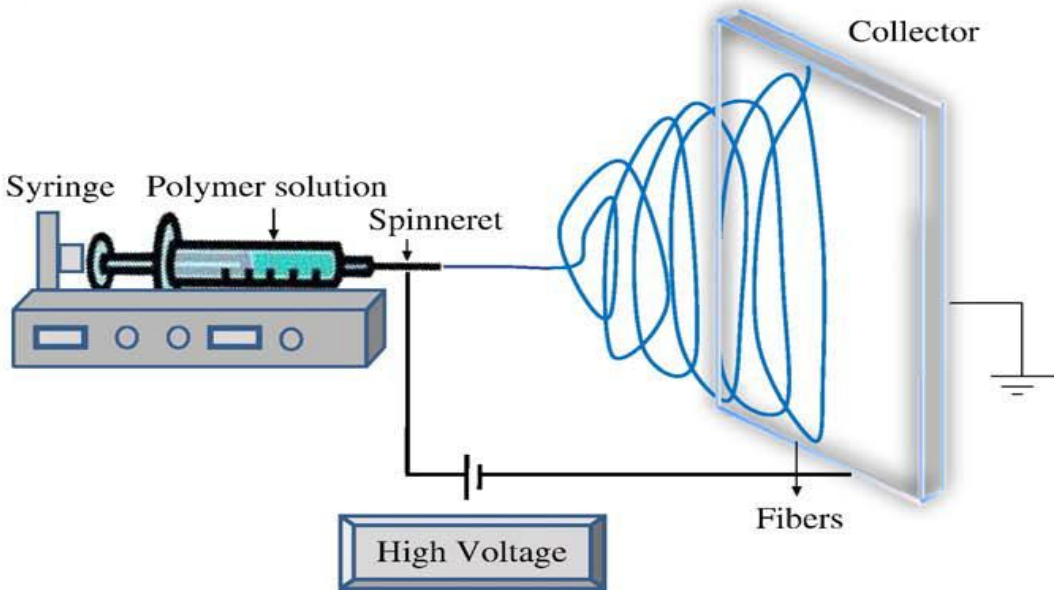


Figure 2.1 Schematic diagram of set up of electrospinning apparatus (a) typical vertical set up and (b) horizontal set up of electrospinning apparatus. (Source: Parven *et al*, 2012)

2.2 Drug Delivery Technology

Researches on drug delivery system (DDS) have many benefits on pharmaceutical industry nowadays. According to Swatantra *et al*., (2012), some of the benefits are include improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved targeting for a specific site to reduce unwanted side effects. Those researches also state about the challenge for both drug and drug delivery companies is to deliver both existing and emerging drug technologies in a manner that improves the benefits to the patients.

Over the past several years, great advances have been made on development of novel drug delivery systems of anticancer drug. According to Ajazuddin *et al*., (2010), in phyto-formulation research, developing nanodosage have a number of advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophages distribution, sustained delivery, protection from physical and chemical degradation. Some of the examples for the nanodosage polymeric are nanoparticles and nanocapsules, liposomes, solid lipid nanoparticles, phytosomes and nanoemulsion (Ajazuddin *et al*., 2010).

Injectable polymers that have biocompatibility and biodegradability are important biomaterials for drug delivery system (DDS) and tissue engineering (Swatantra *et al*., 2012). Accoring to Medina *et al*., (2004), multiple synthetic and natural biodegradable polymers have been investigated for these purposes, including polyesters, polyethers, poly amino-acids, polysaccharides, and proteins. These polymers are employed as injectable drug delivery system, and especially as

injectable drug delivery system for cancer chemotherapy, and have been investigated actively so as to minimize the toxic side effects and increase the carcinostatic pharmaceutical effects (Heller *et al*., 2004). Methods of local administration of drug delivery system, nanoparticles, microspheres, polymeric micelles, liposomes, and hydrogel systems, for targeting and controlled release have been investigated with nonand biodegradable polymers. However, the targeting drug delivery system has not been satisfactorily achieved.

2.3 Nanoparticles in Drug Delivery

Drug delivery is an interdisciplinary and independent field of research and is gaining the attention of pharmaceutical researchers, medical doctors and industry as stated by Singh et al., (2009). A safe and targeted drug delivery could improve the performance of some classic medicines already on the market, and moreover, will have implications for the development and success of new therapeutic strategies such as anticancer drug delivery, peptide and protein delivery and gene therapy. According to Parveen et al., (2012) several drug-delivery technologies have emerged and a fascinating part of this field is the development of nanoscale drug delivery devices in the last decade.

Nanoparticles (NPs) have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and more recently, nucleotides. NPs and other colloidal drug-delivery systems modify the kinetics, body distribution and drug release of an associated drug. This review article focuses on the potential of nanotechnology in medicine and discusses different nanoparticulate drug-delivery

systems including polymeric NPs, ceramic NPs, magnetic NPs, polymeric micelles and dendrimers as well as their applications in therapeutics, diagnostics and imaging (Parveen *et al*., 2012). The applications of nanotechnology in various disciplines and specifically in healthcare are becoming increasingly common and the process of replacing traditional medicines has already begun. Thus, although efficient drug delivery is one of the most prominent problems faced by the biotechnological and pharmaceutical industries, nanotechnology can promote the innovative utilization of the myriad existing drugs produced by these industries.

Nanotechnology focuses on formulating therapeutic agents in biocompatible nanocarriers, such as NPs, nanocapsules, micellar systems and dendrimers (Figure 2.2). Moreover, one of the major advantages that nanotechnology offers is targeted drug delivery to the site of disease. This can be achieved either through passive targeting of drugs to the site of action or by active targeting of the drug (Parveen *et al*., 2012)

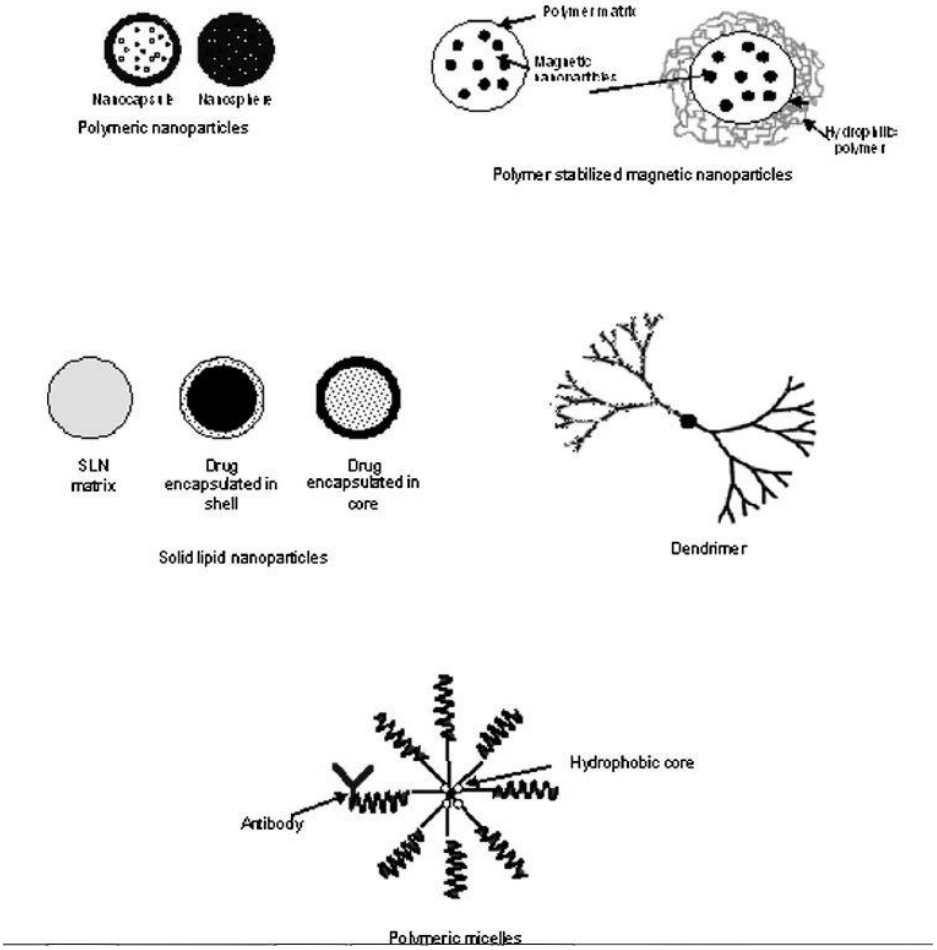


Figure 2.2 Schematic representation of different nanotechnology based drug delivery systems. (Source: Parveen *et.al*, 2012)

2.3.1 Therapeutics

Nanoparticles (NPs) have widespread use in drug delivery as already discussed with regard to the various types of NPs (Parveen *et al*., 2012). Some recent applications of NP in therapeutics are discussed, possible offering insights to the applications of NPs in therapeutics. According to Suphiya *et al*., (2012), the therapeutic applications of NPs are diverse, ranging from cancer therapeutics,

antimicrobial actions, vaccine delivery, gene delivery and site-specific targeting to avoid the undesirable side effects of the current therapeutics. Many chemotherapeutic drugs such as carboplatin, paclitaxel, doxorubicin and etoposide, etc., have been successfully loaded onto NPs and these nanoparticulate systems are very potent against various cancers as demonstrated by the studies of various research groups. In additions, multifunctional NPs with surface functionalized biomolecules are also being synthesized and serve as potential therapeutic agents (Suphiya *et al*., 2012). Functionalized NPs are also being used for targeted gene silencing because these offer exciting prospects and have garnered the attention of researchers. Many NPs are also useful as therapeutics due to their antimicrobial properties.

2.4 Polyethylene Oxide as Core Solution

Polyethylene oxide has a very high molecular weight compare to other polymer. Thus it will make the structure more suitable for nanoparticles and giving the supporting characteristics. In this study, there are two types of PEO used that were differed in molecular weight. PEO is used as core solution of electrospun nanobeads. This is important in studying the surface morphology of nanobeads formation as the phase change of nanobeads can be alter by changing the composition of PEO solution as stated by Chen *et al*., (2011). This will determine the optimal nanobeads formation that using PEO as core solution.

There are a lot of parameter that affect the surface morphology of nanobeads formation using electrospinning such as viscosity, concentration, needle size, voltage and etc. However, according to Frenot and Chronakis (2003), they claimed that the

morphology of electrospun beads is strongly affected by viscosity, equivalent concentration and temperature. In this research, temperature is remaining constant at ambient state. This parameter will affect the fabrication of nanobeads when the analysis of morphology will be done using FE-SEM and EDX-SEM. These parameters are the important advantage from the aspect of industrial application since high temperature makes electrospinning process quickly (Frenot and Chronakis, 2003). PEO has a lot of advantages as core solution compared to other polymer. As the temperature is kept constant throughout the experiment, thus PEO is suitable because it has a good capability to regulate their interior temperature if the ambient temperature keeps changing (Chen *et al*., 2011).

As PEO of different molecular weight will result in the different of morphology and characteristics of electrospun produced, thus it is important to use certain molecular weight. Certain molecular weight of PEO will give the best characteristic of nanobeads and the drug delivery system. According to Hill *et al*., (2008), electrospun nanofibers from PEO/water solutions containing various PEO concentrations, it is found that solutions that contains the viscosity less than 800 centipoises (cp) will broke up into droplets upon electrospinning while solutions with viscosity greater than 4000 cp were too thick to electrospin.

CHAPTER 3

METHODOLOGY

3.1 Materials

Polyethylene Oxide (PEO, MW: 400 000 and 900 000) was purchased from Sigma Aldrich (St. Louis, USA). Ethanol 99.8% (purity) was obtained from Sigma Aldrich (St.Louis,USA).Whey protein for United Kingdom. All the chemicals used are of analytical grade and used without further purification.

3.2 Core Solutions Preparation

a) *1.5% w/v of 400 000 MW and 900 000 MW of PEO*

1.5% w/v for PEO was prepared. 10 mL of distilled water was measured and poured in 50 mL beaker. 150 mg of PEO was weighted and diluted in 10 mL distilled water in the beaker. The solution then was stirred overnight under normal stirring condition and at room temperature. Those steps were same for both 400 000 MW and

900 000 MW of PEO. Make sure to cover the opening of the beaker using aluminium foil in order to avoid oxidization of polymer.

b) *1.5% w/v of 400 000 MW and 900 000 MW of PEO with addition of 50 % v/v of Ethanol*

1.5% w/v for PEO was prepared. 10 mL of distilled water was measured and poured in 50 mL beaker. 150 mg of PEO was weighted and diluted in 10 mL distilled water in the beaker. The solution was mixed with 10 mL of Ethanol of 99.8% purity. The solution then was stirred overnight under normal stirring condition and at room temperature. Those steps were same for both 400 000 MW and 900 000 MW of PEO. Make sure to cover the opening of the beaker using aluminium foil in order to avoid oxidization of polymer.

c) *1.5 % w/v of 400 000MW and 900 MW of PEO with addition of 1.5 % w/v Whey Protein*

1.5% w/v for PEO was prepared. 10 mL of distilled water was measured and poured in 50 mL beaker. 150 mg of PEO was weighted and diluted in 10 mL distilled water in the beaker. The solution was mixed with 150 mg of whey protein. The solution then was stirred overnight under normal stirring condition and at room temperature. Those steps were same for both 400 000 MW and 900 000 MW of PEO. Make sure to cover the opening of the beaker using aluminium foil in order to avoid oxidization of polymer.

d) *1.5 % w/v of 400 000 MW and 900 000 MW of PEO with addition of 50 % v/v of Ethanol and 1.5 % w/v of Whey Protein*

1.5% w/v for PEO was prepared. 5 mL of distilled water was measured and poured in 50 mL beaker. 150 mg of PEO was weighted and diluted in 5 mL distilled water in the beaker. It is then was mixed with 5 ml of Ethanol. After that, the solution was mixed with 150 mg of whey protein. The solution then was stirred overnight under normal stirring condition and at room temperature. Those steps were same for both 400 000 MW and 900 000 MW of PEO. Make sure to cover the opening of the beaker using aluminium foil in order to avoid oxidization of polymer.

d) 3.0 % w/v of 400 000MW and 900 000MW of PEO with addition of 1.5 % w/v of Whey Protein

3.0 % w/v for PEO was prepared. 5 mL of distilled water was measured and poured in 50 mL beaker. 300 mg of PEO was weighted and diluted in 5 mL distilled water in the beaker. After that, the solution was mixed with 150 mg of whey protein. The solution then was stirred overnight under normal stirring condition and at room temperature. Make sure to cover the opening of the beaker using aluminium foil in order to avoid oxidization of polymer.

3.3 Measurement of Solution Viscosity

The viscosities of the polymer solutions, used for electrospinning, were measured using Physica MCR501 Rheometer (Anton Paar® GmBH, Austria). The zero-shear viscosities were recorded in frequency sweep mode at room temperature (25 °C) using spindle type 31 and rotational speed of 50 RPM. In order to prevent rapid oxidization of polymer, a polymer trap was use around the sample during measurements.

3.4 Electrospinning of Polymer

Positive high-voltage supply was used to maintain the voltage of 15kV. Spindle collector of electrospinning was covered using aluminium foil. The rotation speed maintain at 1200RPM. The distance between the tip of needle to the spindle was 15cm (150mm). The solution flowrate remain constant for 0.8 ml/h. Humidity of the surrounding kept at 35% to 55% using humidifier. All the parameters was kept constant for other solution in the electrospinning process. The electrospinning was run about 3-4 hours of each sample. The sample was collected and kept in dissicator. The electrospinning machine was set up as below;

Rotation : 1200 RPM

Distance : 150 mm/15 cm

Flow rate : 0.8 mL/h

Voltage : 15 KV

Humidity : 35-55%

Needle size : 21G ½

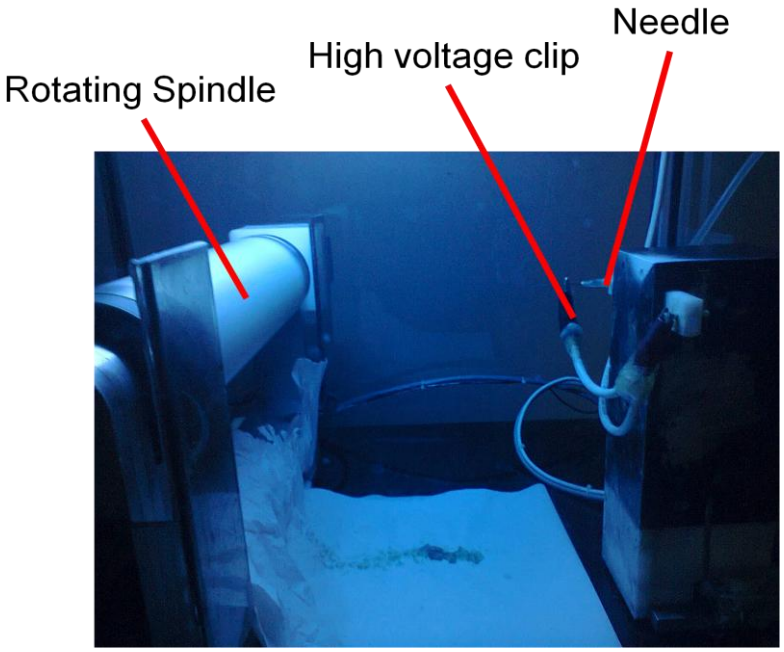


Figure 3.4 Electrospinning machine set up

3.5 Field Emission Scanning Electron Microscope (FE-SEM)

Morphology of the nanobeads were characterized by Field Emission Scanning Electron Microscope (FE-SEM, JEOL JSM-7500F). Samples were mounted on a metal holder using double sided tape and coated with gold using sputter coater followed by observation at accelerating voltages of range between 5-10 kV. There were three values of magnifications that had been captured for further comparison. The magnification of 1000, 5000 and also 20 000 were captured and saved as references for surface morphology comparison.

3.6 Energy Dispersive X-Ray (EDX)

Energy Dispersion X-ray Scanning Electron Microscope (EDX-SEM, JEOL JSM-4500E). The FE-SEM is equipped with EDX spectroscopy system. It is able to take part for quantitative analysis, digital imaging, and X-ray mapping. Using this machine, the sulphur in amino acid contained in whey protein were determined.

3.7 Summary of Workflow

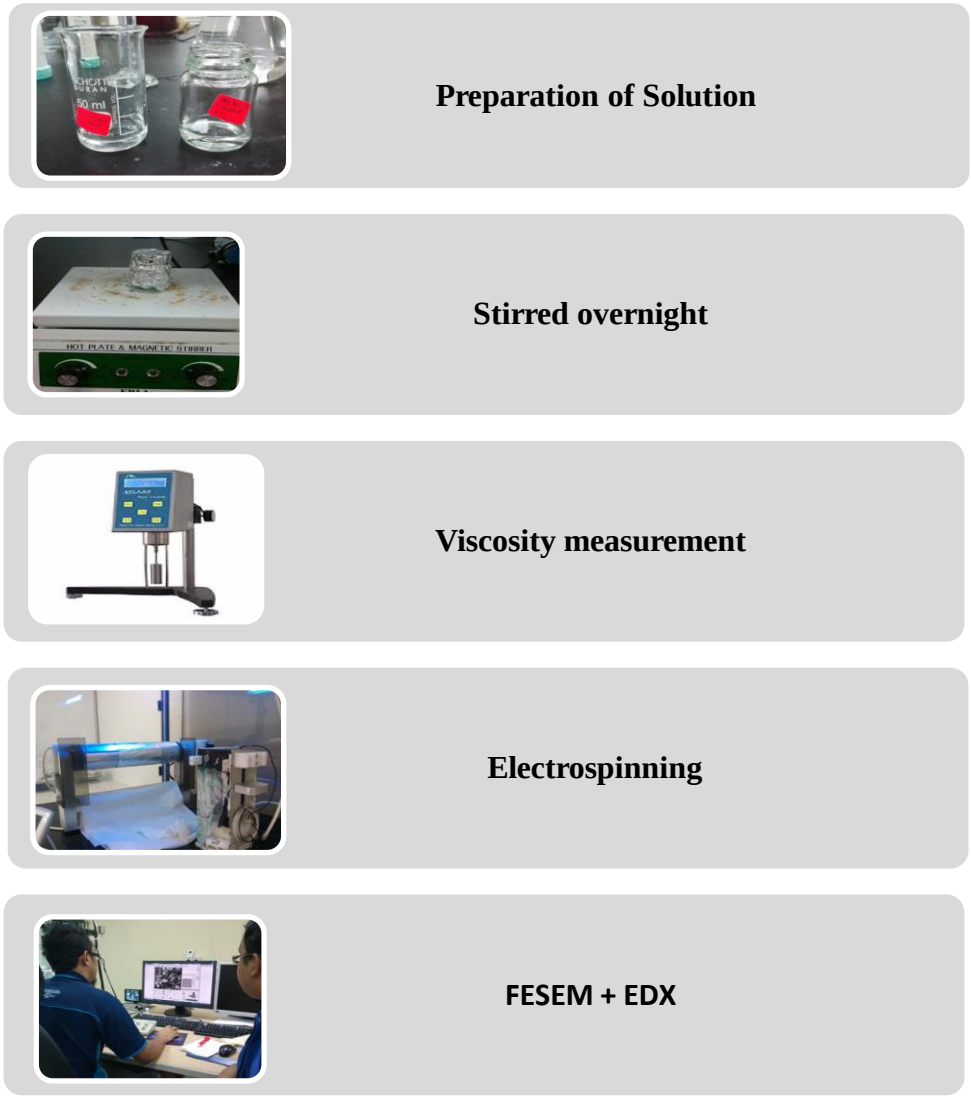


Figure 3.7 The summary of workflow

CHAPTER 4

RESULTS & DISCUSSIONS

4.1 Viscosity Measurement

Viscosity test was done using Programmable Rheometer using below parameter.

- Spindle type: 31
- Rotational speed: 50 RPM

Table 4.1 Viscosity measurement for each sample

NO	SAMPLE	VISCOSITY (Pa.s)
1	1.5% PEO 400K	32.50
2	1.5% PEO 400K + Etoh	55.83
3	1.5% PEO 400K + Protein	65.71
4	1.5% PEO 400K + Etoh + Protein	88.34
5	3.0% PEO 400K + Protein	96.78
6	1.5% PEO 900K	75.60
7	1.5% PEO 900K + Etoh	101.21

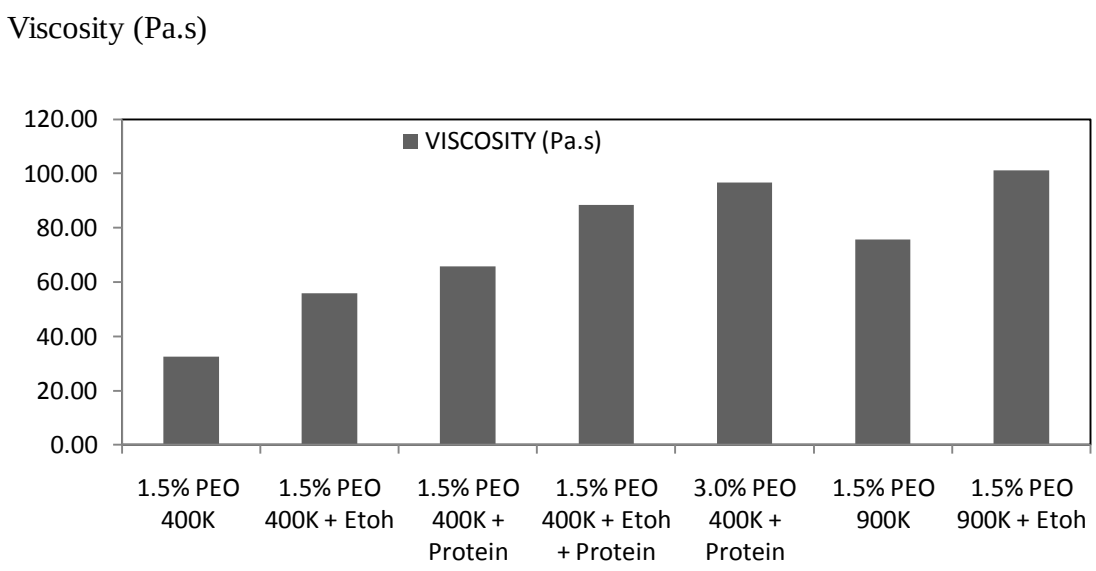


Figure 4.1 Graph of Viscosity over Samples

The Table 4.1 above showed that the value of viscosity from each samples that had been prepared throughout the whole research. The different molecular weight of Polyethylene oxide gave the different value of viscosity. The concentration also can be one of the factors that affect the viscosity. Henceforth, in order to make the analysis much easier, the graph of viscosity against the samples was plotted as shown in Figure 4.1. The highest value of viscosity from the viscosity analysis was sample containing 1.5% w/v 900 000 MW PEO with 50% v/v Ethanol with 101.21 Pa.s. It was followed by 3.0% w/v 400 000 MW PEO with addition of 1.5% w/v whey protein that had the value of 96.78 Pa.s. The other two samples that showed the higher value than others were 1.5% w/v 400 000 MW PEO with addition of 50% v/v Ethanol and 1.5% w/v whey protein with viscosity value of 88.34 Pa.s and 1.5 % 900 000 MW PEO . The sample that gave the lowest value of viscosity was 1.5 % w/v 400 000 MW PEO with 32.50 Pa.s.

4.2 Morphology Study of Electrospun Nanobeads (FESEM)

4.2.1 Effect of Different Molecular Weight on The Surface Morphology of Nanobeads

a) *1.5 % w/v 400 00 MW PEO*

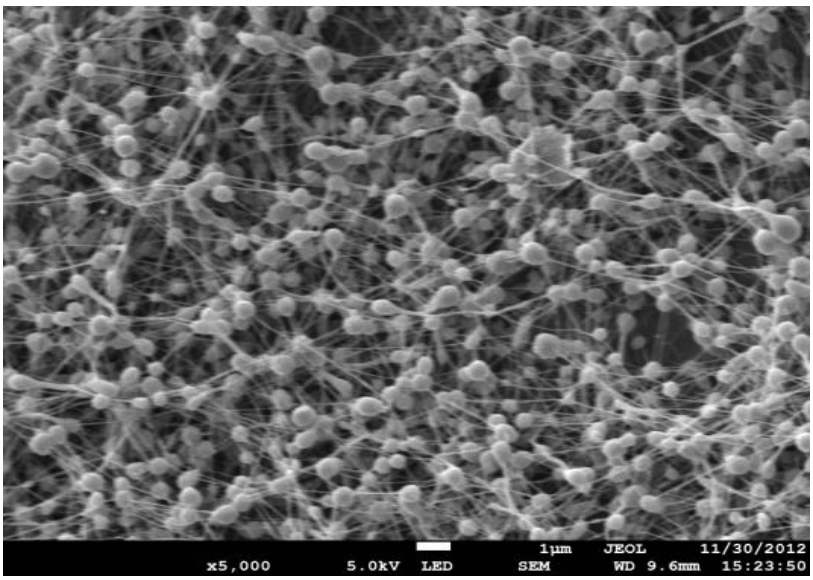


Figure 4.2.1a 1.5 % w/v 400 00 MW PEO under 5000 magnification

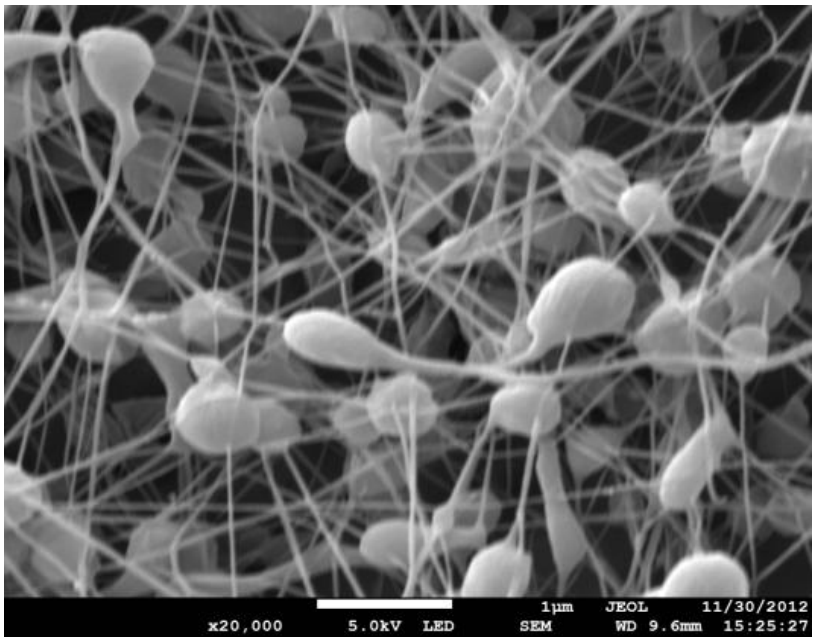


Figure 4.2.1b 1.5 % w/v 400 00 MW PEO under 20 000 magnification

b) *1.5 % w/v 900 00 MW PEO*

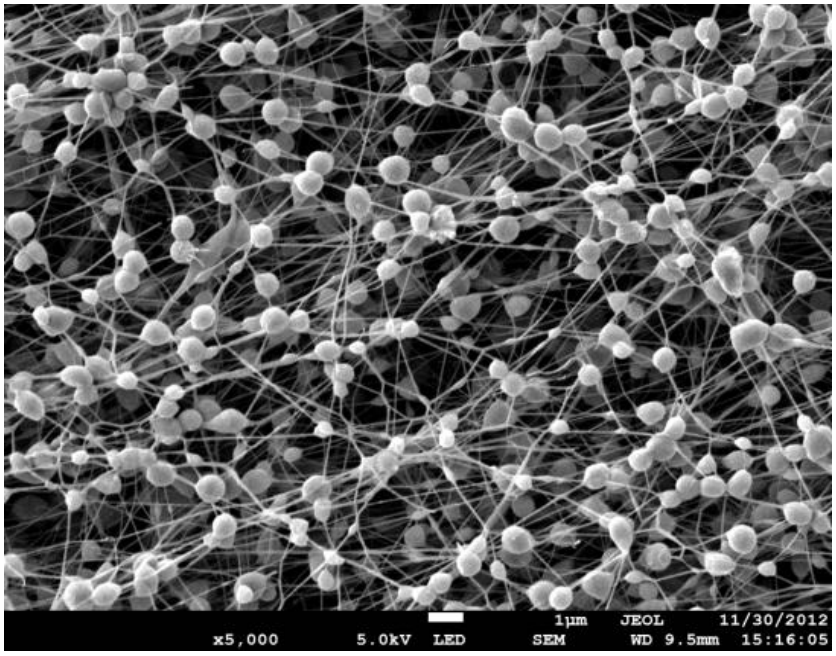


Figure 4.2.1c 1.5 % w/v 900 00 MW PEO under 5000 magnification

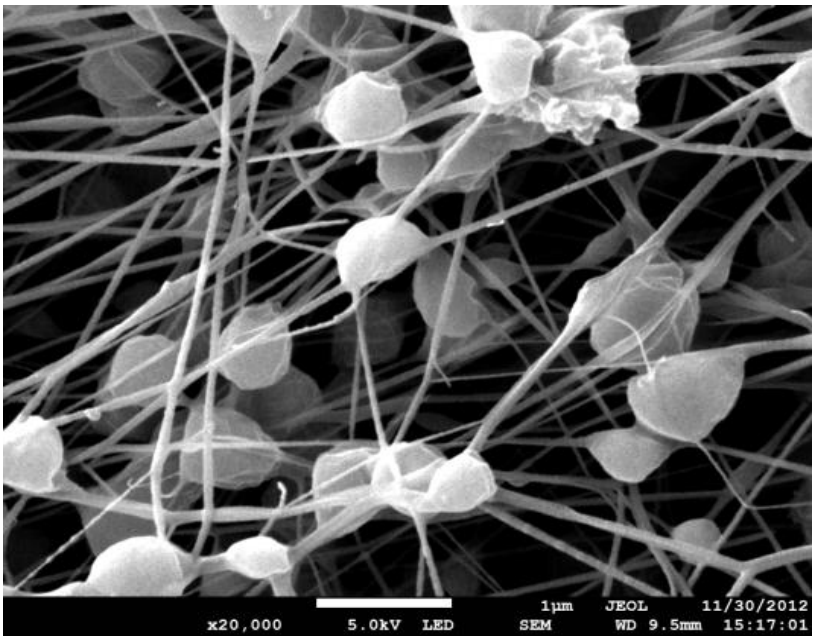


Figure 4.2.1d 1.5 % w/v 900 00 MW PEO under 20 000 magnification

Based on the four figure above (Figure 4.2.1a- 4.2.1d), those showed that the surface morphology of nanobeads from two different samples. In Figure 4.2.1a, it showed that the nanobeads formation of 1.5 % w/v 400 00 MW PEO under 5000 magnification using FE-SEM. It can be observed that there were a lot of nanobeads formed. Based on Figure 4.2.1b under 20 000 magnification using FE-SEM, we can clearly see the beads were formed through the nanofibers web. All the beads were attached to the nanofibers. The sizes of nanobeads were slightly not uniform. The average size of nanobeads formed for 1.5% w/v 400 00 MW PEO was about 450 nm. This is much higher than expected size of beads formation that is below 100 nm.

In Figure 4.2.1c and Figure 4.2.1d, those showed that the morphology of nanobeads formation using 1.5 % w/v 900 00 MW PEO. From Figure 4.2.1c, it can be observed that the formation of nanobeads using 900 000 MW PEO is slightly different from 400 000 MW PEO. The configuration of nanobeads in Figure 4.2.1a showed that it was more compact than in Figure 4.2.1c. This is because, the lower the molecular weight, the more the production of beads. According to He *et al.*, (2011), that molecular weight is strongly correlated with the formation of bead defects in the fibers, the smaller molecular weight, the more beads defect density. As a result, the fibers have beads-in-string structures. Henceforth, based on the observation, it can be clearly said that, the molecular weight affect the beads formation in term of the density or the configuration per area.

4.2.2 Effect of Viscosity on Nanobeads Formation

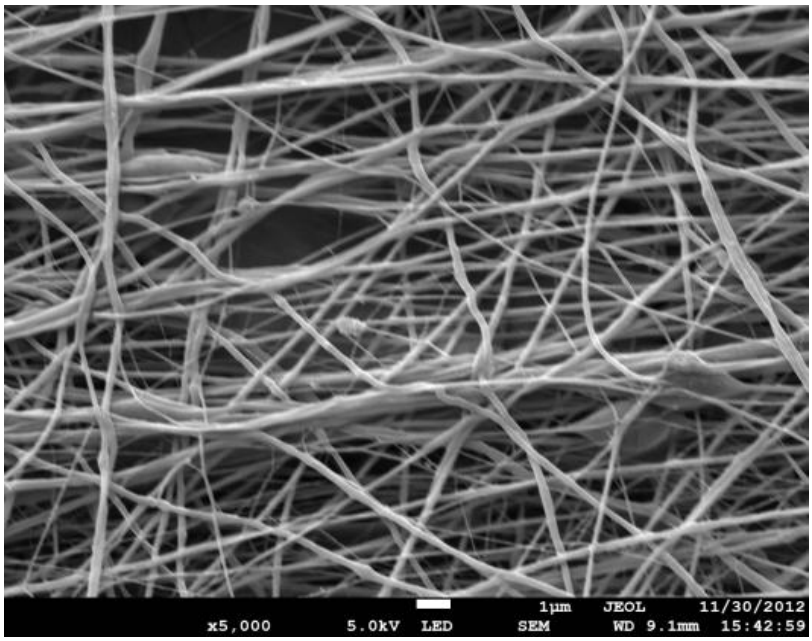


Figure 4.2.2a 1.5% PEO 400K + Ethanol under 5000 magnification

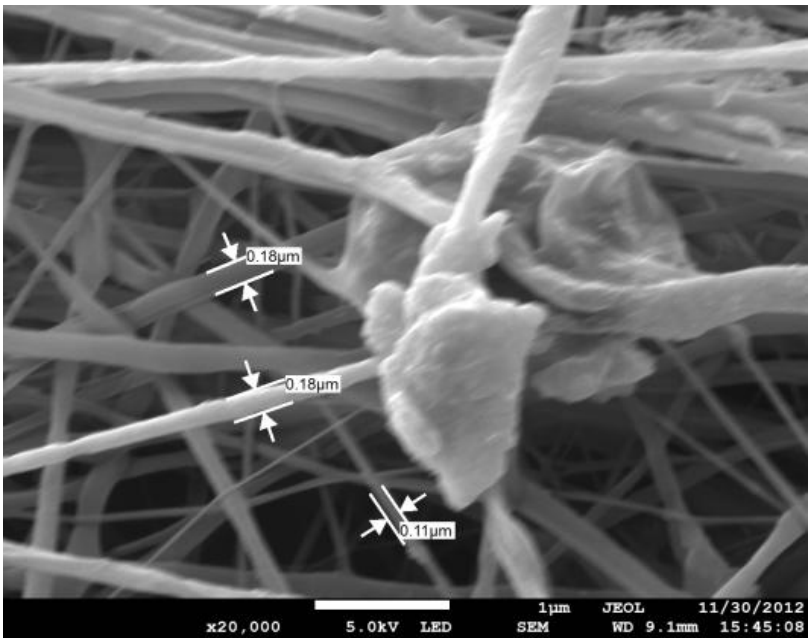


Figure 4.2.2b 1.5% PEO 400K + Ethanol under 20 000 magnification

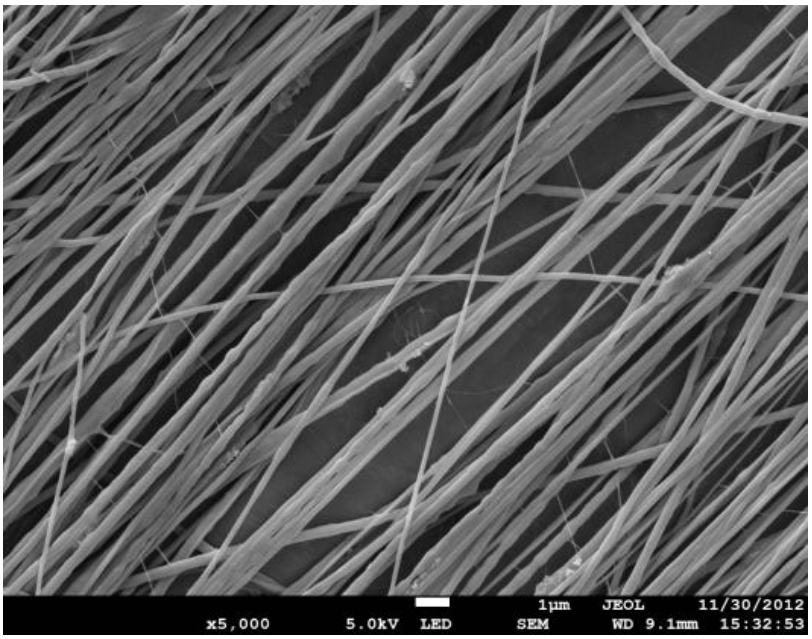


Figure 4.2.2c 1.5% PEO 900K + Ethanol under 5000 magnification

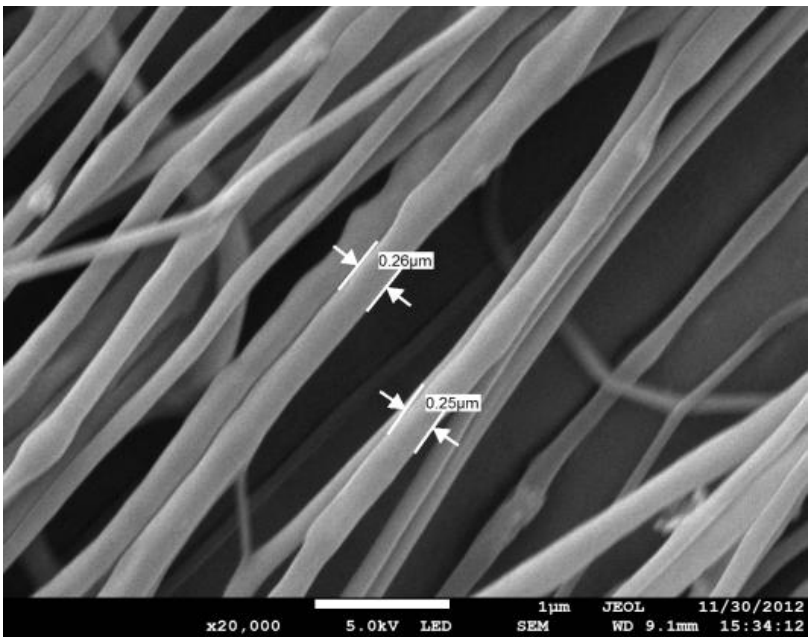


Figure 4.2.2d 1.5% PEO 900K + Ethanol under 20 000 magnification

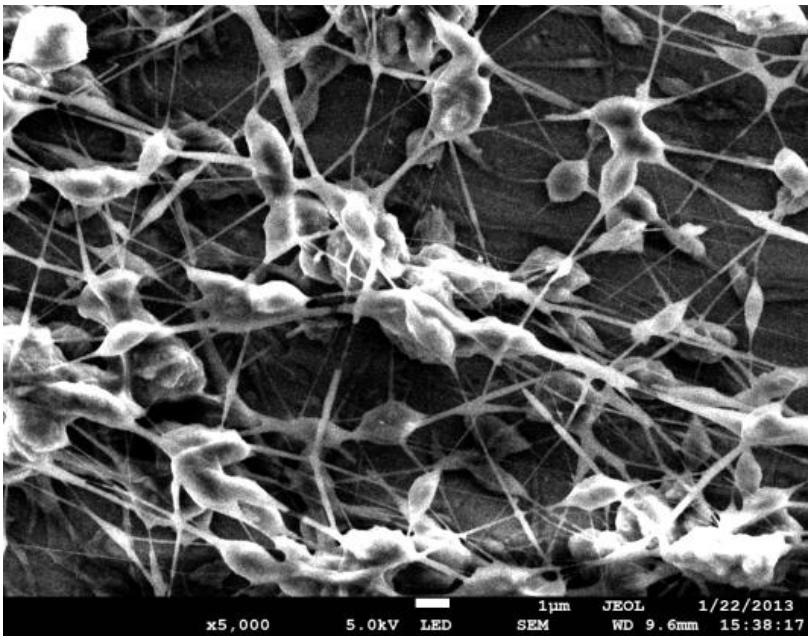


Figure 4.2.2e 1.5% PEO 400K + Protein under 5000 magnification

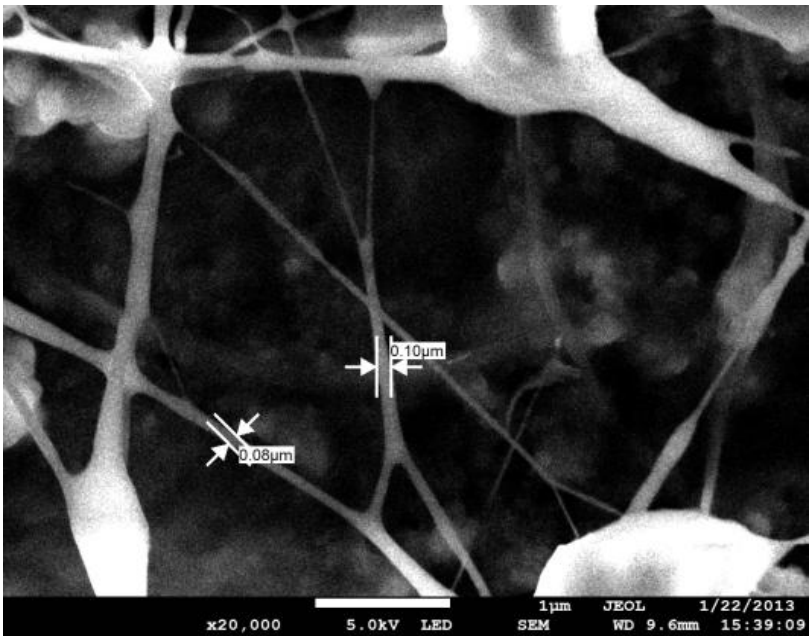


Figure 4.2.2f 1.5% PEO 400K + Protein under 20 000 magnification

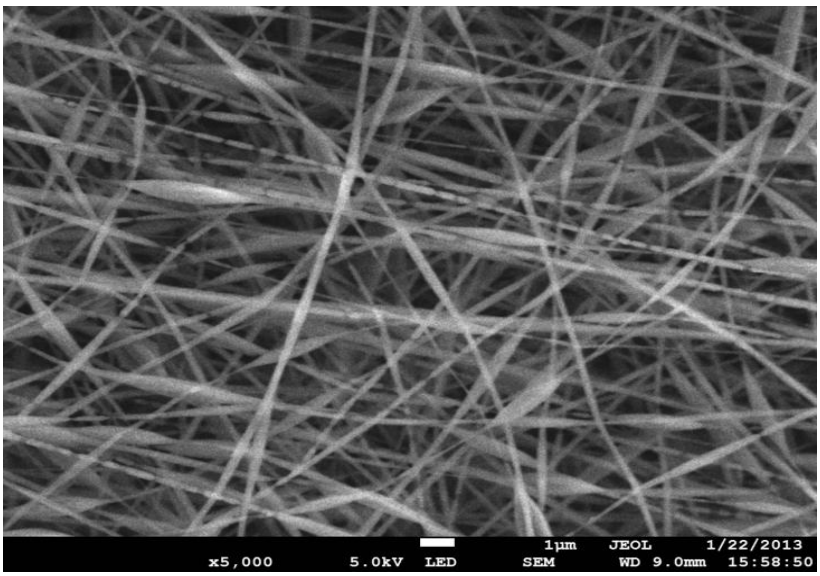


Figure 4.2.2g 3.0% PEO 400K + Protein under 5000 magnification

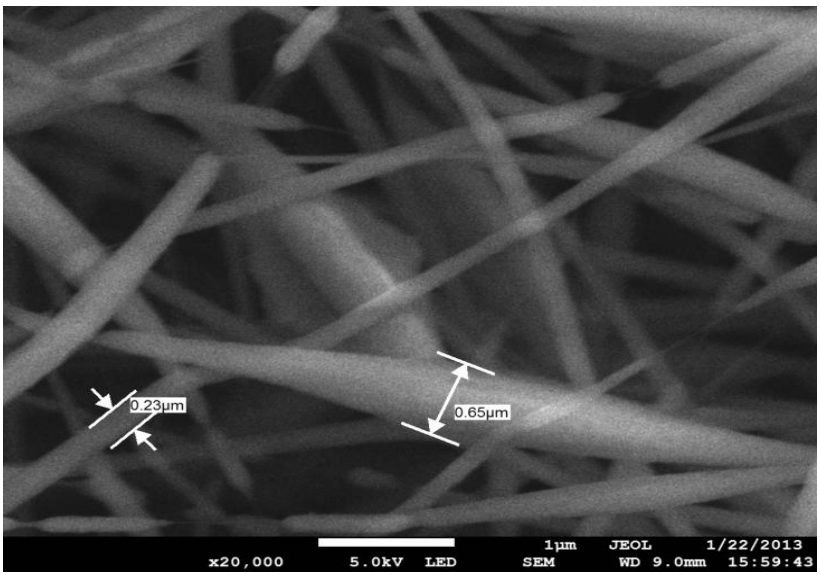


Figure 4.2.2h 3.0% PEO 400K + Protein under 5000 magnification

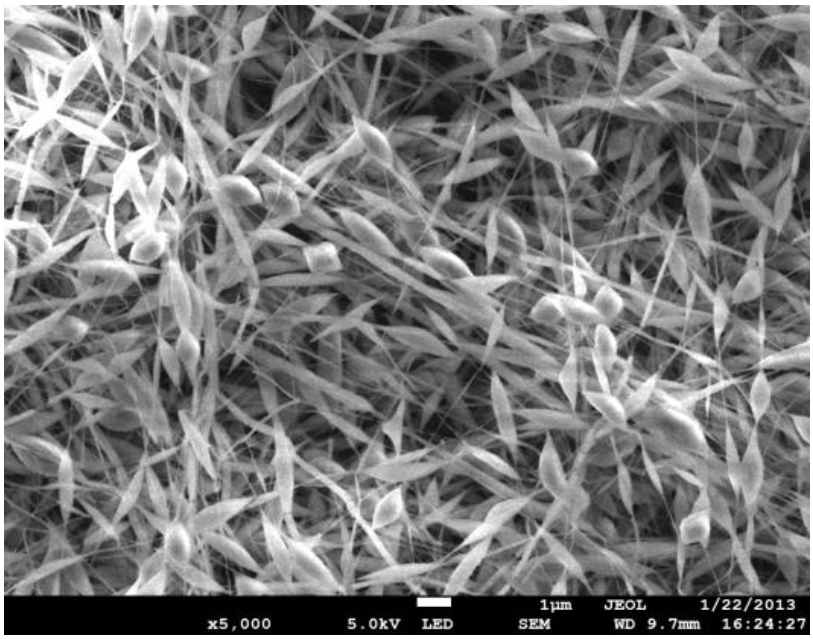


Figure 4.2.2i 1.5% PEO 400K + Protein + Ethanol under 5000 magnification

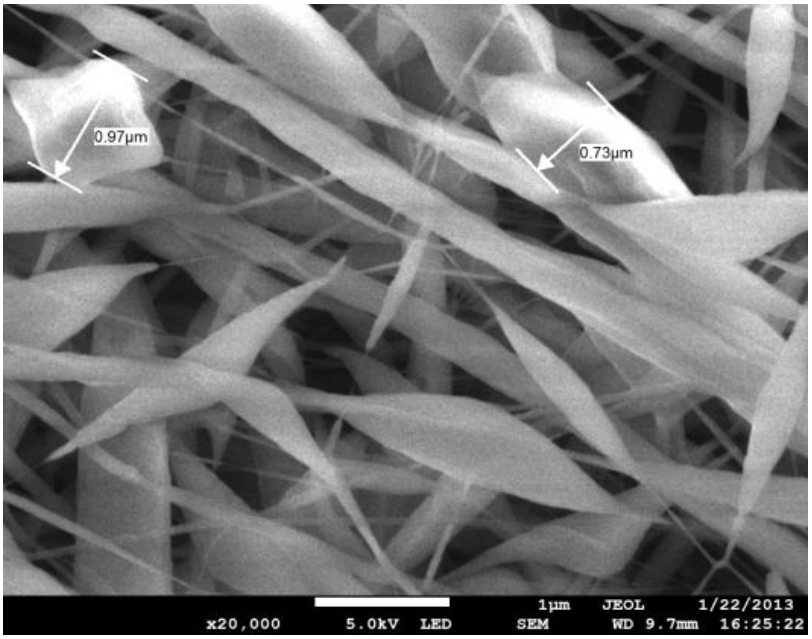


Figure 4.2.2j 1.5% PEO 400K + Protein + Ethanol under 20 000 magnification

The viscosities of the solution for all samples were done by using Programmable Rheometer. The data showed several differences in the value of viscosities. Those were due to the different molecular weight and concentration of solutions used. As we can see from the above figures (Figure 4.2.2a until Figure 4.2.2j), there surface morphology were very different from each other. Those showed that viscosity had given some effect on the formation of nanoparticles. Based on the previous figures (Figure 4.2.1a until Figure 4.2.1d) the formation of nanoparticles was very uniform and can be clearly seen under FESEM analysis caption. The reason behind those was because the viscosities of the solutions are lower than others. Ethanol and water act as solvents for PEO. As Ethanol gave the higher value of viscosity, henceforth addition of ethanol had made the solutions had the higher value of viscosity.

The result in Table 4.1 showed that 1.5% PEO 400K with ethanol gave the higher value of viscosity compared to the sample that used water as solvent. The effect can be observed from Figure 4.2.2c, as there was no nanoparticles can be seen under 5000 magnification. Compared to Figure 4.2.1a under 5000 magnification also, there were a lot of nanobeads were formed. As the viscosity increase, more fibers are formed. In Figure 4.2.2e, the FESEM analysis showed that there were some beads formations. Although the viscosity is higher than sample 1.5% PE0 400K with ethanol, but there is some beads instead of nanofibers. This is due to presence of protein that affects the surface morphology. This is same with 1.5% PEO (Figure 4.2.2i- Figure 4.2.2j) 400K with protein and ethanol, that having nanobeads although the viscosity is high due to the presence of protein. However, for 3.0% PEO 400K, there was no formation of nanobeads. This is because the higher concentration of PEO made the viscosity higher.

4.3 FE-SEM EDX Analysis

4.3.1 1.5% 400 000 MW PEO + Protein

Table 4.3.1 Summary of element in 1.5% 400 000 MW PEO + Protein sample

Element	Weight %	Atomic %	Compound %	Formula
C K	27.07	33.13	99.17	CO2
S K	0.33	0.15	0.83	SO3
O	72.6	66.72		
Totals	100			

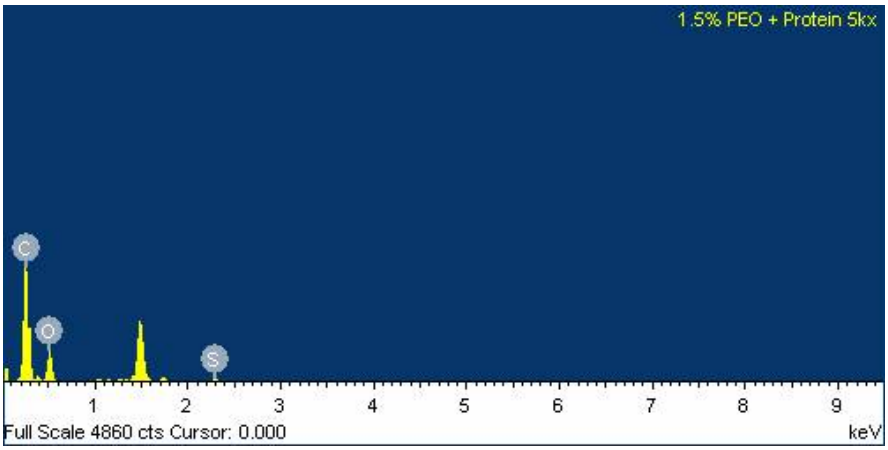


Figure 4.3.1 EDX Spectrum of the component in 1.5% 400 000 MW PEO + Protein

Based on the Table 4.3.1, it showed that the element existed in the sample of 1.5% 400 000 MW PEO + Protein. In this table, there was some percentage of sulphur existed. As whey protein used is containing amino acid cystein, henceforth it clearly approved that cystein that containing sulphur were bind at the outer surface of

elctrospun. This is important in drug delivery technology as existence of sulphur at the outside surface will affect the delivery system. Sulphur was used as it is one of the antitoxification agents.

4.3.2 1.5% 400 000 MW PEO + Ethanol + Protein

Table 4.3.2 Summary of element in 1.5% 400 000 MW PEO + Etoh + Protein

Element	Weight %	Atomic %	Compound %	Formula
C K	25.85	32.23	94.71	CO1
Na K	0.82	0.54	1.11	Na2O
Si K	1.02	0.54	2.17	SiO2
Cl K	1.15	0.48	0	
K K	0.72	0.27	0.86	K2O
O	70.45	65.94		
Totals	100			

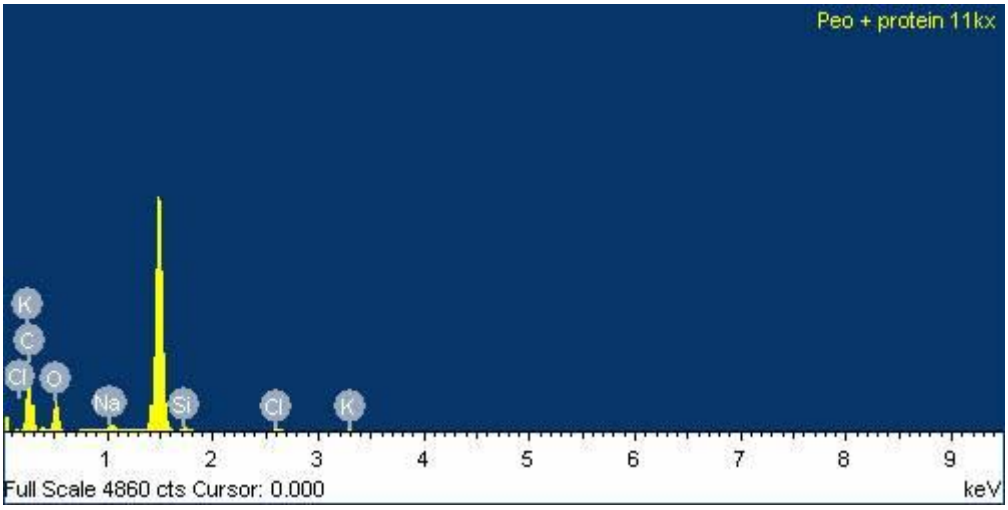


Figure 4.3.2 EDX Spectrum of the component in 1.5% 400 000 MW PEO + Etoh + Protein

In this sample, there was no sulphur presence detected. It should had sulphur in this since there was addition of whey protein to this sample earlier. However, based on the EDX analysis, it showed no presence of sulphur. There may be two assumption can be made from this result. First, the sulphur maybe degraded due to high concentration of solvent (Ethanol) that had been mixed together in the sample. Next is maybe the sulphur was embedded in the nanostructure of the molecule since the concentration of solvent and PEO is quite high.

4.3.3 3.0% 400 000 MW PEO + Protein

Table 4.3.3 Summary of element in 3.0% 400 000 MW PEO + Protein

Element	Weight %	Atomic %	Compound %	Formula
C K	27.29	33.33	100	CO2
O	72.71	66.67		
Totals				

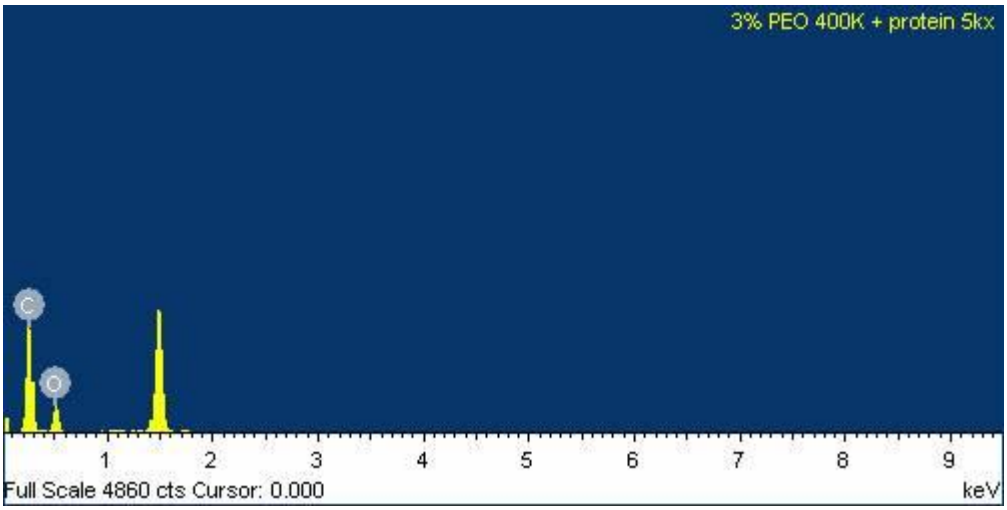


Figure 4.3.3 EDX Spectrum of the component in 3.0% 400 000 MW PEO + Protein

Based on the Figure 4.3.3, there is no spectrum of the sulphur showed during the EDX analysis. This is due to higher concentration of PEO that had been prepared for this sample. Henceforth, the high concentration of PEO resulted in failure of EDX to analyze the location of sulphur in the protein. The assumption can be make from this analysis is the sulphur was embedded in the PEO electrospun and thus made it not possible to be detected at the outside of the fiber and nanobeads.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

There are several parameters that affect the formation of nanobeads using electrospinning. Most of the biggest variable is viscosity of solution. Different molecular weight resulted in different total of beads formation, ratio to surface area. Surface morphology of nanoparticles from electrospinning showed that various sizes of nanofiber and nanobeads were formed depends on different viscosity. The more higher the viscosity value, the less the formation of nanobeads until there is no presence of beads instead of nanofiber.

Analysis on FESEM+ EDX showed that there is existence of Sulphur that come from whey on 1.5% PEO 400K + Protein. This analysis showed that the concentration of PEO affect the location of sulphur and the presence of ethanol also will degraded the sulphur. One of the conditions for electrospinning process is that the polymer should have sufficient molecular weight and viscosity to prevent the breakage of the fibers formed. Higher the molecular weight represents higher the

length of the polymer chain and viscosity and hence more entanglements are presents which prevents of the breakage of the fibers**.**

5.2 Recommendation

In this recommendation, there are some suggestions that can be considered in future study regarding this topic. In order to get the best nanobeads formation is by using low viscosity of solution. Thus, viscosity should be considered first before running the electrospinning. In addition, there are other parameter that should be consider in order to get the best formation of nanobeads such as voltage, needle size, distance between syringe and collector, flow rate of injection and also the solution conductivity. Running the electrospinning longer in order to get uniform size of nanobeads also should be taken as consideration. After the completion of electrospinning process, the sample should be place in desiccators if immediately before going to FE-SEM EDX analysis.

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Appendix A Solution preparation

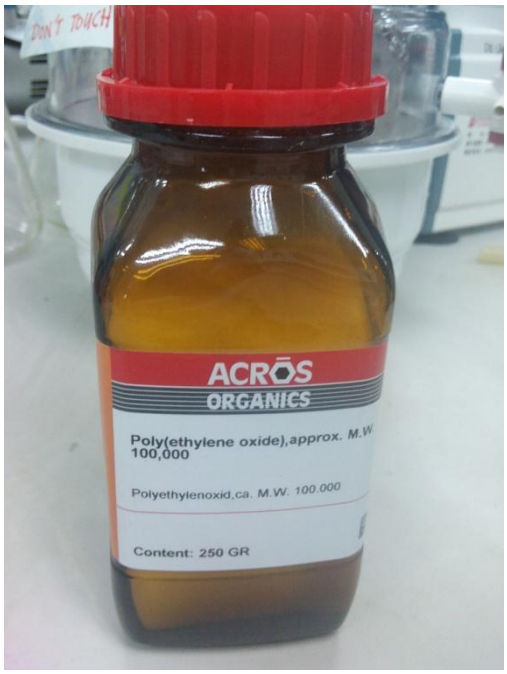


Figure A.1 Polyethylene Oxide

Figure A.2 Solution prepared

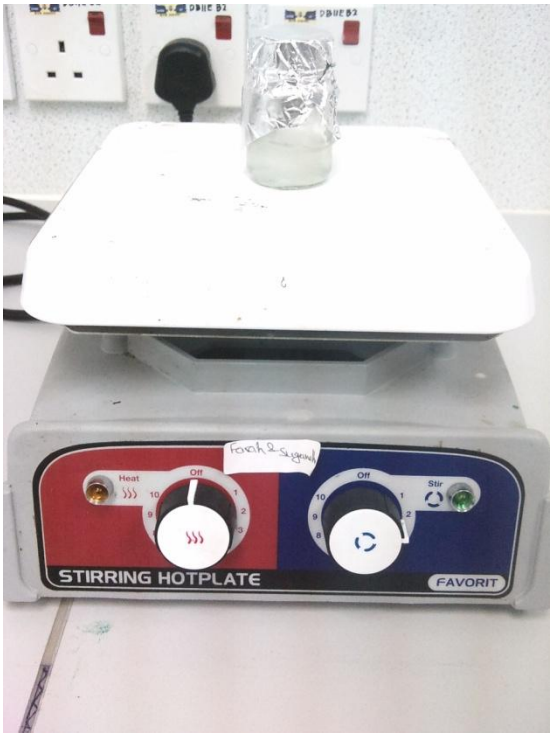


Figure A.3 Solution was stirred overnight

Figure A.4 Insertion of syringe into electrospinning machine

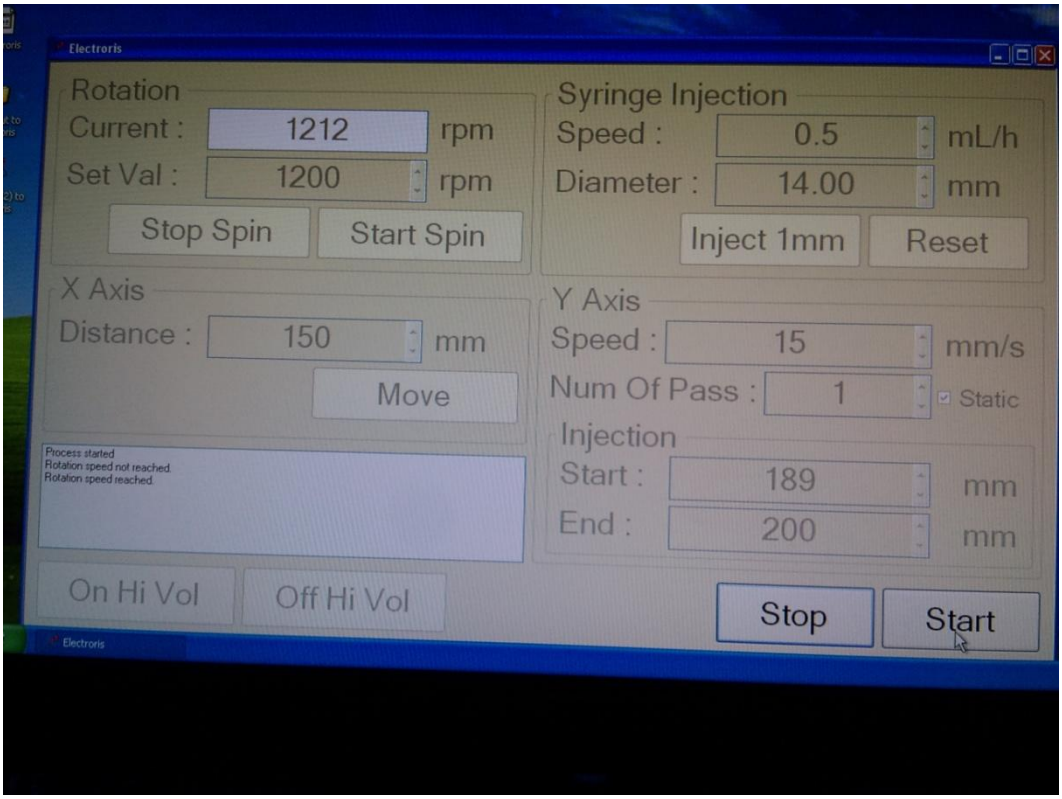


Figure A.5 Electrospinning parameter set up

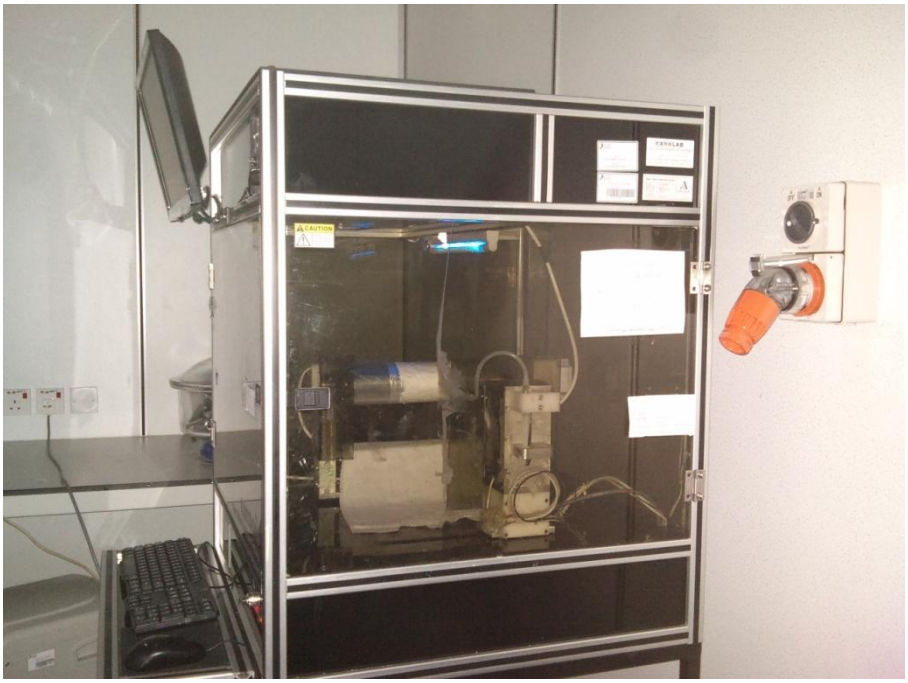
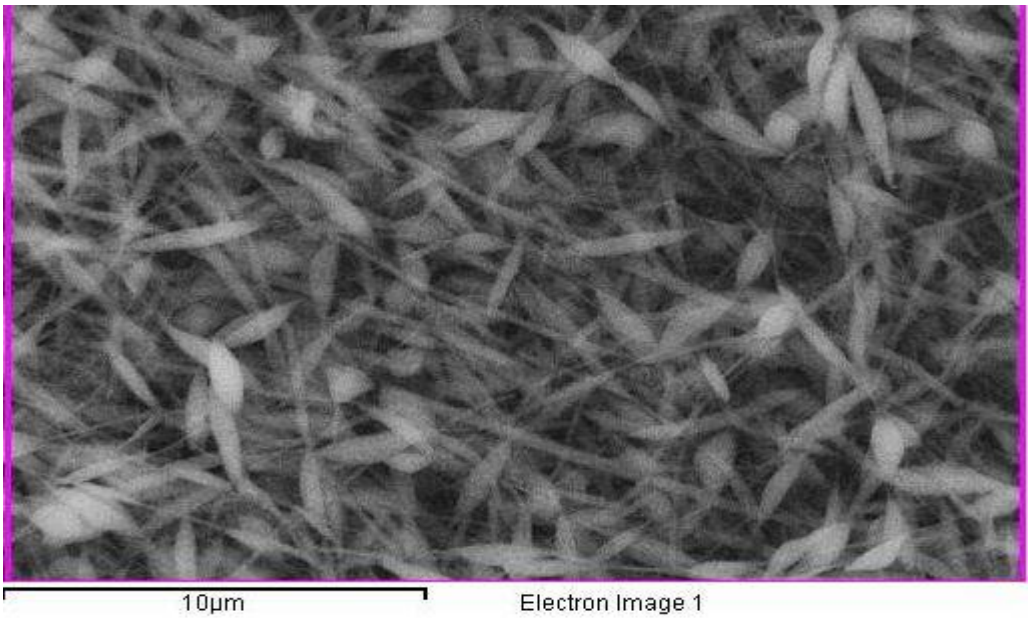


Figure A.6 Electrospinning machine

Appendix B Field Emission Scanning Electron Microscope

Figure B.1 Field Emission Scanning Electron Microscope

Appendix C Energy Dispersive X-Ray



Spectrum processing :
Peaks possibly omitted : 1.488, 1.740 keV

Processing option : Oxygen by stoichiometry (Normalised)
Number of iterations = 2

Standard :
C CaCO3 1-Jun-1999 12:00 AM
S FeS2 1-Jun-1999 12:00 AM

Element	Weight%	Atomic%	Compd%	Formula
C K	27.07	33.13	99.17	CO2
S K	0.33	0.15	0.83	SO3
O	72.60	66.72		
Totals	100.00			

Figure C.1 Example of EDX Spectrum data