CRANFIELD UNIVERSITY

Yanqi Sun

# Studies of PLGA Nanoparticles for Pharmaceutical Applications

School of Engineering
Nanomedicine

MRes
Academic Year: 2013 - 2014

Supervisor: Dr Yi Ge (Cranfield University)

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This thesis is submitted in partial fulfilment of the requirements for the degree of MRes

***(NB. This section can be removed if the award of the degree is based solely on examination of the thesis)***

# ABSTRACT

PLGA have already been successfully applied for controlled drug delivery systems by the pharmaceutical industry due to its biocompatibility, biodegradability and ease of processing. It has recently further been developed and formulated into a form of nanoparticle.

The single emulsion evaporation method was used to prepare nanoparticles in this study. By varying different parameters such as the concentration of regents, the type of surfactant and emulsion method, different particle sizes and size distribution of PLGA nanoparticles could be obtained.

The stability of PLGA nanoparticles was further investigated by assessing their thermal property over a certain period of time using DSC. The decrease of Tg confirmed the hydration and degradation of PLGA polymers and nanoparticles. The changes of surface morphology showed that the nanoparticles were in spherical shape and maintained smooth surface before the storage, whereas they started to lose their original shapes as well as agglomerate to each other after 2-week storage. These results suggested that there was an erosion and degradation of PLGA nanoparticles during storage.

Ibuprofen-loaded PLGA nanoparticles have been successfully prepared by o/w single emulsion evaporation method. During the stability study, a faster degradation rate compared to non-loaded PLGA nanoparticles was exhibited, showing that Ibuprofen increased the degradation rate of PLGA nanoparticles. According to the results of drug releasing study, PLGA nanoparticles exhibiting a slower drug release rate than pure drug which proved that drug-nanoparticule system could effectively increase the stability of drugs. PLGA polymer is a potential material for drug delivery system.



Keywords:

*Poly (lactic-co-glycolic acid); nanoparticles; Ibuprofen; drug loading; characterisation; stability; drug releasing*

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **AFM** | Atomic force microscopy |
| **BBB** | Blood brain barrier |
| **cryo-TEM** | Cryogenic transmission electron microscopy |
| **DLC** | Drug loading content |
| **DLS** | Dynamic light scattering |
| **DMSO** | Dimethyl sulfoxide |
| **DSC** | Differential scanning calorimetry |
| **DTA** | Differential thermal analysis |
| **EE** | Encapsulation efficiency |
| **EFR effect** | Enhanced permeability and retention effect |
| **ESEM** | Environmental scanning electron microscope |
| **EtOH** | Ethanol |
| **FDA** | Food and Drug Administration |
| **GPC** | Gel permeation chromatography |
| **HBcAg** | Hepatitis B core antigen |
| **IBU-NP50** | Ibuprofen-loaded PLGA 50:50 nanoparticles |
| **IBU-NP75** | Ibuprofen-loaded PLGA 75:25 nanoparticles |
| **IDDM** | Insulin-dependent diabetes mellitus |
| **IFN-γ** | Interferon-γ |
| $M_n$ | Number-average molecular weight |
| $M_w$ | Weight average molecular weight |

| | |
|---|---|
| **NP50** | PLGA 50:50 nanoparticles |
| **NP75** | PLGA 75:25 nanoparticles |
| **NSAID** | Nonsteroidal anti-inflammatory drug |
| **PCS** | Photon correlation spectroscopy |
| **PDI** | Polydispersity |
| **PDLLA** | Poly- DL-lactide |
| **PDLA** | Poly D-lactic acid |
| **PGA** | Poly (glycolic acid) |
| **PHNP** | PLGA-Hp55 nanoparticles |
| **PLA** | Poly (lactic acid) |
| **PLGA** | Poly (lactic-co-glycolic acid) |
| **PVA** | Polyvinyl alcohol |
| **R-ROP** | Random ring-opening copolymerization |
| **RT** | Room temperature |
| **SAP** | Segmer-assembly polymerization |
| **SEM** | Scanning electron microscope |
| ***Tc*** | Crystallization temperature |
| **TEM** | Transmission electron microscopy |
| ***Tg*** | Glass transition temperature |
| **TGA** | Thermogravimetric analysis |
| ***Tm*** | Melting temperature |
| **THF** | Tetrahydrofuran |

| | |
|---|---|
| **WHO** | World Health Organization |

# 1 Introduction

## 1.1 Project overview

This research study is designed and supervised by the School of Engineering at Cranfield University (UK)

This project mainly focuses on the studies of biodegradable poly lactic-co-glycolic acid (PLGA) nanoparticles for pharmaceutical applications. Due to its biocompatibility, biodegradability and ease of processing, PLGA have been extensively used for controlled drug delivery systems by the pharmaceutical industry. Biodegradable polymeric nanoparticles may provide many advantages in drug delivery systems such as the improvement of bioavailability of poorly soluble drug and prolongation of drug release. Moreover, the polymeric nanoparticles could deliver the drug more efficient to the targeted tissue as a result of improved permeability and drug diffusion .

## 1.2 Initiation & Motivation

The raise of nanotechnology has been helping people to gain the capacities to observe and manipulate at a scale of atom and molecule. Nanotechnology is a very diverse discipline where it interacts and merges with many other subjects such as medicine, engineering and biology. Nanomedicine, the applications of nanotechnology in medicine, is an emerging interdiscipline growing at an amazing rate.

A report published by BBC research in 2012 showed that the market value of the worldwide nanomedicine industry was undergoing a rapid upward trend. The total market value in 2003 was only $500 million, while it expanded to $63.8 billion and $72.8 billion in 2010 and 2011, respectively. It also estimated that the market would reach $130.9 billion by 2016.

One of the greatest values of nanomedicine could be its applications in new medical treatments and more effective drugs. For example, it can make drug more stable and improve its bioavailability especially for poorly soluble drug, as well as controlled release and delivery to the specific target site. Nanoparticles

made with biodegradable polymers haven been widely exploited in drug delivery system due to their high biocompatibility and low side effect.

PLGA is a degradable biopolymer applied in drug delivery system. It has further been developed and formulated into a form of nanoparticle (Makadia and Siegel 2011). While the preparation methods and many characteristics of PLGA nanoparticles have been widely investigated, the study of the stability of PLGA nanoparticles and their thermal properties, particularly in relation to the pharmaceutical manufacture condition, still remains underdeveloped. Therefore, this research intends to first evaluate the stability of PLGA nanoparticles which will be prepared with the modified emulsion-solvent evaporation method and freeze-drying. The properties of those PLGA nanoparticles will be further investigated, followed by a preliminary study of controlled drug release.

Currently, cancer is still a big threaten to human health. It is difficult to find out a good way for cancer therapy. Chemotherapy is the most common way which causes severe side effect on human body(Zhang, Gu et al. 2008). Therefore, scientists and researchers all put great efforts on developing effective method for cancer therapy with low side effects. Many literatures have stated that ibuprofen is not only the nonsteroidal anti-inflammatory drug, but also shows promising potential on the cancer treatment (Bonelli, Tuccillo et al. 2012, Endo, Yano et al. 2014). However there is still small numbers of studies regarding the Ibuprofen-loaded PLGA nanoparticles. Thus ibuprofen is chosen as the drug to be loaded into PLGA nanoparticles in this study. In order to explore the feasibility to deliver ibuprofen in PLGA matrix, the preparation and characterization of IBU-loaded PLGA nanoparticles, the studies of stability and drug release profile are conducted.

# 2 Literature review

## 2.1 Nanotechnology

“ Nano” originates from the Greek word “dwarf”. Nanotechnology was first proposed in 1959 in the lecture of “there’s plenty of room at the bottom” by physicist Richard Feynman, who looked into the future and predicted how technology might make things smaller and smaller. In 1974, the Japanese researcher Norio Taniguchi used the term “nanotechnology” to describe the semiconductor processes in nanometer level. People initially paid much attention to the fabrication and purity of nanomaterials themselves, but the focal point has now been shifted to apply nanomaterials to various life-related applications such as drug delivery system, DNA self-assembly and proteins delivery.

It is undoubted that nanotechnology not only has a huge potential to be applied in different kinds of areas, but also can create bigger profits and stronger technological competitiveness. Due to the rapid development of nanotechnologies, nanotechnology is regarded as the technology which might lead to the next industrial revolution in 21$^{st}$ century (Andrew D 2007).

### 2.1.1 Nanotechnology & Nanomedicine

Nanotechnology is a multidiscipline with an integration of traditional sciences such as chemistry, physics, biology and materials science. It can be defined as “the science and engineering involved in the design, synthesis, characterization, and application of materials and devices, whose smallest functional organization in at least one dimension, is on the nanometer scale or one billionth of a meter” (Emerich and Thanos 2003). In simple terms, nanotechnology is a science at a very small scale. At this scale, various possibilities can be made.

Nowadays, nanotechnologies are applied in various areas from manufacturing to computing, and even to medicine and cosmetics. Most of applications of nanotechnology are based on the fact that nanomaterials can exhibit unique

properties which are quite different from their bulk properties. With reduction of the size, the surface-to-volume ratio of materials increases. Therefore, a bigger area of surface can be exposed and the materials become more active. As a result, various functionalization can be made on the surface of nanomaterials. Besides the larger surface-to-volume ratio, quantum size effect is another important property of nanomaterials. By utilizing this property, nanotechnology can be an effective tool for imaging. An example of the materials applied in nanotechnology is carbon, which is the basic element in the nature world. It can be fabricated into various kinds of nanostructures such as carbon nanotubes, carbon dots, carbon fibres, which own higher strength and strong fluorescence. Due to their properties, these carbon nanomaterials are widely applied in microelectronics, aerospace industry and medicine.

Nanofabrication could be achieved via two approaches: ‘top-down’ and ‘bottom-up’. The ‘top-down’ approach starts with a block of material followed by crafting into desirable structures by etching or milling. The main challenge for top-down manufacture is how to reduce the size of structures while insure the high accuracy. In contrast, the ‘bottom up’ approach fabricates nanomaterials by assembling the small molecules into bigger shapes. How to effectively build up molecules to a bigger size is the crucial problem for this approach. So far, these two approaches have been extensively exploited to achieve various nanofabrications. The combination of these two approaches now appears to be a new trend to modify the preparation techniques (The Royal Society, 2004).

Nanotechnology undergoing rapid advances triggers a large amount of researches and developments in human health. Consequently, a new discipline, nanomedcine, emerges from nanotechnology and becomes a hot topic. Nanomedicine is the medical application of nanotechnology to disease treatment and drugs optimization. Our body consists of different kinds of organs which are made up with cells. The major constituent of cells is DNA, proteins and some other biological molecules and they are all within nano-scales. Applying nanotechnology to medicine makes it possible to let people to better observe cells and tissues as well as to design corresponding materials and

devices to interact with them where the bulk materials cannot reach. (Silva 2004).

### 2.1.2 Nanoparticles in pharmaceuticals

Nanoparticles are colloidal polymeric particles with a size between 10 to 1000nm (Brigger, Dubernet et al. 2012). Nanoparticles may exhibit many extraordinary properties such as quantum size effect, surface effect, volume effect and macroscopical quantum tunnel effect which cannot be observed in fine particles or bulk materials. These properties enable naoparticles to have bigger surface to mass ratio, visual effects and advanced bulk materials properties. Due to the lager surface to mass ratio, nanoparticles are able to encapsulate drugs, proteins and probes as well as bind and adsorb them on the surface (De Jong and Borm 2008). Because of their submicron size, nanoparticles are small enough to reach the tissues and cells, which can be used in drug delivery system, gene delivery, and bio-imaging etc. It is even possible for nanoparticles to permeate the blood brain barrier (BBB) by coating with different surfactants.

As shown in ***Table 2.1,*** there are different kinds of nanoparticles, including liposome, dendrimers, solid lipid nanoparticle, and polymeric nanoparticles etc., made from nature materials to synthetic materials. Due to the diversity of nanoparticles, they can be designed into different kinds of vehicles for therapeutic purpose. Among these applications, drug delivery is the major interest group and has received an increasing interest and investments from the pharmaceutical industry. The nanoparticles-associated drug delivery could target organs directly and specifically and further reduce the toxicity caused by free drugs to non-target organs. As a result, the bioavailability of the drugs could be increased.

**Table 2.1 Overview of some nanoparticles and their medical applications (De Jong and Borm 2008)**

| Particle class | Materials | Application |
|---|---|---|
| Natural materials or derivatives | Chitosan<br>Dextrane<br>Gelatine<br>Alginates<br>Liposomes<br>Starch | Drug/Gene delivery |
| Dendrimers | Branched polymers | Drug delivery |
| Fullerenes | Carbon based carriers | Photodynamics<br>Drug delivery |
| Polymer carriers | Polylactic acid<br>Poly(cyano)acrylates<br>Polyethyleinemine<br>Block copolymers<br>Polycaprolactone | Drug/gene delivery |
| Ferrofluids | SPIONS<br>USPIONS | Imaging (MRI) |
| Quantum dots | Cd/Zn-selenides | Imaging<br>In vitro diagnostics |
| Various | Silica-nanoparticles<br>Mixtures of above | Gene delivery |

Liposome is the first materials applied in the pharmaceutical field for drug delivery. Due to its similarity to biological cells, liposome is biocompatible and biodegradable to human body, which shows a promising potential to be applied as a vehicle for drug delivery. A wide range of agents have been demonstrated that can be effectively encapsulated into liposomes to achieve a better therapeutic treatment (Sharma and Sharma 1997). However, liposomes also have some limitations, such as poor storage stability, low encapsulation efficiency and hard control for drug release. In order to overcome these limitations, biodegradable nanoparticles have been widely applied in this field and they have shown better results than liposomes (De Jong and Borm 2008). Meanwhile, surface modification is another effective way to solve these problems.

## 2.2 Biodegradable polymers

### 2.2.1 Properties & applications

Biodegradable materials can be obtained both from the nature and synthesis. They are able to be degraded in human body either with enzyme or not. The degradation products they produced which are biocompatible and toxicologically safe are further eliminated by the normal metabolic pathways. During past decades, the applications of biodegradable polymers in controlled drug delivery system were undergoing a dramatic development. An increasing number of researches have been conducted for biodegradable polymers (Makadia and Siegel 2011).

Biodegradation is generally defined as the process of biomaterials being solubilized, hydrolyzed or resolved by enzymes or chemical substances to formulate CO2, H2O, CH4 and other low molecular-weight products (Wang, Wu et al. 2000). The mechanism of biodegradation can be divided into four steps, namely water sorption, reduction of mechanical properties (modulus & strength), reduction of molar mass, and weight loss. During the process of degradation, its speed can be influenced by PH, temperature and humidity (Kronenthal 1975).

According to Ikada Y, biodegradable polymers have been mainly applied in two areas, namely biomedicine and ecology (Ikada and Tsuji 2000). For ecological applications, a large amount of agriculture products and plastics have been designed to be biodegradable in order to reduce their pollutions to ecological environment (Gross and Kalra 2002). For biomedicine applications, they have been widely applied in surgery and pharmacology due to their properties of biodegradability and low-toxicity by-products. There are many advantages of biodegradable polymers. Unlike biocompatible materials, biodegradable polymers could avoid foreign-body reactions since they only stay in the body during a short period and then disappear without any traces. As no one want to take a risk to carry any foreign materials permanently, biodegradable polymers are undoubtedly a good choice for health application (Ikada and Tsuji 2000).

### 2.2.2 PLA, PGA, PLGA polymers

The thermoplastic aliphatic polymers such as poly (glycolic acid) (PGA) and poly (lactic acid) (PLA), and their copolymer poly (lactic-co-glycolic acid) (PLGA), have been extensively studied and widely used as biodegradable polymers.

PGA (***Figure 2.1***) is the simplest linear polyester with high crystallinity (45%-55%), which lead to its low-solubility in various organic solvents. It can be degrade easily by hydrolyzing the ester bond. Surgical suture and bone internal fixation devices have been widely fabricated by PGA to ease the pain for patient and to get these things out of our body because of its biodegradability. However, the low solubility of PGA restricts its application in a wider field (Middleton and Tipton 2000).

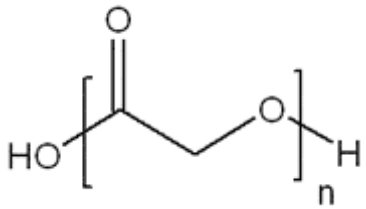



**Figure 2.1 General Structure of Poly(glycolic acid) (PGA)**

In comparison, PLA (***Figure 2.2***) is a chiral molecule with two stereoisomers L-lactic acid and D-lactic acid (Park 1995). As a result, PGA has three forms of polymer, namely poly D-lactic acid (PDLA), poly L-lactic acid (PLLA) and poly D, L-lactic acid (PDLLA). PDLA is high-crystalline and hard to be processed to various structures. For semi-crystalline PLLA, it is degraded in a slow speed and can be utilized to bone internal fixation devices. PDLLA is more flexible than other two forms and it is amorphous. Therefore it can be easily degraded and extensively used in drug delivery system.

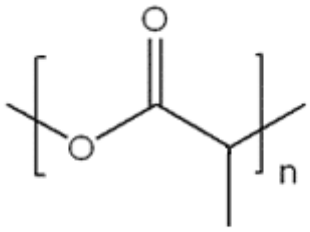
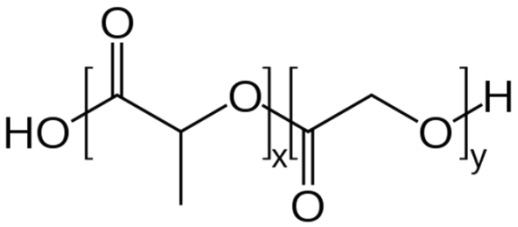


**Figure 2.2 General Structure of Poly (lactic acid) (PLA)**

PLGA (***Figure 2.3***) is co-polyester approved by the American Food and Drug Administration (FDA) and European Medicine Agency, which means PLGA is safe enough to be used as biomedical products such as sutures and pharmaceutical materials especially in drug delivery system (Zolnik and Burgess 2007). While PLGA copolymers are amorphous, their crystallinity is reduced and can be hydrolyzed greater than its monomers, PLA and PGA, in the presence of water (Park 1995). Furthermore, different ratios of PLGA exhibit different properties and degradation times. By changing its ratios, degradation time and drug release kinetics can be controlled (Makadia and Siegel 2011). Among these, 50:50 PLGA is the most common one and own the fastest degradation rate (Lu, Wang et al. 2009).



*x*= number of units of lactic acid; *y*= number of units of glycolic acid

**Figure 2.3 The structure of PLGA copolymer;**

## 2.2.3 Synthesis of PLGA polymers

Currently, the most common method to synthesize PLGA polymers is to condense the glycolic acid, lactic acid and their oligomers directly or random ring-opening copolymerization(R-ROP) between two monomers, the cyclic

dimers (1, 4-dioxane-2, 5-diones) of glycolide and lactide. ***Figure 2.4*** illustrates the basic polymerization process of synthesis. The first method is to simply condense the oligomers of glycolic acid and lactic acid, which means only 50:50 PLGA polymers can be produced, whilst ROP method is the other method shown on ***Figure 2.4***.

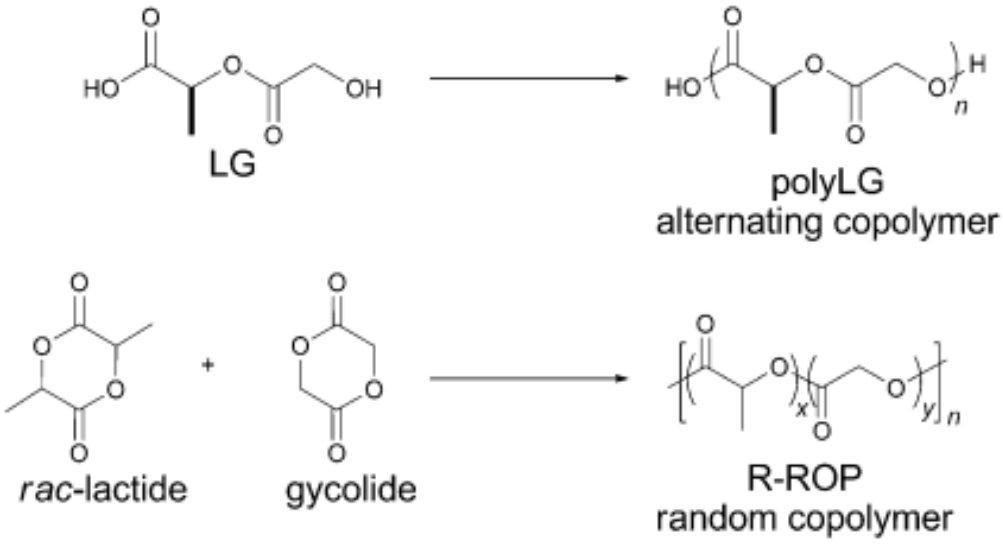



**Figure 2.4 Molecular structure prepared by SOP(Thomas and Lutz 2011)**

However, ROP method shows a broad polydispersity indexes (PDIs) of the molecular weight and wide composition range (Qian, Wohl et al. 2011, Thomas and Lutz 2011). Many studies have demonstrated that both molecular weight and composition play significant roles in the determination of degradation properties and thermal properties of PLGA polymers. In order to achieve narrower PDIs and more homogeneous co-polymers, various optimization are designed. Stayshich and Meyer has successfully prepared the PLGA polymer by segmer-assembly polymerization (SAP) method and demonstrated that repeating sequence of PLGA co-polymers exhibit better properties with uniform degradation and linear weight loss (Stayshich and Meyer 2010). The synthesis method is shown in ***Figure 2.5***, a condensation of fixed sequence of oligomers (LG, LL, GG and GL), which can effectively control the sequence of the co-polymer and obtain varying composition of PLGA. At the same time, differet kinds of catalysts are also founded to optimize the fabrication method of PLGA polymers.

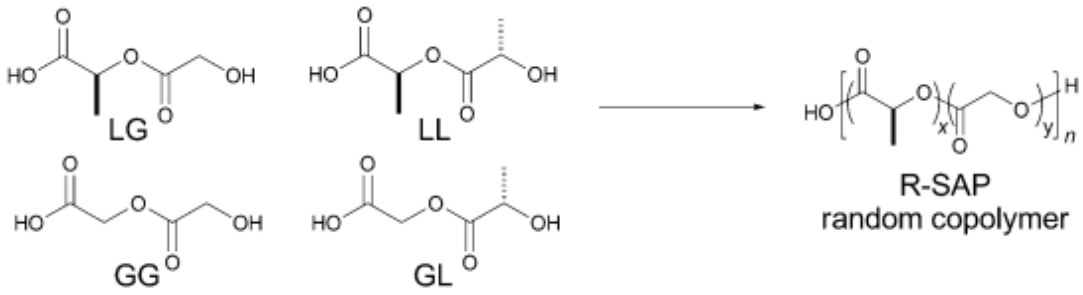



**Figure 2.5 Molecular structure prepared by SAP(Thomas and Lutz 2011)**

**(L=lactic unit, G=glycolic unit)**

## 2.3 PLGA nanoparticles

### 2.3.1 Physicochemical properties of PLGA nanoparticles

The physicochemical properties of PLGA nanoparticles such as surface area, size, molecular weight and crystallinity can affect the biodegradation and the kinetics of drug release (Zolnik and Burgess 2007).

Materials can exhibit different properties when they are in the nano-size. As discussed before, nanomaterials are able to bind, adsorb and carry other compounds more chemically reactive than bulky materials. Their strength and/or electrical properties could also be heavily effected (The Royal Society, 2004).

PLGA is made up with PLA and PGA and it is generally an acronym for poly D, L-lactic- co – glycolic acid where D- and L- lactic acid forms are in equal ratio (Makadia and Siegel 2011).PLGA is an amorphous (non-crystalline or semi-crystalline) material in nature. Thus it can be easily produced into any structures and are soluble in common organic solvents (e.g. acetone and ethyl acetate) (Makadia and Siegel 2011). Tg is the major characteristic transformation temperature of the amorphous phase, which is defined as the temperature when a material transforms from glassy structure to rubbery liquid (Sichina W J, 2000). PLGA copolymers have a glass transition temperature between 40 and 60 °C higher than physiological temperature (37°C), which means it is glassy in nature and have a fairly rigid chain structure which is strong enough to be formulated as drug delivery devices (Wang, Wu et al. 2000). The molecular weight is also a crucial parameter for determining the mechanical strength of the polymer (Wang, Wu et al. 2000).

Size and size distribution are also of significant importance for nanoparticle-aided drug delivery systems. The nanoparticle sizes are closely related to drug release properties. If the particle size is too small, it may degrade before drugs reach the target organs thus causing undesired toxicity. On the contrary, nanoparticle may not be able to permeate into cells effectively with bigger size (Li, Wang et al. 1997).

Meanwhile, different composition of PLGA polymers can exhibit different physicochemical properties. 50:50 is the most commonly used composition of PLGA co-polymers and it has the shortest biodegradation time lasting only 50-60 days. In comparison, other copolymer composition, such as 75:25, leads a longer time to degrade (Mundargi, Babu et al. 2008).

Compared to PGA, PLA is more hydrophobic and more difficult to be hydrolyzed because of its additional methyl side groups which hinder the water molecular to attack. Therefore, glycolic-rich PLGA co-polymers are more soluble to water and have a faster degradation (Wang, Wu et al. 2000, Muthu 2009). Their molecular weight, Tg, melting point and crystallinity percentage are also altered by various ratios of PLGA (Muthu 2009). Due to the lack of any methyl side groups in PGA, lactic-rich PLGA co-polymers have a higher molecular weight and increased Tg.

### 2.3.2 Stability of PLGA nanoparticles

According to Abdelwahed, the instability of PLGA nanoparticles is a major issue for pharmaceutical applications, particularly for the drug delivery system (Abdelwahed, Degobert et al. 2006). In order to increase the stability of PLGA nanoparticles, proper storage conditions should be employed with a monitoring system to track the physiochemical changes. The stability of nanoparticles usually refers to physical stability and chemical stability.

Physical stability is mainly based on the characters of colloid. Due to the size of colloids and their high polydispersity, colloidal particles are in the Brownian motion, resulting in the suspension of particles in the solution. On the other hand, colloid has high surface to mass ratio and large surface area, which cause particles to agglomerate together. This is known as the thermodynamic instability. Adding stabilizers during the preparation is a good way to avoid aggregation (Abdelwahed, Degobert et al. 2006). PVA (polyvinyl alcohol) is the most common stabilizing agent for the preparation of PLGA nanoparticles.

Chemical stability is affected by many parameters such as temperature, pH, type/composition of polymers, etc. Lemoine D et al studied the stability of PLGA

with different composition ratios by measuring their molecular weight. They have found that bigger composition ratios of PLA result in more stable particles. The research showed that the molecular weight of 50:50 PLGA decreased faster than that of 75:25 PLGA (Lemoine, Francois et al. 1996). The rate of degradation remains low at a pH of 7.4 and at a storage temperature of 4 ℃. The extreme pH levels (either in strong acid or strong base) can further enhance the degradation rate and reduce the stability (Abdelwahed, Degobert et al. 2006). In addition, the type of delivered/encapsulated drugs is also an important parameter which may affect the physiochemical properties and degradation rates PLGA (Makadia and Siegel 2011).

## 2.4 Preparation of PLGA nanoparticles

Various methods can be applied for manufacturing synthetic polymeric nanoparticles. Since the way of preparation might determine the properties of nanoparticles, it is essential to optimize the preparation method by adjusting the parameters to make desirable PLGA nanoparticles (Nagavarma, Yadav et al. 2012).

Preparation of PLGA nanoparticles can be made by two approaches： bottom – up approach and top down approach (***Figure 2.6***). The bottom-up approaches include emulsion/microemulsion polymerization, interfacial polymerization, and precipitation polymerization, while the top-down approaches involve emulsion diffusion, solvent displacement, salting out method and emulsion evaporation (Astete and Sabliov 2006, Nagavarma, Yadav et al. 2012).

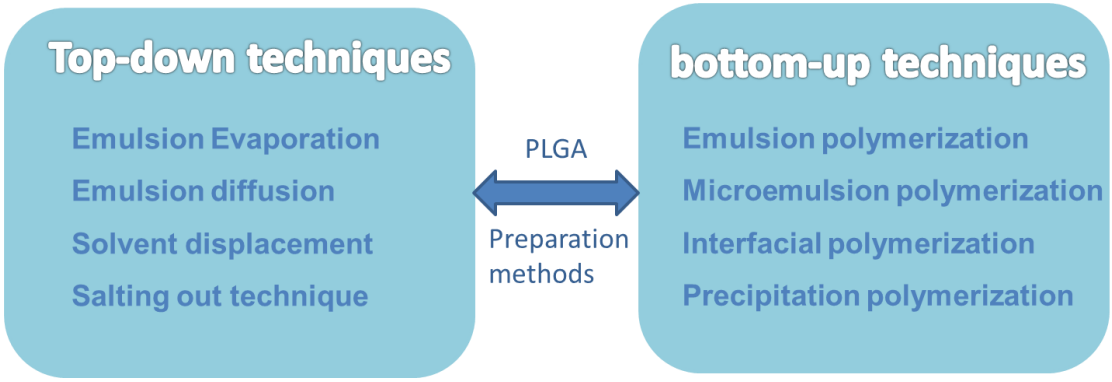



**Figure 2.6 Preparation methods of nanoparticles**

Particle size is of significant importance in the preparation of nanoparticles. Different particle sizes can be obtained by using different kinds of preparation methods. Meanwhile, the variety of particle size also appears in the same preparation methods. By modifying some parameters, particle sizes can change dramatically. ***Table 2.2*** simply summarizes the PLGA nanoparticles prepared by various methods. It also compares the variation of particle size based on one method with different parameters (e.g. ratio of PLGA polymer, molecular weight of polymer, surfactant and drugs)

**Table 2.2 Summary of important parameters of PLGA nanoparticles by different preparation methods**

| PLGA | Mw (Da) | preparation method | Drug | paticle size(nm) | Techniques for particle size | reference |
|---|---|---|---|---|---|---|
| 50/50 | 45000 | solvent evaporation | Gyslosporine | 120±15 | Dynamic light scattering | (Chacón, Molpeceres et al. 1999) |
| 50/50 | 8000 | emulsion-solvent evaporation | BSA model protein | 168±60 | Dynamic light scattering | (Feczkó, Tóth et al. 2011) |
| 50/50 | 13500 | solvent evaporation | Gentamicin | 266±100 | Dynamic light scattering | (Friess and Schlapp 2002) |
| 50/50 | 36200 | solvent evaporation | Gentamicin | 553±207 | | |
| 50/50 | 6000 | interfacial deposition method | Paditaxel | 122±3 | Photon correlation spectroscopy | (Fonseca, Simoes et al. 2002) |
| 50/50 | 14500 | interfacial deposition method | Paditaxel | 133±2 | | |
| 75/25 | 63600 | interfacial deposition method | Paditaxel | 160±2 | | |
| 50/50 | 10000 | Nanoprecipitation | procaine hydrochloride | 198±3.4 | Photon correlation spectroscopy | (Govender, Stolnik et al. 1999) |
| 50/50 | 11000 | Nanoprecipitation | Rhodamine | 300±85 | Scanning electron microscope y | (Betancourt, Shah et al. 2009) |
| 50/50 | 40,000–75,000 | emulsion/solvent evaporation | Dexamethaso-ne | 500±100 | Scanning electron microscope | (Kim and Martin 2006) |
| 50/50 | | Nanoprecipitation | Sparfloxacin | 198 ± 4.4 | Photon correlation spectroscopy | (Gupta, Aqil et al. 2010) |

### 2.4.1 Emulsion evaporation Method

Emulsion evaporation is the oldest method that has existed for over 30 years and it is the most commonly-used method to prepare PLGA nanoparticles (O'Donnell and McGinity 1997). Emulsion evaporation includes single-emulsions (oil in water (o/w) or water in oil (w/o)) and double-emulsions (w/o/w). Single-emulsion (o/w) is mainly used for hydrophobic drugs which can increase the solubility of drugs. In contrast, double-emulsions are suitable to encapsulate water-soluble drug (Astete and Sabliov 2006). ***Figure 2.7*** illustrates the procedures of emulsion evaporation method. Firstly, oil phase is prepared by dissolving the polymer in an organic solvent like chloroform, ethyl acetate, or dichloromethane. Then the oil phase is dropped into an aqueous solution with a high speed stirring to create an oil-in-water (o/w) emulsion by using a surfactant such as poly (vinyl alcohol). After the emulsification between these two phases, agitation is continued until the evaporation of the organic solvent. Reducing pressure can also be used to evaporating the solvent (Nagavarma, Yadav et al. 2012).

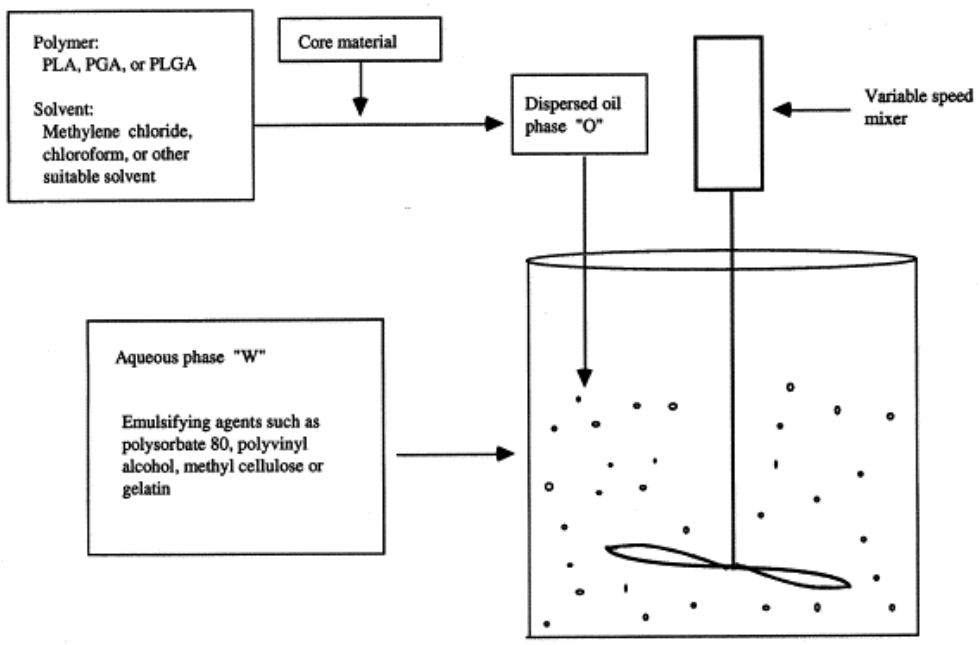



**Figure 2.7 Schematic diagram of O/W emulsion solvent evaporation**

**(O'Donnell and McGinity 1997)**

Parameters like variety of surfactants, homogenizer speed, organic solvents and polymer concentration are crucial to determine the size of PLGA nanoparticles (Astete and Sabliov 2006, Nagavarma, Yadav et al. 2012). For example, homogenization with a high speed is an effective way to reduce the particle size. A research conducted by Lemoine and Francois observed that with increased surfactants concentration, the size of nanoparticle can have a distinct reduction (Lemoine, Francois et al. 1996). Surfactants are used to help stabilize the emulsion for the purpose of avoiding aggregation. PVA is a surfactant that has been generally employed. Although surfactants play important roles in nanoparticles preparations, it is hard to remove them from the surface of nanoparticles, leading to potential side effects and toxicity in the human body.

### 2.4.2 Emulsion Diffusion Method

In this synthetic scheme, the polymer and the drug are dissolved in a partially water-miscible solvent. The organic phase is added onto an aqueous phase prepared by a suitable surfactant (e.g. PVA, anionic sodium dodecyl sulphate (SDS)) under a mild stirring. The resulted emulsion is diffused into water under a moderate stirring to obtain nanoparticles (Astete and Sabliov 2006).

Parameters such as surfactant, polymer concentration, polymer molecular weight and stirring speed are required to be considerate during the nanoparticle preparation. Although the stirring speed does not influence the formation of nanoparticles, it can determine the particle size of nanoparticles. The higher stirring speed is, the smaller the particle size is (Astete and Sabliov 2006).

Many literatures have shown that emulsion diffusion method is energy-saving because it does not require a high speed stirring. However, the main drawback of this method is the difficulty to remove the large amount of water. A higher centrifugation speed and longer centrifugation time are needed to separate nanoparticles and water (Astete and Sabliov 2006, Pinto Reis, Neufeld et al. 2006).

### 2.4.3 Nanoprecipitation(solvent displacement or solvent diffusion) Method

Nanopercipitaion method is also called as solvent displacement of solvent diffusion method. The method is based on the interfacial deposition. Water-miscible solvent such as acetone, methanol was used to dissolve the polymer and drug. Then the solution is poured into an aqueous phase either with surfactant or non-surfactant. Nanoparticles are generated spontaneously with the organic solvent diffusing into aqueous phase. Normally, water is used as aqueous phase. PVA or Poloxamer are the most frequently-used surfactant for the nanoprecipitaion method. Both hydrophilic and hydrophobic drugs can be applied for preparation the drug-loaded nanoparticles (Astete and Sabliov 2006, Rao and Geckeler 2011).

There are many parameters affecting the physicochemical propertied of polymeric nanoparticles. The way to inject the organic phase into aqueous is a key procedure. A constant and wise dropping is of importance to determine the size and morphology of nanoparticles (Rao and Geckeler 2011). Meanwhile, the type of surfactant and organic solvent, the aqueous agitation rate and the concentration of polymer are all the critical parameters for the nanoprecipitation method (Astete and Sabliov 2006).

### 2.4.4 Salting-out

In order to fabricate the nanoparticles, the polymer and drug are dissolved in water-miscible solvent. Then, the prepared solution is added to an aqueous phase which prepared with a high-concentrated salt and emulsifier under a high stirring speed.

***Figure 2.8*** exhibit the process of the salting out method.Salting out is similar to nanoprecipitation method. However, compared with nanoprecipitaion, salting out does not need solvent diffusion that the water-miscible solvent transform to the aqueous phase. Due to the existence of salt, nanoparticles can directly formulate in the aqueous phase(Pinto Reis, Neufeld et al. 2006).

Parameters like concentration of polymer or surfactant, type of surfactant or salt and stirring speed all play important roles in the process of salting out preparation (Astete and Sabliov 2006).

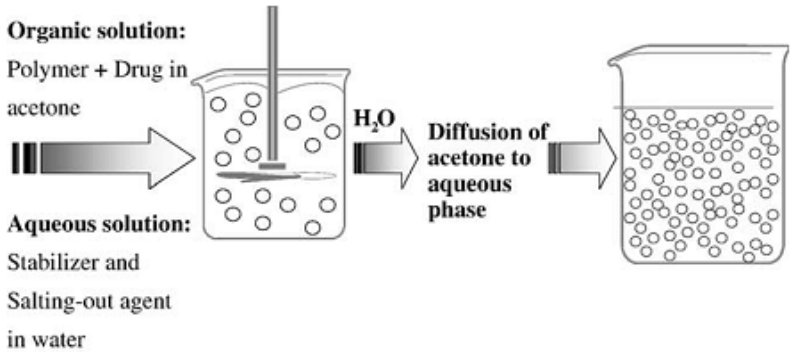



**Figure 2.8 The basic process of salting out method**

**(Pinto Reis, Neufeld et al. 2006)**

## 2.5 Drug Delivery

“Drug do not delivery themselves”(Bala, Hariharan et al. 2004). In order to delivery drug directly and effectively to the target site, a suitable drug delivery system is of the essence. The drug delivery system plays an indispensable role in the pharmaceutical field, which is able to improve the performance of the drug and increase the possibilities for other innovative therapies (Bala, Hariharan et al. 2004). Recently, there is a growing interest on the development of polymeric drug delivery. Due to the low toxicity, biodegradable polymer is preferred materials for the application of drug delivery. Furthermore, various forms of drug delivery agents can be prepared with biodegradable polymer (e.g. films, cubes, nanospheres and nanocapsules), which enlarge the choices of the methods to deliver drugs. Last but not the least, it has great potential to achieve the controlled release and site pacific delivery by using polymers for drug delivery.

PLGA nanoparticle is one of the hot topics in the drug delivery system. The main advantage of PLGA nanoparticles is bio-degradation. Therefore, it is easy to control the drug release rate by varying the degradation time of polymers. There are large numbers of methods to vary the degradation time. Parameters like composition of PLGA, particle size, PH and molecular weight are required to be considered to determine the degradation rate of PLGA, which will be detailedly discussed in the sections below.

### 2.5.1 Drug loading

There are two ways to load drug onto nanoparticles. One is entrapping drugs in the core of nanoparticles. The other one is absorbing drugs on the surface of nanoparticles. The methods to prepare drug-entrapped PLGA nanoparticles have been introduced in the ***Section2.4***. Both hydrophobic and hydrophilic drugs can be encapsulated into PLGA nanoparticles by different preparation method. The surface-absorbed nanoparticles are achieved by adding drugs into a solution containing pre-prepared PLGA nanoparticles or adding them during the process of the polymerization (Soppimath, Aminabhavi et al. 2001).

High loading capacity is an important property for a successful drug delivery system (Kumari, Yadav et al. 2010). Due to the high loading capacity, a reduction of the quantity of PLGA polymers and a increase of drug does can be achieved, which reduce the toxicity of materials to human. The drug loading content (DLC) is used to measure how many drugs have been loaded into nanoparticles. In contrast to theoretical weight of drugs, the amount of the drug loaded into nanoparticles is determined by encapsulation efficiency (EE).The calculation of DLC and EE are shown below.

$$\mathrm{DLC}(\%) = \frac{\text{Drug weight in nanoparticles}}{\text{nanoparticles weight}} \times 100\% \qquad \textbf{Equation 2.1}$$

$$\mathrm{EE}\ (\%)\ = \frac{\text{actual weight of Drug}}{\text{theoretical weight of Drug}} \times 100\% \qquad \textbf{Equation 2.2}$$

However, it is difficult to precisely measure the drug load content of nanoparticles due to the problems to completely remove the unloaded-drugs. The most common method to remove the unbound drugs from nanoparticles is high-speed centrifugation, dialysis method or gel filtration.

### 2.5.2 Degradation

It is commonly acknowledged that the aliphatic polyester nanoparticles degrade via a hydrolytic mechanism. PLGA undergoes hydrolysis degradation as well as a possible enzymatic degradation which still has not been completely proved by the researchers. As shown in ***Figure*** 2.9, PLGA polymer degrades with the uptake of water and then hydrolysis into lactic acid and glycolic acid.

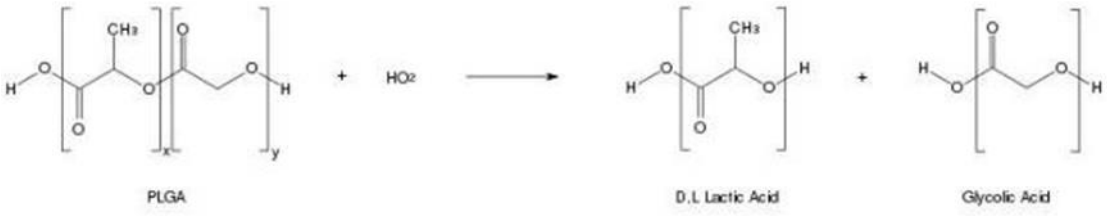



**Figure 2.9 Hydrolysis of poly lactic-co-glycolic acid(Makadia and Siegel 2011)**

The detailed process of PLGA degradation contains three steps. In the first phase of hydrolysis, the molecular weight of PLGA co-polymers reduces dramatically with continuously cleavage and solubilization of low molecular weight fragments. In this process, the structures of polymer remain the same and no monomers are produced. In the second phase of degradation, the D, L-lactic and glycolic acid monomers and soluble oligomer are formulated, resulting in significant loss of mass. Due to the formulation of the acid monomers, the pH reduces rapidly and autocatalyses further degradation. In the final phase, the soluble oligomers are completely hydrolysed into soluble monomers(Bala, Hariharan et al. 2004).

There are two ways for degradation, namely heterogeneous degradation and homogenous degradation. Heterogeneous degradation means that the degradation undergoes a more rapidly rate in the core of PLGA nanoparticles than at the surface. On the other hand, homogeneous degradation means nanoparticles degrade in a same rate both inside and outside. Many studies have suggested that the dynamic of PLGA degradation has a heterogeneous mechanism. (Park 1995, Athanasiou, Niederauer et al. 1996, Zolnik and Burgess 2007). As illustrated above, normally the degradation order of PLGA nanoparticle is inside-out. The reason for this phenomenon is that the degradation production oligomeric acid, which gathers within the microsphere, could decrease the pH and create an acid environment leading to autocatalysis. However, when at PH 2.4, the degradation order changes from inside-out to outside-in (Zolnik and Burgess 2007).Therefore, the degradation order of PLGA nanoparticles greatly depends on the PH.

Due to the bio-degradation properties of PLGA, PLGA is able to degrade in the body under the presence of water. The two hydrolysis products of PLGA, lactic acid glycolic acid, are both easily absorbed and metabolized by human body. Lactic acid is the basic product of metabolism in the body through anaerobic glycolysis and it converts into water and CO2 after Krebs’s cycle. Glycolic acid is also the by-product of various metabolic pathways in the body under normal physiological conditions such as excretion through urine (Abdelwahed,

Degobert et al. 2006). Thus, a minimal systemic toxicity is associated with PLGA nanoparticles for drug delivery.

### 2.5.3 Factors Affecting Degradation

In order to design a more efficient drug delivery system, it is essential to understand the factors affecting the process of PLGA degradation. By varying these factors, different drug-releasing time and degradation properties can be obtained. There are many factors influencing the biodegradation of PLGA nanoparticles both in vivo and in vitro, including preparation method, physicochemical properties of PLGA polymer (composition of polymer, glass transition temperature, molecular weight, particle size, shape and morphology), nature of hydrolysing media (PH, temperature) (Wu and Wang 2001, Bala, Hariharan et al. 2004, Makadia and Siegel 2011).

**Effect of Composition** The ratio of PLGA polymer is of significant importance during the whole process of degradation, which greatly influences the hydration of the polymer matrices. Due to the lack of one methyl comparing to lactic acid, glycolic acid is more hydrophilic, which leads to an increase of the weight loss of polymer. Many studies have demonstrated that PLGA polymers with a higher content of glycolic acids own a faster degradation (Park 1995, Wu and Wang 2001). Therefore, the proportion of glycolic acids determines the degradation and drug release rate.

**Effect of Crystallinity (or $T_g$)** As PLGA polymer is amorphous in nature, glass transition temperature is a key physicochemical properties for PLGA polymers. Ikada and Tsuji proposed that semi-crystalline polymer owns a greater hydrophobicity, leading to a higher rate of degradation (Ikada and Tsuji 2000). Therefore, the crystallinity of lactic acid increases the biodegradation rate of PLGA.

**Effect of Molecular Weight (Mw)** There are two different opinions to the effects of molecular weight ($M_w$).Some studies shows that PLGA with higher molecular weight degrade faster. Due to the longer polymer chains of high molecular weight polymer, the chance of water molecule attack to polymer chain

increase resulting in a higher degradation rate.(Wu and Wang 2001). While other studies show that it may spend a longer degradation time with a longer polymer chains (Makadia and Siegel 2011).

**Effect of Size** The nanoparticle with small particle size has a higher surface volume ratio which increases the chances for nanoparticles to exposure in water. Therefore, the degradation rate of PLGA nanoparticle increase with the reduction of particle size (Makadia and Siegel 2011).

**Effect of pH** Both alkaline and strongly acidic media accelerate the degradation rate (Zolnik and Burgess 2007).

**Effect of Drug Type** The degradation rate of PLGA matrices vary with different kinds of drugs. Meanwhile, the drug release profile and the steady-state rate also vary dramatically. However, the relationship between the drug types and degradation rates are still under investigation (Siegel, Kahn et al. 2006).

**Effect of Drug Load** Matrices with bigger amount of drug loading possess a faster burst release compared to those with lower drug content. However, this load effect only suitable to nanoparticles with certain levels of drug content (Makadia and Siegel 2011).

### 2.5.4 Drug Release Behaviour

As described before, different degradation time could be specified by varying ratios of PLGA composite. Hence, the drug release in specific time could be controlled and achieved (Lewis, 1990).

When drug is released from PLGA nanoparticles, a tri-phasic drug release pattern is normally observed. The pattern shows a high burst effect at the onset, a lag phase and a secondary apparent-zero-order release phase (Zolnik and Burgess 2007). The high initial burst phase is characterized by the dissolution of the drug located near the nanoparticle surface. This dissolution loses the binding with PLGA, resulting in more surface pores and more drugs contacting to the fluid. Nearly 60% of the drugs are usually released during the first phase. Therefore, how to reduce the drugs release in this phase before they arriving

the targets seems to be the main challenge for a more efficient drug delivery system (Kirby, White et al. 2011). After the first phase, the degradation of PLGA nanoparticles occurs and the drug diffuses slowly outside the particles. Finally, with the increased solubilization and erosion of the matrix, the secondary burst release of drug appears (Friess and Schlapp 2002).

There are five factors which determine the drug release rate (Soppimath, Aminabhavi et al. 2001, Kumari, Yadav et al. 2010):

- Desorption of drug bound to the surface of nanoparticles
- Diffusion through the nanoparticle matrix
- Diffusion through nanocapsules
- Nanoparticle matric erosion
- Process with combined erosion and diffusion

According to the five factors, it can be concluded that drug release rate greatly depends on the diffusion and biodegradation of the matrix. Therefore, it is important to choose a proper polymer matrix to delivery drugs.

Drug release mechanism are affected by the type of drugs, the particle size of nanoparticles, molecular weight of the polymer and drug loading content. Nanoparticles with a small particle size have a faster initial burst rate than the ones with big size. Because the smaller the particle is, the bigger surface to mass ratio it has, which means more drugs might be bound to the surface leading to a higher rate of initial burst. Kumari and Yadav disscued the molecular weight in their literature, which said that polymer with higher molecular weight might have a faster initial burst(Kumari, Yadav et al. 2010). Polakovic et al demonstrated that most of drug is undergoing diffusion model once the drug loaded content is below 10% (Polakovič, Görner et al. 1999).

There are many methods used for the in vitro release study such as biological membranes, dialysis bag/tube and ultrafiltration. Among these, dialysis bag/tube is the most commonly-used one. In order to separate released-drugs from nanoparticles, dialysis tubes/bags are utilized. For the release study, dialysis bags with a suspension of nanoparticles are incubated into PBS, which is able

to trap high-molecular-weight nanoparticles in the bags as well as release drugs into the dissolution medium (Kumari, Yadav et al. 2010).

## 2.6 Pharmaceutical Applications of PLGA nanoparticles

### 2.6.1 Cancer treatment

Nowadays, cancer still keeps on a top position to threaten our health. Although chemotherapy is the most traditional way for the cancer therapy, it destroys not only the tumour cells but also the normal tissues which could generate great adverse effect on human body (Zhang, Gu et al. 2008).

Due to its small size, PLGA nanoparticle is able to permeate deeper in the tissues. Further with the advantage of controlled release, it can prolong drug residence time. Therefore, PLGA nanoparticles have been successfully applied in the anti-cancer drug delivery system by delivering accumulate therapeutic agents with enhanced permeability and retention effect (EPR effect) (Lu, Wang et al. 2009, Danhier, Ansorena et al. 2012).

PLGA nanoparticles can either be the matrix to encapsulate the drugs or have functionization on their surface. For example, cancer related drugs, such as 9-nitrocamptothecin, paclitaxel, and cisplatin, have been encapsulated in PLGA nanoparticles successfully. For 9-nitrocamptothecin and paclitaxel, PLGA is used to enhance their low solubility and increase the availability of drugs. Despite the high toxicity of cisplatin, the encapsulating PLGA nanoparticles can make good effect on the target tissues specifically and reduce its toxicity to other normal organs (Kumari, Yadav et al. 2010). When PLGA nanoparticles are linked with biotargeting ligands, such as hormones, cytokines, chemotherapeutic agents and vaccines, it is feasible to make drugs target malignant tumour with high affinity and specific (Zhang, Gu et al. 2008).

### 2.6.2 Diabetes

Nowadays, insulin injection is the most common way for the drug therapy of patients with insulin-dependent diabetes mellitus (IDDM or Type I diabetes). The multiple treatment and high-does injection reduce the life quality of patients and may cause severe adverse effects. As a solution, insulin-contained PLGA nanoparticles can maintain a long-time release which prolongs the release of

insulin from several hours to 1-9 days. The time could be further varied with different preparation methods (Takenaga, Yamaguchi et al. 2002).

The oral delivery system has been developed to bing in convenience and exclude potential side effect caused by insulin injection. Cui F and his colleagues found that the PLGA-Hp55 nanoparticles (PHNP) could be used in oral insulin delivery effectively and successfully. The in vivo tests on rats showed that PHNP reduced the serum glucose level over 24 h. The PLGA nanoparticles coated with Hp55 has a better drug encapsulation efficiency and an increased drug bioavailability by reducing the initial burst of insulin (Cui, Tao et al. 2007).

### 2.6.3 Vaccine

PLGA nanoparticles applied in vaccine delivery system can increase the uptake of both antibodies and adjuvants. Beside the uptake, it is realizable to combine different kinds of antibodies or adjuvants in one particle to exhibit multiple functions and control the dose to reduce inflammatory responses and enhance the efficiency of vaccines (Danhier, Ansorena et al. 2012).

The viral nucleocapsid hepatitis B core antigen (HBcAg) is a useful antigen for patients infected by HBV. Chong and Cao et al. have found that a vaccine formulation containing MPLA + HBcAg encapsulated PLGA nanoparticles showed a stronger Th1 cellular immune response with a predominant interferon-γ (IFN-γ) profile than those induced by HBcAg alone, suggesting that the vaccine-loaded PLGA are more effective in terms of the therapeutic effect (Chong, Cao et al. 2005).

## 2.7 Ibuprofen-loaded PLGA nanoparticles

### 2.7.1 Ibuprofen

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) (Bonelli, Tuccillo et al. 2012, Dian, Yang et al. 2013), which is generally used to reduce the inflammation as well as relieve the pain, especially for the pain caused by inflammation. It has been proved that ibuprofen is effective to treat rheumatoid arthritis, rheumatoid osteoarthritis, ankylosing spondylitis etc. Moreover, ibuprofen has been approved by World Health Organization (WHO) and Food and Drug Administration (FDA) as a necessary medicine for the basic health system and the preferred anti-inflammatory drug for children (Dian, Yang et al. 2013).

Many studies have observed that the nonsteriodal anti-inflammatory drugs (NSAIDs) are able to inhibit cellular proliferation of some tumors effectively within the concentration from 400 to 800 μM. Ibuprofern as one of the NSAIDs, have been proved by Patrizia Bonelli and his colleagues having the potential to anti-proliferate the human gastric cancer cell line MKN-45 (Bonelli, Tuccillo et al. 2012). Meanwhile, Endo H et al. have also demonstrated that ibuprofen can be used as a chemotherapeutic agent for the lung cancer. The downregulation of Hsp70 in cancer cells can prevent the cancer cell from progressing. Ibuprofen enables to suppress the Hsp70. Therefore, the combination of ibuprofen and cisplatin as a chemotherapeutic agent can not only enhance the antitumoral activity of cisplatin to fight against the lung cancer cells, but also can reduce the doses of cisplatin, which lead to a higher efficiency of chemotherapeutic and lower toxicity to patients (Endo, Yano et al. 2014).

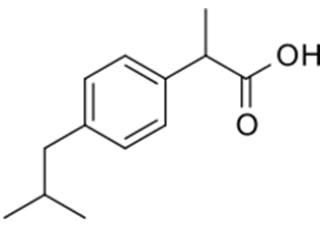



**Figure 2.10 Structure of Ibuprofern**

However, the main obstacle for the application of ibuprofen in pharmaceutical field is its low water solubility. ***Figure 2.10*** shows the structure of Ibuprofen. Functional groups such as phenyl group and isobutyl group both are the hydrophobic groups, which demonstrate the hydrophobicity of the drug. Therefore, an increasing number of researches are conducted to increase the solubility and bio-availability of Ibuprofen.

### 2.7.2 Ibuprofen-loaded nanomaterials

In order to enhance the solubility and bioavailability of ibuprofen, various methods have been found and studied, such as the use of permeation enhancers, solid dispersion, nanoparticles, nanocubes and films. Different materials are chosen as vehicles for the delivery of Ibuprofen

Lipid-based cubic liquid crystalline nanoparticles have been designed for drug delivery. Due to their lipid matrix, cubic nanoparticles are biocompatible and able to encapsulate varied drugs from hydrophilic to hydrophobic, to amphiphilic drugs. Meanwhile, cubic phase nanoparticles can increase the stability of drugs. Dian and his colleagues have prepared the ibuprofen-loaded cubic phase nanoparticles by liquid phytrantriol for the oral delivery system, which has proved that ibuprofen-loaded cubic nanoparticles has a longer release time than pure ibuprofen, showing sustained releasing profile (Dian, Yang et al. 2013). Jiang, Hu et al. used DEAE dextran as a stabilizer to prepare the ibuprofen-loaded nanoparticles by co-precipitation method. The nanoparticles have been successfully prepared with an average size of 14.7nm and have been proved more stable than the pure drug (Jiang, Hu et al. 2005).

### 2.7.3 Ibuprofen-loaded PLGA nanoparticles

Using PLGA polymeric nanoparticles as vehicles to delivery drugs is one of promising methods in the pharmaceutical areas. Many studies have been proved that the advantage of nanoparticles is that poorly water-soluble drugs can be delivered in higher efficiency in the aqueous environment when entrapped in the nanoparticles (Bonelli, Tuccillo et al. 2012). Moreover, the

nanoparticles can increase the stability of drugs as well as controlled-release the drug in the pacific target.

Meanwhile, there are many advantages to use PLGA as the material for preparing the drug-loaded nanoparticles. PLGA, which have been introduced in precious section, is a biodegradable and nontoxic polymer approved by FDA. Therefore it is a suitable material for drug delivery. Secondly, due to the hydrophobicity of ibuprofen, it is easy to load the drug into the hydrophobic material---PLGA. Both Single emulsion evaporation method and emulsion diffusion method can be used to prepare the ibuprofen-loaded PLGA nanoparticles. Only a certain amount of drug is needed to be added in the oil phase during the preparation, and then the drug-loaded PLGA nanoparticles can be obtained (Soppimath, Aminabhavi et al. 2001). Numbers of research have shown the potentials to utilized PLGA nanoparticles as drug carries for ibuprofen delivery (Siegel, Kahn et al. 2006, Bonelli, Tuccillo et al. 2012).

According to the crucial role of ibuprofen in the treatment of some tumors, increasing the solubility as well as the bioavailability of the drugs seems to be an urgent problem needed to be tackled. Therefore, encapsulating ibuprofen into the PLGA nanoparticles is one of the powerful and effective ways to delivery drugs.

## 2.8 Characterization Techniques

Adequate and proper characterization of PLGA nanoparitcles is essential for its quality control. The important parameters which need to be evaluated for the PLGA nanoparticles are particle morphology, particle size and size distribution, molecular weight and thermal analysis (e.g. crystallinity and Tg). When PLGA nanoparticles are produced by different preparation methods and with different composition ratios of PLGA co-polymer, these changes may cause varieties of its physicochemical properties and affect the degradation time and drug release kinetics (Hausberger and DeLuca 1995).

On the other hand, since PLGA is polyester, the major challenge for using this polymer is its chemical and physical instability during storage and/or drug delivery. During storage, aggregation and unwilling degradation may take place. Characterization techniques are used to track these changes and ensure the quality of the products used in drug delivery system (Abdelwahed, Degobert et al. 2006).

### 2.8.1 Particle morphology

The particle morphology can be observed by scanning electron microscopy (SEM), transmission electron microscopy (TEM), cryogenic transmission electron microscopy (cryo-TEM) and atomic force microscopy (AFM) (Astete and Sabliov 2006). TEM and SEM are both very useful for producing the particle images. TEM is commonly applied to see the shape, aggregation and internal details of particles (Astete and Sabliov 2006). TEM can provide a broad image of a sample which has up to 50 million magnification level with a high resolution. When preparing the samples of TEM， nanoparticles should be diluted in water. During the capture of images, the electron can be transmitted through the particles. Thus a detailed internal image of nanoparticles can be obtained by TEM. Due to the understory of samples and the high resolution, TEM techniques are more suitable to biologic samples. The limitation of TEM is that it only can show a two-dimensional structure of the image.

In comparison, SEM is usually applied for observing sample's surface and monitoring the degradation process (Park 1995, Zolnik and Burgess 2007). Since it can provide a three-dimensional and deeper image, SEM can further be used to estimate the size distribution (Hausberger and DeLuca 1995). However, samples are required to be conductive when using SEM. The non-conductive samples should be coated by gold leading to destroy and non-recovery of the samples. In order to avoid the non-conductive to be destroyed, environmental scanning electron microscopy (E-SEM) is designed which introduce small amount of water in the vacuum making the non-conductive samples detected by electron. Due to its low resolution, only a bigger size of particles can be imaged by E-SEM.

AFM is another powerful technique to analyze the 3-D topology of a surface. AFM is used for qualitative and quantitative study of properties like surface area, size and volume distribution. For example, Ravi Kumar et al. has applied AFM to observe the size and surface morphology of PLGA, which clearly showed the electrostatic interaction between positively charged PLGA nanospheres and negatively charged DNA (Ravi Kumar, Bakowsky et al. 2004).

### 2.8.2 Particle size and distribution

Dynamic light scattering (DLS), analytical ultracentrifugation, and transmission electron microscopy (TEM) have been broadly used for analyzing particle size and size distribution. TEM not only can detect the particle morphology, but also be used in size distribution measurement.

DLS, also known as (PCS) photon correlation spectroscopy, is the most common technique for determining the size distribution profile of small particles in suspension or polymers in solution. For the analysis of PLGA nanoparticles, they normally should be sonicated in advance in order to reduce aggregation (Astete and Sabliov 2006, Holzer, Vogel et al. 2009). DLS is a technique measuring the variation of intensity fluctuations in the scattered light over time. Once nanoparticles are suspended in a liquid, they will move in irregular Brownian motion. Based on the Brownian motion, the speed of particle movement is closely related to the particle sizes. Particles with smaller sizes

can move faster than those with bigger sizes, which lead to a slower fluctuation in the scattered intensity, showing the difference in the intensity fluctuation among different sizes. Finally, particle sizes can be calculated according to the rate of the intensity fluctuation. DLS is an effective method to measure particle sizes, especially for the particles in micro and nano scales.

Analytical ultracentrifugation measures the size distribution based on sedimentation velocity analysis. In a research conducted by Holzer M and his colleagues, due to the higher resolution of analytical ultracentrifugation, which is able to measure the size distribution of the samples even if it is in an extended size range, this technique was employed to get more detailed information about particle size distribution and support the results measured by DLS (Holzer, Vogel et al. 2009).

### 2.8.3 Molecular weight

Molecular weight plays an important role in the process of nanoparticle preparation and the studies of degradation. Static Light Scattering (SLS) is the technique that can be applied to measure the molecular weight of the sample in solution. The Zetasizer Nano S from Malvern is one of facilities that have the technique of SLS. It can measure the intensity of scattered light ( $\frac{K}{CR_\theta}$ ) under different concentrations© of one sample. ***Equation 2.4*** is the Rayleigh equation.

$$\frac{KC}{R_\theta} = \frac{1}{M} + 2A_2C \qquad \textbf{Equation 2.3}$$

( $R_\theta$: The Rayleigh ratio; C: concentration; K: constant; M: molecular weight; $A_2$: 2$^{nd}$ virial coefficient)

According to the equation, the relationship between molecular weight and intensity of scattered light ( $\frac{K}{CR_\theta}$ ) is linear.

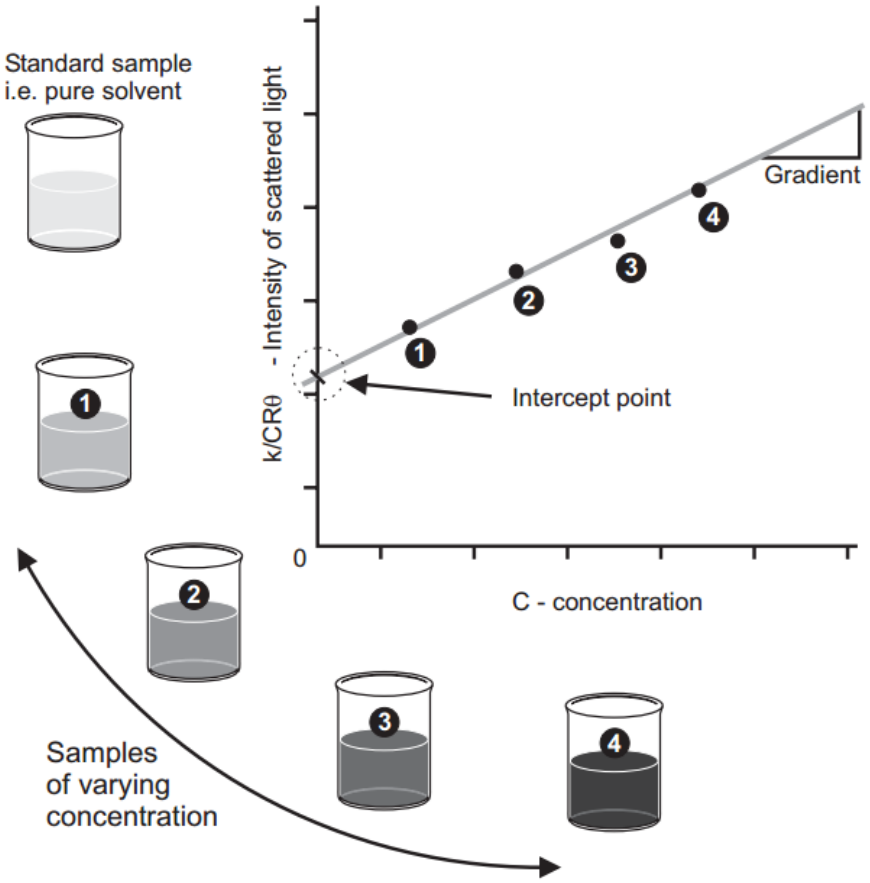



**Figure 2.11 The example of the Debye Plot**
**(Malvern Instruments Ltd. 2003, 2004)**

Therefore, a Debye Plot can be obtained based on their relationship. The intercept point on the X axis can be measured as the molecular weight, meanwhile the gradient of the line stands for the 2$^{nd}$ virial coefficient, which "describing the interaction strength between the particles and the solvent or appropriate dispersant medium." (Malvern Instruments Ltd. 2003, 2004)

Although the basic principle of SLS is easy to understand, a high standard of sample preparation is required. Furthermore, the molecular weight measured by SLS is only the relative molecular weight and it is hard to obtain molecular distribution by SLS, which are the main reasons for the uncommonly application of this technique.

Gel-permeation chromatography (GPC), also known as size exclusion chromatography (SEC), is another technique to determine the molecular weight.

The basic principle of GPC is the separation of molecules by different size. The columns are used to achieve the size exclusion. The pores in the columns are able to trap small particles inside which leads to bigger molecules being removed earlier than small ones. Due to the size exclusion from big molecules to small ones, a distribution of molecular weight can be obtained (Hausberger and DeLuca 1995). Therefore, GPC can not only measure the number average molecular weight ($M_n$) and the weight average molecular weight ($M_w$), but also can provide a basic idea of the distribution of molecular weight. It has been demonstrated as a suitable and reliable technique to determine the molecular weight.

### 2.8.4 Thermal analysis

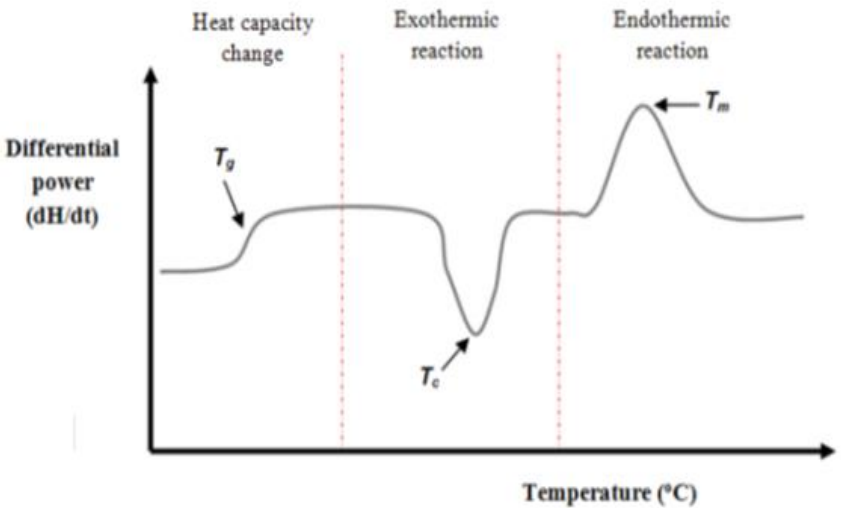
Thermal analysis is a group of techniques that study the relationship between a sample property and its temperature, which can be characterized by thermo gravimetric analysis (TGA), differential scanning calorimetry (DSC) and differential thermal analysis (DTA) (Rouquerol, Rouquerol et al. 2006).

DSC is the most widely used thermo analytical technique since it is easy to be operated and it enables user to obtain the results quickly and precisely. It not only can be used to measure various temperatures, such as glass transition temperature ($T_g$), melting temperatures ($T_m$), crystallization temperature and phase transition temperature, but also is an effective techniques for the degradation study of polymers (Hausberger and DeLuca 1995). Since most of the PLGA copolymers are amorphous in nature, they only have $T_g$ without having $T_m$. The change of $T_g$ during storage could well reflect the degradation process (Hausberger and DeLuca 1995).

According to Rouquerol J et al., DSC is defined as “a technique where the heat flow rate difference into a sample and a reference material is measured”(Rouquerol, Rouquerol et al. 2006). The physical transformations of samples are either exothermic or endothermic resulting in less or more heating. Therefore they are required more or less heat flow to keep the same temperature as the reference. For example, when PLGA polymers change from glass to rubbery which is an endothermic phase transition, it leads to an

increase of heat flowing through the samples to balance the temperature(Hausberger and DeLuca 1995). As shown in

***Figure 2.12,*** when reaching the glass transition temperature, amorphous polymer changes from glassy polymer to rubbery polymer. The heat flows into the sample results in an increase of heat capacity and a significant change in DSC curve.




**Tg: glass transition temperature, Tc: crystallization temperature, Tm: melting temperature**

**Figure 2.12 Features of a DSC curve (Menczel, Judovits et al. 2008)**

The method to calculate the $T_g$ is illustrated in ***Figure 2.13***. First, three tangents should be drawn, namely at the beginning, at the turning point and at the end of the curve which enable us to get the crossover point representing $T_1$ and $T_2$ respectively. Tg is the average of these two temperature. Normally, the onset point ($T_1$), the temperature first occurs when polymer become glassy, is used for data analysis since it is more convenient and precise than $T_g$ which may cause some errors during calculation.

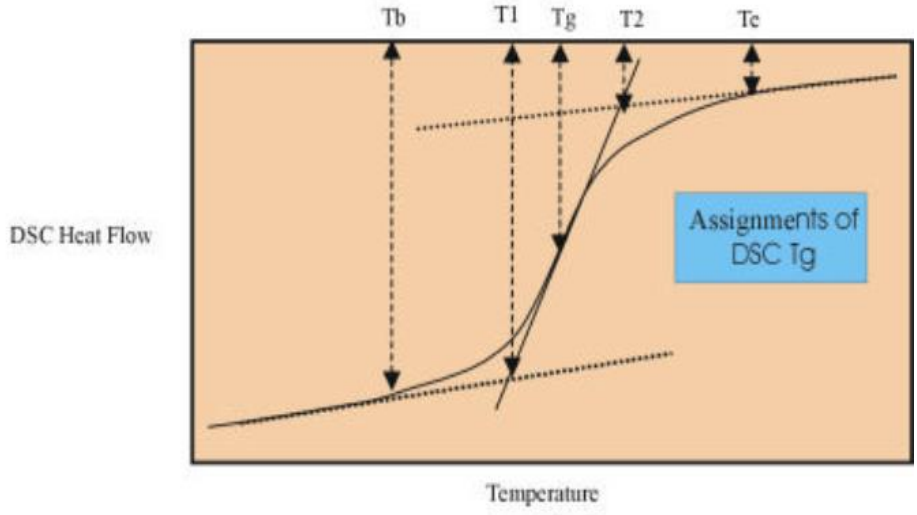



**Figure 2.13 Assignments of DSC $T_g$ (Sichina W J, 2013)**

# 3 Project overview

## 3.1 Overall aims

Nanomedicine is an emerging multiple-discipline and it has become a buzz word in pharmaceutical industry. Nanoparticles, as a novel vehicle to carry drugs, show great potentials for advanced drug delivery system.

PLGA is a biodegradable co-polymer and it has been applied as a biocompatible material used in pharmaceuticals in the form of nanoparticle. It is acknowledged that parameters like particle sizes, molecular weights and thermal properties could greatly affect the mechanical strength and controlled-release properties of various drug delivery devices. This study thus aims to study the stability of PLGA nanoparticles by monitoring their physiochemical properties (e.g. size and size distribution, molecular weight, shape and Tg), followed by an evaluation on their controlled release performance. A primary study of drug-loaded PLGA nanoparticles is conducted later. Ibuprofen, as a potential drug for cancer theraoy is applied in this research. In order to gain a basic view of the properties and stability of Ibuprofen-loaded PLGA nanoparticles, the storage stability and drug releasing properties is studied.

## 3.2 Specific objectives

The overall aims could be realized after achieving the following specific objectives:

I. Fabrication of PLGA nanoparticles and optimization

II. Characterization(particle sizes, molecular weight, surface morphology)

III. Degradation and storage study

IV. Preliminary study of drug-loaded nanoparticles

- Preparation and characterization of Ibuprofen-loaded PLGA nanopartilces
- Storage stability
- Drug releasing

# 4 Materials and methodology

The research was conducted at the School of Engineering, Cranfield University (UK).This chapter presents the methods and materials used in the research. Major materials are presented in ***Section 4.1.1***. Other general materials are described in ***Appendix A.1***.

## 4.1 Preparation of PLGA nanoparticles

### 4.1.1 Materials

- Two PLGA polymers with different ratios were used to prepare nanoparticles. PLGA 50:50(Mw 24000-38000) & PLGA 75:25(Mw 76000-115000), CAS 26780-50-7, from Sigma Aldrich UK
- Polyvinyl alcohol (87-89% hydrolyzed, Mw 31,000-50,000) (PVA), CAS 9002-89-5, from Sigma Aldrich UK
- Dichloromethane (DCM): from Fisher Scientific UK Ltd
- Dimethyl sulfoxide (DMSO) anhydrous, ≥99.7%, CAS 76-68-5, Mw 78.13, Fisher Scientific UK Ltd
- Ethanol absolute 99.9%, CAS 64-17-5, Mw 46.06, VWR Prolab
- Ibuprofen ≥98%, Mw 206.28, CAS 15687-27-1, Sigma Aldrich UK

### 4.1.2 Equipment

Materials were weighted by using analytical balance with accuracy of 0.1mg. In order to aid to dissolve the PVA completely, the water bath and ultrasonic bath (Patternson Scientific) were used. For high speed emulsification, a Silverson SL2T homogenizer (Kinematica AG. Luzern, Switzerland) was applied. The homogenizer was fitted with a Polytron PT-DA 3030/2 (Kinematica AG. Luzern, Switzerland) homogenizer generator. Centrifugation was obtained by Heareus Megafuge 16R (Thermo Scientific). A freeze-dryer (Edwards Modulyo) was used to remove the water from the samples.

### 4.1.3 Methodology

#### 4.1.3.1 Single emulsion evaporation method

PLGA nanoparticles were prepared via a modified single emulsion evaporation method (see the general method below). Several conditions were further modified and assessed in order to optimize the preparation method of making PLGA nanoparticles. The variations and modification were focused on homogenization (step3) and centrifugation (step 5).

### General method

1. 200 mg PLGA polymer in 5 m DCM.
2. A 20 ml aqueous solution of 1 % PVA (1g in 100 mL water) was prepared. The mixture was placed in a water bath (40---60 ˚C) to improve the solubility.
3. Homogenization and emulsion formation
   The aqueous PVA solution was first transferred into a 500ml beaker. The PLGA solution was then drip manually added drip wise into the PVA solution while homogenizing. The adding time was approximately 2.5 minutes and the speed of homogenization was set at 9,000 rpm. After the adding of PLGA solution, the mixture was further left homogenizing for another 5 minutes at 9,000 rpm.
4. The resulting mixture was stirred for 15 hours at 300 rpm on a magnetic stirrer at room temperature (RT) in order to evaporate DCM completely.
5. In prior to centrifugation, the mixture was vortexed. After a centrifugation at 3,000 rcf at RT for 10 minutes, the supernatant was removed by pipette. Fresh deionized water (45ml) was added to the precipitate and the mixture was vortexed followed by another centrifugation. This procedure was repeated for 3 times. Finally the precipitate was transferred into an anti-crack vial/container.

The sample in the anti-crack vial was frozen by liquid nitrogen and then placed in a freeze-dryer overnight. The dried sample (white power) was collected and weighed.

### Modified method A

**Step3:** The period drip of adding PLGA solution into PVA solution was increased from 2.5 minutes to 6 minutes. After that, the mixture was left homogenizing for 7 minutes at 9,000 rpm.

### Modified method B

**Step3:** Very similar to the above modified method A but a constant dripping rate (0.5ml/min) was set by applying a syringe pump to drip add the PLGA solution drip.

### Modified method C

**Step3:** A smaller size beaker (250ml) was used and placed on an ice bath. PLGA solution was added into PVA solution manually within a period of 6 minutes with homogenization followed by another 7-minute homogenization. The speed of homogenizer was set at 9000 rpm.

**Step5:** A longer period of centrifugation and lower temperature were applied. The first centrifugation was set at 3,000 rcf at 5 °C for 30 minutes. Then the supernatant was removed by glass pipette. Fresh deionized water (45ml) was added to the precipitate and the mixture was vortexed followed by another centrifugation for 10 min. This procedure was repeated for 2 times.

### Modified method D

**Step3:** A higher homogenization speed was applied, which increased to 15000rpm.

***Table 4.1*** summarizes the varying parameters when using the single emulsion evaporation for preparation based on methodologies shown above.

**Table 4.1 Single Emulsion Evaporation Method by varying different parameters**

| Method / Parameters | General method | Modified Method A | Modified Method B | Modified Method C | Modified Method D |
|---|---|---|---|---|---|
| Homogenization time(min) | 2.5 | 6 | 6 | 6 | 6 |
| Dropping method | manual | manual | syringe pump | manual | manual |
| Stirring speed(rpm) | 9000 | 9000 | 9000 | 9000 | 15000 |
| Ice bath | (-) | (-) | (-) | (+) | (+) |
| Volume of beaker(ml) | 500 | 500 | 500 | 250 | 150 |
| centrifugation time(min)/temperature (℃) | 10min/20℃ | 10min/20 ℃ | 10min/20 ℃ | 30min/5℃ | 30min/10 ℃ |
| Centrifugation speed(rpm) | 3000 | 3000 | 3000 | 3000 | 5000 |

#### 4.1.3.2 Emulsion Diffusion Method

1. 100mg PLGA were co-dissolved in a binary organic solvent which was prepared by 4ml DMSO: EtOH (50:50, v/v).
2. 8ml 0.5% (w/w, 0.5g in 100 mL water) PVA water solution was prepared as the aqueous phase.
3. The organic phase was dropped into aqueous phase under 500rpm magnetic stirring/ 15000rpm homogenizer to get the nanoemulsion.
4. In order to form the nanoprecipitaion, the nanoemulsion was poured into 160ml fresh deionized water under the 500rpm magnetic stirring/ 15000rpm homogenizer.
5. The resulting mixture was then obtained by centrifugation at 9,000 rpm at 5 °C for 20 minutes. The supernatant was removed by pipette to get the solids which were washed by fresh deionized water (45ml) subsequently. Another two centrifugations were repeated to remove the PVA. Finally the precipitate was transferred into an anti-crack vial/container.

6. The sample in the anti-crack vial was frozen by liquid nitrogen and then placed in a freeze-dryer overnight. The dried sample (white power) was collected, weighed.

#### 4.1.3.3 Preparation of drug-loaded PLGA nanoparticles

The preparation method of drug-loaded PLGA nanoparticles was developed based on Modified single emulsion evaporation method D.

1.40mg Ibuprofern and 200 mg PLGA polymer were co-dissolved in 5 ml dichloromethane (DCM) as the organic solution.

2. 20 ml aqueous solution of 1 % PVA (1g in 100 mL water) was prepared.

3. The aqueous PVA solution was transferred into a 250ml beaker. The organic solution was dropped manually into the PVA solution under a high speed homogenization (15,000rpm) for approximately 6 minutes. Then the mixture was kept being homogenized for another 7 minutes.

4. The resulting mixture was stirred on a magnetic stir plate for 15 hours at 300 rpm at room temperature (RT) to achieve the complete solvent evaporation.

5. In prior to centrifugation, the mixture was vortexed. Then the resulting mixture was recovered by centrifugation at 3,000 rcf at 5 ˚C for 30 minutes. The supernatant was removed by pipette to get the solids which were washed by fresh deionized water (45ml) subsequently. Another two centrifugations were repeated to remove the PVA and unencapsulated drugs. Finally the precipitate was transferred into an anti-crack vial/container.

6. The sample in the anti-crack vial was frozen by liquid nitrogen and then placed in a freeze-dryer overnight. The dried sample (white power) was collected, weighed.

## 4.2 Characterizations

The main physicochemical properties studied in this project include the particle size, thermal properties (e.g. glass transition temperature), molecular weight

and particle morphology. ***Table 4.2*** shows the techniques which have been utilized in the project.

**Table 4.2 Characterization techniques used in the project**

| | Property | Technique |
|---|---|---|
| 1 | Particle size | Dynamic light scattering |
| 2 | Glass transition temperature | Differential scanning calorimetry |
| 3 | Molecular weight | Static light scattering UV spectroscopy |
| 4 | Morphology | Scanning electron microscope |

### 4.2.1 Particle size & morphology

**1. Dynamic light scattering (DLS)**

Dynamic light scattering technique was used to measure the mean particle size and size distribution (Holzer M et al., 2009; Astete C E, 2006). In this project, the particle sizes of PLGA nanoparticles were obtained by using a Malvern Zetasizer Nano S (Malvern UK) with a HeNe laser (633nm 'red' laser). The software used to analyze and exhibit the result is DTS (Nano) software.

**Methodology**

- The nanoparticles were evenly suspended in the water by mixing the nanoparticles and deionised water with a ratio of 1:600 (1g: 600 mL).
- The solution of reconstituted nanoparticles was placed in a sonic bath for sonication for 5-10 minutes to prevent aggregation.
- 1 mL sample solution was pippetted into a disposable cuvette for the measurement.
- The average of three measurements was taken and then calculated.

**2. Scanning Electron Microscope (SEM)**

SEM can not only be used to observe the shape and the morphology of PLGA nanoparticles, but also can be applied to measure the particle size. The samples were firstly gold-coated and then analyzed by a Carl Zeis HD15 EVO Scanning Electron Microscope. The resolution of the SEM was 10Kv.

### 4.2.2 Molecular weight

Zetasizer Nano S (Malvern) combines both dynamic and static light scattering (DLS & SLS) techniques, which is able to measure the particle size and molecular weight. DLS is proved to be a powerful technique to obtain the particle size, while the SLS can be used for the measurement of molecular weight.

#### Methodology

1. All glassware and apparatus were washed with water and rinsed with acetone. Then these glassware and apparatus were flashed with nitrogen gas and kept dry in a dust free place at 25°C.
2. The PLGA solution was prepared in toluene. The concentration of PLGA is 0.5mg/mL, 1mg/mL and 1.5mg/ml. The prepared samples were standed for 1 hour to ensure complete solubilization of the polymer in the solvent.
3. Sample solution was pippetted into a quartz cuvette for the measurement.
4. Different concentrations of samples were respectively inserted into machine to get the Debye plot

### 4.2.3 Thermal analysis

The thermal analysis was accomplished by using the Jade Differential Scanning Calorimeter (DSC) (Perkin-Elmer, UK) with nitrogen gas as the inert gas.

#### Methodology

1. The samples were loaded into aluminum pans before the pans were sealed by crimper.
2. The samples were placed in the DSC and then scanned from -30 ˚C to

180 °C with a heating rate of 20 °C $min^{-1}$

3. The curve was shown in the Pyris Software.

### 4.2.4 Drug loading

**Measurement of calibration curve for Ibuprofen in THF**

1. Three concentrations of Ibuprofen were prepared in THF, 0.1mg/ml,0.3mg/ml and 0.5mg/ml.

2. Different UV absorptions were measured by UV spectrophotometer at wavelength 264nm (the typical absorption of IBU in THF). According to the Beer–Lambert law, the standard line could be obtained. The X-axis stands for the concentration of IBU, while the y-axis is the UV absorptions at λ=264nm.

**Drug loading**

1. An accurately weighted amount of nanoparticles (approximately 10mg) was dissolved in 5ml acetonitrile and then detected by UV spectroscopy under λ=264nm.
2. The concentration of the IBU in nanoparticles can be measured according to the standard line. The encapsulation efficiency (EE) and Drug loading content (DLC) were determined using the following equations.

DLC (%) = (IBU weight in nanoparticles/nanoparticles weight) ×100%

EE (%) = (actual weight of IBU/theoretical weight of IBU) × 100%

## 4.3 Storage stability study

PLGA nanoparticles were stored in 75% humidity and 40 °C, which is the standard storage condition for the study of storage stability in pharmaceutical industry. The samples were reserved in a small bottle put into dessicator with saturated NaCl solution to ensure the constant humidity. Then they were stored in the oven for two weeks. After two-week storage, the samples were analyzed by SEM and DSC for the surface morphology and $T_g$ respectively to study their stabilities. The picture of the setting is shown in ***Appendix A.2***.

## 4.4 In vitro releasing study

### 4.4.1 Measurement of calibration curve for Ibuprofen in PBS (PH=7.4)

1. Four concentrations of Ibuprofen were prepared in PBS, 6μg/ml, 8μg/ml, 10μg/ml and 12μg/ml

2. Different UV absorptions were measured by UV spectrophotometer at wavelength 221nm (the typical absorption of IBU in PBS). The calibration curve could be obtained based on the Beer–Lambert law

### 4.4.2 Drug releasing study

1. 10mg Ibuprofen-loaded nanoparticles were suspended in 1ml phosphate buffered saline (PBS) in a sealed filter membrane. The sealed membrane was immersed into PBS (50ml, PH7.4) which was stored at 37 °C with moderate shaking(100rpm). The facilities are shown in ***Appendix A.3***.
2. For comparison, 10mg of pure Ibuprofen and raw PLGA nanoparticles were weighted and stored in the same condition.
3. At the time intervals of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0 and 24.0 h, 32h, 40h, 48h, 72h ,2ml of medium were withdrawn. At the same time, the same volume of PBS solution was added in order to maintain the volume of the medium at 50ml.
4. The concentration of released Ibuprofen was then analyzed by UV spectroscopy under λ=221nm.

# 5 Results & Discussion

## 5.1 Fabrication of PLGA nanoparticles

Two approaches were used to prepare the PLGA nanoparticles, namely single emulsion evaporation method and emulsion solvent diffusion method. ***Section 5.2.1*** shows the results of nanoparticles prepared by single emulsion evaporation method. ***Section 5.2.2*** summary the results of nanoparticles prepared by nanoprecipitation

### 5.1.1 Single Emulsion Evaporation Method

#### 5.1.1.1 Observation of Nanoparticles

The product of PLGA nanoparticles looks to be in the form white powder. After freeze-drying, the PLGA nanoparticles takes a cotton-like appearance. The process of removing the nanoparticles from its beaker is a tedious process. It had to be separated by spatula and removed into bottles piece by piece. However, after being grinded and stored in the freezer, the PLGA nanoparticles transform a white powder form. While PLGA nanoparticles were insoluble in the water, it could completely disperse in water after sonication. Thus, water can be used as a liquid phase when utilizing the DLS.

#### 5.1.1.2 Particle size

***Table 5.1*** shows the particle sizes and polydispersity (PdI) of PLGA 50:50 and PLGA 75:25 nanoparticles prepared with different parameters. According to the table below, it could be noted that the preparation method plays an important role in terms of the size and polydispersity of PLGA nanoparticles. It has been argued that by varying different parameters such as the concentration of regents, the surfactants and emulsion method, different particle sizes and size distribution could be obtained (Astete and Sabliov 2006, Nagavarma, Yadav et al. 2012). ***Figure5.1*** to ***Figure 5.7*** shows examples of DSC results, which all shows a single peak of size distribution. More results of particle sizes are shown in ***Appendix A.4***.

**Table 5.1 Summary of the particle size and PdI of NP50:50 and NP75:25**

| Method | Ratio of PLGA | Sample | Particle size(nm) | Polydispersity(PdI) |
|---|---|---|---|---|
| General method | 50:50 | SYQ_50_2 | 3340 | 0.246 |
| Modified Method A | 50:50 | SYQ_50_3 | 746.1 | 0.584 |
| | | NP50-1 | 585.9 | 0.696 |
| | | NP50-3 | 439.8 | 0.533 |
| | | NP50-4 | 1872 | 0.180 |
| | 75:25 | NP75-1 | 630.3 | 0.568 |
| | | NP75-3 | 2164 | 0.258 |
| Modified Method B | 50:50 | NP50-2 | 1570 | 0.842 |
| | 75:25 | NP75-2 | 1903 | 0.199 |
| Modified Method C | 50:50 | NP50-4 | 548 | 0.027 |
| | | NP50-5 | 555.1 | 0.057 |
| | | NP50-6 | 591.2 | 0.099 |
| | 75:25 | NP75-4 | 656.1 | 0.042 |
| Modified Method D | 50:50 | NP50/15000-1 | 420 | 0.099 |
| | 75:25 | NP75/15000-1 | 509.8 | 0.052 |

As shown in ***Table 5.1*** the size of some PLGA particles, such as SYQ_50_2, NP50-2, NP50-4, NP75-2, NP75-3, are not within the range of normal size of nanoparticles which is 100-1000nm. The rest of products are all within 100-1000nm size range, well within what could be defined as nanoparticles.

Product SYQ_50_2, which is a microsphere, was prepared by the general method, due largely to it being the biggest in particle size. Whilst, the size distribution range is wide and the single peak shown on DLS report is

dissymmetry. Current literature have suggested that the key step in the whole procedure lies in the emulsion formation stage, which have a great impact on the particle size (O'Donnell and McGinity 1997, Rosca, Watari et al. 2004). Thus a longer period of dripping organic phase into liquid phased and prolonged homogenization time (modified method A) could significantly affect the particle size. Based our study and research, product SYQ_50_2, produced by using the modified method A, show a much smaller size of 746.1nm.

Particles SYQ_50_3, NP50-1, NP50-3, NP50-4, NP75-1 and NP75-4 were all prepared by modified method A. However, the DLS results (***Table 5.1***) seem to suggest that these samples are too polydisperse. Polydispersity (PDI) could be defined as the width of particle size distribution in the field of light scattering. The smaller the PDI is, the better the samples are. In normal circumstances, if the PDI is less than 0.04, samples is considered to be monodisperse (Arzenšek D, 2010). As given in ***Table 5.1***, the PDI of these particles are from 0.199 to 0.842. ***Figure 5.8*** is the quality report to illustrate that a high degree of polydispersity could cause problems to the distribution and accumulation analysis, resulting in a poor DLS result. Besides the problem of high polydispersity, the poor reproducibility in terms of the particle sizes from batches to batches, was also observed while using modified method A. The size of particles varies from 439.8nm to 1872nm. Thus, modified methods B & C were developed to overcome these shortcomings.

In order to introduce the constant rate of dropping the PLGA solution for a better control and reproducibility of results, a syringe pump was introduced into the process. However, the particle sizes of (NP50_2: 1570nm, NP75_2: 1903nm) were found to be unsatisfactory. The fixed position of syringe and needle for dripping PLGA solution in this method, may have caused the homogenization of PLGA and PVA solutions to be lacking when compared to the general method. It appears that the constant dripping rate does not have great impact on the size of resulting nanoparticles. Thus, further investigation is needed.

Modified method C was developed based on a method reported by Javadzadeh, Ahadi et al. (Javadzadeh, Ahadi et al. 2010). The ice bath was used during the

process of emulsion and a smaller beaker was chosen. The results improved significantly, as shown in the DLS reports (***Figure 5.4-5.7***). The results quality were better with the PDI being reduced drastically and were all below 0.1. Therefore, this seems to suggest that the ice bath could be an effective method to produce a better, more sufficient emulsion process which in turn generate a more homogeneous particles. Literatures have shown that temperature plays a significant role during the preparation of the nanoemulsion, which has an effect on the viscosity of the dispersed phase, the Ostwsld ripening and the evaporation speed of the solvent (Yang, Chia et al. 2000, Tadros, Izquierdo et al. 2004). Yang,Chia et al. have studied the effect of temperature on the emulsion evaporation method. They have demonstrated that the smaller particle sizes and lower polydispersity can be obtained under the lower temperature preparation (Yang, Chia et al. 2000). Another possible reason could be due to Ostwald ripening (the tendency of the small particles redeposit onto large particles). Ostwald ripening increases with the temperature. With an increase in temperature, the smaller droplets decrease in size and the bigger ones get bigger, which leads to a higher polydispersity (Tadros, Izquierdo et al. 2004).

Besides the importance of the homogenization time and the preparation temperature, stirring speed is another key factor for the particles size (Jain 2000). It has been demonstrated that using a higher stirring speed can effectively reduce the size of the particles (Astete and Sabliov 2006, Nagavarma, Yadav et al. 2012). Therefore, modified method D was utilized to get a smaller size of nanoparticle. As shown in the ***Table 5.1***, the stirring speed was increased from 9000rpm to 15,000rpm, which the particle sizes of PLGA 50:50 and PLGA 75:25 have both reduced over 100nm as compared to the nanoparticles prepared by modified method C.

According to the different ratios of the PLGA polymer, it could be noted that the particle sizes of PLGA 75:25 nanoparticles are bigger than PLGA 50:50 nanoparticles based on the same preparation method. This could be due to the fact that they have different ratio of co-polymers. PLGA 75:25 contains more lactic acid than PLGA 50:50, resulting in a higher molecular weight than PLGA

50:50. The higher molecular weight, the bigger the particle size. Moreover, ***Table 5.1*** also indicate that nanoparticles with bigger sizes could gain a higher yield. This is mainly so because bigger particles are easy to be precipitated during centrifugation, while the smaller ones may be suspended in water leading a poor precipitation. Even longer centrifugation time and lower temperature have been adapted in modified method C; the products with smaller size were still in a low yield.

It has been reported that a wide range of particle sizes of PLGA nanoparticles can be obtained by single-emulsion evaporation method, varying from 200nm to 750nm (Astete and Sabliov 2006, Kim and Martin 2006). All the products prepared by Method C & D falls within this range. Another point of note is that DSL results of these nanoparticles all showed a single and fairly narrow peak with a low polydispersity. Therefore, it can be concluded that Method C & D are the moderate method for generating PLGA nanoparticles in uniform sizes.

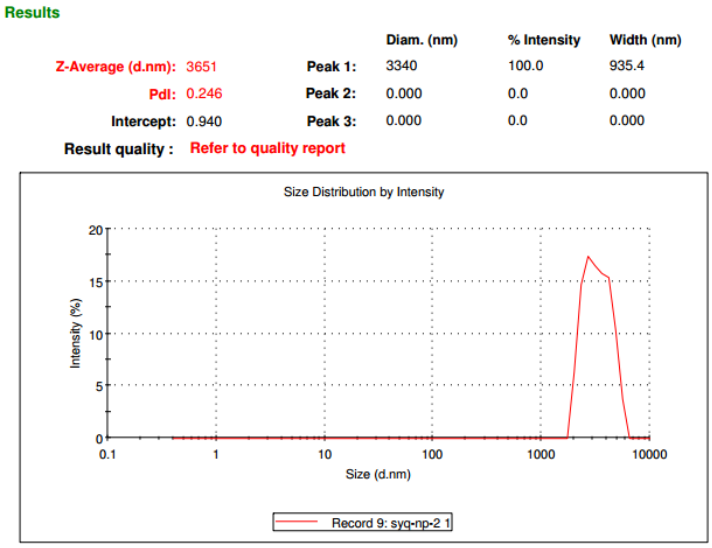



**Figure 5.1 Size Distribution by Intensity for SYQ_50_2 prepared by General Method**

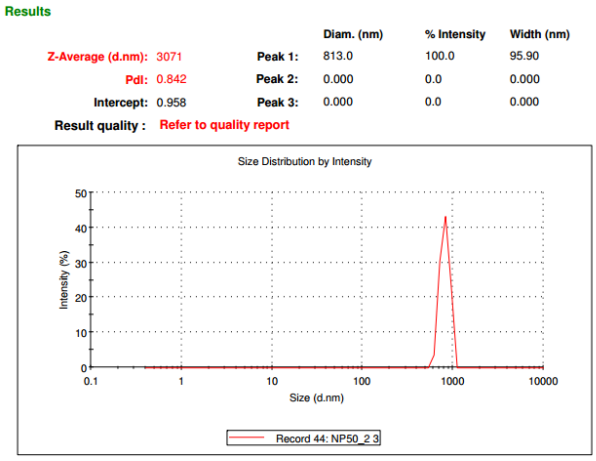



**Figure 5.2 Size Distribution by Intensity for NP50-2 prepared by Method A**

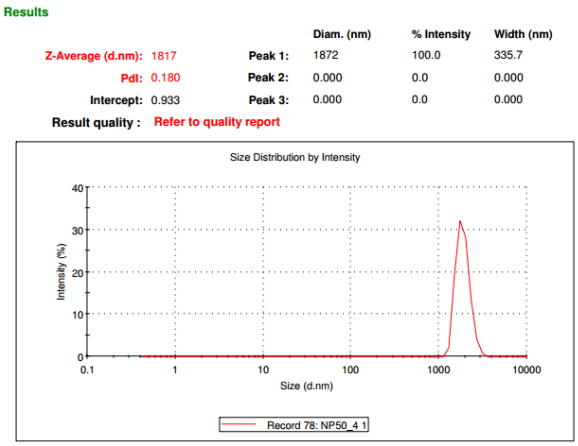



**Figure 5.3 Size Distribution by Intensity for NP50-4 prepared by Method B**

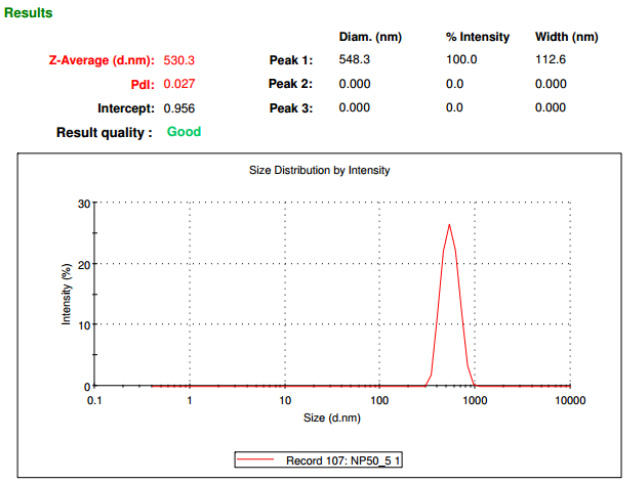



**Figure 5.4 Size Distribution by Intensity for NP50-5 prepared by method C**

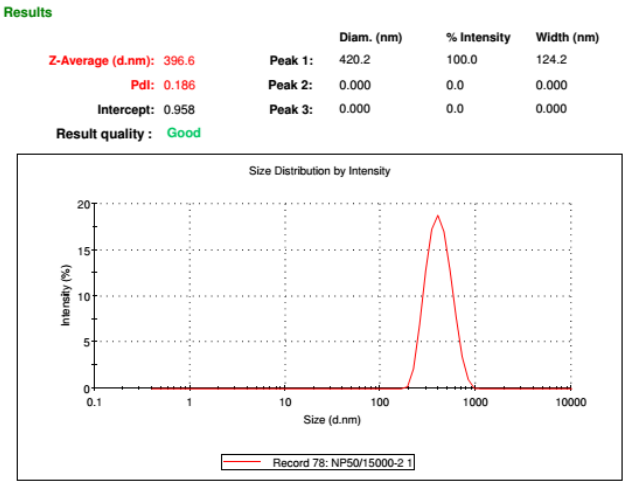



**Figure 5.5 Size Distribution by Intensity for NP50/15000-1 prepared by method D**

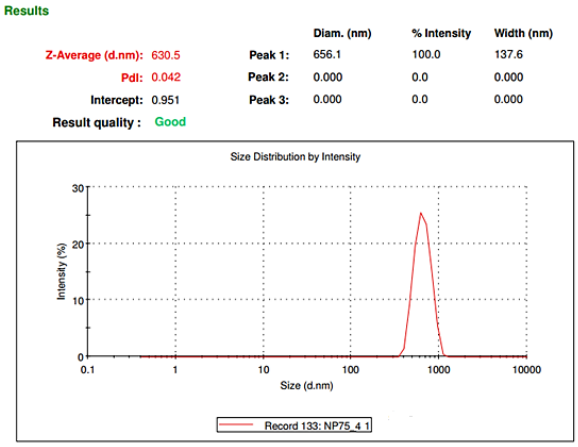



**Figure 5.6 Size Distribution by Intensity for NP75-4 prepared by Method C**

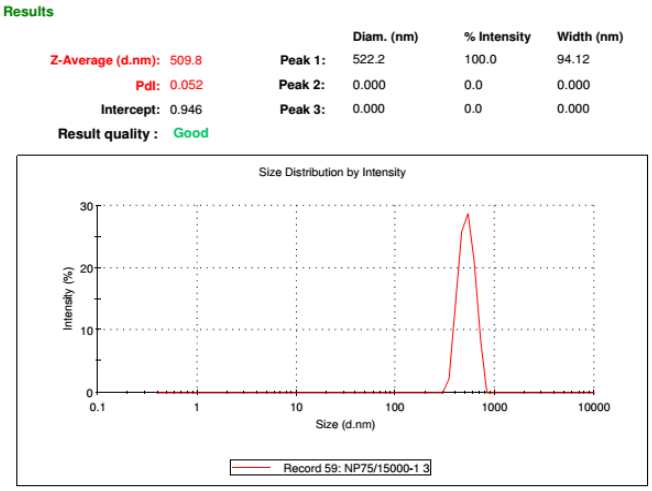
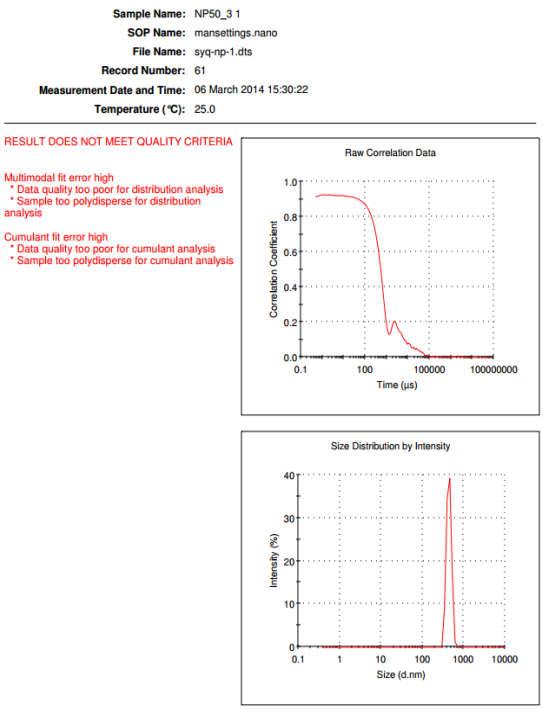



**Figure 5.7 Size Distribution by Intensity for NP75/15000-1 prepared by Method C**



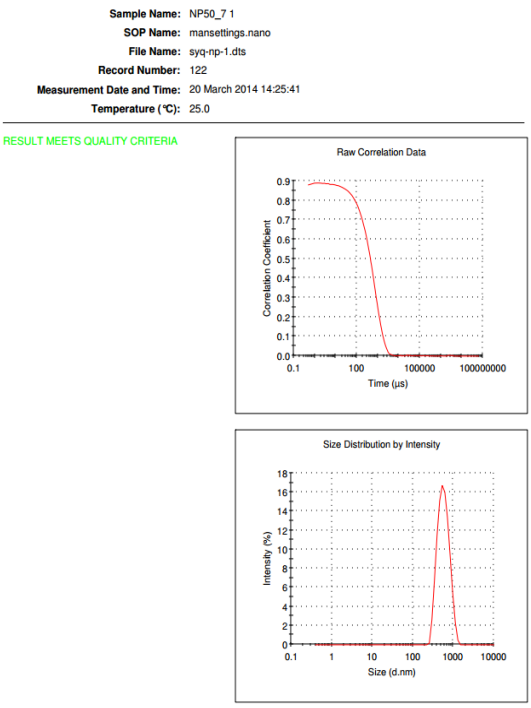




**Figure 5.8 Quality report for NP50-3** **Figure 5.9 Quality report for NP50-7**

### 5.1.1.3 Surface morphology

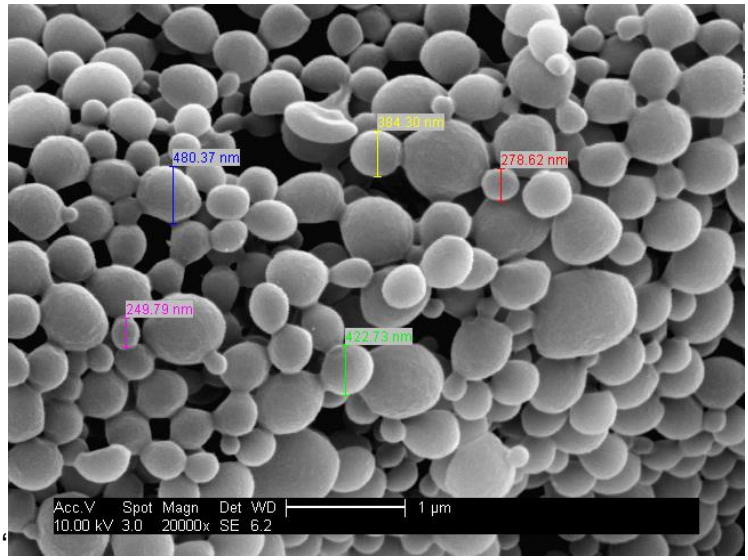



**Figure 5.10 SEM image of NP50-5**

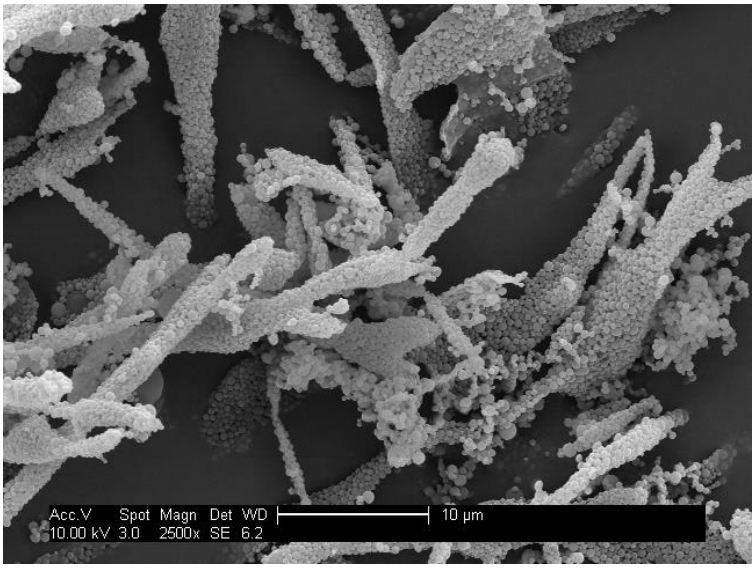



**Figure 5.11 SEM image of NP50-5**

Due to the large numbers of prepared samples, a series of SEM images have been captured. According to the SEM images, the nanoparticles prepared by single emulsion evaporation method all showed similar SEM results. Therefore, NP50-5 was chosen as an example to show the surface morphology of PLGA nanoparticles prepared by single emulsion evaporation method.

It can be seen in ***Figure 5.10*** that PLGA nanoparticles showed relatively smooth surface and are spherical in shape. ***Figure 5.11*** captured the nanoparticles in a larger magnification. The arrangement of nanoparticles shown in ***Figure 5.11*** was mostly in piece and in chain. As seen in a study by De and Robinson, all nanoparticles are dispersive entities initially and closely arranged as fibre after freeze-drying, which explain the SEM result of PLGA nanoparticles after the procedure of freeze-drying (De and Robinson 2004).

Meanwhile, some of particle sizes have been labelled in ***Figure 5.10***. Most of them were in a uniform size. Compared with the DLS results of NP50-5 (***Table 5.1, 555.5nm***), the particle sizes shown on the SEM is smaller than 555.5nm from 279nm to 480nm, There are two reasons causing the difference in particle sizes. One is that PLGA nanoparticles may have shrunk during the sample preparation for SEM. In order to make PLGA nanoparticles conductive, Au-shadowing is required, which carries the risk that the particles might shrink (Bootz, Vogel et al. 2004).The other possibility is that some particles may be aggregated together leading to bigger particle sizes when using the DLS technique (Bootz, Vogel et al. 2004). Although the results of particle sizes are different between two techniques, DLS technique remains more reliable than SEM. While DLS is able to aggregate the average size of nanoparticles, SEM only captured a small part of image which is difficult to be repeated and involves more uncertainties.

### 5.1.1.4 Thermal Analysis

Differential Scanning Calorimeter (DSC) was used for thermal analysis which yield the DSC thermogram showing the change at the glass transition temperatures of PLGA polymers and nanoparticles. The onset value is taken as the standard of the Tg.

**Table 5.2 Glass transition temperature of PLGA 50:50 and PLGA 75:25 polymer and nanoparticles**

| Sample | Glass transition temperature(℃) | |
|---|---|---|
| | PLGA 50:50 | PLGA 75:25 |
| PLGA polymer | 46.40 | 50.98 |
| PLGA nanoparticles | 45.59 | 50.76 |

As PLGA are amorphous in nature, glass transition temperature represents its major temperature characteristic. ***Table 5.2*** summarized the $T_g$ values of PLGA 50:50 and 75:25 calculated after freeze-drying.

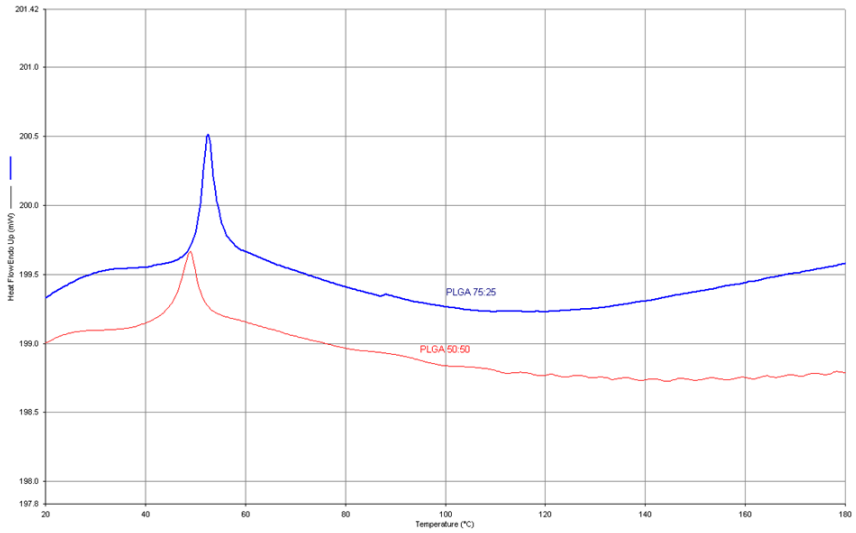



**Figure 5.12 DSC result of PLGA polymers**

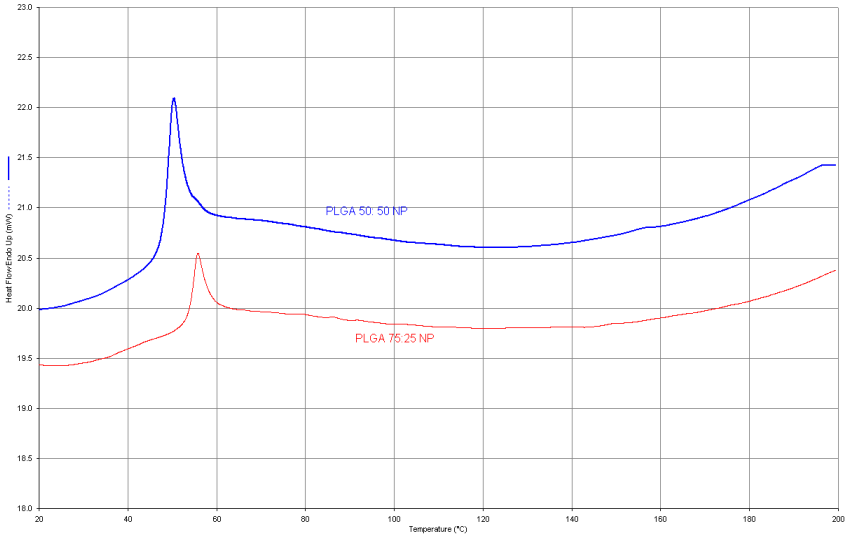



**Figure 5.13 DSC result of PLGA nanoparticles**

***Figure 5.12*** exhibited the $T_g$ of the PLGA raw material, which demonstrated that PLGA 75:25 had a higher $T_g$ as compared PLGA 50:50. The difference of ratios was considered to be the main reason. Lactic acid has one more methyl side group when compared to glycolic acid. The higher contents of lactic acid in PLGA 75:25 leads to a higher molecular weight. According to Wang N et al., the molecular weight can determine the mechanical strength of the polymer (Wang N et al., 1995). PLGA 75:25 owns a higher molecular weight, which has a stronger mechanical strength. Thus, PLGA 75:25 is more difficult to transform from glassy state to rubber state which results in a higher $T_g$. Moreover, the $T_g$ of the initial PLGA 75:25 and PLGA 50:50 polymers shown on the ***Figure 5.12*** confirmed to the range of $T_g$ provided from the manufacturer SIGMA-Aldrich (Polysciences Inc.2013).

In ***Figure 5.12*** and ***Figure 5.13***, both PLGA 50:50 and PLGA 75:25 nanoparticles showed a decrease in Tg after single emulsion. The decrease of the Tg proved the fact that the hydration has an effect on the Tg. During the preparation of PLGA nanoparticles and freeze-drying, it was hard to remove all the water. Some of moisture still remained in the nanoparticle products leading to a slight decrease in Tg as shown in the ***Table 5.2.***

### 5.1.2 Emulsion Diffusion Method

**Table 5.3 Summary of particle sizes prepared by Emulsion Diffusion Method**

| Ratio of PLGA | stirring speed(rpm) | Particle size(nm) |
|---|---|---|
| 50:50 | 500<br>magenative stirring | 685 |
| 50:50 | 15000<br>generator | 314 |

Although nanoparticle with a size of 200—780 nm is able to pass through the vascular endothelium of tumor or inflammatory tissue for cancer treatment, a smaller size from 70 to 200nm is desirable as it could provide for better treatment (Gaumet, Vargas et al. 2008). As stirring speed is one of the key factors to reduce the particle size for the single emulsion evaporation method. A higher stirring speed might be effective in reducing particle size. However, due to the restriction of the homogenizer, 15000rpm is the maximum stirring speed. Therefore, in order to reduce the particle size, an emulsion diffusion method is applied, and this allows the process to yield nanoparticles between 95nm and 300nm (Astete and Sabliov 2006, Ye and Squillante 2013).

Two different stirring speeds (500rpm, 15000rpm) are applied in this method. According to the results of size distribution, both samples showed single peak. The particle size of nanoparticles prepared by 15000rpm stirring speed produce products that are half the size of those prepared by magnetic stirring. However, the size remains over 300nm, which was not in the desirable size range of 70 to 200nm. Therefore, a further modification to variables such as solvent, concentration of PLGA and the way in which oil addition phase to aqueous phase, can be applied to generate smaller particle size.

The results of particle sizes showed a significant reduction (***Table 5.3***), which process that stirring speed is an effective method to reduce the particle size in either the homogenizer step or during the step of water addition for emulsion diffusion method (Astete and Sabliov 2006). Nonetheless, the SEM images (***Figure 5.16, Figure 5.17***) were still not desirable enough. The prepared samples are in irregular shape, which is more likely to fibre. The surface of

products were rough and uneven. The irregular shape of samples may be attributed to the recovery procedure. The organic solvent might not have been thoroughly removed and washed out by water during centrifugation step, which caused the nanoparticle coalescence (Bilati, Allémann et al. 2005). The potential reasons for such an occurrence requires further investigation.

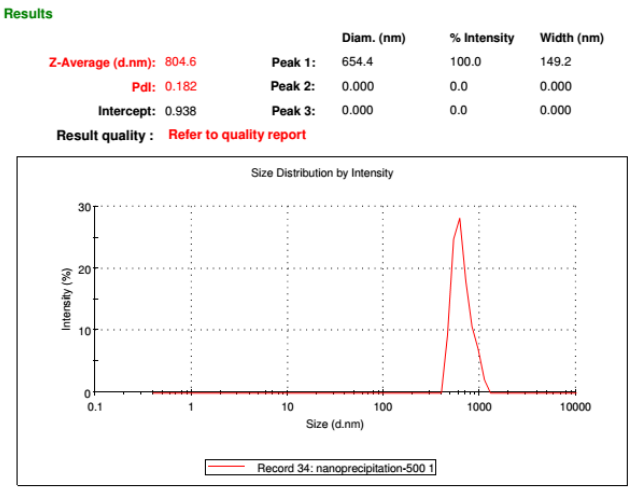



**Figure 5.14 DSC result of samples prepared under 15000rpm**

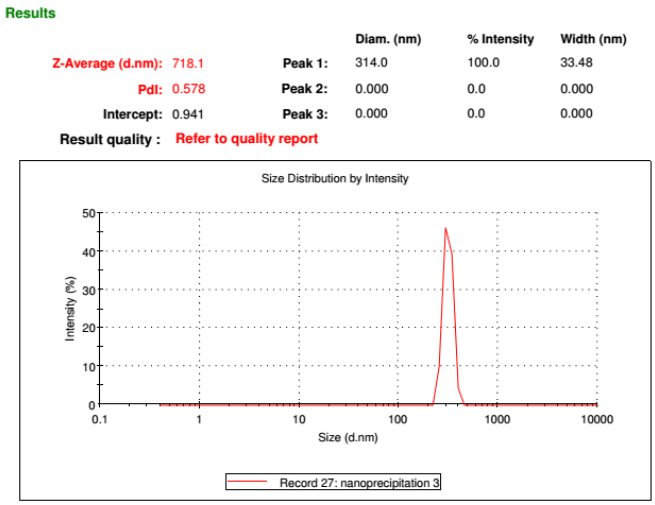



**Figure 5.15 DSC result of samples prepared under 15000rpm**

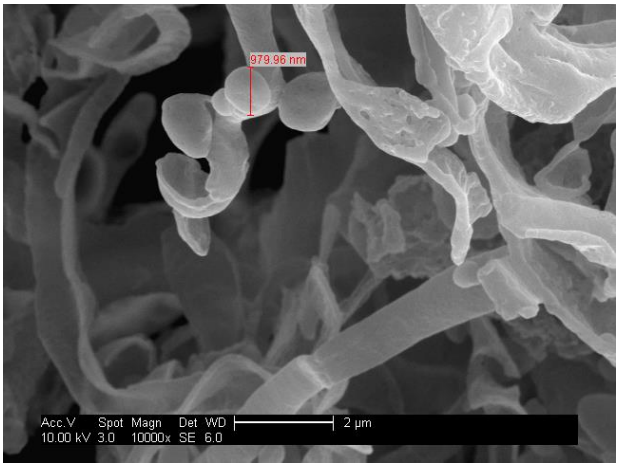



**Figure 5.16 SEM image of sample prepared by 500 rpm**

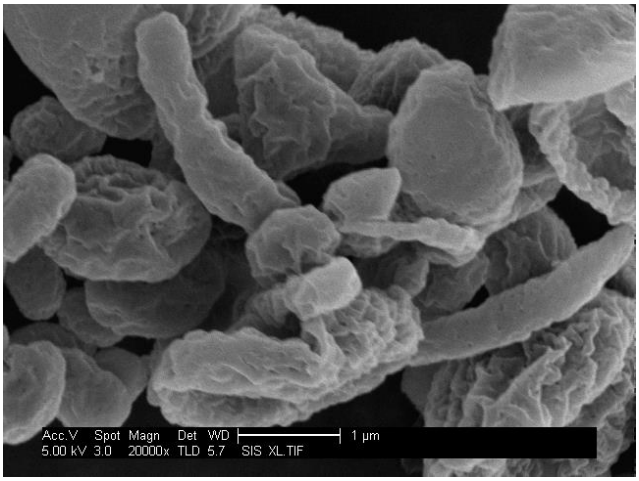
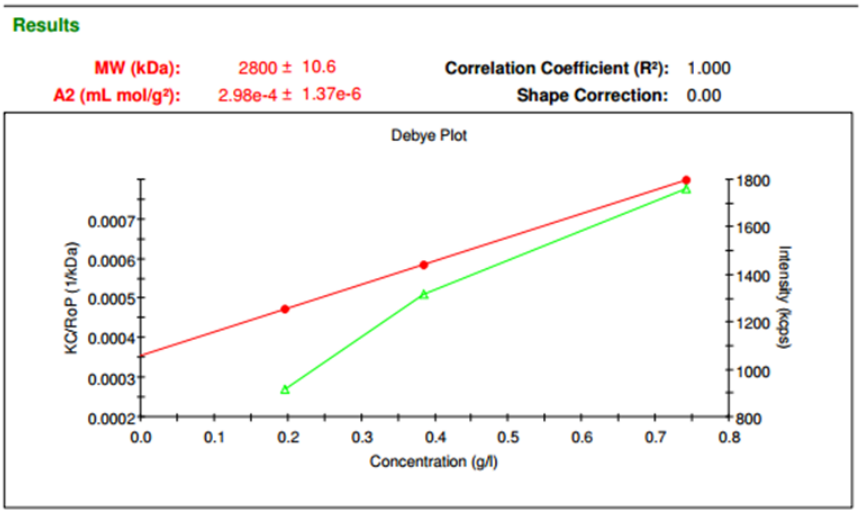



**Figure 5.17 SEM image of sample prepared by 15000rpm**

## 5.2 Molecular weight of PLGA nanoparticles




| Conc. g/l | Intensity kcps | KC/RoP 1/kDa |
|---|---|---|
| 0.196 | 920.4 | 4.74e-4 |

| Conc. g/l | Intensity kcps | KC/RoP 1/kDa |
|---|---|---|
| 0.385 | 1319.4 | 5.85e-4 |

| Conc. g/l | Intensity kcps | KC/RoP 1/kDa |
|---|---|---|
| 0.741 | 1761.8 | 7.99e-4 |

| Conc. g/l | Intensity kcps | KC/RoP 1/kDa |
|---|---|---|
| | | |

**Figure 5.18 Debye Plot of PLGA 50:50 nanoparticles**

***Figure 5.18*** exhibited the debye plot of PLGA 50:50 nanoparticles. As shown in the figure, the molecular weight of PLGA 50:50 was 2800±10.6 kDa meanwhile the 2$^{nd}$ virial coefficient was around 0.0546 above zero which means the particles are stable in the solution. According to the manual of the Zetasizer nano series, the intensity of scattered light is proportional to the sample concentration, the result of the Debye Plot concur with the study.

As mentioned in ***Section 2.6.3***, the standards required in the sample preparation in SLS technique is extremely strict. Sample should be monomodal which means the intensity particle size distribution (PSD) of samples can only have one peak. In order to ensure that only one mode is present, samples should be filtered before measurement. However, the filtration will remove some of the material. Therefore, the concentration of the filtered sample needs to be determined, as this is one of the factors affecting the accuracy of the results.

For the SLS technique, the purity of the sample plays a key role in the whole experiment procedures. Even small dust particualte would affect the results. Due to the obstacles in preparing samples as well as the sample loss during the filtration, the SLS method of molecular measurement is hard to execute and

repeat. Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) might be more suitable choices to measure the molecular weight.

## 5.3 Drug-loaded nanoparticles

### 5.3.1 Particle Size

**Table 5.4 Summary of particle size of ibuprofen-loaded nanoparitcles**

| Ratio of PLGA | Sample | Particle size(nm) | |
|---|---|---|---|
| | | peak 1 | peak 2 |
| 50:50 | IBU-NP50-1 | 570.2(94.8%) | 4854.0(5.2%) |
| | IBU-NP50-2 | 705.6(95.3%) | 5024.0(4.7%) |
| | IBU-NP50-3 | 591.8(94.5%) | 4885.0(5.5%) |
| | IBU-NP50-4 | 587.1(91.0%) | 4776.0(7.0%) |
| 75:25 | IBU-NP75-1 | 545.2(94.2%) | 4962.0(5.8%) |

A number of literature have shown that o/w single emulsion method could be applied to encapsulate hydrophobic drugs. While w/o/w double emulsion method is suitable to hydrophilic drugs (Soppimath, Aminabhavi et al. 2001)(Astete and Sabliov 2006). Due to the hydrophobicity of ibuprofen, modified single emulsion evaporation method D was applied in this study for the preparation of IBU-loaded PLGA nanoparticles.

***Table 5.4*** summarized the particle sizes of different batches of samples. ***Figure 5.2019-23*** shows the results of DLS report. As shown in figures, the size distributions for the samples all have two peaks. One is within the range of nanoparticles, the other one is around 5000nm which might be the sizes of unloaded drugs. Excluding IBU-NP50-2, the particle sizes of samples were all around 570±20. Meanwhile, the proportion of peak1 is also similar to each sample. Therefore, the preparation method used in the study shows a relatively uniform size.

Results

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 565.0 | Peak 1: | 570.2 | 94.8 | 199.2 |
| PdI: 0.227 | Peak 2: | 4854 | 5.2 | 697.7 |
| Intercept: 0.961 | Peak 3: | 0.000 | 0.0 | 0.000 |

Result quality : Good

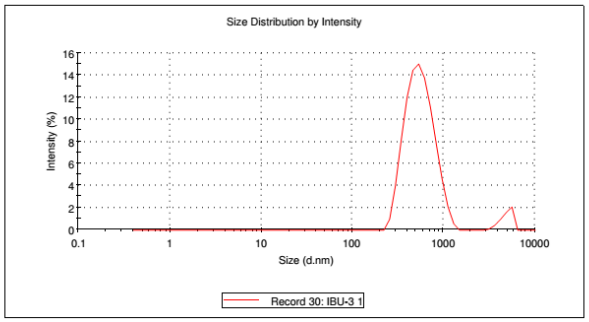



**Figure 5.19 Size Distribution by Intensity for IBU-NP50-1**

Results

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 757.1 | Peak 1: | 705.6 | 95.3 | 243.3 |
| PdI: 0.279 | Peak 2: | 5024 | 4.7 | 669.2 |
| Intercept: 0.941 | Peak 3: | 0.000 | 0.0 | 0.000 |

Result quality : Good

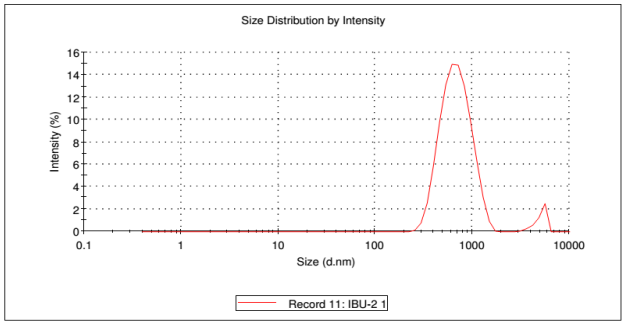



**Figure 5.20 Size Distribution by Intensity for IBU-NP50-2**

Results

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 623.4 | Peak 1: | 591.8 | 94.5 | 198.0 |
| PdI: 0.255 | Peak 2: | 4885 | 5.5 | 737.3 |
| Intercept: 0.937 | Peak 3: | 0.000 | 0.0 | 0.000 |

Result quality : Good

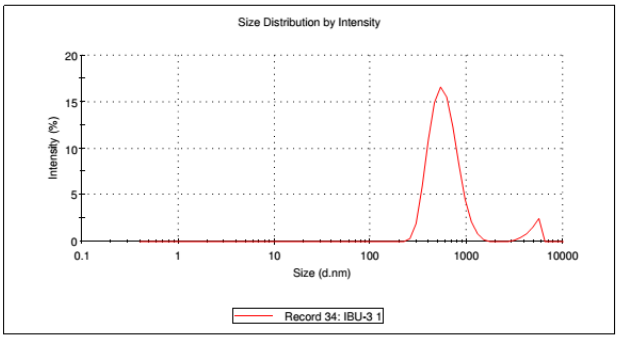



**Figure 5.21 Size Distribution by Intensity for IBU-NP50-3**

Results

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 587.0 | Peak 1: | 587.1 | 91.0 | 241.0 |
| PdI: 0.312 | Peak 2: | 4776 | 7.0 | 850.2 |
| Intercept: 0.942 | Peak 3: | 164.1 | 2.0 | 21.90 |

Result quality : Good

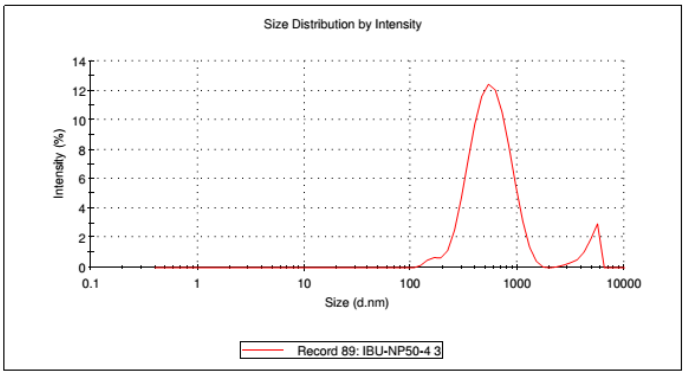



**Figure 5.22 Size Distribution by Intensity for IBU-NP50-4**

Results

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 522.9 | Peak 1: | 545.2 | 94.2 | 260.9 |
| PdI: 0.286 | Peak 2: | 4962 | 5.8 | 649.4 |
| Intercept: 0.937 | Peak 3: | 0.000 | 0.0 | 0.000 |

Result quality : Good

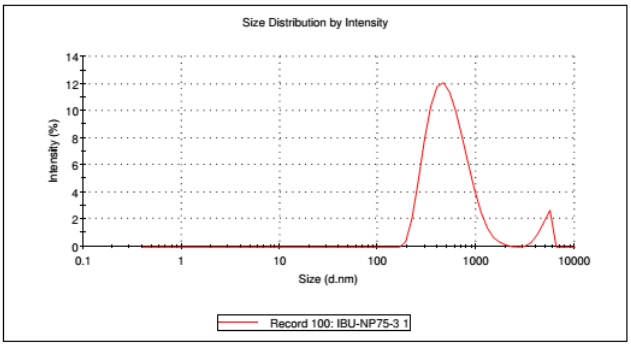



**Figure 5.23 Size Distribution by Intensity for IBU-NP75-1**

### 5.3.2 Drug Efficiency

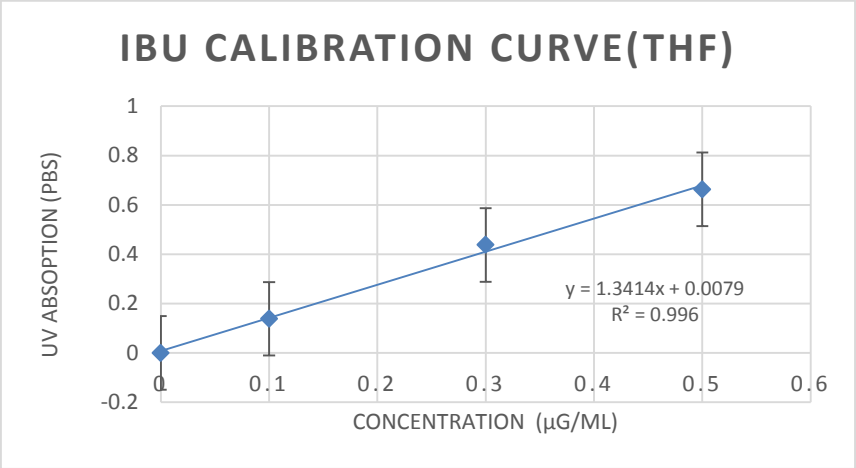



**Figure 5.24 calibration curve for Ibuprofen in THF**

**Table 5.5 Drug loading content and Encapsulate efficiency for ibuprofen-loaded nanoparticles**

| Sample | UV Absorption | Drug loading(%) | Encapsulate Efficiency(%) |
|---|---|---|---|
| IBU-NP50-1 | 0.418 | 16.17% | 80.85% |
| IBU-NP50-2 | 0.533 | 20.74% | 103.73% |
| IBU-NP50-3 | 0.598 | 23.41% | 117.07% |
| IBU-NP50-4 | 0.440 | 16.93% | 84.63% |
| IBU-NP75-1 | 0.406 | 15.53% | 77.65% |

UV was utilized for the study to measure the drug loading content. Different UV absorptions were obtained and compared against ibuprofen concentration ranging from between 0.3 to 1 mM. The calibration curve (A=0.8884C+0.2308, $R^2$=0.9983) was calculated and fits the Lambert and Beer's Law: the concretion of drugs is proportional to the UV absorption. Therefore, the concentration of ibuprofen in PLGA nanoparticles can be calculated according to the calibration curve.

The results of drug loading and encapsulation efficiencies of each batch are exhibited in ***Table 5.5***. It is notable that the drug loading content for samples

(IBU-NP50-1, IBU-NP50-4, IBU-NP75-1) exhibit satisfactory results of more than 75%. However, the drug loading content for IBU-NP50-3 and IBU-NP50-3 are larger than the theoretic loading (20%). A likely reason is that unloaded drugs could not be completely washed out by water, resulting in a higher concentration of ibuprofen. With a comparison between NP50 and NP75, the drug loading content of NP50 is higher than NP75 which is in line with the study by Fernadez-Carballido et al. (2004), whose studies point out that drug loading content increases with a decrease of PLGA molecular weight.

### 5.3.3 Thermal Analysis

There are two ways for drugs to be loaded on PLGA nanoparticles, absorbing on the surface of nanoparticles or encapsulating inside the PLGA matrix. Therefore, DSC technique were carried out to determine whether ibuprofen was bounded on the surface or incorporated in the PLGA matrix. At the same time, it is also able to determine the physical form of ibuprofen existing in the PLGA matrix whether in a crystalline form or amorphous form. Table 5.6 exhibits both onset Tg and Tm of samples, which gives a comparison of each samples.

**Table 5.6 Summary of Tg and Tc for PLGA nanoparticles and Ibuprofen**

| Sample | Glass transition temperature(℃) | Melting temperature(℃) |
|---|---|---|
| Ibuprofen | - | 77.85 |
| PLGA NP50 | 45.59 | - |
| IBU-NP50 | 43.24 | 72.06 |
| PLGA NP75 | 50.76 | - |
| IBU-NP75 | 42.06 | - |

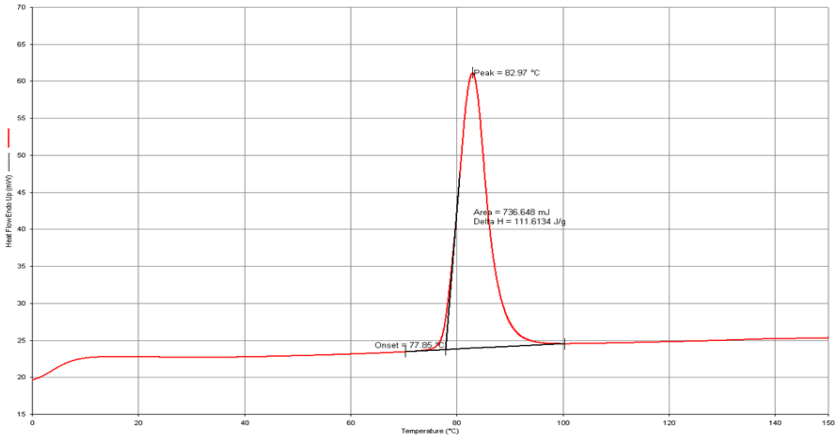



**Figure 5.25 DSC result of ibuprofen**

Ibuprofen exists in crystalline form and has a melting point under an increase of temperature. ***Figure 5.25*** is the DSC result of ibuprofen and it shows that Ibuprofen's melting point stands at 77.85℃. As stated in the Material Safety Data Sheet from Sigma-Aldrich, the melting point of ibuprofen is between 77℃ and 78℃. Thus result in the study is within the range of its standard melting point.

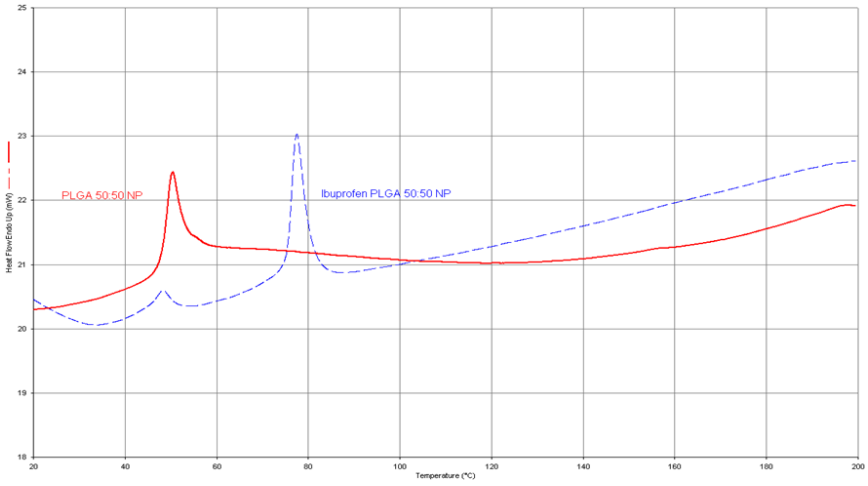



**Figure 5.26 DSC result of Ibuprofen-loaded PLGA 50:50 nanoparticles**

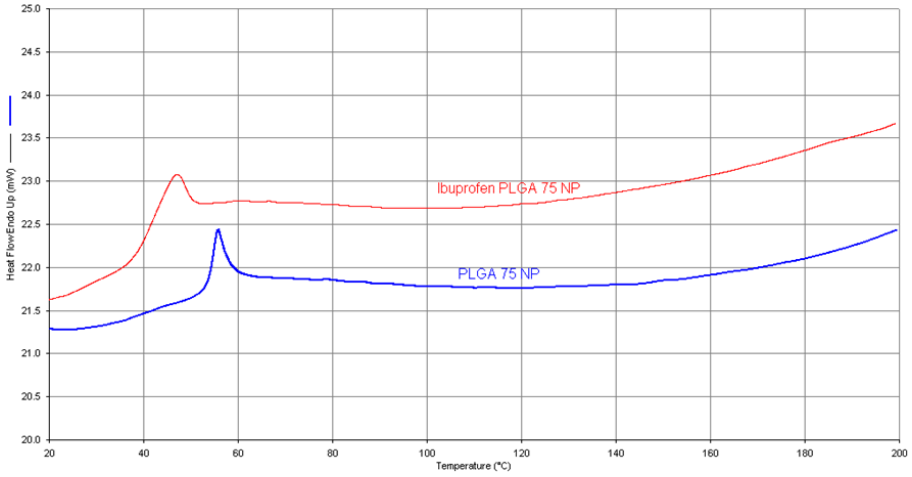



**Figure 5.27 DSC result of Ibuprofen-loaded PLGA 75:25 nanoparticles**

***Figure 5.26*** and ***Figure 5.27*** draws comparisons between ibuprofen-loaded PLGA nanoparticles and non-loaded PLGA nanoparticles. According to ***Figure 5.26***, there were two peaks shown in the DSC thermogram which respectively represented the glass transition temperature of PLGA and the melting temperature of ibuprofen. The two temperatures were lower than their original value. The DSC curve for PLGA 75:25 nanoparticles with ibuprofen showed a lower Tg for PLGA 75:25 nanoparticles, but no characteristic ibuprofen peak is observed in the thermogram.

It is notable that both ibuprofen-loaded PLGA 50:50 nanoparticles and ibuprofen-loaded PLGA 75:25 nanoparticles showed peak shift in the DSC thermogram compared to the non-loaded nanoparticles. This confirmed the existence of drug-polymer interaction. As mentioned in study of Fernández-Carballido et al., due to the structure of PLGA and ibuprofen, carboxylic acid end groups in ibuprofen might form hydrogen bonding with PLGA polymer showing the drug-polymer interaction (Fernández-Carballido, Herrero-Vanrell et al. 2004). The polymer peak for ibuprofen PLGA75 NP in DSC curve showed a bigger temperature shift than ibuprofen PLGA50 NP, which indicated a higher interaction between PLGA 75:25 and ibuprofen.

As mentioned above, due to the different composition of PLGA polymer, PLGA75:25 NP exhibited a higher Tg than PLGA50:50 NP. However， ibuprofen-loaded PLGA NP shows a reverse result. This might also associate

with the drug-polymer interaction. PLGA 75:25 with a higher molecular weight might increase the chance to interaction with acid drugs. The study to illustrate the specific interaction between ibuprofen and PLGA polymer is still limited. Therefore, a further study is needed to demonstrate it.

The two curves for ibuprofen-loaded nanoparticles show different results. The thermogram of Ibuprofen PLGA50 NP shows both polymer peak and drug peak, while thermogram of Ibuprofen PLGA75 NP does not show the drug peak. The result of Ibuprofen PLGA75 NP is in accordance with previous studies that drug peak is not seen in the DSC curve for drug-nanoparticule system (Fernández-Carballido, Herrero-Vanrell et al. 2004, Agnihotri and Vavia 2009, Gupta, Aqil et al. 2010). As stated in the study of Gupta, Aqil et al., the diminished drug peak attributes to the dilution effect and entrapment of ibuprofen in PLGA matrix.(Gupta, Aqil et al. 2010). Due to the lower drug loaded content of PLGA 75:25 than PLGA 50:50, a smaller concentration of ibuprofen in PLGA75:25 polymer exhibits, resulting in the suppression of ibuprofen peak. Furthermore, the result of IBU PLGA75:25 nanoparticles proved that most of ibuprofen was encapsulated in the matrix of PLGA 75:25.

The two endothermic peaks of IBU PLGA50:50 NP, which is indicated that some of the ibuprofen is absorbed on the surface of PLGA50:50. This can be attributed to the fact that nanoparticles were broken and ibuprofen leaked out under the high speed homogenization and centrifugation during the process of sample preparation. The molecular weight is a crucial parameter for determining the mechanical strength of the polymer. Thus PLGA 50:50 with a lower molecular weight leads to a weaker mechanical strength which is easier to be crushed by physical stress.

The results for the two ibuprofen-loaded PLGA nanoparticles with different compositions showed that some of the drugs were absorbed on the surface of PLGA50:50 nanoparticles, whereas PLGA 75:25 successfully encapsulated drugs into the polymer matrix. This was both attributed to the composition of PLGA polymer and the preparation procedure. The peak shifts of both samples demonstrated the interaction between the drugs and PLGA polymers.

## 5.4 Stability study

Sample NP50/15000-1, NP75/15000-1, IBU-NP50-4 and IBU-NP75-1 were used for the degradation study. Due to the limitation of time, storage condition of 40℃ and 75% relative humidity were applied to shorted the degradation time.

### 5.4.1 PLGA nanoparticles

#### 5.4.1.1 Surface morphology

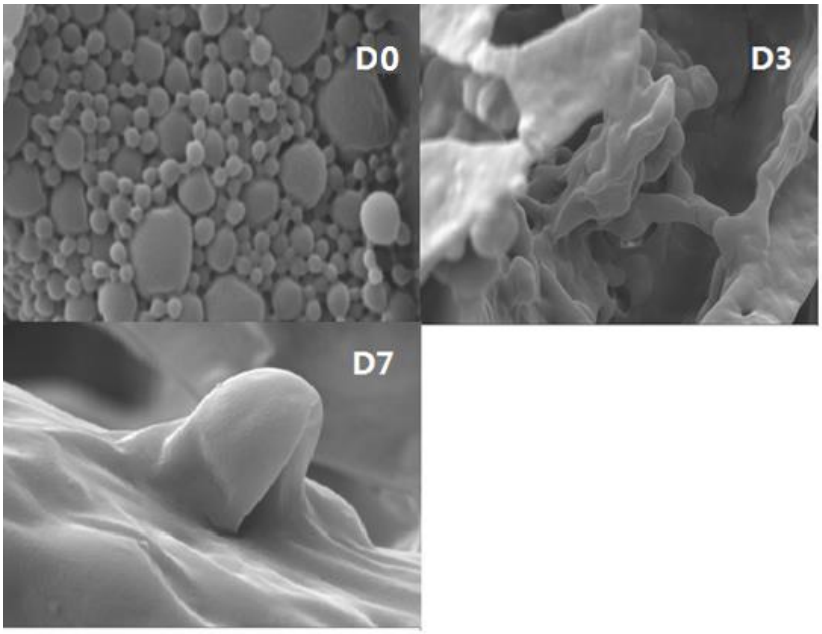



**Figure 5.28 SEM images for NP50/15000-1**

**(D0: after freeze-drying; D3: 3-day storage; D7: 7-day storage)**

The Scanning electron microphotographs about PLGA 50:50 stored in 40℃ and 75% relative humidity are presented in ***Figure 5.28***. The three pictures were respectively captured when samples prepared after freeze-drying (D0) and stored for 3 days and 7 days in certain storage condition. According to the variation of the morphology of PLGA nanoparticles, the degradation of PLGA as well as the difference between PLGA 50:50 and PLGA 75:25 could be observed.

As shown in ***Figure 5.28***, the PLGA nanoparticles after freeze-drying (D0) had a spherical geometry. Meanwhile, the surfaces of the nanoparticles were smooth. After 3 day storage, PLGA nanoparticles started to agglomerate and

fuse together. Most of nanoparticles were lost their shapes. Only some big particles remained their spherical geometries. At the same time, the chain arrangement of PLGA polymer still existed. By day 7, nanoparticles had completely lost their original shape and changed into block polymer. All the small particles agglomerated into big particles as shown on the ***Figure 5.28***

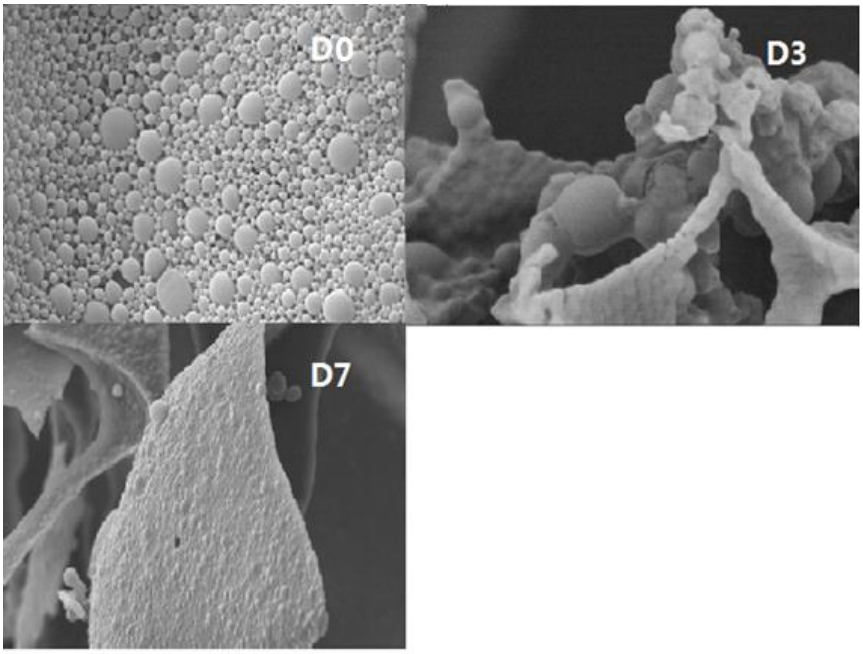



**Figure 5.29 SEM images for NP75/15000-1**

**(D0: after freeze-drying; D3: 3-day storage; D7: 7-day storage)**

***Figure 5.29*** shows PLGA 75:25 nanoparticles prepared after sing emulsion method and stored in stated condition. It can be seen in ***Figure 5.29(D0)*** that NP75 before storage exhibits spherical shape with smooth surface. By Day 3, NP75 showed a similar degradation state as NP50 which nanoparticles sticked to each other in a form of chain or piece. However, after a seven-day storage, NP75 shows a different SEM result as shown in ***Figure 5.29(D7)***. Although most of particles agglomerated together, there still a small numbers nanoparticles existed showing part of spherical geometry as well as remaining the chain arrangement.

The changes of surface morphology showed that the nanoparticles were in spherical shape and maintained smooth surface before the storage, whereas

they started to lose their original shapes as well as agglomerate to each other after 1 week storage. These phenomena were the signs of the erosion and degradation of PLGA nanoparticles. The degradation process of PLGA is undergoing an uptake of water. Therefore, the hydrophilic of polymer is an important factor needed to be considered. It is notable that PLGA 75:25 owns a slower degradation rate than PLGA 50:50. As stated by (Mundargi, Babu et al. 2008), PLGA 50:50 is the most common composition owning a shortest degradation time in comparison to other composition of PLGA polymer, which has been demonstrated in this study. Due to the fact that PLGA 75:25 has more lactic acid content, PLGA 75:25 is less hydrophilic than PLGA 50: 50 leading to a slower degradation rate.

The storage condition is another fact that influences the degradation rate of PLGA. It is reported that a higher storage temperature and relative humidity increase the moisture content. More water is able to attach the chain of PLGA polymer for the hydrolysis which results in a shorter degradation time and higher degradation rate. (De and Robinson 2004). Therefore, in order to accelerate degradation speed, 40℃ and 75% relative humidity is used in this study. PLGA 50:50 nanoparticles have completely lost their original shape and become block polymer within seven days. At the same time, PLGA 75:25 nanoparticles have sticked to each other and start to fuse into block polymer. Both samples showed a faster degradation speed under 40℃ in comparison to those stored in 4℃ and 25℃ shown in the study of (De and Robinson 2004).

In conclusion, both storage condition and PLGA composition are of importance to polymer degradation rate. The degradation rate is increased with the storage temperature, relative humidity and the lactic acid content. A further study can be conducted to investigate the effect of PH and molecular weight to the degradation rate.

#### 5.4.1.2 Thermal analysis

Differential Scanning Calorimeter (DSC) was used for thermal analysis which yielded the DSC thermogram showing the change of glass transition

temperature of PLGA polymers and nanoparticles. The onset value is taken as the standard of the Tg.

**Table 5.7 Glass transition temperature of PLGA 50:50 and PLGA 75:25 nanoparticles**

| Sample | Glass transition temperature(℃) | |
|---|---|---|
| | PLGA 50:50 | PLGA 75:25 |
| PLGA nanoparticles | 45.59 | 50.76 |
| PLGA nanoparticles (D7) | 44.53 | 49.82 |

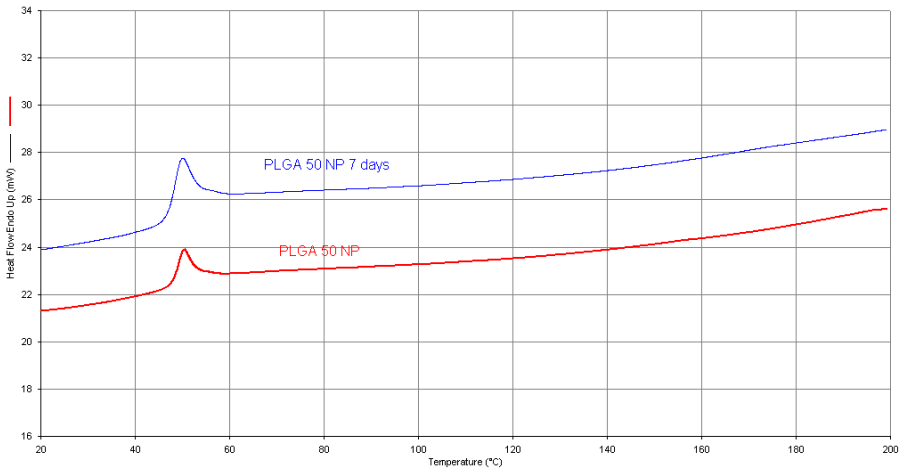



**Figure 5.30 DSC result of PLGA 50:50 and PLGA 50:50 nanoparticles**

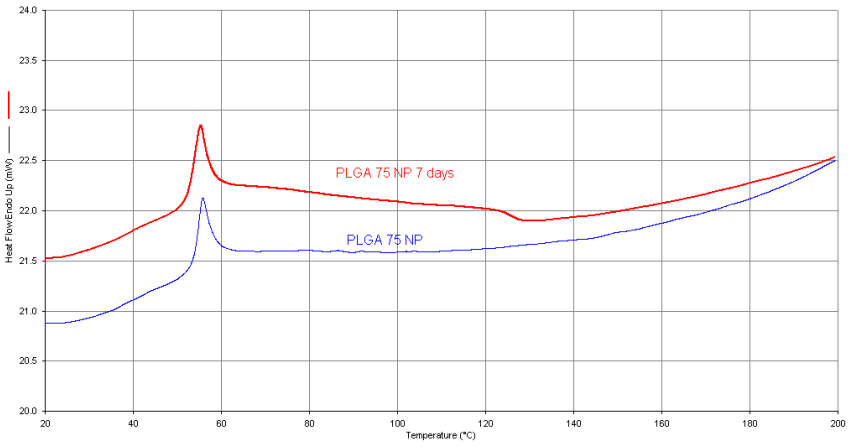



**Figure 5.31 DSC result of PLGA 75:25 and PLGA 75:25 nanoparticles**

Thermal analysis was an indispensable property for the stability study of PLGA. As shown in the ***Table 5.7,*** $T_g$ exhibited slight decline after one-week storage,

The degradation of PLGA polymers under the exposure of water was accountable for the decrease. Furthermore, with observing the red curve, it is notable that some fluctuations showed at the end of curve. This result might account to the fact that PLGA nanoparticles started to degrade into highly crystallisable lactic acid and amorphous glycolic acid oligomers. Therefore, the crystalline melting peaks might appear on the DSC thermogram (over 100℃) during the degradation of PLGA polymers, which was also another fact of the degradation of the polymers.

With comparison the Tg variation of two polymers, PLGA NP 50:50 underwent a bigger decline than PLGA NP75:25. The higher ratios of glycolic acid in PLGA 50:50 increase the hydriphility of the polymer making it easier to be degraded under the exposure of water resulting in a bigger extent of decline. Accordingly, PLGA 75:25 has a higher content of lactic acid hence absorbing less water and reducing the rate of degradation. The SEM images also can support the DLS results, which PLGA 75:25 degraded in slower speed.

## 5.4.2 IBU-loaded nanoparticles

### 5.4.2.1 Thermal analysis

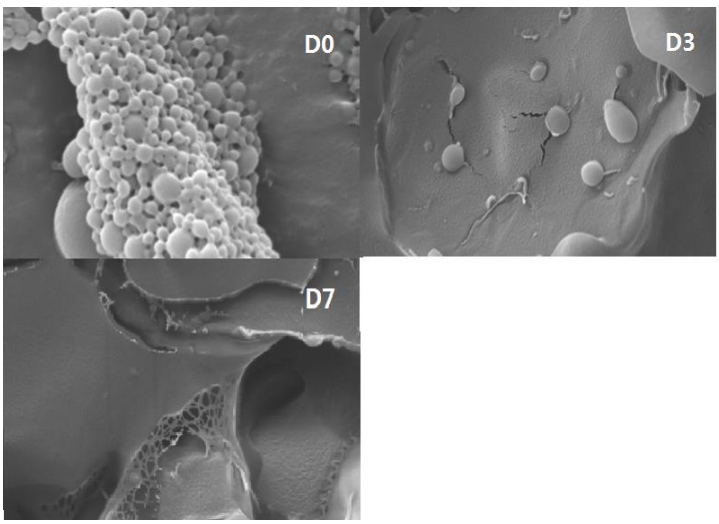



**Figure 5.32 SEM images for ibuprofen-loaded PLGA 50:50 nanoparticles**

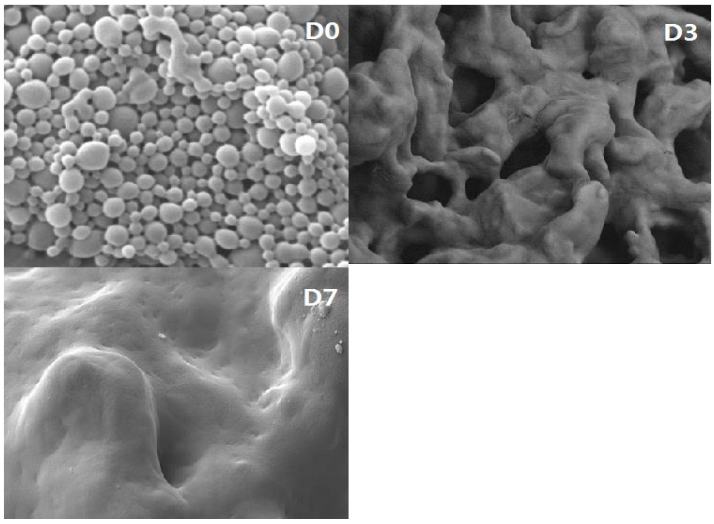



**Figure 5.33 SEM images for ibuprofen-loaded PLGA 75:25 nanoparticles**

***Figure 5.32*** and ***Figure 5.33*** respectively show the degradation process of IBU-NP50 and IBU-NP75. Both two samples own spherical geometry with a low surface roughness after freeze-drying. By Day 3, most of IBU-NP50 had lost their chain-like arrangement and fused into block polymer, whereas IBU-NP75 showed a lower extent of degradation, which still remained the chain-like arrangement. By the seventh day, all the IBU-NP50 particles have collapsed as well as IBU-NP75.

With tracking the SEM image, it is concluded that IBU-NP50 degrade faster than IBU-NP75 which is the same result as non-loaded PLGA nanoparticles. However, in comparing to non-loaded PLGA nanoparticles, it is observed that Ibuprofen-loaded PLGA nanoparticles exhibit a faster degradation rate than non-loaded ones, which can be proved on Day 3 when NP50 still arrange in chain while IBU-NP50 has collapsed into block polymer.

### 5.4.2.2 Thermal analysis

**Table 5.8 Summary of Tg and Tm for Ibuprofen-loaded PLGA nanoparticles and Ibuprofen**

| Sample | Ibuprofen | IBU-PLGA 50:50 | IBU-PLGA 50:50(D7) | IBU-PLGA 75:25 | IBU-PLGA 75:25(D7) |
|---|---|---|---|---|---|
| Glass transition temperature(℃) | - | 43.24 | 38.53 | 42.06 | 40.88 |
| Melting temperature(℃) | 77.85 | 72.06 | 72.06 | - | - |

It was seen in ***Table 5.8*** that the change of Tg for ibuprofen-loaded PLGA nanoparticles shows a similar trend to non-loaded nanoparticles. Both samples showed a decline in their Tg and PLGA 50:50 underwent a bigger decline than PLGA 75:25. The reasons have been mentioned in ***Section 5.4.1.2*** which is associated to their composition. However, with a closer look at ibuprofen-loaded PLGA 50:50 nanoparticles, it can be found that the characteristic drug peak exhibited the same temperature after storage 7 days showing that ibuprofen is relatively stable in the PLGA matrix.

It was noted that Ibuprofen-loaded nanoparticles experienced a bigger decline of Tg than non-loaded nanoparticles, which is also observed in the results obtained by SEM, showing that Ibuprofen increased the degradation rate of PLGA nanoparticles. This result is agreement with the study of Siegel et al. and Li et al, who stated that the drugs in PLGA matrix were able to affect the degradation rate of polymer (Li, Girod-Holland et al. 1996, Siegel, Kahn et al. 2006). It has been stated that the chemical structures of drugs affect both matrix degradation rate and water absorption rate. Therefore, a faster degradation rate compared to non-loaded PLGA nanoparticles was shown in this study.

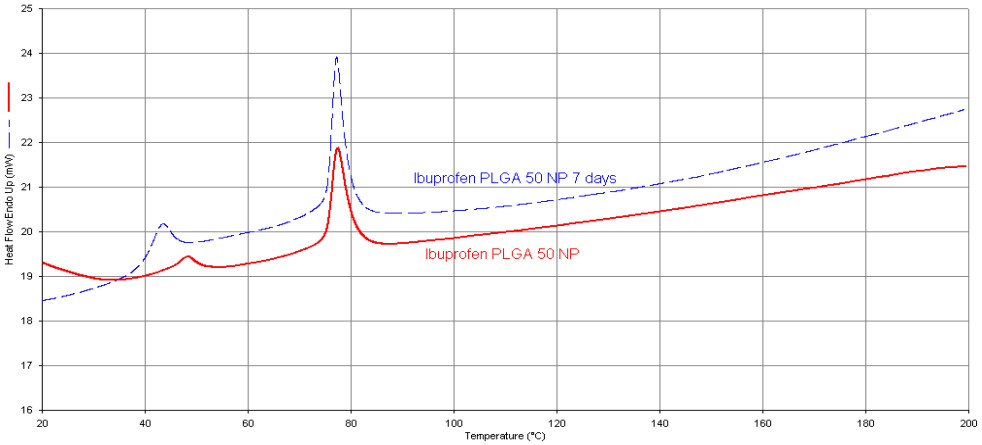



**Figure 5.34 DSC result of ibuprofen-loaded PLGA 50:50 nanoparticles for 7-day storage**

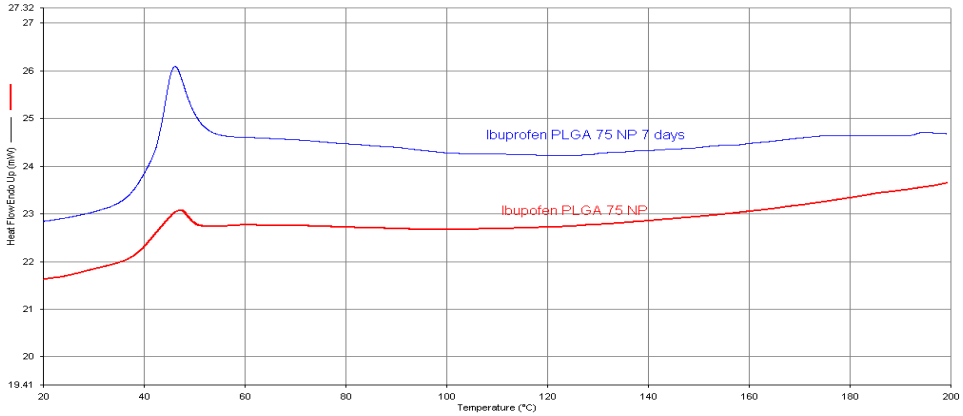
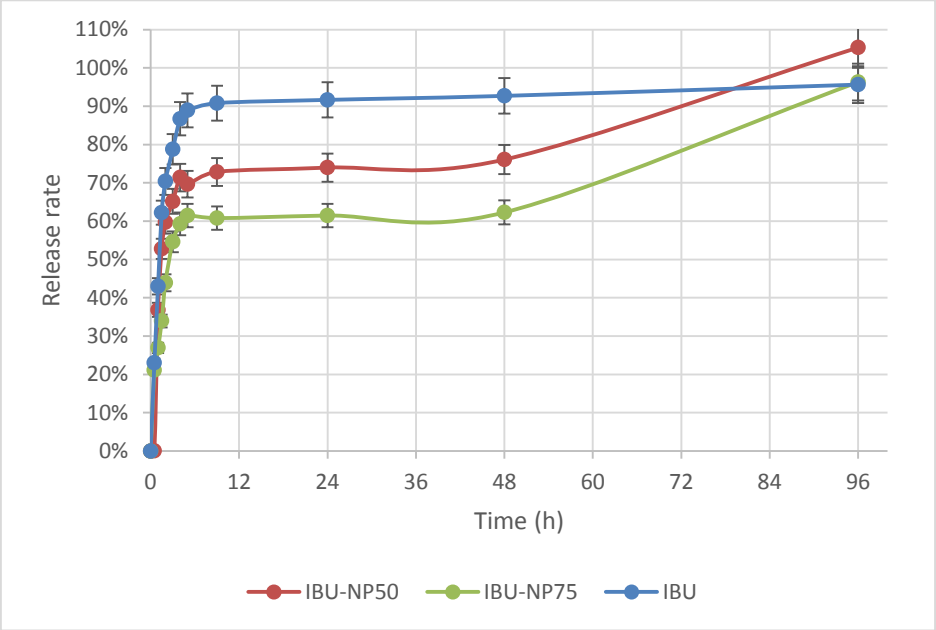



**Figure 5.35 DSC result of ibuprofen-loaded PLGA 75:25 nanoparticles for 7-day storage**

## 5.5 In vitro releasing study




| Time（h） | Durg releasing Rate（%） | | |
|---|---|---|---|
| | Ibuprofen | IBU-NP50 | IBU-NP75 |
| 0.5 | 23.00 | 0.00 | 21.11 |
| 1 | 43.02 | 36.83 | 26.92 |
| 1.5 | 62.20 | 52.80 | 33.64 |
| 2 | 70.38 | 59.74 | 43.93 |
| 3 | 78.76 | 65.18 | 54.59 |
| 4 | 86.73 | 71.38 | 59.32 |
| 5 | 88.93 | 69.64 | 61.48 |
| 9 | 90.82 | 72.86 | 60.80 |
| 24 | 91.66 | 73.98 | 61.48 |
| 48 | 92.70 | 76.08 | 62.29 |
| 96 | 95.63 | 105.30 | 96.30 |

**Figure 5.36 Drug releasing curve for ibuprofen-loaded PLGA nanoparticles**

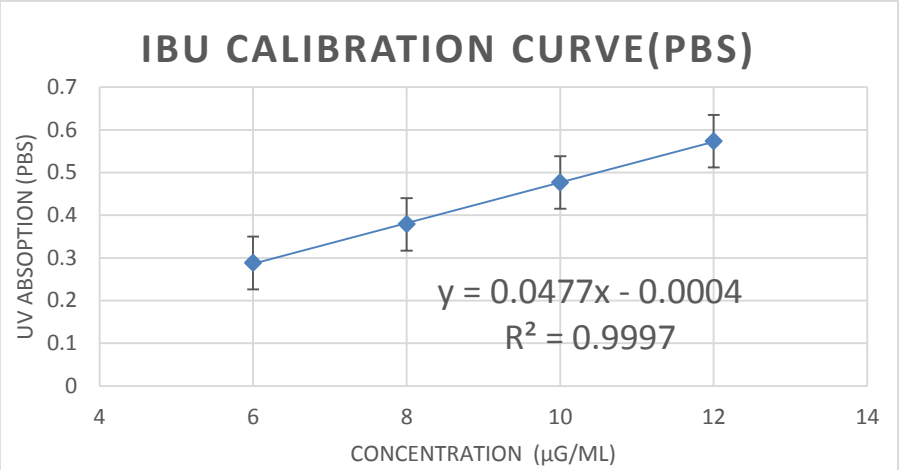



**Figure 5.37 Calibration curve for Ibuprofen in PBS**

The drug releasing study was evaluated by UV technique. Ibuprofen calibration curve was obtained to calculate the concentration of ibuprofen in PBS buffer (PH7.4) which is used as the dissolution medium.

Ibuprofen-loaded nanoparticles were prepared with different polymer compositions, PLGA 50:50 and PLGA 75:25. ***Figure 5.36*** shows the cumulative ibuprofen release rate. According to ***Figure 5.37***, three curves respectively represent IBU-NP50, IBU-NP75 and ibuprofen. All the three samples were shown 100% drug release. Among these three curves, pure ibuprofen showed the fastest drug releasing rate, which was seen to be completely released after 9 hours. PLGA 50:50 and 75:25 nanoparticles showed 72.9% and 60.8% release of total drug within 9 hours respectively, which indicated that PLGA 50:50 nanoparticles underwent a higher release rate than PLGA 75:25 nanoparticles. It can be observed that drug release profile for both PLGA50:50 and PLGA75:25 suggested the existence of three zones, namely the first 4h, 4h-24h and 24h-96h.The ibuprofen release rate increased dramatically in the first 4h which can be attributed to diffusion of ibuprofen from the surface of nanoparticles. The rate of drug releasing slowed down between 4h to 24h indicating that most of drugs absorbed on the surface have diffused into PBS. From 24h to 96h, a climb in drug release was observed suggesting the PLGA degradation. It is reported that drug release undergoes three steps: dissolution of drug absorbed on the nanoparticle surface, drug diffusion outside the

particles with the degradation of nanoparticles and the erosion of polymer matrix (Soppimath, Aminabhavi et al. 2001, Kumari, Yadav et al. 2010). In this study, drug release profile exhibited the same procedures as shown in the literatures.

It is notable that PLGA with an increase in lactic acid content exhibited a reduction in drug release rate. This observation can be explained by the composition of PLGA polymer, the degradation rate increase with the reduction of lactic acid content (Mundargi, Babu et al. 2008). Therefore, a faster drug releasing rate can be obtained by PLGA 50:50, which was well agreed with the results of stability study in former section.

These results showed that PLGA nanoparticles exhibiting a slower drug release rate than pure drug are able to effectively increase the stability of drugs. Thus controlled drug release can be achieved by drug-nanoparticles system and PLGA polymer is a suitable carrier for the drug delivery.

# 6 Conclusions

In this study, various method modifications have been developed and assessed based on the general single emulsion evaporation method to prepare PLGA nanoparticles. It was found that longer homogenization time and lower temperature during the process of emulsion could effectively reduce the polydispersity of PLGA nanoparticles. The particle sizes of nanoparticles produced by modified method C could be successfully controlled between 548 to 591nm. Modified method D increased the stirring speed to 15000rpm, which demonstrated that a higher homogenization speed could effectively reduce the particle size. Meanwhile, according to the SEM image, nanoparticles prepared by single emulsion evaporation method showed spherical geometry with smooth surface.

In order to gain a smaller particle sizes, emulsion diffusion method was applied for PLGA nanoparticles preparation, which is a two stage method combined with emulsion and diffusion. Although smaller size of particles (314nm) was obtained when using the same stirring speed (15000rpm) as emulsion evaporation method, the surface morphology didn't show a desirable result which might relate to incompletely removal of the solvent.

The stability of PLGA nanoparticles was further investigated by assessing their thermal property over a certain period of time using DSC. The decrease of Tg confirmed the hydration and degradation of PLGA polymers and nanoparticles. Furthermore, the figure of SEM also has demonstrated that PLGA nanoparticles showed degradation and aggregation after two-week storage under 40℃ and 75% humidity. PLGA 75:25 with higher lactic acid content showed slower degradation rate compared to PLGA 50:50.

Ibuprofen-loaded PLGA nanoparticles have been successfully prepared by o/w single emulsion evaporation method. Nanoparticles ranging from 500nm to 700nm with relatively high drug loading content were generated by this method. The reduction of lactic acid content showed the increase of drug efficiency. DLS SEM and DSC were utilized for the characterization of Ibu-loaded nanoparticles.

According to the DSC result, a slight peak shift of Tg can be observed in the thermograms compared to non-loaded nanoparticles. It can be proved the existence of drug-polymer interaction between the ibuprofen and PLGA polymer. During the stability study, it is notable that IBU-loaded nanoparticles underwent a faster degradation rate than non-loaded ones. Thus drug would influence the stability of PLGA polymer due to the drug-polymer interaction which was well agreement with the results of DSC. Meanwhile, the diminishment of the drug peak in thermogram for IBU-loaded PLGA 75:25 demonstrated that drug was encapsulated in the PLGA matrix rather than absorbed on the surface.

In the drug releasing study, all the samples showed initial burst release in the first 4 hours. The slower release rate in the following hours was the sign of PLGA degradation. With a comparison to two samples, ibuprofen released in slower rate from PLGA 75:25 nanoparticles due to its low degradation rate. The results of drug releasing study showed that both PLGA nanoparticles underwent a slower release rate than pure drug which has potential application to the drug delivery system.

Further work would involve the modification of emulsion diffusion method to get better shapes of nanoparticles and more uniform sizes. Meanwhile, the measurement of molecular weight and the characterization under the various storage conditions are also essential for the stability study of PLGA nanoparticles. Moreover, the interaction between polymer and different drugs are required for the further study.

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# APPENDICES

## A.1 Reagents and chemicals

**PLGA 50:50** Mw 24000-38000, CAS 26780-50-7, Sigma Aldrich UK

**PLGA 75:25**, Mw 76000-115000, CAS 26780-50-7, Sigma Aldrich UK

**Polyvinyl alcohol** (PVA) 87-89% hydrolyzed, Mw 31,000-50,000, CAS 9002-89-5, Sigma Aldrich UK

**Dichloromethane** (DCM) Mw: 84.93, Fisher Scientific UK Ltd

**Dimethyl sulfoxide** (DMSO) anhydrous, ≥99.7%, CAS 76-68-5, Mw 78.13, Fisher Scientific UK Ltd

**Ethanol absolute** 99.9%, CAS 64-17-5, Mw 46.06, VWR Prolab

**Ibuprofen** ≥98%, Mw 206.28, CAS 15687-27-1, Sigma Aldrich UK

**Laboratory Reagent Grade Sodium Chloride** (NaCl), Fisher Scientific UK Ltd

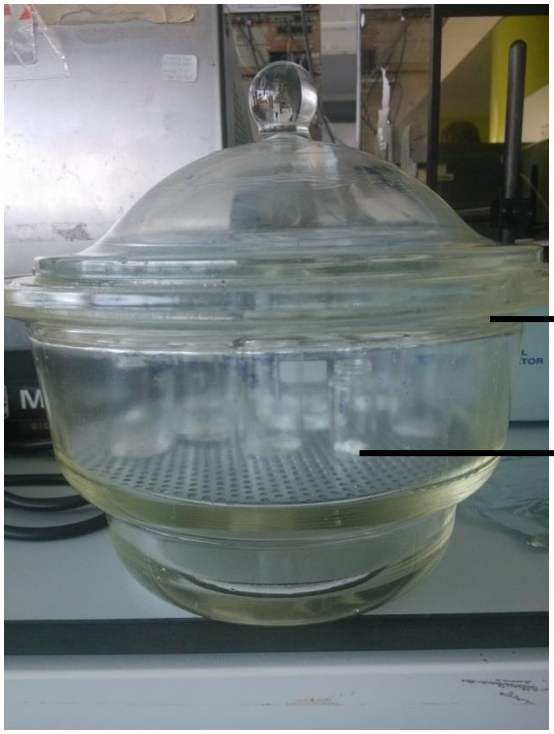
**Phosphate buffered saline tablet** (PH=7.4) (PBS), Sigma- Aldrich. MO, USA

**Tetrahydrofuran** (THF) 99.8+%, Fisher Scientific UK Ltd

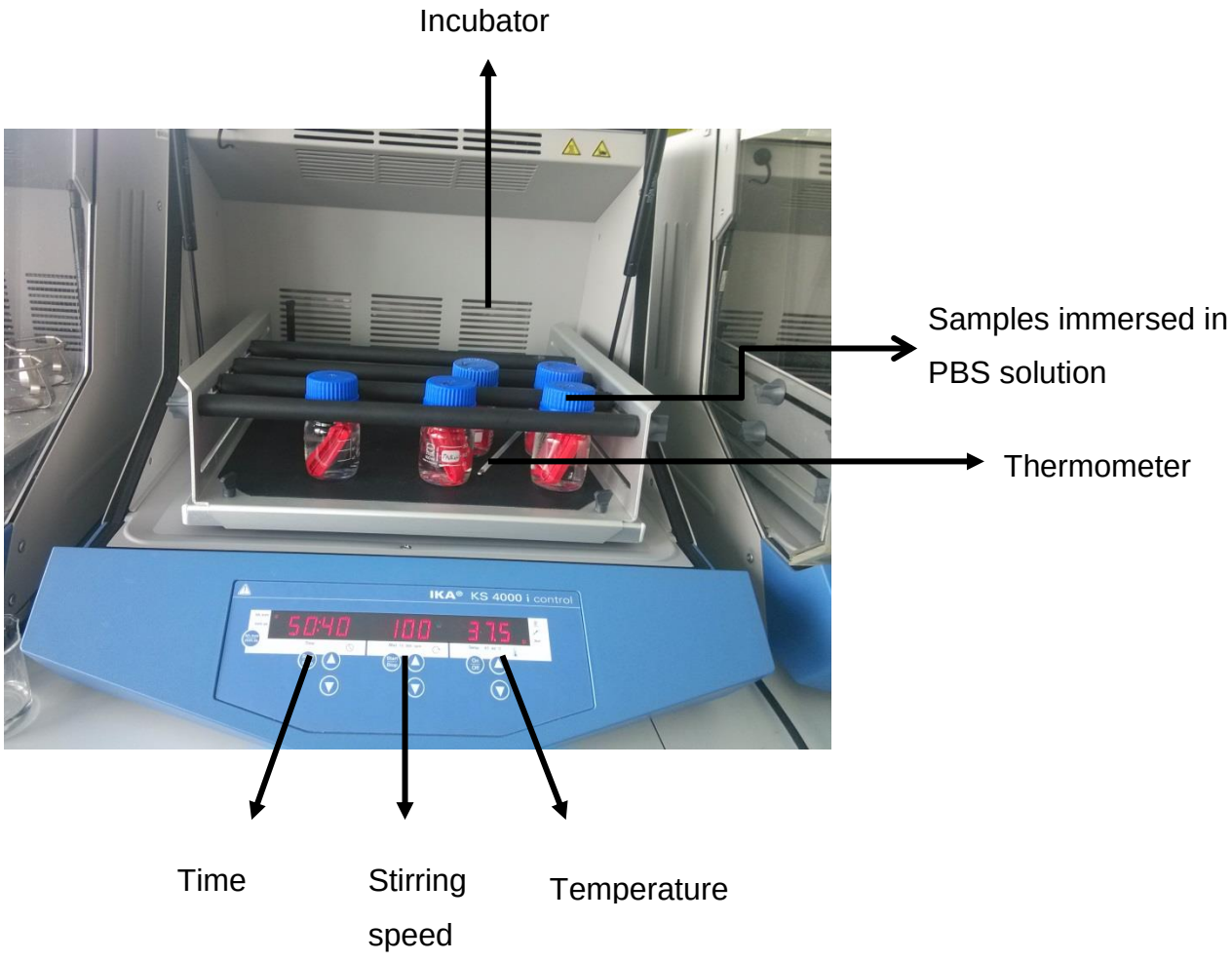
**Toluene** anhydrous, 99.8%, Mw 92.14, CAS 108-88-3, Sigma Aldrich UK

**Water**, deionized water

## A.2 Storage study



## A.3 Drug releasing study

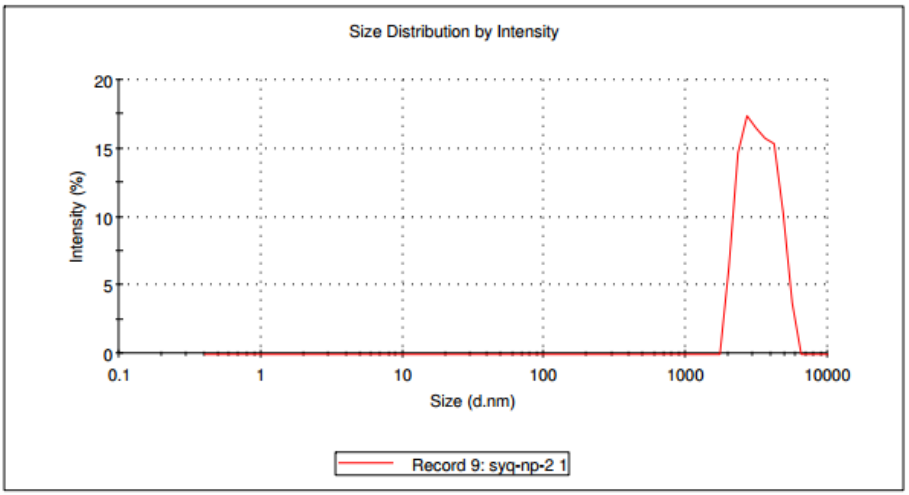


## A.4 Results

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 3651 | Peak 1: | 3340 | 100.0 | 935.4 |
| PdI: 0.246 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.940 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

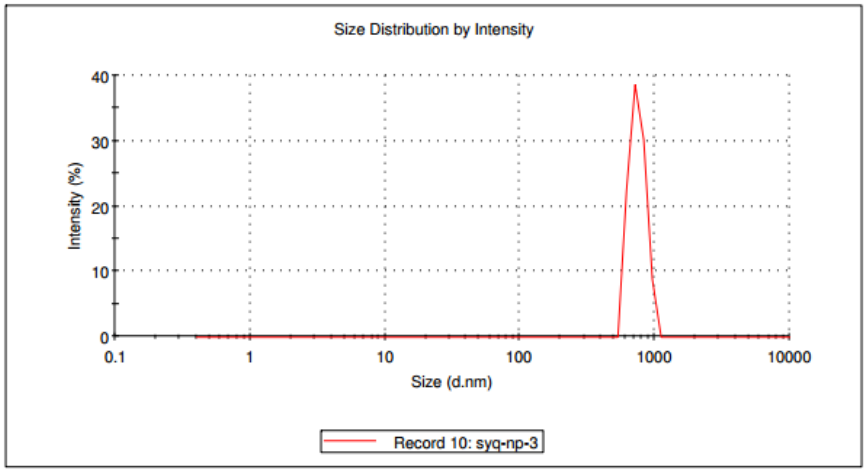



Size Distribution by Intensity for SYQ_50_2

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 1210 | Peak 1: | 746.1 | 100.0 | 100.2 |
| PdI: 0.584 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.913 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

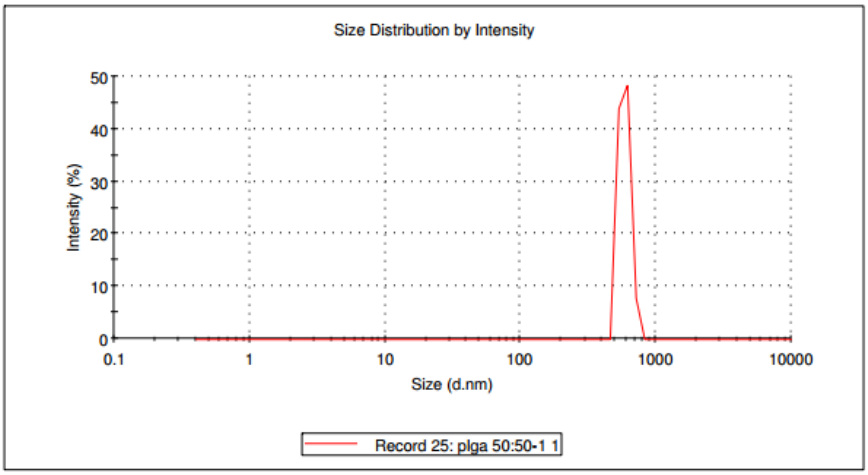



Size Distribution by Intensity for SYQ_50_3

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 1625 | Peak 1: | 585.9 | 100.0 | 54.57 |
| PdI: 0.696 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.918 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

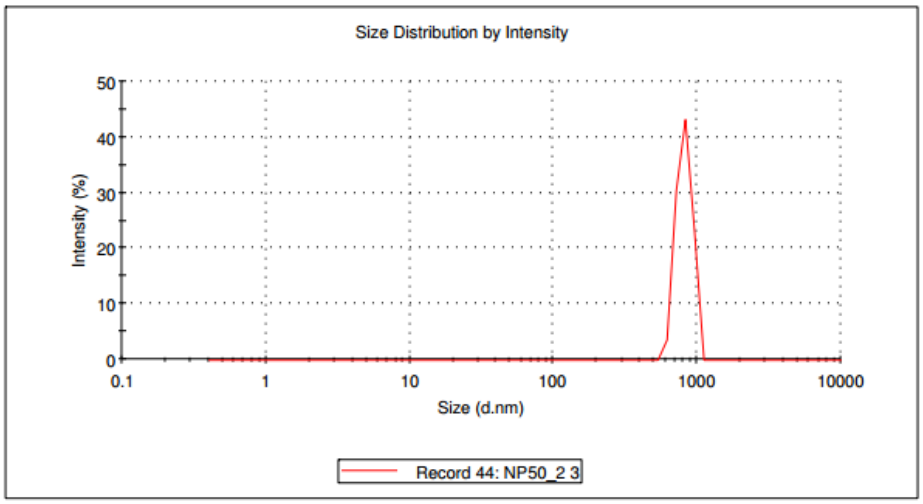



Size Distribution by Intensity for NP50-1

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 3071 | Peak 1: | 813.0 | 100.0 | 95.90 |
| PdI: 0.842 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.958 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

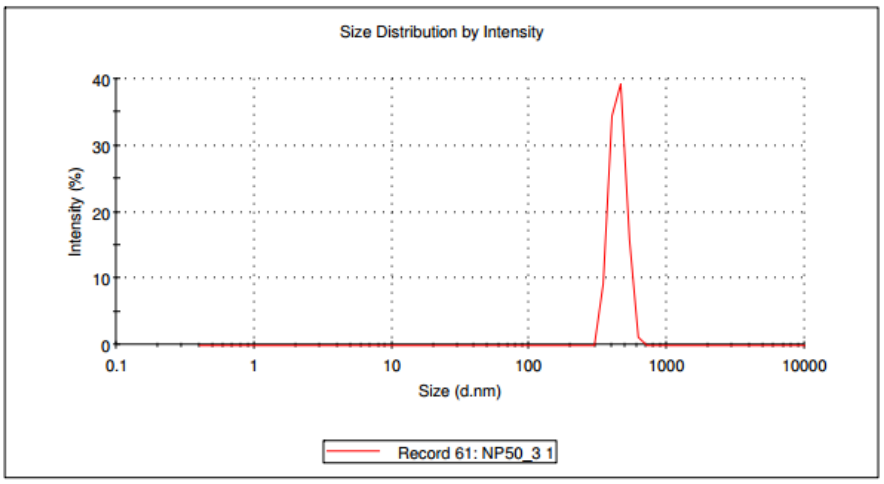



Size Distribution by Intensity for NP50-2

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 586.4 | Peak 1: | 439.8 | 100.0 | 58.25 |
| PdI: 0.533 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.960 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report




Size Distribution by Intensity for NP50-3

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 1817 | Peak 1: | 1872 | 100.0 | 335.7 |
| PdI: 0.180 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.933 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

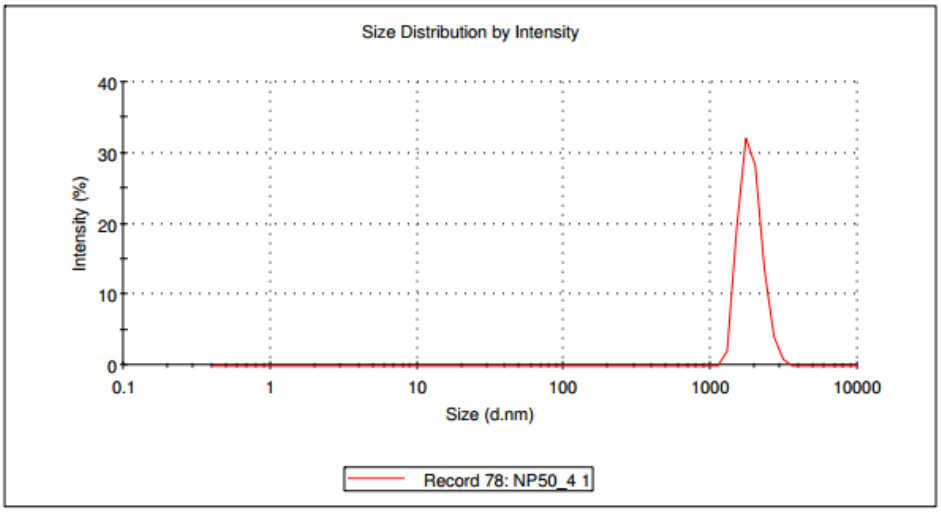
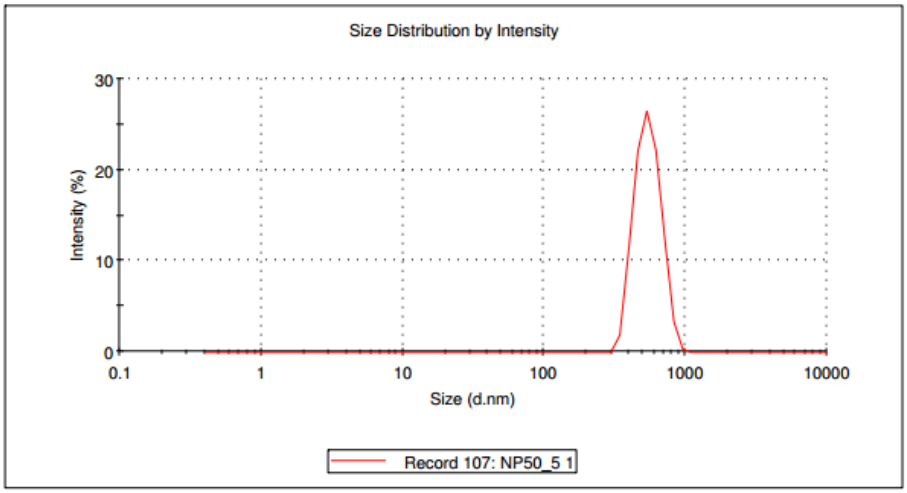



***Figure5.7*** Size Distribution by Intensity for NP50-4

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 530.3 | Peak 1: | 548.3 | 100.0 | 112.6 |
| PdI: 0.027 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.956 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Good

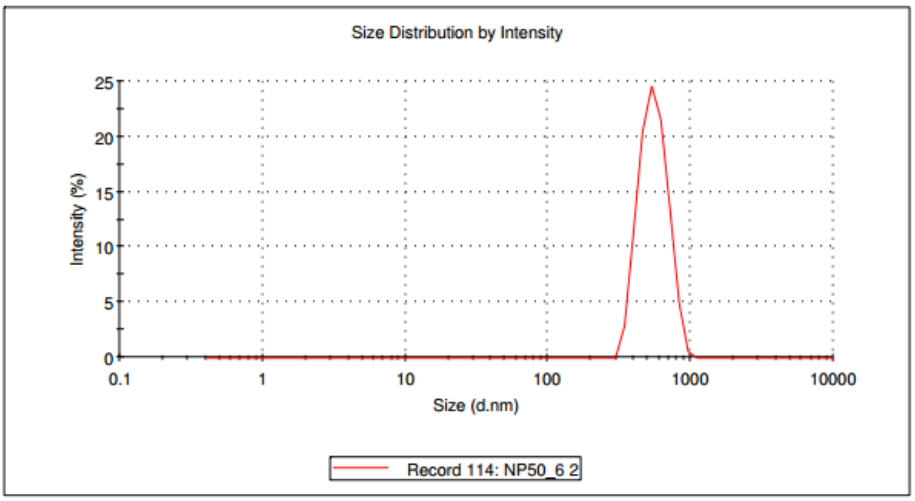



Size Distribution by Intensity for NP50-5

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 526.6 | Peak 1: | 555.1 | 100.0 | 122.3 |
| PdI: 0.057 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.949 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Good

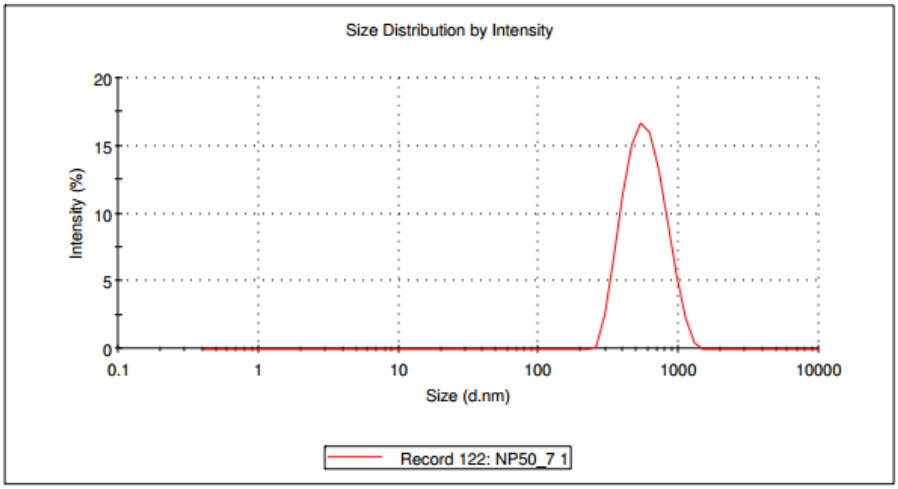



Size Distribution by Intensity for NP50-6

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 534.3 | Peak 1: | 591.2 | 100.0 | 193.5 |
| PdI: 0.099 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.942 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Good




Size Distribution by Intensity for NP50-7

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 1817 | Peak 1: | 630.3 | 100.0 | 65.80 |
| PdI: 0.568 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.919 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

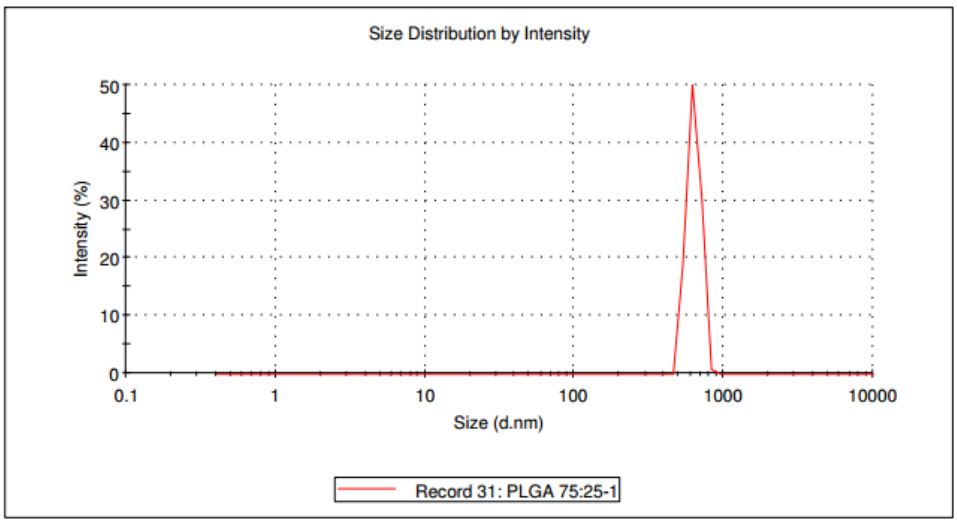
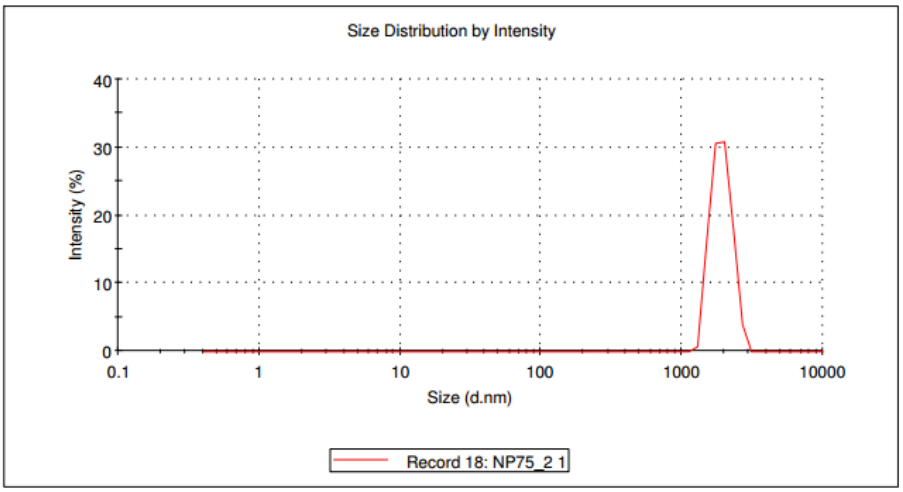



***Figure 5.11*** Size Distribution by Intensity for NP75-1

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 1916 | Peak 1: | 1903 | 100.0 | 310.0 |
| PdI: 0.199 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.943 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

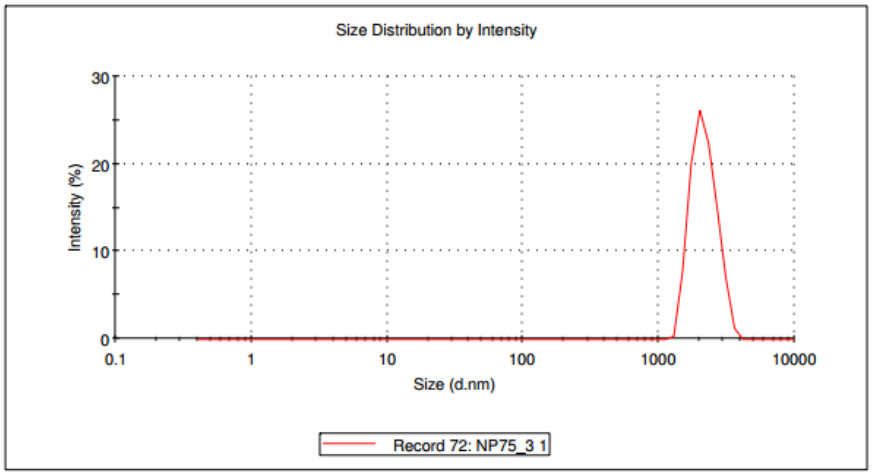



Size Distribution by Intensity for NP75-2

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 2485 | Peak 1: | 2164 | 100.0 | 464.1 |
| PdI: 0.258 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.926 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Refer to quality report

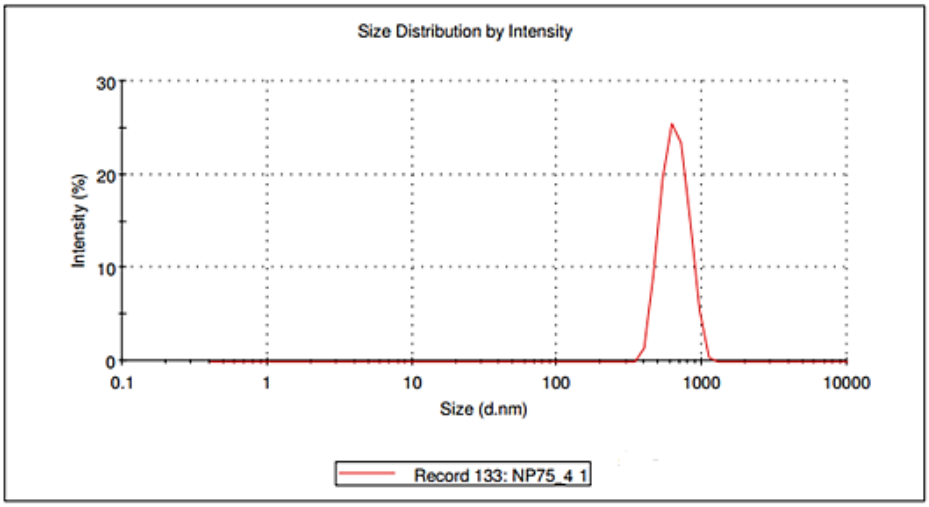



Size Distribution by Intensity for NP75-3

**Results**

| | | Diam. (nm) | % Intensity | Width (nm) |
|---|---|---|---|---|
| Z-Average (d.nm): 630.5 | Peak 1: | 656.1 | 100.0 | 137.6 |
| PdI: 0.042 | Peak 2: | 0.000 | 0.0 | 0.000 |
| Intercept: 0.951 | Peak 3: | 0.000 | 0.0 | 0.000 |

**Result quality :** Good




Size Distribution by Intensity for NP75-4