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Biodegradable Nano-Clusters as Drug Delivery Vehicles

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ABSTRACT

Goal of work (key words): The use of polymer nano particles (NPs) is of potential interest in the field of targeted drug delivery systems owing to multiple degrees of freedom like bio-degradability, hydrophobicity and the form (particles, capsules) in which they can be produced. The overall goal was to synthesize and characterize stable, monodisperse polymeric NPs loaded with drugs using flash nanoprecipitation in a Multi Inlet Vortex Mixer (MIVM) which could be used subsequently to build nano-clusters (NCs) comprising different drugs. The work further aimed at studying and optimizing drug encapsulation, loading and release kinetics from the synthesized NPs for different polymers.

Conclusions and recommendations (key words): The MIVM setup is found to produce stable polymeric NPs as small as 50nm and as large as 155nm depending on polymer concentration and nature of polymer. The results indicate that this setup is capable of producing drug(ibuprofen) loaded NPs with high drug loading efficiencies varying between 75% and 88% differing with polymers. This has been established to be both reproducible and valid for a wide range of polymers (PLA, PLGA, PCL functionalized with different groups and their blends). On the contrary, the release kinetics from almost all the different types of polymeric systems is slow; lasting over several days and moreover, it is not possible to release the entire loaded drug. It is claimed that either the chemical interaction of the polymers with ibuprofen or the location of the drug inside the polymeric NPs is the potential reason for extremely slow release kinetics. It is therefore suggested that further investigation is needed for the same system with another drug to confirm the observed behavior or even a completely different synthesis method for drug loaded polymeric NPs using ibuprofen to substantiate the observed results.

I declare that this is an independent work according to the exam regulations of the Norwegian University of Science and Technology

Date and signature: 28.06.2012 ..... Sulalit Banerjee

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Abstract

The use of polymer particles as tailored drug delivery systems is becoming more and more attractive. The reason for such growing interest is related to the many degrees of freedom which polymer materials offer (biodegradable vs non-degradable polymers, hydrophobic vs. hydrophillic) as well as the form in which they can be produced (particles, capsules). Even though this approach was successfully applied for producing particles containing single drug, the situation becomes much more complicated when several drugs should be delivered at the same time or when drug in combination with imaging agent would be of interest. In this case newly developed nano-clusters (NCs) can serve as a delivery device. In particular, such NCs are composed of many small nano-particles (NPs) loaded with different drugs, which can be prepared independently and combined thereafter. NCs can be efficiently produced through aggregation followed by breakup, or self-assembly of oppositely charged NPs, or aggregation driven by polyelectrolyte. To ensure biocompatibility of the produced NCs, primary particles need to be produced out of biodegradable polymer. In order to synthesize stable, multifunctional NCs loaded with several drugs; the constituent primary NPs have to be stable and show considerably fast release kinetics.

The master thesis has investigated primarily on the synthesis of different polymeric NPs viz PLA (synthesized in house as well as commercial grade), PLGA (commercial) and PCL(synthesized in house, having different functionalities –COOH, -PEG and their blends) employing flash nano-precipitation technique in a multi inlet vortex mixer (MIVM), previously optimized in the Morbidelli group at ETH. These NPs were characterized using DLS (Dynamic Light Scattering) and Zeta-Sizer to report the variation of the sizes and zeta potentials respectively of the NPs as a function of polymer molecular weight and initial concentration of polymer. The lowest possible sizes of the NPs were then selected for further studies as the overall motivation of the work is to synthesize NCs composed of primary particles and thereafter compare and contrast drug loading, encapsulation efficiencies and release kinetics of a model drug between the two. Ibuprofen (model drug) was loaded into the primary NPs using the MIVM setup, following which drug loading and encapsulation efficiencies were measured using High Performance Liquid Chromatography (HPLC). The release kinetics experiments were performed at 37°C and also studied at room temperature (25°C) and 45°C to evaluate the effect of temperature on release

mechanism. The drug-loaded NPs were separated from the free drug in solution at different times using centrifugal filtration. The amount of drug released over time was measured by analyzing these supernatants using HPLC.

The MIVM setup is found to produce stable polymeric NPs as small as 50nm and as large as 155nm depending on polymer concentration and nature of polymer. The results indicate that this setup is capable of producing drug loaded NPs with high drug loading efficiencies varying between 75% and 88% differing with polymers. This particular aspect has been established to be both reproducible and valid for a wide range of polymers through subsequent experiments. On the contrary, the release kinetics from almost all the different types of polymeric systems is slow; lasting over several days and moreover, it is not possible to release the entire loaded drug. It is claimed that either the chemical interaction of the polymers with ibuprofen or the location of the drug inside the polymeric NPs is the potential reason for extremely slow release kinetics. It is therefore suggested that further investigation is needed for the same system with another drug, having similar solubility parameters as ibuprofen to confirm the observed behaviour or even a completely different synthesis method for drug loaded polymeric NPs using ibuprofen to substantiate the observed results.

1 Introduction

Targeted drug delivery and controlled release processes have become important and popular in medical science because it becomes possible to transport hydrophobic low molecular weight drugs, to enhance the efficiency of drugs and cure diseases like cancer or tumor faster than before. Due to the large variety of surface functionalities that NPs can possess, they are widely being used in health care and biomedicine, as clinical imaging agents and pharmaceutical delivery carriers.

The design and development of bio-degradable carriers containing biologically active agents like drugs for therapeutic application require not only a fundamental understanding of the in vivo bio-degradation phenomena but also of cellular and tissue responses which determine the biocompatibility of the constructs [1]. The use of biodegradable polymers is very attractive because controlled drug release can not only be optimized by suitable degradation strategie but it also allows clearance of the polymeric material from the body, avoiding its accumulation and possible toxicity [2][3]. Polymers like poly (DL-lactic acid), poly-(L-lactic acid) and poly (lactide-co-glycolide) are just few examples that are widely used in drug delivery applications. In most cases, the drug carriers are synthesized in the form of micro-spheres. Macrophages are capable of phagocytosing very small particles (< 5 μm) while larger particle sizes (> 10 μm) induce the formation of foreign body giant cells [4]. Particles larger than 200 nm are sequestered by phagocytotic cells of the spleen, while particles smaller than 5.5 nm are rapidly removed through renal clearance [5]. Thus, ideally most drug delivery constructs need to be size-optimized between 10nm and 200nm to avoid both macrophages and foreign body giant cells.

Bio-degradation mainly occurs through a homogeneous hydrolytic chain cleavage mechanism as reported by Vert et al [6]. The rates of polymer degradation are the same for the bulk and surface of the drug carriers, however, it is affected by their size, the nature of the drug incorporated, the molecular weight and molecular weight distribution of the polymer [7]. On the other hand, bio-compatibility of the drug delivery systems is explained based on inflammation, wound healing and foreign body responses [1].

Colloidal drug delivery is advantageous in many ways as it can deliver poorly water-soluble drug molecules, settle stability issues typical of biological drug molecules, design

parenteral sustained release forms and provide functionalized soft particles that are very effective in drug targeting [8]. Further, these also act as continuous sources of drugs, therefore supplying the subject with a continuous dose as opposed to sudden variations in concentration as injection or pills do. It is a regular practice to incorporate multiple functionalities in NPs to achieve various goals. For example, core-shell NPs are widely used in the field of medical diagnostics and drug delivery as multimodal agents uniting various functionalities like contrasting, targeting, imaging into a single nano-structured system.

On the other hand, the use of NCs has found large attention in the production of sensors and microelectronics as well as in cellular imaging and therapy due to particles' segregation and enhancement of functionality, such as the enhanced absorbance used in biomedical imaging and therapy [9]. In principle, there exist two strategies for NC preparation, viz thermodynamically-driven self assembly and kinetically-driven self assembly of primary particles. Thermodynamic self assembly is achieved through specific interactions between NPs and templating agents like polymers and is also affected by parameters that affect self-assembly like temperature, pH and ionic strength [10]. Owing to the fact that large amounts of these agents are retained in the NCs, the metal loading is substantially reduced [11]. The size of the NCs is thus exclusively controlled by specific interactions due to surface/bulk properties until a minimum energy configuration is reached. On the other hand, kinetic self-assembly is obtained through the manipulation of Van der Waal's and electrostatic interactions, by varying solution's pH or ionic strength, and steric interactions between particles [11]. In this case, the aggregation process is stopped by the addition of a stabilizer. Since aggregation is intrinsically a function of the NP surface and nature, NC size is highly controlled knowing the aggregation kinetics [12]. However, the main drawback is uncontrolled growth that often yields irregularly shaped aggregates greater than several hundred nanometers in diameter owing to rather open structure [11].

The overall goal of this work was to synthesize and characterize stable, monodisperse polymeric NPs loaded with drugs using flash nanoprecipitation in a MIVM setup which could be used subsequently to build NCs comprising different drugs. The work aimed at

studying and optimizing drug encapsulation, loading and release kinetics from the synthesized NPs for different polymers.

The subsequent chapters describe the techniques used for synthesizing polymeric NPs, polymeric NPs loaded with model drug ibuprofen, characterizing these synthesized drug-free and drug loaded polymeric NPs, measuring drug loading and encapsulation efficiencies and release kinetics in the same chronological order. Furthermore, different modifications of the parent process are also discussed in the light of the observed effects and recommendations are drawn for future work to achieve the overall aim of the project.

2 Materials and Methods

2.1 Polymer synthesis and characterization

For the poly-(DL-lactic acid) synthesis (hereafter referred to as PLA), DL lactide was purchased from PURAC (The Netherlands), initiator DL-lactic acid from Fluka (purity ~90%) and catalyst 2-Ethylhexanoic acid tin (II) salt ($Sn(Oct)_2$,95% purity) from Sigma Aldrich. The solvents THF, toluene and dichloromethane, were purchased from Sigma Aldrich.

PLA samples were synthesized in bulk by ring opening polymerization (ROP) of DL lactide catalyzed by $SnOct_2$ and initiated by DL lactic acid. Since ROP of lactide is a semi-living process characterized by reversible catalyst activation, samples with different molecular weights are obtained by varying the catalyst to co-catalyst ratio [13]. Briefly, 25 g of lactide were melted at 130 °C in a closed 50 ml glass flask. The temperature was controlled by means of an external oil bath. Then, 0.07 g of catalyst and 0.034g of co-catalyst (initiator) were added and the reaction was carried out overnight. The polymer was finally purified by dissolution in 25ml of dichloromethane, followed by drop-wise addition of the same in excess of methanol resulting in precipitation of the polymer; in order to remove species with lower molecular weights such as residual monomer, impurities and reaction side products [14].

The molecular weight distributions were characterized by Size Exclusion Chromatography (SEC Agilent, 1100 series) equipped with two detectors; ultraviolet and differential refractive index. An oligopore column (Polymer Laboratories; length, 300 mm; diameter, 7.5 mm; measuring range, 0 - 4500 Da) was used. Chloroform was used as eluent at a flow rate of 1 mL/min and at temperature of 30°C. The column was previously calibrated in the group with Polystyrene standard. PLA was dissolved in chloroform to make a concentration of 1mg/ml. This sample was injected into the SEC setup for analysis of the MW. The molecular weights are reported in Section 3.1 and are relative to poly (styrene) standards.

Besides PLA, other polymers have been used for the purpose of comparing and contrasting the drug loading, drug encapsulation and drug release kinetics measurements. Poly(lactic-*co*-glycolic acid) (PLGA), Polycaprolactone (PCL) functionalized with COOH (PCL-COOH) and Poly(epsilon-caprolactone)-co-poly(ethylene glycol) (PCL-MPEG) and a blend of the two (PCL-COOH/MPEG) with a PEG ratio of 0.25 have been used in order to study the drug loading, encapsulation and release kinetics from individual polymeric NPs.

As mentioned earlier, PLA was synthesized in-house while another grade of PLA (referred hence forth as PLA_SA, Mw 18kDa-24kDa) was purchased from Aldrich. PLGA (referred hence forth as PLGA_SA, acid terminated, 65:35, Mw 24kDa-38kDa) was also purchased from Adrich. The PCL polymers were used from samples synthesized previously in the group.

2.2 Synthesis and Characterization of Polymer NPs

As highlighted before, bio-degradable nano-spheres forms the most common form used in bio-medical applications. To produce spherical polymer NPs, there exist two different methods, the Emulsification-Diffusion method and the Solvent-Displacement method.

The Emulsification-Diffusion process [15] involves the formation of oil in water (o/w) emulsion with a partially water-soluble solvent, which contains the biodegradable polymer, and with an aqueous phase containing a stabilizer. The subsequent addition of water to the system causes the diffusion of the solvent into the external phase carrying with it some polymer and thus forming a three-component phase in the immediate vicinity of the interface. As the solvent diffuses further into the water, the associated polymer is thrown out of solution, and is "stranded" in the water in the form of fine emulsion drops. Thus, this “diffusion-stranding” mechanism explains the formation of NPs owing to solvent diffusion thereby producing regions of local super-saturation from which NPs are formed due to phase transformation in these regions [16].

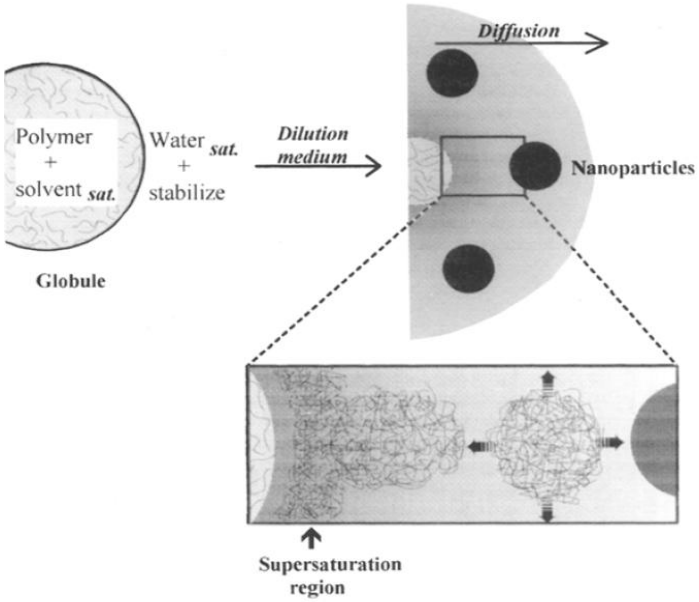

Figure 1 Schematic description of the formation mechanism of PLA NPs by the emulsification-diffusion method based on the diffusion stranding mechanism[16]

The solvent displacement method or nano-precipitation is in contrast a one step method and requires only two phases, a solvent and an anti-solvent phase. The solvent phase, usually organic includes the polymer, whereas the anti-solvent phase is usually water and if necessary a surfactant. The particle formation occurs due to interfacial turbulence or spontaneous agitation of the interface between two non-equilibrated liquid phases [17].However it is difficult to produce very small organic particles with direct nanoprecipitation.

To produce organic NPs of small size and narrow particle size distribution flash nano-precipitation (FNP) in a static mixer using a confined impinging jets (CIJ) mixer is a reported process [18]. The principle underlying the process is the rapid mixing of solvent and anti-solvent at a time-scale shorter than the formation time of the NP. Hence the mixing time τ_{mix} must be less than the induction polymer aggregation time τ_{agg} and the induction time for nucleation and growth τ_{ng}. Mixing in milliseconds ensures that the NP properties depend on the kinetics of polymer aggregation and organic solute nucleation and growth [9]. So in FNP, kinetics instead of thermodynamics, controls the preparation of NPs and leads to a narrow particle size

distribution and the possibility of high drug loading. Later on a new design namely the multi-inlet vortex mixer (MIVM) was presented which enables to control the solvent/anti-solvent input ratio. With the MIVM, NPs of 50-500 nm with narrow size distribution can be produced due to rapid micro-mixing and high super-saturation values [10].

Polymer solutions containing 0.1 to 0.75 % by weight of polymer dissolved in THF were prepared. To precipitate the NPs in the MIVM, the prepared organic solutions were injected at a flow rate of 10 ml/min. The polymer solution was filtered before entering the mixer by passing through a CHROMAFIL Xtra PTFE-45 µm filter to remove any undissolved impurities. The water flow rate was set to 100 ml/min. The whole set up is shown in Figure 2. The system was allowed to equilibrate for 30 seconds before withdrawing samples. In the initial phase of the research work, PLA was the polymer of utmost concern, however, the same process was employed for all the other polymers viz. PLA-HMW (MW 60kDa , synthesized in the group), PCL, PCL-COOH, PCL-MPEG, blends of PCL-COOH and PCL-MPEG, PLA_SA and PLGA_SA to synthesize respective polymeric NPs.

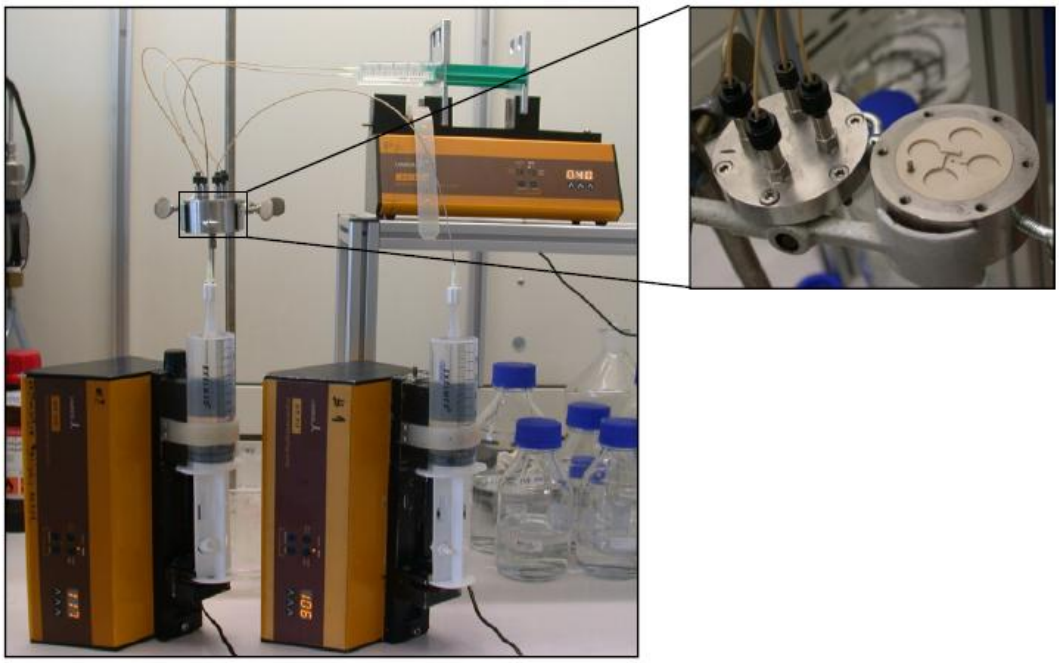

Figure 2 (a) Experimental set-up for flash nanoprecipitation (b) Mixing chamber of Multi-Inlet-Vortex-Mixer.

2.3 Synthesis of drug loaded polymer NPs: Drug Loading and Encapsulation Efficiencies

In order to study drug encapsulation efficiency and release kinetics from NPs, model drug - Ibuprofen was purchased from Sigma Aldrich. Ibuprofen is a non-steroid drug commonly used in the treatment of post-operative, epidural, arthritis, arthragra, dysmenorrhea and dental pain. It is an α-aryl propionic acid drug, shows poor water dissolution and tableting behaviour due to its hydrophobic substituted isobutyl benzene [19].

In order to maintain the composition of the drug (ibuprofen, in this case) in the water phase below the drug's solubility limit, calculated amounts of ibuprofen were added to the polymer solution in THF (Table 1). The MIVM setup described in Section 2.3.1 was used to synthesize PLA NPs loaded with ibuprofen, the only change made being the organic phase, which now contained both the polymer and the drug.

Sample name	Concn of PLA (wt %)	Concn. of Ibuprofen (wt of drug/wt. of polymer)
PLA0.1ibu1	0.10	0.010
PLA0.1ibu5	0.10	0.050
PLA0.1ibu25	0.10	0.250
PLA0.1ibu125	0.10	1.250
PLA0.75ibu1	0.75	0.001
PLA0.75ibu5	0.75	0.007
PLA0.75ibu25	0.75	0.030
PLA0.75ibu125	0.75	0.170

Table 1 Composition of Ibuprofen in drug loaded PLA NPs

These samples were prepared in order to study the drug loading efficiencies (LE) given by equation 2.1 and encapsulation efficiencies (EE) given by equation 2.2.

$$\mathbf{LE} = \frac{\mathbf{Mass\ of\ drug\ in\ NPs}}{\mathbf{Feed\ mass\ of\ drug}} \mathbf{X\ 100} \qquad (2.1)$$

$$EE = \frac{\text{Mass of drug in NPs}}{\text{Mass of polymer}} \text{ X } 100 \qquad (2.2)$$

In order to calculate LE and EE values, centrifugal filtration was employed after THF removal in a rotary evaporator and the supernatant was analyzed thereafter using HPLC. The technique is detailed in the following sub-section, it being the same used for measuring the drug release kinetics.

These drug loading and encapsulation results are presented in Section 3.3. However, it was observed that in cases of drug concentrations more than 0.05 g of drug per g of polymer, drug particles were formed (inferred from DLS results). Thus, the drug concentration was optimized at 0.05 g/g of polymer, as for lower concentrations, errors in weighing accurate drug amounts and detection (HPLC technique) on further dilution would have become problematic.

2.4 Drug Release Kinetics

The release experiments were carried out at room temperature using water as the release medium. The polymeric NPs loaded with drug were synthesized by the methods described above. The final solution was diluted ten times with water to provide a high enough concentration gradient for the drug release. From this stock solution, 500µl of solution was withdrawn at defined time intervals. The sample solution was filtered using Vivaspin centrifugal filters having a molecular weight cut off (MWCO) of 100K at 10,000rpm for 10 minutes. Thereafter, the supernatant was analyzed using HPLC. The HPLC used was an Agilent Technologies 1200 series. An Eclipse XDB-c18 column was used, with 5µm pore diameter and dimensions 4.6 x 150mm (diameter x length). The eluent used was a mixture of THF (90% THF and 10% distilled water by volume) and distilled water, both stabilized with phosphoric acid at pH 2.1. The flowrate used was 1ml/min. The eluent composition was changed in time: linearly from a 0vol% mixture of THF and water to 50vol% THF during the first 10 minutes, then maintained at the same condition for 5 minutes and then changed to 100vol% THF during the last 10 minutes.

Calibration curve was prepared for ibuprofen dissolved in THF. The calibration curve was used to calculate the concentration of the drug in the supernatant. The area under the curve at the drug elution time is a measure of the concentration of the drug in the solution, the calibration curve giving the constant showing relation between the moles of drug and the area. The results are enumerated subsequently in Sections 3.3 and 3.4.

3 Results and Discussion

This section follows the same chronology as used in the previous chapter to discuss the results obtained.

3.1 Characterization of PLA – Size Exclusion Chromatography (SEC)

Molecular weight (MW) of PLA was characterized using SEC (Agilent, 1100 series) equipped with two detectors, ultraviolet (UV) and differential refractive index (RI) as outlined in Section 2.1.

Figure 3 shows the calibration curve used to calculate the MW of synthesized PLA.

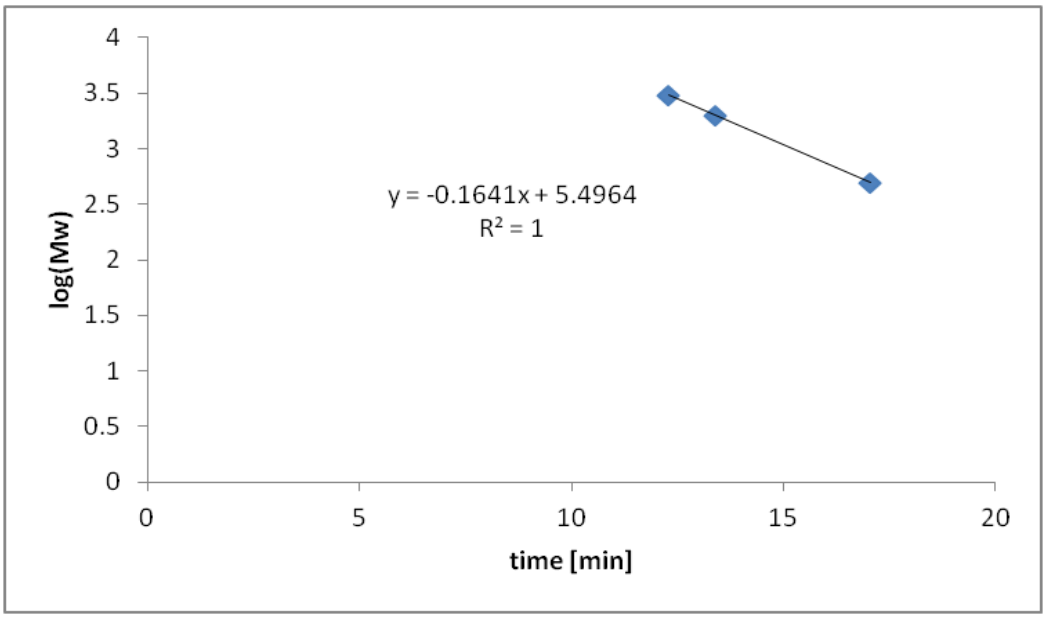

Figure 3 Calibration curve for determination of MW of polymers using SEC

The MW of the synthesized PLA was determined to be 2500 Da.

3.2 Size and Zeta Potential of synthesized NPs

Size measurements were done using a Malvern Instruments' Zeta-Sizer Nano device. The dispersant refractive index was specified as that of water at 25°C. In order to avoid the effect of THF in the latexes on the diffractive properties of the dispersant, each sample was diluted ten times with de-ionised water.

Figure 4 shows a plot of the sizes of the polymeric NPs as a function of polymer concentration synthesized by the MIVM setup. As can be inferred from the graph, with increase in concentration of the polymer weight fraction in THF, there is an increase of the sizes of the synthesized NPs. It is also interesting to note that with increase in the molecular weight of the polymer (PLA, PLA_SA and PLA HMW), for a particular polymer concentration, the size increases. This can be explained by the fact that for higher molecular weight, it is difficult to pack the longer chains closely enough, resulting in increase of size.

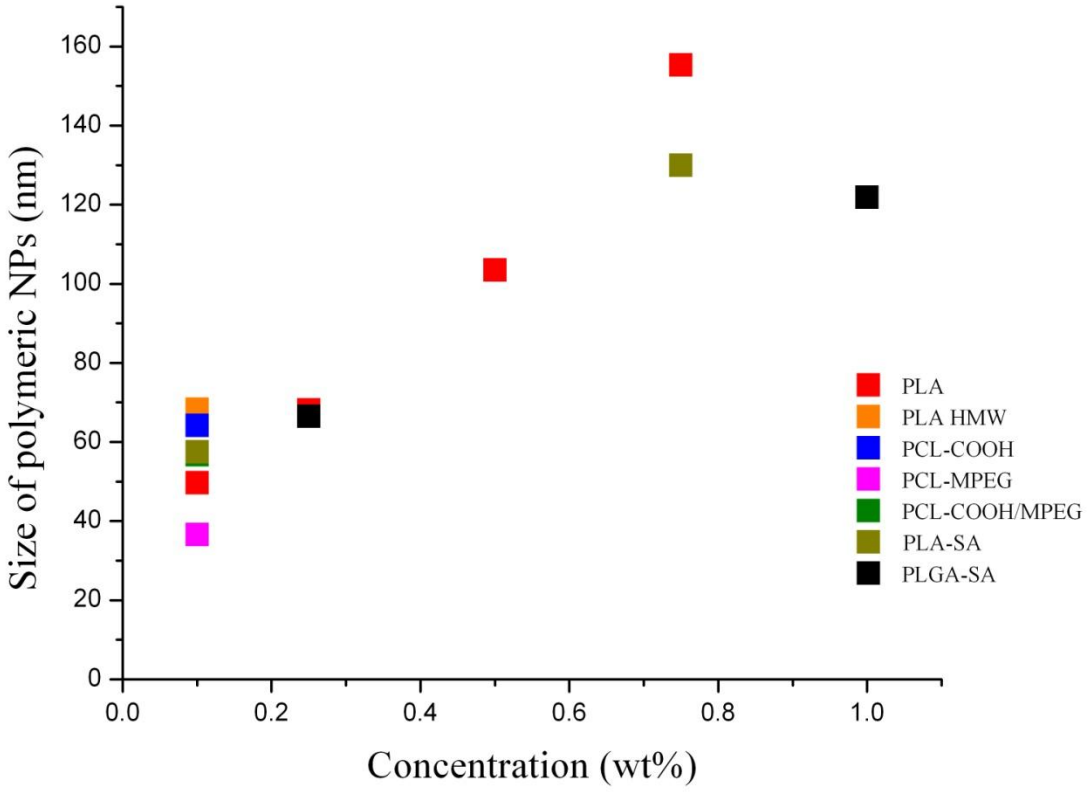

Figure 4 Plot of DLS Size (nm) of polymeric NPs vs concentration

Although the initial aim of the study revolved around studying PLA as the model polymer, different polymers were sequentially investigated to study the effect on the release kinetics from primary particles. However, the guiding principle was to

synthesize NPs as small as possible and therefore the lowest concentration was only chosen for studies of the other polymers.

Figure 5 illustrates the linear variation of the sizes of PLA NPs and concentration. As the underlying motive of the study was to synthesize NCs from primary NPs, the extreme points viz. PLA0.1 and PLA0.75 were selected for further studies.

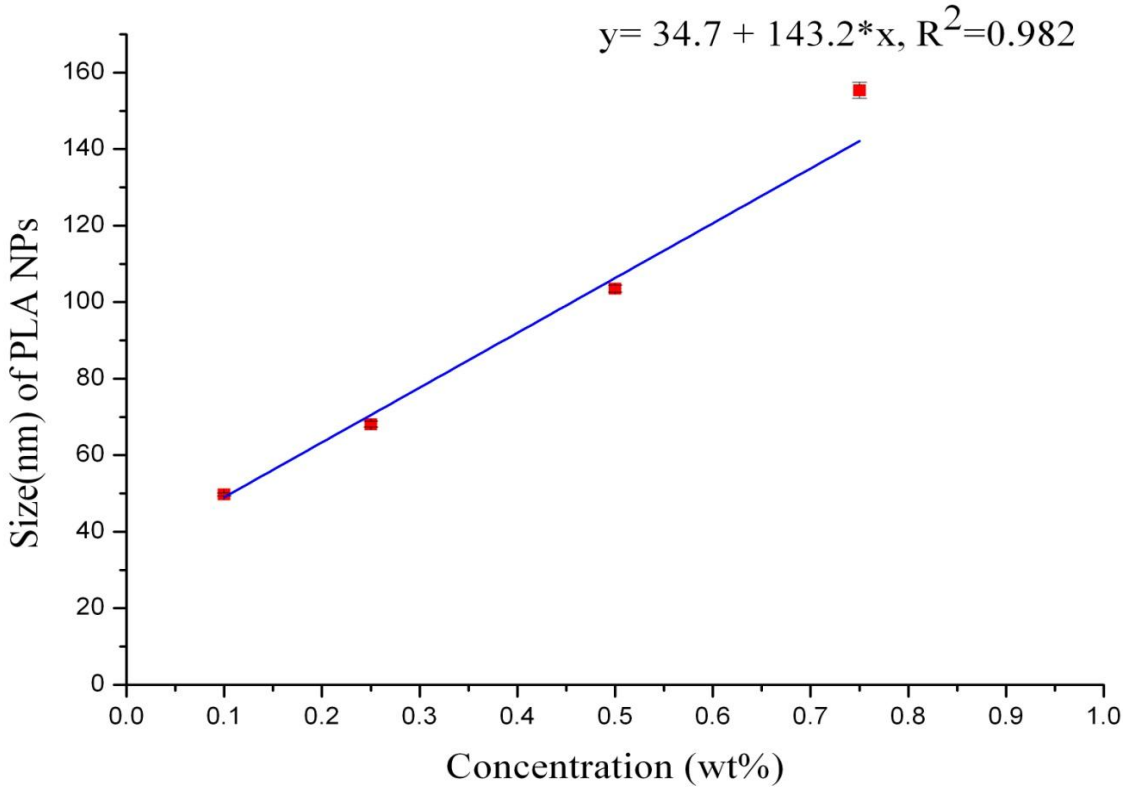

Figure 5 Plot of Size (nm) of PLA NPs vs concentration

The same instrument was also used for the measurement of the zeta potential and Smoluchowski model was used to process the data.

Figure 6 shows the variation of zeta potential of various polymeric NPs with concentration. As can be inferred from the results, for a particular polymer, increase in concentration results in higher absolute zeta potential. For PLA, the negative zeta potential is due to the presence of carboxylic chain end groups, which during polymer precipitation redistribute on the NP surfaces. At lower concentrations, there are smaller number of carboxylic groups which can re-distribute, leading to lower zeta

potentials and lower colloidal stability. This effect increases with increase in concentration.

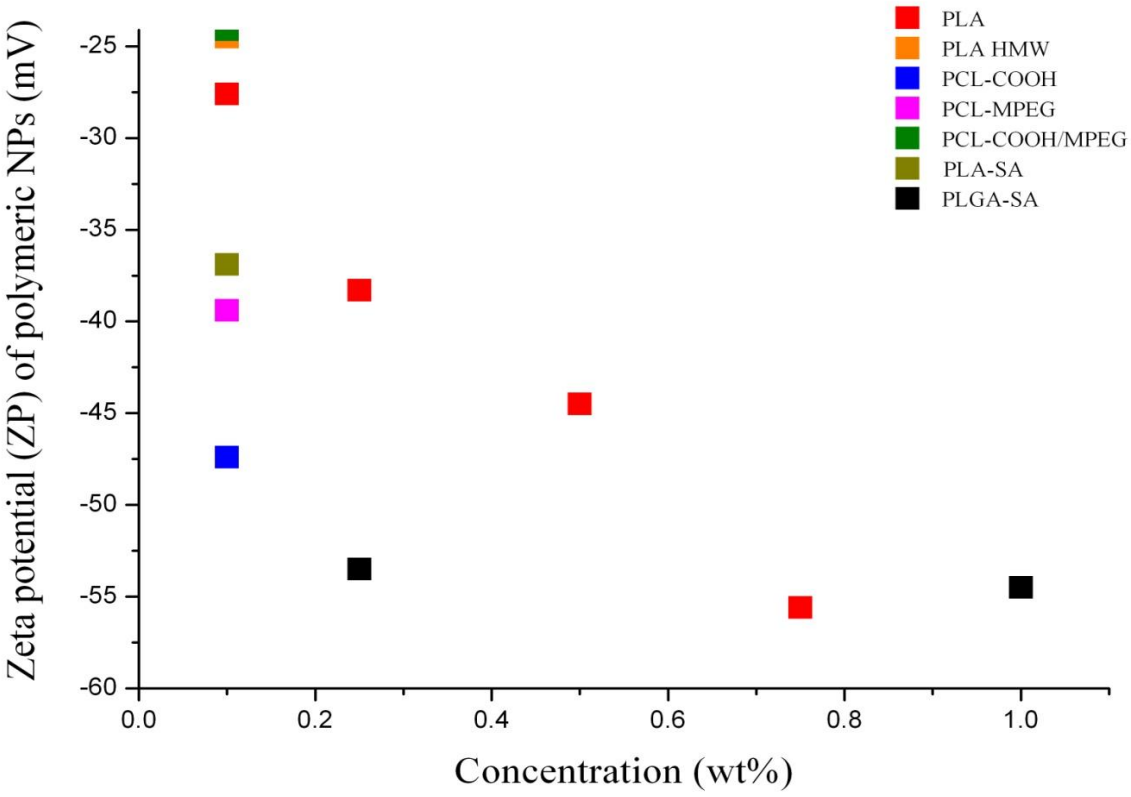

Figure 6 Plot of Zeta potential (mV) of Polymeric NPs vs concentration

It is also worth mentioning that for a particular polymer concentration, the absolute values of the zeta potentials of PCL-COOH, PCL-MPEG and the blend PCL-COOH/MPEG decrease owing to the insertion of uncharged PEG into the polymer backbone. The absolute zeta potential of PLGA is higher than for PLA NPs at the same concentrations. Thus, PLGA NPs have higher colloidal stability than PLA NPs prepared from polymer solutions at same concentrations.

Figure 7 illustrates the linear variation of the zeta potential of PLA NPs and concentration. As the underlying motive of the study was to synthesize NCs from primary NPs, the extreme points viz. PLA0.1 and PLA0.75 were selected for further studies.

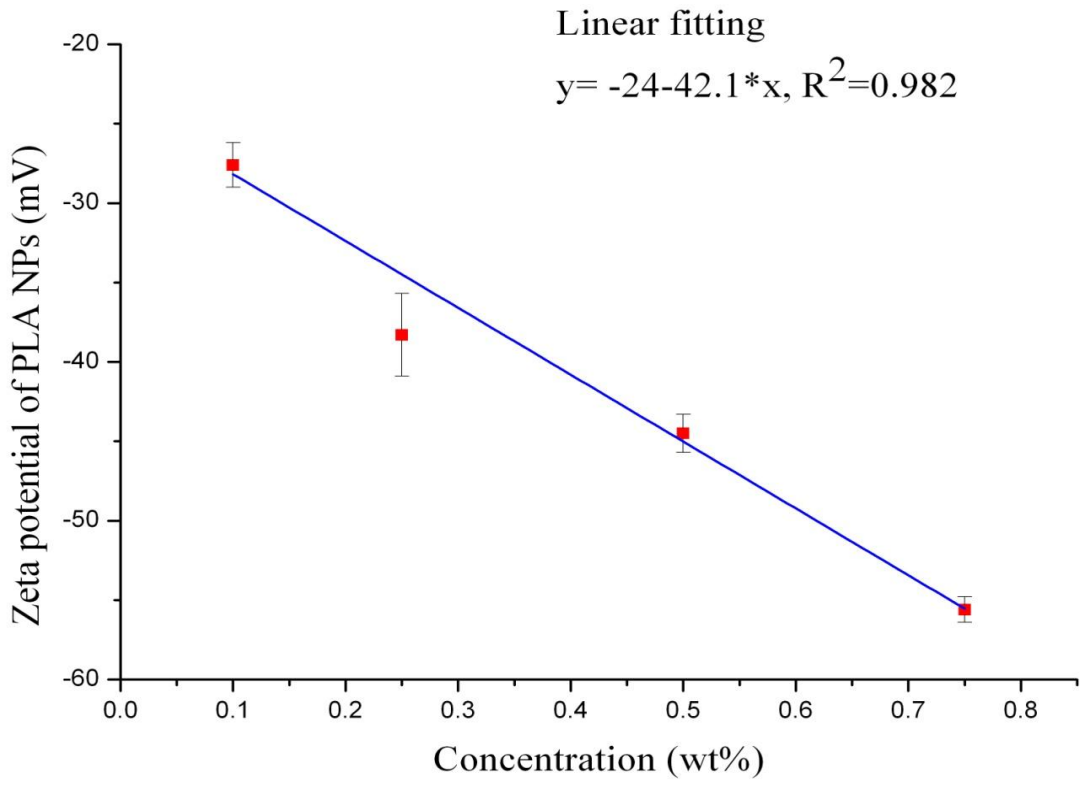

Figure 7 Plot of Zeta potential (mV) of PLA NPs vs concentration

Table 2 illustrates the measured sizes of the synthesized primary PLA NPs before and after solvent (THF) removal.

Concentration of PLA (wt. %)	Average Diameter (nm)	Average Diameter(nm) after THF removal
0.10	49.7 ± 0.4	44.9± 0.1
0.75	155.3 ± 2.1	145.9 ± 2.0

Table 2 Comparison of PLA NP sizes before and after removal of THF

The subsequent decrease in the sizes of the NPs after THF removal could be accounted to the mechanism of THF removal. It is believed from previous experiments in the same line that the NP formation in the MIVM cannot be explained through the classical nucleation theory. Instead, after mixing, the organic phase is dispersed in the form of droplets into the non-solvent and precipitation occurs when

the solubility limit of polymer into the organic phase is reached. An indirect support to the droplet mechanism has also been reported previously in Fabio's PhD thesis.

3.3 Drug loading and Encapsulation efficiencies

As elaborated previously, the HPLC column was calibrated using different weights of ibuprofen dissolved in THF. Figure 8 shows the calibration curve for the same. The drug was eluted around 5 minute while the THF was eluted around 7.5 minute.

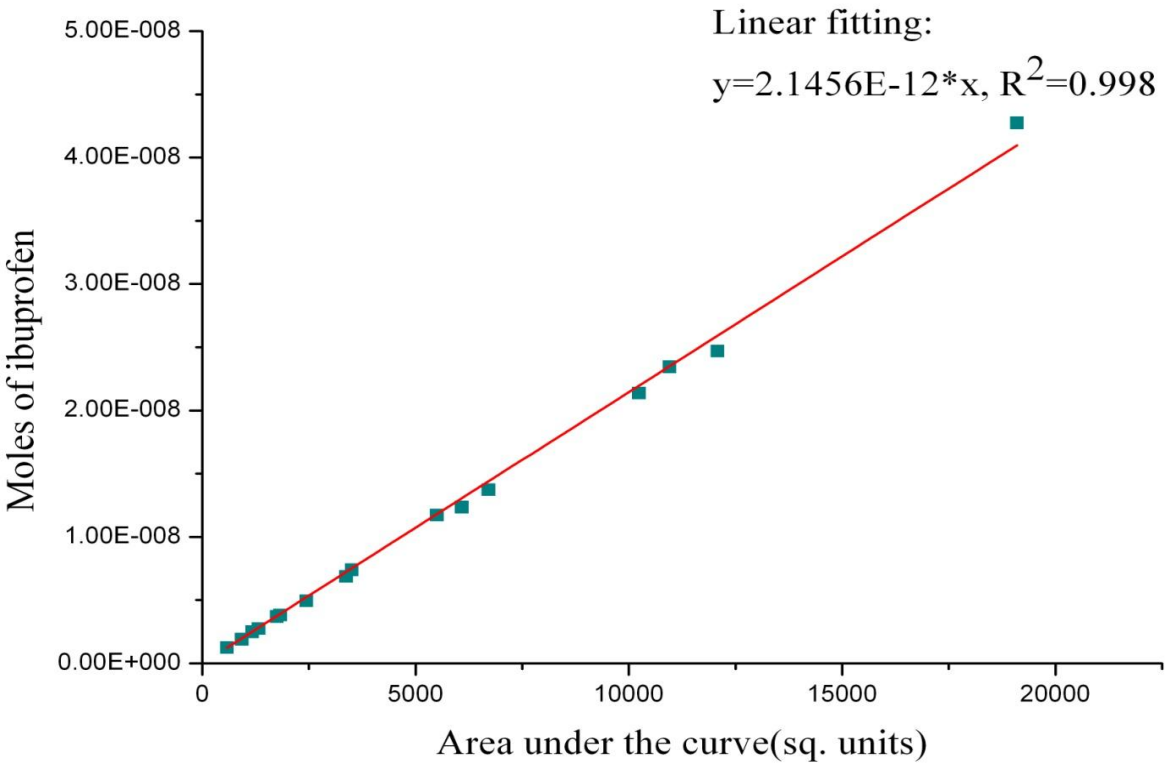

Figure 8 HPLC Calibration curve for ibuprofen

As an initial check to the fact that the ibuprofen used for encapsulation was loaded into the NPs, preliminary experiments were performed as outlined below. After the formation of the drug loaded NPs using the MIVM, both the solvents viz water and THF were removed using a rotary evaporator and the residue was re-dissolved in THF (volume of THF being used to mimic the initial concentration of the PLA and ibuprofen dissolved in THF before the MIVM process). Figure 9 shows a comparison of the chromatograms obtained for this sample (in blue) and the one for the initial

sample before entering the MIVM setup (in red). The green curve is the chromatogram for pure THF. As can be observed, the coincidence of the chromatograms closes the mass balance and is suggestive of the fact that there is essentially no drug loss in the preparation technique.

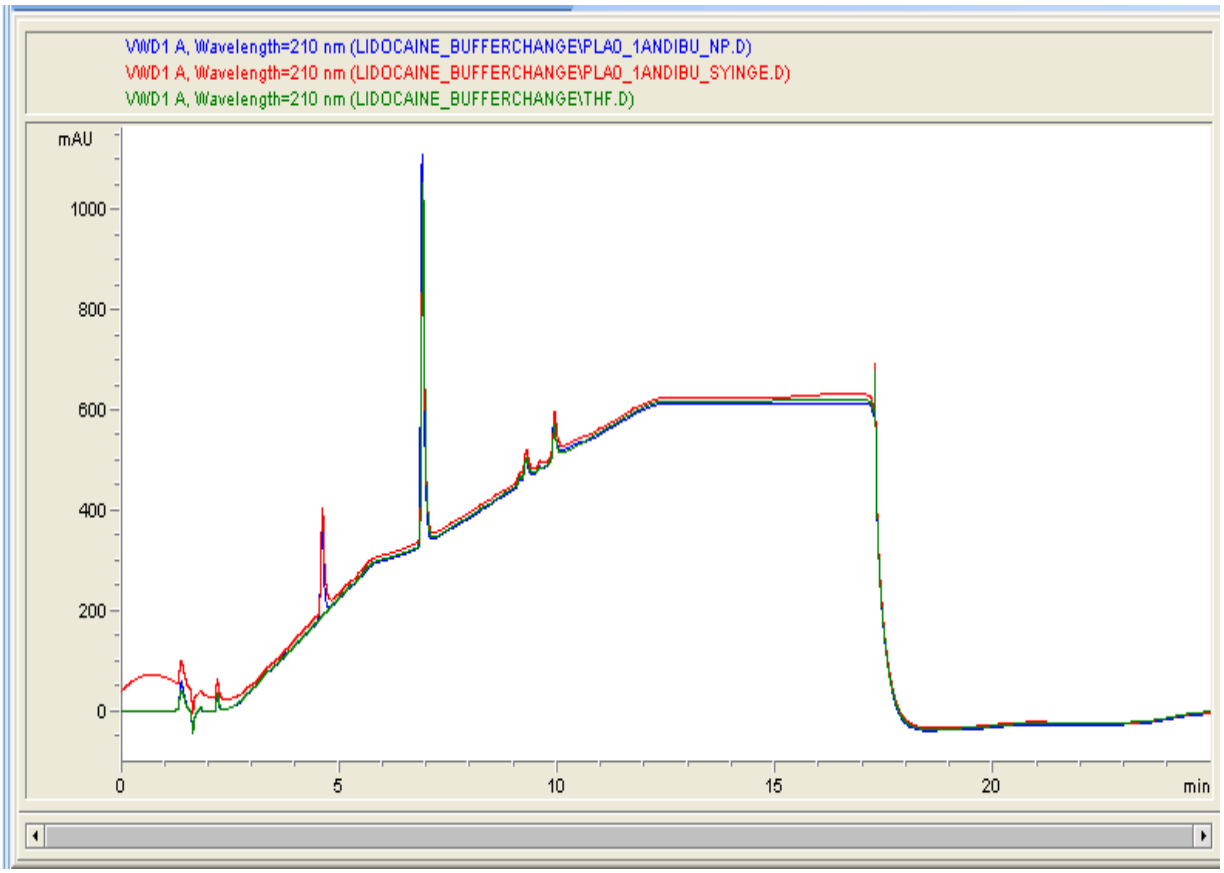

Figure 9 Comparison of chromatograms

Table 3 shows the LE and EE values of ibuprofen loaded PLA NPs synthesized from two different PLA concentrations in THF viz 0.1 and 0.75 wt%. As reflected in green, the optimum initial drug amount was selected to be 5mg per 50mg of the polymer solution. For initial drug concentrations above this, free drug particles were formed in the mixer, confirmed by bi-modal distribution in DLS studies of the samples. It is also reflected in EE values greater than 100% for 0.1PLA, indicating that there is not enough polymer particles to entrap the drugs.

Sample name	Drug loading efficiency (%) LE	Encapsulation efficiency (%) EE
PLA0.1ibu1	82.47	1.65
PLA0.1ibu5	**87.90**	**8.79**
PLA0.1ibu25	94.33	47.17
PLA0.1ibu125	98.78	246.95
PLA0.75ibu1	88.41	0.24
PLA0.75ibu5	**89.37**	**1.19**
PLA0.75ibu25	95.96	6.40
PLA0.75ibu125	98.52	32.84

Table 3 Drug loading efficiencies (LE %) and Encapsulation efficiencies (EE %) of PLA NPs loaded with different drug amounts

Another interesting observation is the similar LE values for both the samples, indicating that drug loading is independent of initial concentration of the polymer but rather dependent on the nature of the drug and the drug loaded NP preparation method employed. This will be further discussed while comparing LE and EE values for different polymeric NPs.

Figure 10 shows the LE values for different polymeric NPs, the initial concentration of the polymer in each case being 0.1wt% except for PLGA for which it is 0.25wt% (as stable NPs could not be obtained below this concentration) and the measurement temperature being 25°C. It is evident from the graph that high values of drug loading are obtained irrespective of the nature of the polymer ranging between 75% and 88%.

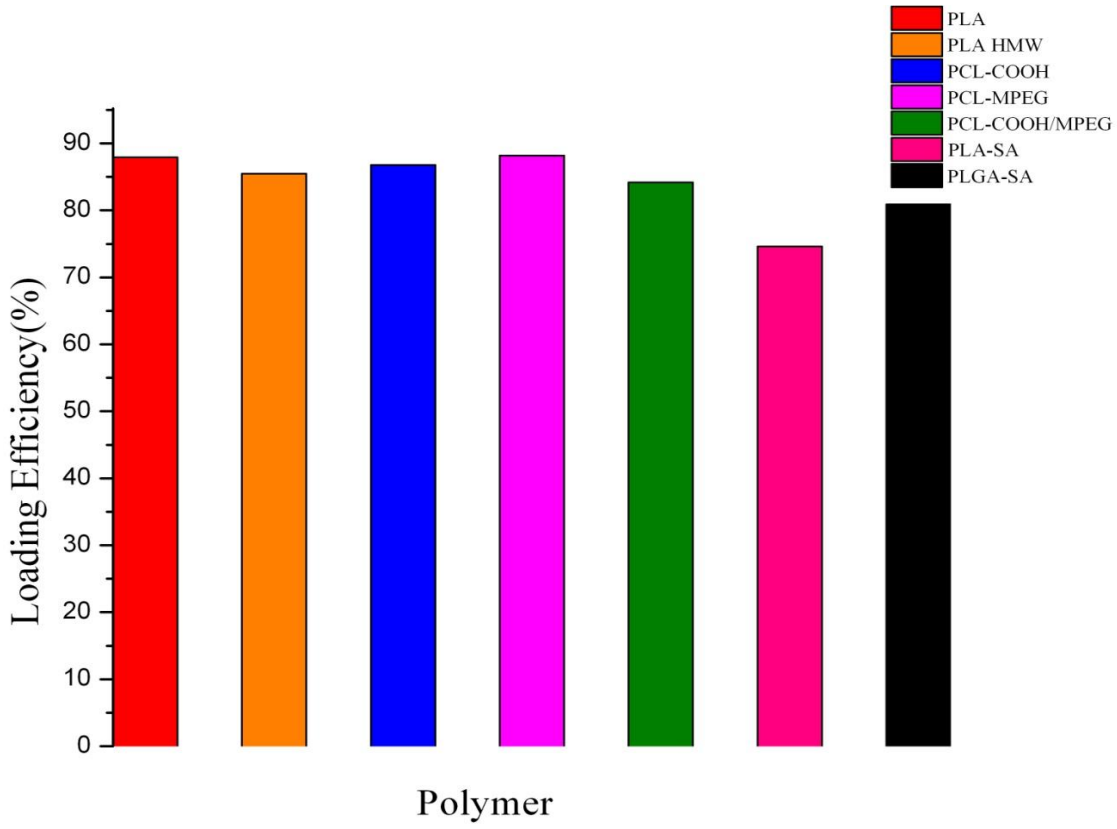

Figure 10 Comparison of drug loading efficiencies (%) of polymeric NPs at 25°C

Figure 11 shows the EE values for different polymeric NPs, the initial concentration of the polymer in each case being 0.1wt% except for PLGA for which it is 0.25wt% and the measurement temperature being 25°C. As reported previously, the higher drug loading trend is also followed in case of encapsulation efficiencies except for PLGA. This can be explained by the fact that initial weight of PLGA in solution is higher than for PLA and PCL polymers and therefore lower drug encapsulation per weight of polymer.

The values when compared to those reported in literature [19-21] are comparatively higher for both LE and EE. This is a remarkable advantage of the MIVM setup for synthesizing NPs loaded with drugs.

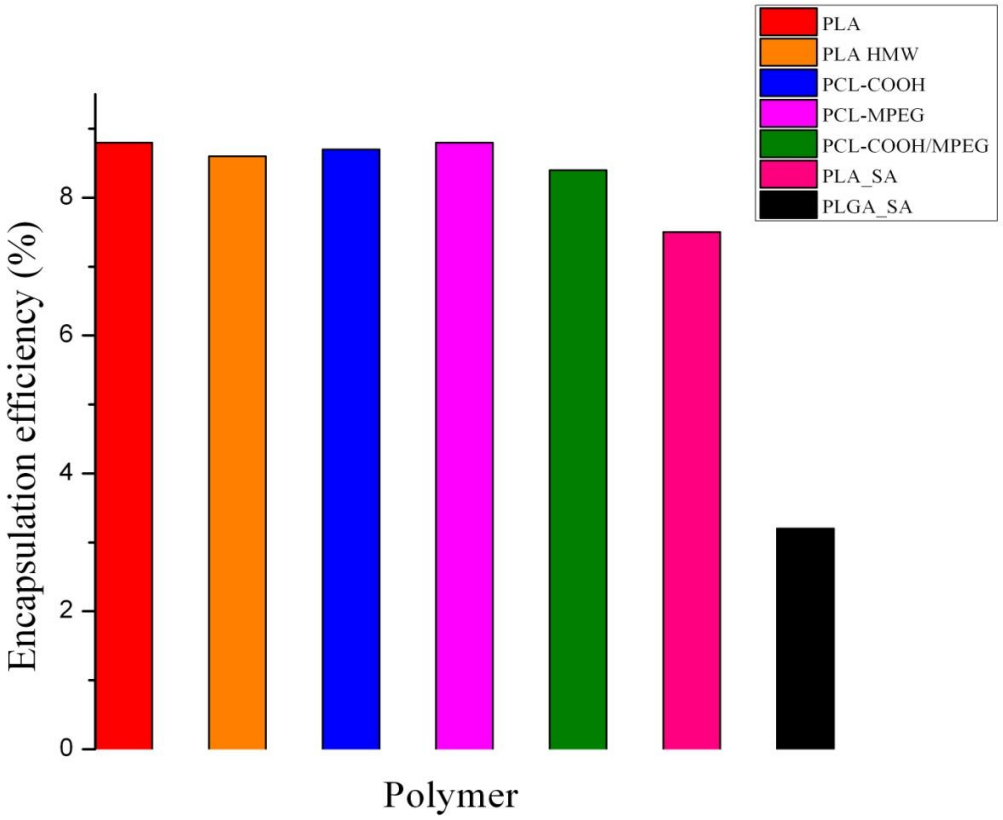

Figure 11 Comparison of encapsulation efficiencies (%) of polymeric NPs at 25°C

In order to re-check the calculated EE values and the veracity of the use of the right MWCO filters, two different sets of experiments were performed.

In the first case, ultracentrifugation was applied for the 0.1PLA NPs loaded with ibuprofen sample to precipitate the NPs loaded with ibuprofen from the supernatant. Ultracentrifugation was carried out using 12ml of the stock solution and at two different centrifugation velocities, viz 15,000 rpm for 1 hour and 41,000 rpm for half an hour. In both cases, the supernatant was analyzed using the HPLC technique outlined in Section 2.4. The EE values for both the cases were obtained to be 93%, slightly higher than the ones obtained under similar conditions while using centrifugal filters. However, this difference is within experimental errors and is a proof of the fact that the technique employed (centrifugal filtration) in separating the NP-loaded with ibuprofen from the solution containing free/released ibuprofen is well justified.

In the second case, a lower MWCO centrifugal filter viz 30k was used instead of the standard 100k filter with all other experimental conditions being kept unchanged. The EE values were obtained to be the same, thereby confirming the judicious selection of the MWCO of the centrifugal filters.

Thus, it is concluded that the centrifugal filtration method employing 100K MWCO used for separating the ibuprofen loaded NPs from the free drug in solution is well substantiated by the control experiments discussed previously. This technique of sampling has been rarely used in drug release kinetics measurement in contrast to the dialysis setup as reported more often in the literature [22-23]. It is however emphasized that this centrifugal separation technique is simpler and equally dependable.

3.4 In vitro release measurements

This section illustrates the release kinetics measurements from various polymeric NPs.

Figure 12 shows the ibuprofen release profiles for different PLA NPs and also the effect of temperature on release of ibuprofen from PLA. As can be observed, the release kinetics from all the different PLA samples were extremely slow and it was not possible to release the entire entrapped drug. Further, a steady state value is reached for each sample which is also observable for higher temperatures. It is observed that ibuprofen release from higher molecular weight PLA (PLA HMW) is even slower than from lower molecular weight PLA. This effect is also observed for PLA_SA and PLA at 37°C. However, this effect could also be attributed to the fact that while PLA_SA is a commercial sample, PLA was synthesized in house and differences in chemical composition of the polymers cannot be completely neglected.

The effect of temperature on the release kinetics is self-explanatory. With an increase in the temperature, the drug diffusion coefficient (D) increases, leading to faster release. An estimate of the D using equation 3.1, where t represents the characteristic time scale and R represents the radius of the NPs, is found to be 1E-21, almost 7

orders of magnitude lower than reported in literature for ibuprofen release from micro-spheres[19].

$$D \sim \frac{R^2}{t} \tag{3.1}$$

The glass transition temperature (T_g) of PLA varies from 52-55°C [24] depending upon molecular weight, synthesis method and other factors. So, the use of temperatures below the T_g ensures the crystallinity of the PLA. However, it is not just to conclude on the crystallinity of the polymer without conducting further microscopy studies. Moreover, it is impractical to design drug delivery vectors at temperatures above normal body temperature of 37°C as they cannot be rendered for real life applications. Thus, investigation into the temperature effect was carried out only to show that indeed ibuprofen is being released, but at extremely slow rates.

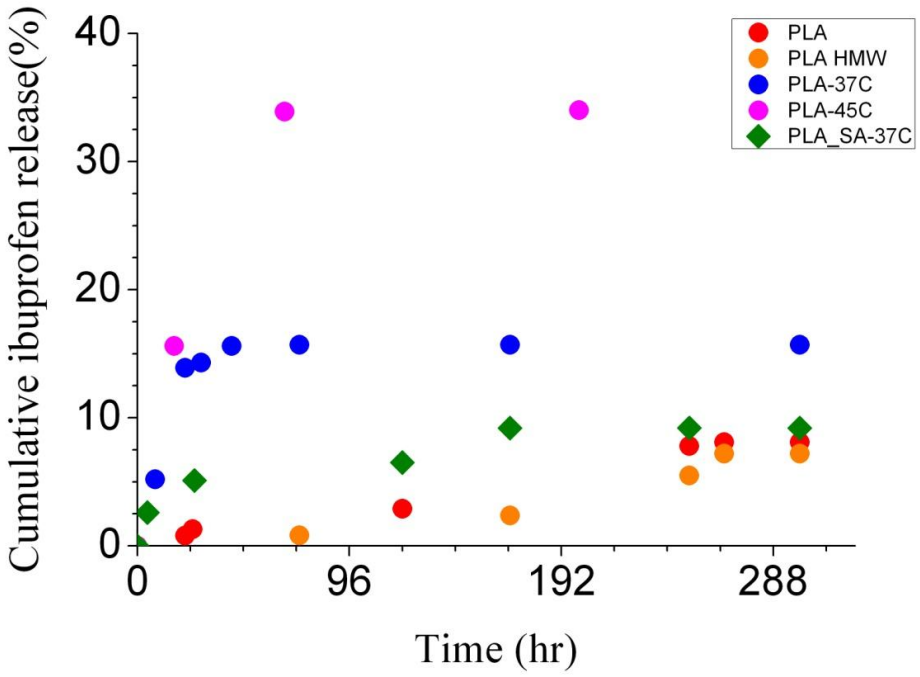

Figure 12 Comparison of Ibuprofen release kinetics from various PLA NPs and effect of temperature on release kinetics.

Figure 13 presents the release kinetics measurements of ibuprofen from PCL-COOH NPs at different temperatures [Figure 13 (a)] and from differently functionalized PCL NPs at room temperature[Figure 13 (b)]. The temperature effect in the case of PCL-COOH NPs is similar to the one observed for PLA. However, the steady state values

of cumulative percentage drug released obtained for PCL-COOH are much lower than that for PLA. Unlike PLA, PCL has a lower T_g (~-70°C) [25] and thus the release is from amorphous polymeric NPs.

On the other hand, changing the functionalities from negatively charged COOH group to neutral PEG or even using a blend of the two does not modify the release kinetics profile of ibuprofen.

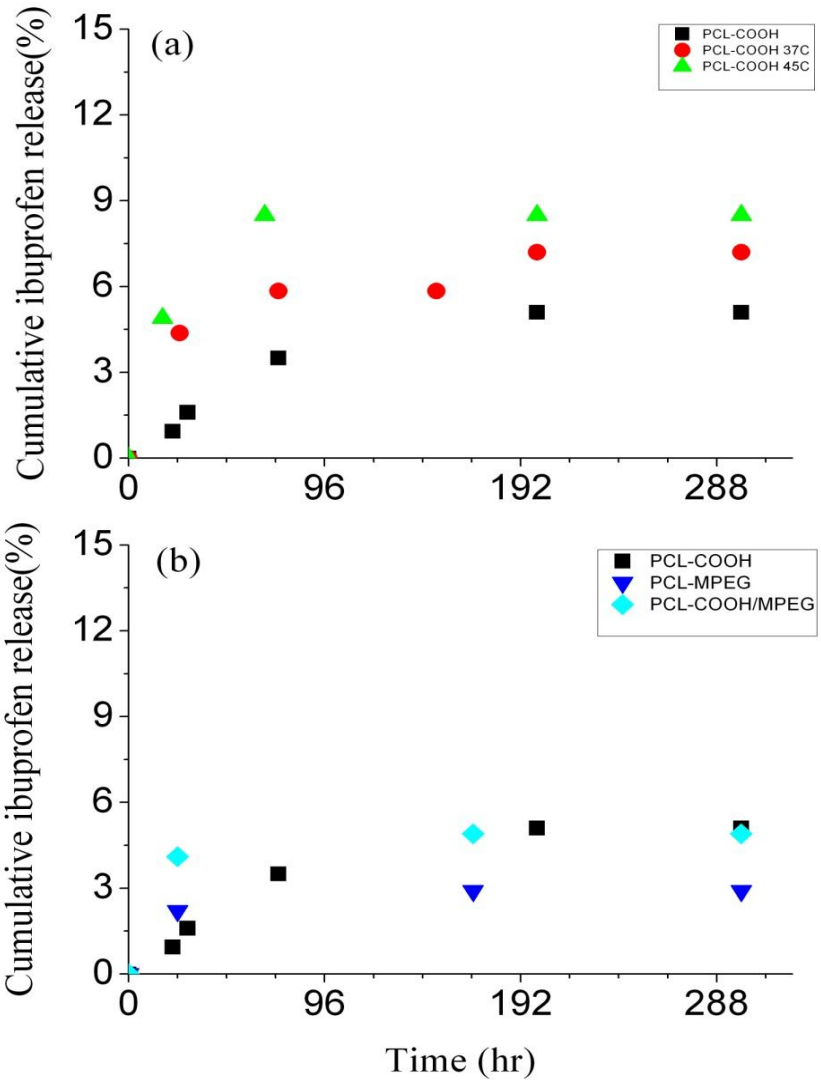

Figure 13 Comparison of Ibuprofen release kinetics from (a) PCL-COOH NPs at different temperatures (b) various PCL NPs at room temperature

From these preliminary experiments, it was concluded that the slow release kinetics could be either due to a chemical interaction between the drug ibuprofen and the polymer, or the fact that ibuprofen is not that a good drug for release kinetics studies

from PLA NPs or even the fact that MIVM setup is not a suitable method. Although, the latter has shown the potential of loading extremely high amounts of drug into the NPs and could therefore be used for coating or even permanent loading of materials into NPs, but it may not be the best method when it comes to synthesize drug loaded delivery vectors which requires substantial release of the loaded material over time.

Systematic investigation into the causes required an elaborate workplan. However, it was possible to investigate whether the nature of the polymer is a causative drawback. As depicted in Figure 14, commercial PLA and PLGA were used to synthesize NPs loaded with drugs. It is observed that their release profiles are comparable to release kinetics from PLA synthesized in house. In actuality, the release profiles from commercial polymeric NPs are even slower, which could be because of different synthesis techniques of the polymers.

One step ahead, the effect of solvent was also checked. Instead of THF, acetone and acetonitrile were used for commercial PLA and PLGA respectively. There was no significant change in release kinetics.

Another possibility to investigate was the location of the drug. As reported in literature [19-21], drug loading experiments have always been carried out for larger NPs extending upto micro spheres using emulsification techniques for synthesis. In almost all the cases, experimentalists have used one or more surfactants for synthesis of drug-loaded NPs. While the use of surfactants has always been reasoned as stabilization of emulsions, its interaction with a hydrophobic drug molecule can never be ruled out completely. In order to check if the use of surfactants does change the morphology of the drug-loaded NPs, 0.1 wt% Tween 80 solution was used instead of the water stream in the MIVM setup, other conditions remaining the same. However, there was no effect observed in the release kinetics.

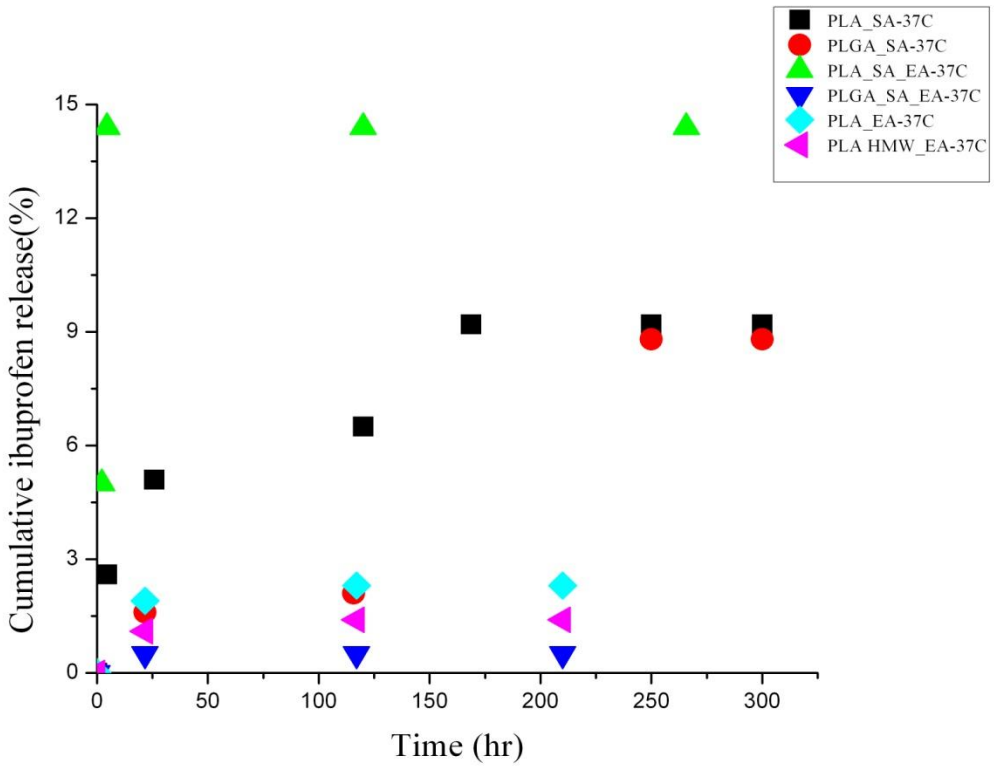

Figure 14 Comparison of Ibuprofen release kinetics from various Polymeric NPs and Polymeric nano capsules

As an alternative to the use of surfactants, a new method was implemented in order to form nano-capsules to decrease the distance of drug diffusion. Based on the method reported by Marchisio et al (http://dx.doi.org/10.1016/j.ces.2012.02.050) for the synthesis of nano-capsules in a very recent publication, Ethyl acetate (EA) was used (1.26 times the mass of polymer) in the solvent stream. The guiding principle being to locate the drug on the outside of the NPs as the EA forms a core in the centre and ibuprofen being hydrophobic would tend to stay with the polymer on the outside. The presence of the polymer on the outside was confirmed by negative zeta potentials as reported in Table 4. Table 4 also reports the DLS sizes of the NPs loaded with ibuprofen.

As observed from the data, the NPs are larger in size when compared to those synthesized without EA.

Sample	Concentration of PLA/PLGA (wt. %)	Average Diameter (nm) after drug loading	Average Zeta potential (mV) after drug loading
PLA_SA_EA	0.10	73.8 ± 0.1	-24.3± 4.5
PLGA_SA_EA	0.25	71.8 ± 0.1	-38.8 ± 5.8
PLA_EA	0.10	88.2 ± 5.9	-16.5 ± 2.3
PLA_HMW_EA	0.10	93.8 ± 8.2	-38.4 ± 1.6

Table 4 DLS Sizes (nm) and Zeta potentials(mV) of polymeric nanocapsules

The release kinetics profiles from these NPs loaded with ibuprofen are also shown in Figure 14. As can be seen, there is significantly fast release kinetics for the commercial PLA, although the steady state value is still low. On the other hand, the other samples show almost no release at all. However, this single set of experiments is not enough to draw conclusions about the method. Possibilities of changing oil to polymer mass ratio, solvents and other parameters could be further investigated.

4 Future Work

The project work has revealed that the MIVM setup is capable of producing drug loaded NPs with very high loading efficiencies for a wide range of polymers. However, it has also been observed that the release kinetics of ibuprofen is extremely slow in most of the cases, reaching a steady state value in few days. This effect has been noted irrespective of the nature of the polymer, whether synthesized in house or bought commercially and also for polymer blends.

Thus, it would be the focus to repeat the whole set of experiments of synthesis, drug loading and release kinetics measurements using a new drug having similar solubility limits as ibuprofen. This would also suffice the claims made regarding interaction between the drug and the polymer. Although, within the scope of this work, two other drugs namely, Lidocaine and Capsaicin have been identified to repeat these experiments. Different analytical protocols have to be developed to use the drugs in the future work.

Moreover, it is felt necessary to repeat a different technique altogether namely emulsification method to synthesize drug loaded NPs and study the release kinetics. This is important to justify the usage of the MIVM method in synthesizing such constructs.

Once this incipient problem of slow release kinetics from the NPs is solved, efforts will be concentrated towards making NCs either through aggregation followed by breakup, or self-assembly of oppositely charged NP, or aggregation driven by polyelectrolyte. In the final part, two different populations of NPs would be loaded with different drugs and synthesized into NCs, whereby having capability of releasing two drugs simultaneously which may also be triggered by different physical conditions.

A schematic of the future work is presented in Figure 15. The present idea upholds the prospect of synthesizing NCs composed of the smallest primary NPs and compare the encapsulation efficiencies and release kinetics of the two. However, the same could also be compared to those obtained for largest size of NPs to investigate into the mechanism of

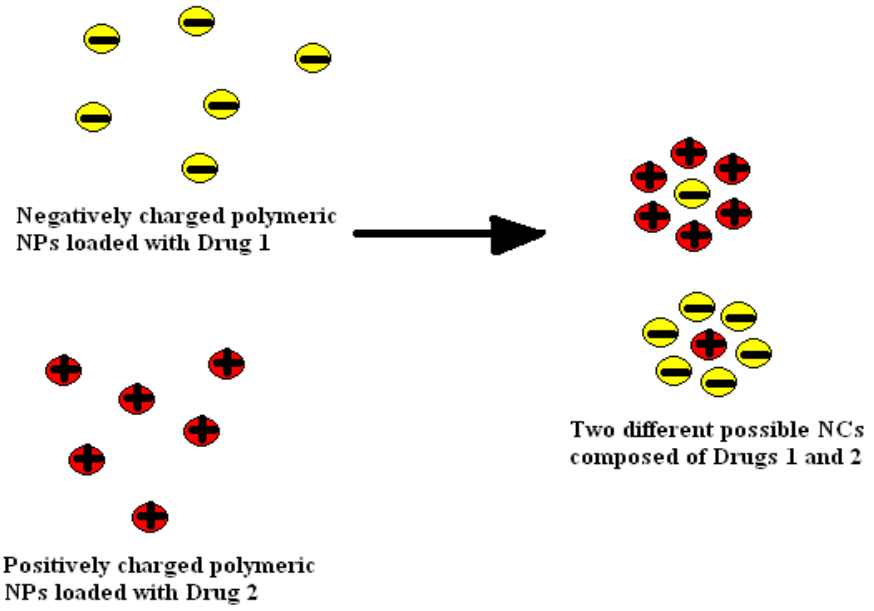

Figure 15 Schematic of synthesis of NCs composed of two drugs

formation of NCs and drug location. While there exist many options to tune the drug release, the primary objective would be to trigger the release mechanisms using different stimuli – heat, pH and so on. In a further step, superparamagnetic Fe NPs or even core-shell Fe-Au NPs may be integrated into these polymeric systems to aid magnetic or even optical detection. These would pave the way for synthesis of multimodal drug delivery vectors loaded with multiple drugs and equipped with targeting as well as imaging possibilities.

5 Conclusion

This work has primarily concentrated on the synthesis of different polymeric NPs viz PLA (synthesized in house as well as commercial grade), PLGA (commercial) and PCL (synthesized in house, having different functionalities –COOH, -PEG and their blends) employing flash nano-precipitation technique in a MIVM setup, previously optimized in the Morbidelli group at ETH. These NPs were characterized using DLS (Dynamic Light Scattering) and Zeta-Sizer to report the variation of the sizes and zeta potentials of the NPs as a function of polymer molecular weight and initial concentration of polymer. The MIVM setup is found to produce stable polymeric NPs as small as 50nm and as large as 155nm depending on polymer concentration and nature of polymer.

The lowest possible sizes of the NPs were then selected for further studies as the overall motivation of the work is to synthesize NCs composed of primary particles. Ibuprofen (model drug) was loaded into the primary NPs using the MIVM setup, following which drug loading, encapsulation efficiencies were measured using High Performance Liquid Chromatography (HPLC). The results indicate that this setup is capable of producing drug loaded NPs with high drug loading efficiencies varying between 75% and 88% differing with polymers.

The release kinetics experiments were performed at 37°C and also studied at room temperature (25°C) and 45°C to evaluate the effect of temperature on release mechanism. The drug-loaded NPs were separated from the free drug in solution at different times using centrifugal filtration. The amount of drug released over time was measured by analyzing these supernatants using HPLC. The release kinetics from almost all the different types of polymeric systems were slow; lasting over several days and moreover, it is not possible to release the entire loaded drug. Various other alternatives including change of solvent, use of surfactant, use of Ethyl acetate to synthesize nano-capsules, whereby changing the drug location were also tried out.

It is claimed that either the chemical interaction of the polymers with ibuprofen or the location of the drug inside the polymeric NPs is the potential reason for extremely slow

release kinetics. It is therefore suggested that further investigation is needed for the same system with another drug to confirm the observed behavior or even a completely different synthesis method for drug loaded polymeric NPs using ibuprofen to substantiate the observed results.

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