HIGH INTENSITY ULTRASONICATION-ASSISTED PICKERING EMULSIONS FOR THE FABRICATION OF MONODISPERSE COMPOSITE NANOPARTICLES

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High Intensity Ultrasonication-assisted Pickering Emulsions for the Fabrication of Monodisperse Composite Nanoparticles

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CERTIFICATE OF ORIGINALITY

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WANG Jiaxin

Abstract

Abstract of thesis entitled "High Intensity Ultrasonication-assisted Pickering Emulsions for the Fabrication of Monodisperse Composite Nanoparticles" submitted by WANG Jiaxin for the degree of Master of Philosophy at The Hong Kong Polytechnic University on 1st August 2018.

In this study, a method has been developed to improve Pickering emulsions and solvent evaporation using high intensity ultrasonication (HIU) to produce nanoparticles for controlled drug release. By applying the proposed method, fabrication of core-shell structured composite nanospheres composed of biodegradable polymers with a high level of monodispersity based on soft colloid-stabilized Pickering emulsions have been successfully implemented. It overcomes the limitations of methods based on Pickering emulsions, that are, (i) the resulting systems size ranges are normally limited to the micron-scale and (ii) the high dependence of the use of bulky or custom-made auxiliary equipment, such as ceramic membranes or microchannel devices, to achieve high levels of uniformity and monodispersity. Therefore, it can exploit the advantages offered by Pickering emulsions and concurrently produce uniform and monodisperse nano-sized drug delivery systems (DDSs) with controllable size ranges. Moreover, the proposed method can also be further developed to become an alternative drug release strategy due to its ability in enabling a facile fabrication of monodisperse composite DDSs in nanoscale, with no use of molecular surfactants or crosslinkers.

The experimental results demonstrated that the introduction of HIU mainly contributes to two aspects: (i) depolymerization of soft colloidal stabilizers and (ii) well-dispersion of emulsion. With such basis, well-dispersed self-assembled chitosan colloid-stabilized emulsion droplets were resulted and further facilitated the preparation of monodisperse poly(lactic-co-glycolic acid)–chitosan core-shell composite nanospheres (PLGA-CS). Moreover, drug-loaded PLGA core with chitosan as one layer of coating, can be formed simultaneously by polymer precipitation resulting from solvent evaporation at room temperature. The key conditions influencing the proposed fabrication process were also investigated. Particle size of PLGA-CS within the range of 255 nm to 830 nm can be controlled by adjusting the amplitudes of HIU and the initial molecular weight of stabilizers. For instance, a low amplitude of 20% of the total power could be used to control drug-loaded PLGA-CS to an average particle size of 255 nm and a very low level of polydispersity index of 0.078. Results from scanning electron microscopy, Fourier transform infrared spectrometry, zeta potential measurement and drug release study further confirmed the successful implementation of complementary functionalities, such as positive-charged chitosan giving rise to enhanced dispersion in aqueous solution and modulation of *in vitro* drug release of the PLGA-CS.

The requirements of the preparation of biodegradable polymeric DDSs, with regard to tunable sizes, enhanced solubility and potential for controlled release and targeted

delivery, were met by applying the proposed method. Investigations on further improving the proposed method, to achieve simultaneous loading of varied therapeutics in single systems with programmed drug release behaviours, are recommended for future studies.

Publications

Some findings from this study gave rise to the following research outputs:

Journal paper:

1. **Wang J**, Law WC, Chen L, Chen D, Tang CY. Fabrication of monodisperse drug-loaded poly(lactic-co-glycolic acid)-chitosan core-shell nanocomposites via Pickering emulsion. Composites Part B: Engineering. Vol. 121, 2017, 99-107.

Conference paper:

1. **Wang JX**, Chen L, Law WC, Chen DZ, Tang CY. Facile fabrication of biodegradable drug-loaded chitosan/poly(lactic-co-glycolic acid) microspheres via O/W Pickering emulsions. 24th Annual International Conference on Composites or NanoEngineering (ICCE-24), Haikou, China, July 17-23, 2016

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Table of Contents

List of Abbreviations

AA	Acetic acid
CS	Chitosan
CH_2Cl_2	Dichloromethane
DA	Degree of acetylation
DDA	Degree of deacetylation
DDSs	Drug delivery systems
DL	Drug loading capacity
DLS	Dynamic Light Scattering
DME	Direct membrane emulsification
DOX	Doxorubicin
EE	Encapsulation efficiency
EPR	Enhanced permeability retention
FA	Folic acid
FDA	Food and Drug Administration
FTIR	Fourier transform infrared spectrometry
LbL	Layer-by-Layer
LMWC	Low molecular weight chitosan
M_w	Average molecular weight
MAA	Methacrylic acid

MC	p(NIPAM-co-MAA)-core/P(NIPAM)-core
MDR	Multidrug resistance
mPEG-b-PLA	Methoxy-poly(ethylene glycol)-block-poly(D,L-lactic acid)
MPTMS	ethacryloxypropyltrimethoxysilane
MS	p(NIPAM)-core/P(NIPAM-co-MAA)-shell
NaOH	Sodium hydroxide
O/W	Oil-in-Water
p	Laplace pressure
PACA	Poly (alkyl cyano acrylates)
PDMS	Polydimethylsiloxane
PEG	Poly(ethylene glycol)
PEMs	Polyelectrolyte multilayers
PGA	Polyglycolic acid
PLA	Polylactic acid
PLGA	Poly(lactic-co-glycolic acid)
PLGA–CS	Poly(lactic-co-glycolic acid)–chitosan core-shell composite nanospheres
PME	Premix membrane emulsification

pNIPAM	Poly(N-isopropylacrylamide)
p(NIPAM-co-MAA)	Poly(N-isopropylacrylamide-co-methacrylic acid)
PS	Polystyrene nanoparticles
PTX	Paclitaxel
PVA	Polyvinyl alcohol
HIU	High intensity ultrasonication
R	Radius
RESS	Rapid Expansion of a Supercritical Solution
RME	Rotational membrane emulsification
scCO_2	Supercritical carbon dioxide
SCFs	Supercritical fluids
SCME	Stirred cell membrane emulsification
SEM	Scanning electron microscopy
SiO_2	Silica nanoparticles
SPG	Shirasu Porous Glass
UV	Ultra-violet
UV-vis	Ultraviolet and visible
VPTT	Volume phase transition temperature
W/O	Water-in-Oil

ZrP	Zirconium phosphate

List of Figures

Chapter 1 Introduction

1.1 Background and Significance

The significance of this study lays mainly in solving the current limitation of Pickering emulsions in producing particles of micron-scale with relatively high polydispersity. By introducing high intensity ultrasonication, the proposed method was able to fabricate monodisperse composite particles with controllable size ranges in nano-scale.

Compared to conventional emulsion stabilized by molecular surfactants, Pickering emulsions stabilized by colloidal particles have been extensively studied for the fabrication of drug delivery systems (DDSs) due to their preferable properties of higher biocompatibility and better emulsion stability (Wu et al. 2016). However, most of them are stabilized by inorganic particles that are biologically incompatible. Soft material-stabilized Pickering emulsions have therefore attracted intensive research interest because they allow the use of soft materials and provide broadened options for fabrication. It has been reported that soft colloidal stabilizers such as chitosan colloids can be used to fabricate a variety of composite DDSs (Wang et al. 2016). However, methods based on soft colloid-stabilized Pickering emulsions that can lead to the fabrication of monodisperse systems with nanometer size have barely been reposted.

Most systems resulting from current methods based on Pickering emulsions are limited to the micron-scale and a broad size distribution. Nevertheless, in biomedical applications, monodispersity is one of the most important requirements of DDSs for precise design of medical dose arrangement. To overcome such limitation, some fabrication methods have been studied for preparing monodisperse DDSs (Bala et al. 2004). Recent methods based on Pickering emulsions have been employed with different techniques, such as microchannel technique and membrane emulsification, to narrow the size distribution of desired DDSs. However, such fabrication methods for producing uniform DDSs is usually established on the sacrifice of operation time as well as the need of delicate and tailor-made devices (Wu et al. 2016). Although the as-obtained DDSs possess high level of monodispersity, the resulting size ranges are still limited to the micron-scale. Besides, as the majority of the applied stabilizers are non-biodegradable solid inorganic colloids, such as silica particles, applications of most as-produced DDSs are therefore limited (Larson-Smith et al. 2012). In this regard, growing attention has been shifted to the application of soft colloid-stabilized Pickering emulsions for developing fabrication methods of monodisperse DDSs.

Modifications are needed to be implemented for fabrication methods based on soft colloid-stabilized Pickering emulsions in order to broaden the size of the resulting systems into nanoscale and narrow their size distribution. HIU is a cost-effective technique which has been widely applied in the food industry for emulsion

homogenization (Abbas et al. 2013). As a kind of powerful mechanical waves, ultrasonication waves generated by HIU can lead to well dispersion of emulsions and depolymerization of carbohydrates. Since the stability of soft colloid-stabilized emulsion is highly dependent on the size of the stabilizers, the benefits of incorporating HIU into Pickering emulsions become more prominent because it can not only conduct homogenization, but also reduce the size of the stabilizers by inducing depolymerizations (Baxter et al. 2005).

Fabrication of DDSs based on Pickering emulsions solvent evaporation represents a straightforward method which enables high versatile designs of composite systems by predetermining the choice of stabilizers. As a kind of soft materials, chitosan colloids resulting from protonation-deprotonation of chitosan, have been used for the fabrication of chitosan/poly(lactic-co-glycolic acid) (PLGA)-based microcapsules (Wei et al. 2012b). Chitosan-coated DDSs with core-shell structure were obtained by evaporating volatile solvent and the feasibility of adopting chitosan soft colloids as stabilizers were confirmed and demonstrated. Hence, it is therefore hypothesized that, by taking the benefits of HIU in (i) depolymerization of soft colloidal stabilizers and (ii) well-dispersion of emulsion, HIU-assisted chitosan-based oil-in-water (O/W) Pickering emulsions can become an alternative strategy for a facile fabrication of monodisperse composite DDSs on the nanoscale.

To date, research on the utilization of soft colloids as stabilizers for the preparation of emulsions is still at its initial stage and a few studies have reported the use of HIU in Pickering emulsions, especially for the preparation of DDSs (Wu et al. 2016). This study aims at fulfilling the knowledge gap of Pickering emulsions in fabricating nano-scale systems. Based on the proposed method, PLGA-chitosan core-shell composite nanospheres (PLGA-CS) with narrow size distribution have been successfully fabricated for controlled drug release.

1.2 Objectives

This research study is focused on the modification of Pickering emulsions to fabricate biodegradable composite nanospheres with controllable sizes and high monodispersity in order to overcome the limitations of current Pickering emulsions on size range. The main objectives of this study are:

(i) to design and modify Pickering emulsions for the fabrication of monodisperse biodegradable drug-loaded composite nanospheres for sustainable drug release;
(ii) to fabricate drug-loaded composite nanospheres with narrow size distribution based on the modified Pickering emulsion method;
(iii) to investigate and study the effect of the processing conditions on the sizes and drug release behavior of the drug-loaded composite nanospheres.

First of all, with the basis of Pickering emulsions, the use of molecular surfactants in conventional emulsions can be avoided. Furthermore, due to the integration of HIU with soft colloid-based Pickering emulsions, composite nanospheres with high monodispersity can be readily and facilely obtained. This circumvents the use of complex auxiliary equipment for narrowing the system size distribution, which are usually required when only Pickering emulsifications are used. Moreover, this study also reveals that the charged surface inherited from the used ionic polymer of both emulsion droplets and particles lays the foundation for the good stability of system during the fabrication process.

Chitosan (CS) and PLGA have been chosen as the model biodegradable materials owing to the following reasons. As a typical ionic polymer, chitosan has been widely paired with PLGA for fabricating composite systems. Moreover, it can shield the hydrophobicity of PLGA when it is deposited as a layer of coating based on its mucoadhesive and hydrophilic nature. On the other hand, in comparison with chitosan matrices, PLGA matrices exhibit less burst-release, due to their hydrophobicity. Therefore, PLGA-CS composite nanospheres fabricated in this study form a core-shell structure.

As the sizes and the surface charge of the stabilizers play dominant roles in determining the stability and size distribution of the obtained composites, both the

effect of the initial molecular weight of chitosan stabilizers and the impact of the applied amplitude of HIU on the properties of resulting DDSs have been investigated. To understand the drug loading and releasing behavior of the prepared biodegradable DDSs, drug-loaded PLGA-CS has been studied in phosphate-buffered saline (PBS) solutions under different pH values at different temperatures.

1.3 Overview of Research Methodology and Layout of Thesis

In order to address the objectives of the research, a method employing high intensity ultrasonication to enhance the Pickering emulsions has been developed. An overview of the methodology is shown in Figure 1, which shows the alignment of research activities and the structure of this thesis.

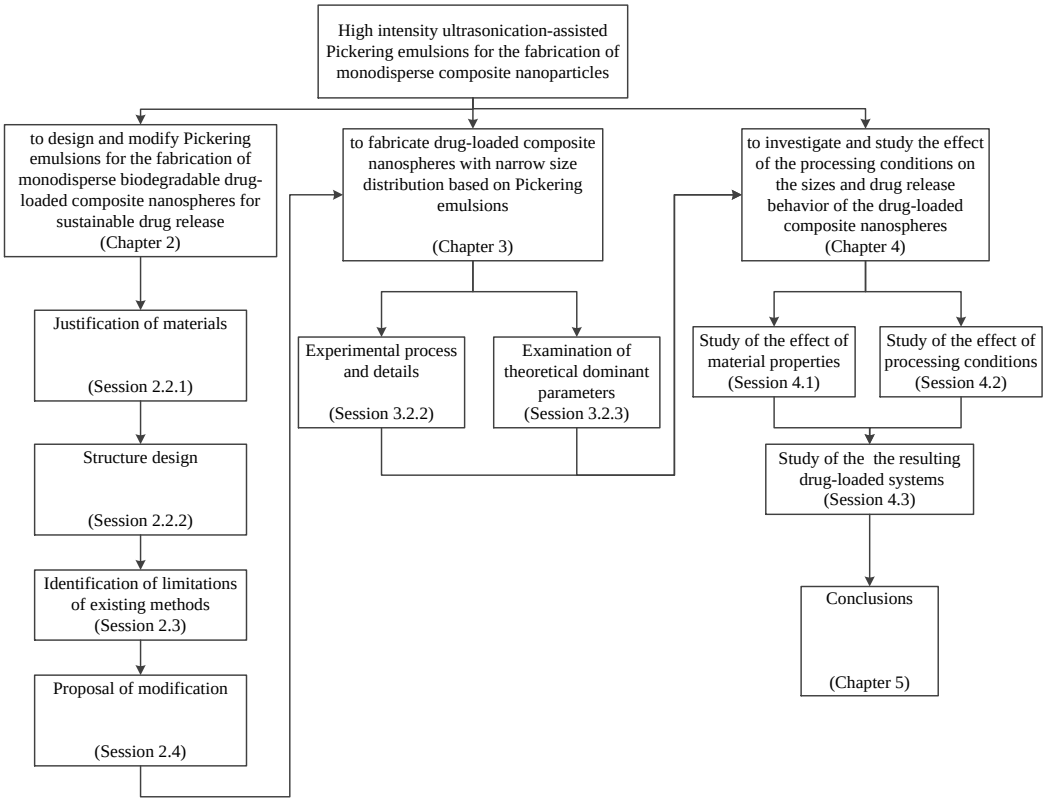

Figure 1 Overview of research methodology

Referring to Figure 1, this thesis, consisting of five chapters, is structured to report the research activities and their results aligning with the research objectives. Guiding by the three objectives, mainly for overcoming the limitations of current Pickering emulsions on size range, modifications of Pickering emulsions were firstly discussed and a proposal (i.e. HIU-assisted Pickering emulsions) was made. Thereafter, the experimental process of the proposed method was presented with investigation details. With such basis, result discussions were conducted in order to verify the

feasibility of the proposed method on the fabrication of drug-loaded monodisperse nanoparticles and conclusions were lastly drawn.

Specifically, chapter one introduces the background, objectives, and significance of the study. Background information is presented with the description of current challenges on fabrication of monodisperse DDSs. The statement of the objectives of this research and scope of this study have been made with the motivation and significance described. The overview of research methodology and the layout of this thesis are thereafter illustrated.

Chapter two presents a comprehensive review on literatures in the specific areas of the fabrication of DDSs. It therefore leads to the justifications for choosing suitable biodegradable polymers and structures for the construction of desired composite systems, and subsequently the fundamentals for developing the fabrication techniques. The HIU-assisted Pickering emulsions were proposed with the illustration of the fabrication process in the end of this chapter.

Chapter three gives a thorough description of the methodology of this project. In this chapter, soft colloid-based Pickering emulsions assisted by HIU are used for the fabrication of composite nanospheres with high monodispersity. The characterization

tests and their results of the composite DDSs prepared by the modified emulsion method are also explained and discussed.

Chapter four is set for discussing the results of this study. At the end, chapter five states the originality and contributions of this research work with suggestions for future investigation.

Chapter 2 Literature Review

2.1 Polymeric Systems for Controlled Drug Release

The evolution of drug delivery relies not only on inventions and investigations of materials, but mostly on the design and development of tailored DDSs with (i) sufficient capacity to deliver defined quantities of a therapeutic payload, (ii) the ability to deliver therapeutics at a specific target site and (iii) the capability to release drug at a controlled release rate (Koch 1991;Yun et al. 2015).

Guiding by these baselines, DDSs have therefore become competitive candidates in overcoming limitations of present therapeutics for the enhancement of treatment strategies regarding to various diseases (Tiwari et al. 2012). As most of currently existing drugs are far from ideal, the use of DDSs should be capable of reducing their toxic side effects and undesirable drug resistance by releasing drug in a controlled manner and in targeting spots (Tibbitt et al. 2016). Especially in cancer therapy, multidrug resistance (MDR) and severe drug toxicity are induced frequently along with the use of chemotherapeutic drugs (Ulbrich et al. 2004). Without effective encapsulation provided by tailored DDSs, their clinical use is unreachable.

Polymers as one of the most versatile class of materials have gained a significant impact in biomedical applications (Fu et al. 2010). Integration between polymer engineering and pharmaceutical science sets a promising stage for the build-up of novel polymeric

DDSs (Uhrich et al. 1999). Notably, the worldwide burden in cancer therapies has led to intensive research in polymeric DDSs for the specific objectives of improvement in the efficacy of drug in parallel with the reduction of undesired side effects. Promising progress has been made in the development of drug delivery strategies based on different types of polymers to realize desired release patterns for efficient drug release and delivery (Tibbitt et al. 2016).

2.1.1 Release Kinetics

Release of therapeutics from polymer-based DDSs in a predetermined manner is conclusive for improving the therapeutic efficacy. It has been proved that simple drug-polymer conjugations can lead to sustained release of drugs (Lankalapalli et al. 2009). However, the lack of protections towards the conjugated drugs can lead to over-exposure and result easily to early decompositions before the arrival at targeted spots, which lower the efficacy of drug delivery (Kamaly et al. 2016). Hence, to overcome such drawbacks, aims of using polymers for the encapsulation of drugs are set to maintain their integrity and to elevate their stability for a prolonged time during the delivery. Driven by these goals, great effort has been devoted to study and understand the mechanisms of controlled drug release from polymeric DDSs for better protection and manipulation (Fu et al. 2010;Kamaly et al. 2016). In this regard, studies focus on release kinetics from matrices composed of different polymer materials have been a significant boost to direct novel design and development of polymeric DDSs that enable

programmed release kinetics under diverse circumstances. Compared to drug incorporations based on chemical conjunction, in polymeric matrix-based systems, therapeutics are combined with polymers by being dissolved or dispersed, which involve less use of additional chemicals and therefore restrict interactions with detrimental chemical residuals (Tiwari et al. 2012). The remainder of this chapter is devoted to give descriptions on typical controlled release mechanisms from polymeric matrices that composed of non-degradable or biodegradable polymers for controlled drug release.

2.1.1.1 Non-degradable Polymeric Matrices

Polymers are classified into non-degradable polymers because of their long duration for complete degradation, which makes the degree of degradation is negligible during drug delivery and release (Prasad Shastri 2003). The utilization of polymeric matrices can facilitate sustained and controlled release by controlling several dominant factors. In general, drug release from polymeric carriers can follow (i) diffusion of payloads, (ii) chemical reaction-caused degradation or cleavage of conjugated drugs and (iii) solvent activation-resulting osmosis and/or swelling of matrices (Fu et al. 2010). It is generally considered that the main driving force for the drug release from non-degradable matrices is Fickian diffusion, as a result of the environmental concentration gradient, diffusion distance and swelling degree of the DDSs (Hombreiro-Pérez et al. 2003). Therefore, most of the non-degradable polymeric matrices for drug delivery

allow near-linear or near-zero order release of the encapsulated therapeutics in a relatively long period of time with short initial burst release. For example, polyurethanes have been well-studied as a type of non-biodegradable polymers with good biocompatibility (Schierholz et al. 1997). It has been used in fabrications of different DDSs for various biomedical applications such as drug delivery implants and skin wound dressings (Zdrahala et al. 1999). Regardless of the diverse geometries of those systems, drug release from polyurethane systems often display a near-linear release pattern after an initial burst release, which is suggested to be driven mainly by diffusion (Fu et al. 2010). Another classic non-biodegradable polymer is polydimethylsiloxane (PDMS). Different from traditional polymers, PDMS is consisted of inorganic Si-O-Si units (Pillai et al. 2001). Interestingly, by preparing PDMS-based systems into different structures, the resulting drug release profile can be altered. Matrix-type PDMS DDSs normally yield a typical first-order release profile (Malcolm et al. 2005). On the other hand, it was reported that by fabricating PDMS into reservoir-type DDSs, in which PDMS matric could be coated by one layer of polymers, such as poly(ethylene glycol) (PEG) on its surface for release regulation. While the matrix core remained a first-order release, the outer layer of PEG coating acted as a solubility enhancing agent. In contrast to matrix-type systems, a near zero-order release profile of such core-shell DDSs was obtained (Maeda et al. 2003).

Nevertheless, non-degradable polymeric DDSs are restricted in drug delivery since their non-degradability usually requires invasion for device removal, which can cause secondary injuries and induce excess risk for treatments (Pillai et al. 2001). Hence, non-degradable polymeric matrices are widely used to fabricate materials for long-term use systems such as transdermal films and implant devices with their inherent characteristics such as good durability and robust structure.

2.1.1.2 Biodegradable Polymeric Matrices

Increased expertise in polymer chemistry resulting in a wide variety of polymers with diverse structures and characteristics. In contrast to non-degradable polymers, biodegradable polymers are originally developed for biomedical applications (Nair et al. 2007). Significant effort has been devoted on using biodegradable polymers to prepare DDSs for controlled release since they have been already medically adapted for their intrinsic biocompatibility and biodegradability, which enable them to be degraded into non-toxic monomers by human body (Jain et al. 2011). Intrinsic characteristics of biodegradable polymers can be implemented into DDSs in terms of (i) biocompatibility and biodegradability to avoid surgical removal and nonessential pain to patients, (ii) ability in realizing internalization and intracellular drug delivery and (iii) capability of releasing drug with minimized drug loss during transition (Heller 1984). Biodegradable polymeric matrices therefore play an indispensable role in the development of DDSs.

The release pattern of encapsulated payload is inevitably associate with the biodegradability of biodegradable polymers. Biodegradability can be attributed to labile bonds in their backbones, including ester-, amide-, and anhydride-bonds that could be hydrolyzed or degraded by enzymes (Fu et al. 2010). Biodegradation can therefore lead to the scission of polymers which further results in reducing of the average molecular weight of polymer. Together with the effect of polymer dissolution in aqueous environment, the release of encapsulated drugs happens along with decomposition of polymer-based matrices (Heller 1980). While degradation represents a chain scission process of polymers that is majorly induced by bond cleavages, mass loss of polymers is referred to erosion. Depending on the difference between erosion rates of polymers and permeation rates of water, erosion can be categorized as two typical modes: (i) surface erosion and (ii) bulk erosion (Kamaly et al. 2016). Generally, as drugs with a certain level of dissolvability are entrapped in polymeric matrices, when the rate of water permeating into a polymer matrix for the dissolution of biodegradable polymer is lower than the polymer erosion rate, it is likely that drugs can be released ideally in zero-order as the erosion rate of the matrix is in dominant and therefore a linear relation to external matrix surface can be obtained. On the contrary, when the permeation of water is quicker than its erosion, such drug delivery matrix may undergo a typical bulk erosion (Uhrich et al. 1999).

It is obvious that in comparison with non-degradable polymers, biodegradable polymers show better potential as building blocks of DDSs due to its incomparable biocompatibility and biodegradability. However, the address of material safety is achieved at the expense of drug delivery efficacy (Fu et al. 2010). Because, most of biodegradable polymeric matrices experience bulk erosion. The drug release kinetics of biodegradable DDSs are therefore more complicated since the release rate of encapsulated drug can be affected not only by polymer erosion, but also polymer degradation, which is easily influenced by solvent penetration which can lead to hydrolysis (Heller 1984). This brings difficulties for the prediction of drug release patterns from biodegradable DDSs. Nevertheless, the exist of the inherent cons of biodegradable DDSs does not inhibit their success in biomedical applications. Instead, their pros are taken maximum advantages in the development of particulate systems with different structures to circumvent material limitations and to release drug in sustained and controlled patterns.

2.1.2 Nano/Microstructural Properties

It is no doubt that DDSs with suitable biocompatibility, biodegradability and the ability of controlled drug release can highly facilitate the realization of desirable drug delivery. However, as discussed, despite of material safety, efficacy in controlled release and targeting delivery towards desired spots is essential to be addressed. In this regard, structural properties of biodegradable DDSs are particularly important.

2.1.2.1 Surface Coating based on Ionic Polymers

For the sake of complementing the limitations of bare biodegradable polymeric matrices, such as burst drug release, as revealed, a core-shell structure of particulate DDSs has non-negligible impacts on this issue to achieve prolonged and controlled drug release (Fu et al. 2010;Tiwari et al. 2012). At the same time, it is also anticipated that before the arrival of targeted spots, formulated DDSs can provide sufficient protection and prevent premature drug loss during drug delivery (Doppalapudi et al. 2016). Hence, another major concerned structural parameter of DDSs is its surface versatility, which is of importance in maintaining the stability and structural integrity of DDSs.

For example, chitosan has been widely applied for surface modifications of PLGA-based DDSs. By blocking electrostatic interactions and hydrophobic interactions, the chitosan coating can function as a diffusion controller for prolonged drug release and a solubility enhancing agent at the same time (Danhier et al. 2012). In vivo studies had been conducted by administrating drug-loaded PLGA/chitosan nanoparticles as DDSs into lungs of guinea pigs (Ungaro et al. 2012). Compared to bare PLGA nanoparticles, it was revealed and confirmed that chitosan-modified PLGA nanoparticles performed in a decreased elimination rate because of the mucoadhesive characteristics of chitosan (Chronopoulou et al. 2013). Besides, promotion of cell internalization can be facilitated by DDSs with suitable surface charge. In vitro studies were conducted on the cellular uptake performance by using chitosan-based nanoparticles (~215 nm) as DDSs. It was

illustrated that interactions between the cells and the positive charge of the surface, which is inherent from the cationic chitosan, facilitated a higher rate and a greater amount of cellular uptake compared to controlled groups (Nafee et al. 2007). Moreover, subsequent results suggested that positively charged nanoparticles could escape from lysosome after cellular internalization for more effective drug delivery.

From the prospective of materials, ionic polymers can provide DDSs with intrinsic surface charge. By definition, polymer contains a net negative or positive charge at near neutral pH is defined as ionic polymer or polyelectrolyte (Lankalapalli et al. 2009). At the initial stage of the development of ionic polymers for drug delivery, it was considered to have a negative impact for pharmaceutical formulations (De Geest et al. 2009). It was concerned that therapeutics could be unintentionally affected by the existence of ionic interactions. Formulations incorporated ionic polymers did not become promising strategies until it was proved that combinations between polyelectrolytes and drugs are capable to aid sustainable and controlled release with the drug integrity preserved. Furthermore, increasing number of studies have revealed that stimuli-responsive drug release can be realized by coating DDSs with ionic polymers that have weak polyelectrolyte sequences and are capable to response to pH changes (Weiren et al. 2014). Additionally, solubility of ionic polymers can be altered based on the pKa of acidic or basic moieties of polymers based on certain types of functional groups, such as amino groups (Ravi Kumar 2000). The equilibrium of protonation and

deprotonation can be manipulated for controlled drug delivery. In such cases, repulsions between each polymer segments are decreased because of the appearance of counterions or molecules with opposite charge can lead to a certain level of expansion of network and entrapped drugs can be therefore released (Onaca et al. 2009). On the other hand, DDSs coated by ionic polymers showed adequate pH sensitivity can be presented at charged state for enhanced solubility or uncharged state for insolubility by simply varying the pH as well (Chen et al. 2014).

As revealed, ionic interactions are preferable between DDSs with positive charged surfaces and negatively charged cell membrane for cellular intake. Moreover, charged DDSs are capable to response to changes of environmental conditions such as pH differences. A good potential of such DDSs is reported for realization of stimuli-responsive delivery (Xu et al. 2008). Recently, it was demonstrated that paclitaxel-loaded low molecular weight chitosan (LMWC)-coated PLGA nanoparticles was capable to achieve sustained and pH-sensitive delivery (Abouelmagd et al. 2015). The PLGA core was designed to serve as protective carrier to retain drug integrity and functionalized by LMWC. The cationic coating could not only increase the hydrophilicity of the polymeric matrix, but also impacted stealth effect and electrostatic interactions under mildly acidic pH of tumors, which further showed less phagocytic uptake for elevated therapeutic effect in comparison to bare PLGA nanoparticles. Therefore, it is prospective that systematic toxicity can be reduced as drug release is

constrained during the delivery and DDSs with desirable surface charge may become more inclined to carry out ideal drug delivery.

2.1.2.2 Particle Size

Despite of the core-shell structure, another crucial condition is the size of DDSs as it is the determinant of the realization of targeting delivery, which could greatly facilitate enhanced therapeutic therapy by delivering drug to the targeted cells instead of healthy cells. According to the underlying mechanisms, targeted delivery can be divided as active and passive targeted delivery (Ferrari 2005). In spite of their different mechanisms, neither active nor passive targeting can be achieved if DDSs are not prepared within a suitable range of sizes because their realization is highly size-reliable (Torchilin 2000;Byrne et al. 2008). Introduced by Ehrlich, systems with the ability of targeted delivery were firstly described in the term of 'magic bullet', which is further suggested as the delivery can be properly targeted to particular disease sites on the premise that material safety and controlled release are guaranteed by using biodegradable polymers and adopting core-shell structure (Lammers et al. 2012). Much research interest has been focused on the understanding of targeted delivery for reasonable design of DDSs to meet diverse requirements.

Passive targeting delivery is realized by taking the advantage of enhanced permeability retention (EPR) effect, which is specifically induced by leaky vessels and high

interstitial pressure in tumorous environment (Torchilin 2000). Particulate DDSs, as one of the most effective forms of drug carriers, with approximate 60 to 400 nm is permeable and is able to be entrapped within solid tumors (O'neal et al. 2004).

Basically, EPR effect occurs when a tumor grows over a volume of 2 mm^3 (Maeda et al. 2000). Under such volume, transportation of both nutrition and waste inside the tumor are gradually hindered along with the occurence of diffusion limitations. Revascularization is therefore programmed by cancer cells to overcome such difficulties. Incomplete vasculatures in the surrounding of tumor occurred because of the fast growth of vessels and this leads to the aforementioned leaky vessels with permeable gaps (Fang et al. 2011). Moreover, the level of interstitial pressure at the central of tumor becomes higher than at its fringe. Supporting by the appearance of the pressure difference, carriers with suitable range of sizes are therefore prone to gain the access inside the tumor with longer retention time than in normal tissues (Maeda et al. 2000). It can be concluded that the combination of leaky vasculature and poor lymphatic drainage shapes EPR effect. Hence, passive targeting can be described as the ability of certain DDSs with suitable range of sizes that can transport through the existed gaps in tumors by being ‘passively’ forced into targeted sites.

Active targeting delivery is different from passive targeting, which counts indispensably on chemical attachments of receptor-identifiable ligands on the surface

of DDSs. The placement of recognition sequences therefore enables surface modified DDSs to function as ‘keys’ for the ‘opening’ of ‘doors’ on specific cells. In order words, the functional surface of DDSs entitles them to facilitate possible cellular uptake actively through bindings between ligands and receptors. Folic acid (FA) is a low-molecular-weight vitamin which exhibits high affinity to folate receptor, a common membrane-anchored protein (Leamon et al. 2001). As a typical targeting ligand, FA has been widely studied for achieving active targeting. It was applied on chitosan (CS) coated-poly(alkyl cyano acrylates) (PACA) via conjugation between CS as an outer layer (Zhou et al. 2010). This study had demonstrated that increased therapeutic effect can be observed by using active targeted DDSs with ligands decorated. However, as reported, the realization of active targeting requires the support of passive targeting. Systematically delivery of DDSs may not be effective in the absence of EPR as a second mechanism, which in other words, DDSs is required to be fabricated in specific ranges of nano-scale sizes (Mahon et al. 2012). It was found that drug loaded liposomes with the attachment of certain anti-bodies as targeting ligands were incapable to accumulate in targeted cells by conducting active targeting solely. In contrast, it could be achieved more effectively by combining EPR effect (Marcucci et al. 2004).

As reported, active targeting should be inefficient for targeted delivery without the support of passive targeting as a second mechanism. As a result, the success of targeting delivery, regardless of active or passive targeting, is highly dependent on the size of

prepared DDSs. It therefore becomes a prerequisite for biodegradable DDSs to be prepared in a suitable nano-scale sizes in order to achieve desired drug release and targeted delivery.

2.2 Recent Development of Polymeric Particulate Systems for Controlled Release

The field of polymeric particulate systems is experiencing a quick expansion with continuous research efforts invested (Tiwari et al. 2012). Being able to deliver a great variety of drugs including hydrophilic small molecular drugs, hydrophobic small molecular drugs, vaccines and biological macromolecules, polymeric particulate systems are beneficial for drug delivery in allowing targeted administration to specific compartments with the ability of controlled drug release. These unique properties guarantee their pivotal role in a wide spectrum of areas ranging from biotechnology to energy technology and enable them to meet various applications and market needs (De Jong et al. 2008;Parveen et al. 2012).

As requirements of different applications varied, properties of systems have to be optimized. For the purpose of obtaining properties of interest, the choose of materials, the design of system structure and the preparation method play dominant roles.

2.2.1 Biodegradable Polymers for Controlled Release

One of the most straightforward strategies to realize controlled drug release from biodegradable polymeric DDSs is the manipulation of degradation rate of biodegradable polymers, as biodegradability is a material-oriented functionality (Uhrich et al. 1999;Pillai et al. 2001). Therefore, the drug release rate can be predetermined for the maintenance of a constant drug concentration in a desired period for minimum side effects by using polymers with different degradation rates (Heller 1984;Jain et al. 2011;Doppalapudi et al. 2016).

2.2.1.1 Poly(lactic-co-glycolic) acid (PLGA)

PLGA as a synthetic biodegradable polymer is one of the most extensively studied and well-documented materials for preparing biomedical materials (Ungaro et al. 2012). Together with polylactic acid (PLA) and polyglycolic acid (PGA), PLGA belongs to polyesters and have been commonly regarded as the best-defined biomaterials by taking design versatility, feasibility and material performance into considerations (Danhier et al. 2012). Among all, PLGA as a hydrophobic copolymer is the first Food and Drug Administration (FDA)-approved material for fabrications of controlled release systems. It also gained the approval from European Medicine Agency for parenteral administration (Lü et al. 2009). Being widely approved as commercially adoptable biomaterial, a number of PLGA-based DDSs have been applied for human clinical trials

(Bala et al. 2004). Thereafter, PLGA has been intensively adopted for the composition of novel DDSs.

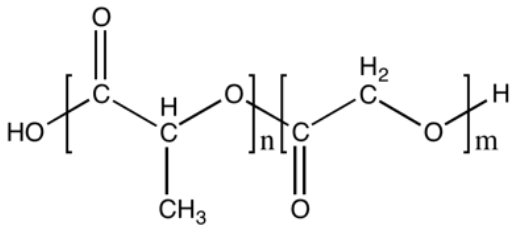

Figure 2 Molecular structure of PLGA (n represents the number of lactic acid units and m represents the number of glycolic acid units)

Solid matrices composed of polymers are capable of loading molecules of nearly any sizes, as introduced. Currently, there are over twenty types of commercial DDSs based on PLGA are available in the market (Bala et al. 2004). It is evident that PLGA has been applied mostly for encapsulation of drugs, especially hydrophobic drugs due to its intrinsic hydrophobicity. In addition, studies have illustrated that a great variety of drugs can be loaded by PLGA-based carriers, including nucleic acids and peptides (Wischke et al. 2008). Properties of PLGA such as molar ratio between lactide (LA) and glycolide (GA), average molecular weight (M_w) and terminal functional groups on its backbone have great impact on its ability of controlled drug release. PLGA is usually identified by its monomers ratio. For instance, PLGA 50:50 represents a copolymer composed of 50% LA and 50% GA. As shown in Figure 2, by simply varying the compositions of PLGA, release rate of DDSs can be altered and controlled. For example, PLGA with certain M_w and a low molar ratio of GA leads to a relatively faster

degradation rate and hence results in rapid drug release compared to PLGA with same M_w but a higher molar ratio of LA. On the other hand, a prolonged drug release from PLGA matrix can be achieved by using PLGA with the same monomers ratio but higher M_w (Fredenberg et al. 2011). However, the carboxylic end group and degradation products (LA and GA) make PLGA hydrophobic and endows it poor solubility in aqueous environment, which can induce inflammatory responses easily and weaken the ability of PLGA matrices to enter targeted organ or cells and therefore lower its drug delivery efficacy (Lü et al. 2009). Solutions in overcoming such limitations have been proposed by modifying the surface of PLGA matrix and applying core-shell structures for PLGA-based DDSs (Nafee et al. 2007;Chronopoulou et al. 2013).

2.2.1.2 Chitosan

Chitosan (CS) as a kind of biodegradable ionic polymers is the second abundant natural polymer and has been extensively used for its outstanding biocompatibility, biodegradability and mucoadhesive ability (Rinaudo 2006). It is originally derived from partial deacetylation of chitin and serves as the only linear cationic polysaccharide on earth (Kumar et al. 2004). Figure 3 illustrates the molecular structure of CS. Particularly, the degree of deacetylation (DDA) and molecular characteristics such as M_w impact significantly on its physicochemical properties, which can be adopted for controlled drug release. For instance, with the same M_w, the higher the DDA, the higher the positive charges that CS presents because of the increased numbers of free amino

groups (Peniche et al. 2003). Moreover, same as PLGA, CS is one of the few FDA-approved polymers. On account of these characteristics, CS has been extensively applied for the development of functional DDSs in divers structures catering requirements of different interest (Nafee et al. 2007;Wu et al. 2010). For example, the cationic nature of CS entitles it to ascribe positive charge to DDSs for enhanced cellular intake and the ability of loading different therapeutics and its mucoadhesive ability has been studied for prolonged drug release (Chronopoulou et al. 2013). It is noteworthy that the free amino groups and hydroxyl groups of CS provide versatilities on chemical modifications with a broad range of molecular segments for DDSs. For instance, chitosan nanoparticles can be modified with both biotin and avidin for active targeting of drug delivery in hepatoma cells (Wang et al. 2011b). Glycyrrhizic acid, as a type of anti-tumor drugs were conjugated to chitosan-based nanoparticles and it was able to strengthen the active targeting of the DDSs towards liver by interacting with specific binding sites (Tian et al. 2010). Nevertheless, the hydrophilicity of CS can induce burst release of bare CS matrices as DDSs more significantly when in comparison with hydrophobic matrices (Rinaudo 2006). It was studied that DDSs with the same particle size around 2.5 μm based on chitosan, PLGA and chitosan coated-PLGA particles released drugs in different patterns (Manca et al. 2008). As a result, chitosan microparticles exhibited a higher burst release in comparison with PLGA and chitosan-coated PLGA microparticles under mild acidic environment which was simulated as tumorous environment. In addition, chitosan-coated PLGA microparticles possessed

the highest drug loading capacity and performed a reduced burst release with enhanced solubility in aqueous solution when compared to PLGA microparticles. Furthermore, the amino groups on glucosamine units of chitosan usually exist a protonation-deprotonation equilibrium, which is responsive to pH differences. Protonated amino groups empower CS to act as a cationic polymer and be dissolved in aqueous solution at pH < pKa (6.5) but insoluble at pH > pKa due to the deprotonation of CS molecules.

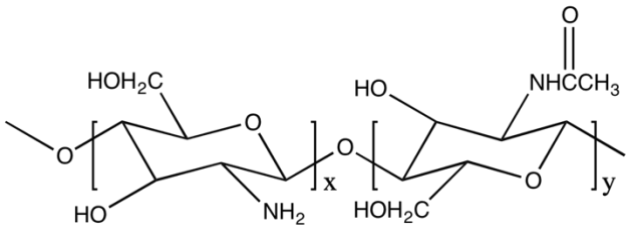

Figure 3 Molecular structure of CS (x is the number of the deacetylated units and y is the number of non-deacetylated units)

Hence, CS is a unique and promising biodegradable ionic polymer as the coating for the fabrication of hydrophobic core-shell structured DDSs with its capability in facilitating controlled and stimuli-responsive drug release.

2.2.2 Composite Systems with Core-Shell Structure for Enhanced Control

Although controlled release and targeted drug delivery can be facilitated by nano-scale biodegradable DDS, sufficient protection for the DDSs is in need during drug delivery in order to minimized undesired release before the reach of destination. Furthermore, as described, confinements of single component coexist with their desirability.

Complements from different materials with advantageous properties are the basis for the promotion of pragmatic design. Generally, DDSs with desirable properties can be formulated mainly by combining corresponding materials via chemical modifications or composite technology.

Chemical conjugations of materials are widely studied as a straightforward strategy for properties integrations in DDSs (Basu et al. 2015). However, the success of chemical modifications relies heavily on mandatory requirements, including (i) the formation of chemical bonds that are strong enough to against hydrolysis, (ii) a high level of re-dispersity of modified products into liquid medium, (iii) repetitive steps for chemical reactions and tedious procedures for residual eliminations, (iv) focused concentration on the maintenance of the dispersion stability during processing (Saito et al. 2003;Allen et al. 2004). It is obvious that the reproducibility of methods involved with chemical modifications could be challenging with all these constrained demands. On the other hand, the use of composite strategies has potential in relieving the burden of conducting complex fabrication procedures for chemical reactions and this could be ridded together with the risks and uncertainties of the introduction of toxic chemicals for biomedical applications (Hughes 2005;Schärtl 2010). By adopting composite technology, desirable properties from different materials can be integrated with rational arrangement by adopting different structures for the systems. Moreover, as the material safety could be secured by using biodegradable polymers, the drug delivery efficacy become the main

concern to achieve controlled drug delivery. However, as discussed, the incorporation of biodegradability for polymeric matrices can suffer from burst release of drugs and frequent drug administrations. In order to circumvent these limitations, different formulations of DDSs based on biodegradable polymers are designed with desired physicochemical and biological characteristics.

Unlike conventional DDSs such as pills or capsules that release loaded drugs once the systems are in contact with aqueous environment, biodegradable composite DDSs with suitable size and structure enable (i) targeted drug delivery in particular body compartment, (ii) preservation and controlled release towards fragile medications such as proteins that can be easily decomposed during drug delivery, (iii) reduction of dosage times and the need for afterwards surgical removal and (iv) improvement in patient compliance (De Jong et al. 2008;Tiwari et al. 2012).

As one of the most quintessential composite DSSs, matrices with core-shell structure allow the inheritance of diverse functionalities of materials directly (Schärtl 2010). For example, by coating ionic polymers on polymeric matrices, DDSs are permitted to conduct further surface modifications that are simply driven by electrostatic interaction (Chronopoulou et al. 2013). Based on the electrostatic attractions between counterions, PLGA nanoparticles were coated by chitosan and alginate alternatively on their outer surface via Layer-by-Layer (LbL) self-assembly (Zhou et al. 2010). It was investigated

that by forming polyelectrolyte multilayers (PEMs), the as-prepared DDSs possessed antifouling properties and hence allow low protein adsorption during drug delivery. PEMs based on chitosan and alginate can therefore be regarded as a potential candidate for being as an alternative of PEG to perform shielding effect. It has been extensively reported that PLGA-based nanospheres coated by CS were successfully exploited for pH-responsive DDSs for controlled drug release. At the same time, ionic repulsion among CS-coated systems is capable to prevent coagulation and elevate the level of system stability. Composite DDSs with surface modified by coating polymers, especially ionic polymers, can thereafter be electrostatically modified with counterpart materials via electrostatic adsorption and release drugs preferentially in tumor environment (Vilela et al. 2016). The control of shell thickness can be achieved by coating different numbers of polymers and applied for manipulation of drug release rate. Aiming at reducing fast burst release, it was reported that compared to bare PLGA particles, chitosan-coated PLGA particles could reduce the burst release from 70% to 36% (Danhier et al. 2012). Hence, drug–loaded nanospheres with core-shell structures were successfully fabricated for controlled drug release. It suggests that DDSs with core-shell structure can benefit from charged coating in respects of not only enhanced cell intake, but also better control of drug release.

2.2.2.1 Polymersomes

Polymersomes are vehicles with intrinsic core-shell structure based on amphiphilic synthetic block copolymers (Discher et al. 2006). Their polymeric shells are inherently resulting from polymer self-assembly, which is similar to the structure of natural phospholipids (Palivan et al. 2016). Studies have been reported that with such bilayer structure, polymersome-based DDSs are enabled to load chemotherapeutics as well as gene materials in single DDSs. For instance, polymersomes were demonstrated to carry anti-tumor drugs with different surface wettability simultaneously. PEG-PLGA co-polymers were firstly fabricated and subsequently, the hydrophobic drugs were loaded in the PLGA shell and hydrophilic drugs were loaded in the PEG core (Wang et al. 2011a). In vivo studies indicated a good potential for using such strategy for realizing multi-drug delivery. In addition, polymersomes based on methoxy-poly(ethylene glycol)-block-poly(D,L-lactic acid) (mPEG-b-PLA) block copolymer were designed to encapsulate both siRNA and anti-tumor drug into single systems (Kim et al. 2013). By loading hydrophobic anti-tumor drugs into the hydrophobic shell and encapsulating siRNA into the hydrophilic hollow core, this study revealed that co-delivery of therapeutics and effective dual therapeutic actions was achieved.

However, although polymersomes possess core-shell structure inherently by using specific co-polymers, polymersomes as DDSs suffer from inevitable drawbacks including poor storage stability as dynamic-affected systems and rapid leakage of

water-soluble drugs (Lee et al. 2012). More importantly, in most cases, tailored co-polymers are needed to be re-designed to cater varied properties of interests for different applications and the specialized chemical process could be labor-consuming and time-costing, not to mention that the possible low yield during various chemical procedures (Meng et al. 2011). Therefore, DDSs with core-shell structure should allow to be fabricated via a more general applicable fabrication method by using commonly approved materials with higher feasibility.

2.2.2.2 Composite Nanospheres

Composite spheres are solid particulate systems for drug delivery (Steichen et al. 2013;Prajapati et al. 2015). As one of the most promising strategies, composite nanospheres attracted considerable attention due to their ability in realizing not only bottom-up, but also top-down designs (Parveen et al. 2012). They can therefore further act as building blocks for DDSs with different structures and diverse functions. Moreover, compared to other forms of DDSs, such as polymersomes, sphere-based systems have improved encapsulation ability in drug loading because their rigid structure ensures the maintenance of stable conformation and the retention of their biological activity for efficient drug release and delivery under environments with different conditions (Gadad et al. 2011).

As a kind of particulate DDSs, composite spheres in nano-scale have been widely studied as one of the most common and feasible drug carriers for targeted delivery of various kinds of therapeutics (Champion et al. 2007). By taking the advantage of its spherical structure, cells can internalize spherical DDSs from any point of attachment due to their symmetry and rigid morphology. Such spherical structure also enable the adoption of various techniques for fabrication (Champion et al. 2007;Gadad et al. 2011). Composite PLGA-based nanospheres developed for delivery of cancer-associated antigen with surface modified by ligands can induce therapeutic anti-tumor effect with no concomitant autoimmunity (Danhier et al. 2012). Moreover, PEG-coated PLGA nanospheres were developed and co-delivery of hydrophilic doxorubicin (DOX) and hydrophobic paclitaxel (PTX), two of the most frequently-used chemotherapeutic drugs in clinical, were successfully achieved in tumor regression (Müller et al. 2003). Additionally, studies revealing performance of DDSs with different ranges of sizes had been extensively conducted. It was found that in inflammatory tissues of the intestinal mucosa of rats, nanoparticles were more effective in performing targeting delivery than microparticles (Lamprecht et al. 2001). It is again supportive to the fact that nanoparticles have indispensable strengths in realizing effective targeting delivery, which cannot easily be achieved by DDSs in micron-scale.

Therefore, composite spheres, especially nanospheres, can be ascribed with a number of desirable advantages such as the ability of achieving targeting delivery as well as

good practicability in adopting different fabrication techniques and methods. Biodegradable composite nanospheres can hence be highly demanded for the build-up of DDSs that could provide sufficient protection for drugs, controllable drug release and good efficacy for drug delivery.

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2.3 Fabrication Techniques for Preparation of Monodisperse Composite Polymeric Particulate Systems

Decision on the choice of appropriate fabrication method is made by taking a number of conditions into considerations, which can be mainly summarized as (i) the area of applications, (ii) size requirement and (iii) the type of polymeric systems. As discussed in previous sections, for controlled drug release and targeted delivery, realization of biodegradable polymeric composite nanospheres as DDSs with core-shell structure in controllable ranges of size is highly desirable (Uhrich et al. 1999;Tiwari et al. 2012). This relies entirely on fabrication techniques and methods for productions.

In fact, a great variety of techniques have been developed for the fabrication of particulate composite DDSs (Lu et al. 2004;Lassalle et al. 2007). In order to choose adaptable techniques to aid the goals, firstly, the attributes of drugs and polymers need to be clarified. As for controlled release and targeted delivery, the site of drug action as well as the expected duration of therapy should be taken into consideration for determining the desirable size ranges and the selection of materials with characteristics

of the DDSs in need (Caldorera-Moore et al. 2010). Aside from biodegradability that can be assured by adopting appropriate materials and surface versatility can be relied on the design of core-shell structures, monodispersity of DDSs that is entirely ruled by the applied fabrication methods is of great importance in applications. Because both particles size and particle size distribution are decisive on particle properties such as viscosity, surface area and packing density. However, in most of current literatures, particles were prepared with broad size distributions (Lassalle et al. 2007). A low level of monodispersity varies particle properties and their performances can be hugely different from the primary design, which could greatly limit their applications in drug delivery as it is confessedly required precise dosage control. For the purpose of improving the monodispersity, different techniques were developed and investigated

2.3.1 Conventional Emulsion Solvent Evaporation

In general, fabrication strategies of polymeric nanoparticles mainly based on (i) the dispersion of preformed polymers and (ii) the polymerization of monomers. For biomedical applications, dominant strategies come from those with the less use of additives in order to avoid any unpredictable side effects (Solans et al. 2005).

As one of the most classic and well-studied techniques of the dispersion of preformed polymers, emulsions stabilized by molecular surfactants are defined as conventional emulsions (Gutiérrez et al. 2008). Most of the current fabrication methods are

developed based on conventional emulsions. Depending on the amphiphilic nature of surfactants, oil or water phase can be stabilized in their counter phase due to the reduction of surface tension. Conventional emulsions are one of the most well-investigated methods and have been extensively adopted for the fabrication of DDSs. With such basis, solvent evaporation is widely preferred for the fabrication of drug-loaded particulate systems (Arunkumar et al. 2009). Generally, a typical oil-in-water (o/w) emulsion is prepared by first dissolving hydrophobic polymer in a water-immiscible, volatile organic solvent. Thereafter, drugs are dissolved or suspended in the prepared oil phase of polymer. By mixing with an aqueous phase containing surfactants, the resulting mixture is then emulsified with the presence of agitation of homogenization. Commonly, o/w emulsion is prepared for the encapsulation of hydrophobic therapeutics and water-in-oil (w/o) is adoptable for the loading of hydrophilic drugs (Shah et al. 2010). Polyvinyl alcohol (PVA) as a typical molecular surfactant was applied to prepare PLGA nanoparticles (60-200 nm) by using two types of volatile solvent dichloromethane (CH_2Cl_2) and acetone in proportion (Astete et al. 2006). Additionally, in order to yield DDSs with different structures and the capability of carrying diverse types of drugs, double emulsions have been developed based on conventional single emulsions (Iqbal et al. 2015).

To harvest solid systems from emulsions, the merits of the utilization of solvents were taken by conducting solvent evaporation. Along with the volatilization of solvent from

the inner phase, polymer precipitation happens. After thorough volatilization, the as-prepared solid DDSs are suspended in the outer phase. Subsequently, collections will be performed after conducting at least three times of centrifugation-redispersion cycles in deionized water in order to remove unformed materials and additives such as molecular surfactants. CH_2Cl_2 as one of the most commonly used volatile solvent has been employed for the fabrication of PLGA nanoparticles (~200 nm) by using either PVA or Span 40 as molecular stabilizers. Emulsions solvent evaporation enables the maximum preservations materials properties and therefore the resulting systems can possess inherent characteristics directly. For instance, chitosan-coated PLGA nanoparticle was prepared based on single emulsion solvent evaporation for DNA/RNA delivery (Nafee et al. 2007). The coating of chitosan was incorporated by adding prepared chitosan solution along with PVA as emulsifiers and deposited on the formed emulsion droplets simultaneously. Another strategy is based on the weak negative charged of PLGA by first formulating PLGA nanoparticles via single emulsion (Chronopoulou et al. 2013). Later, as-prepared nanoparticles were incubated in chitosan solution overnight. One of the major drawbacks of such method is that premature drug loss may occur during a relatively longstanding incubation for chitosan coating.

As illustrated, the employment of conventional emulsions and solvent evaporation that rely on the use of molecular surfactants and volatile solvent is practical for the fabrication of solid particle in nano-scale. Nevertheless, it has been observed that in the

case of single emulsion system, the control of resulting particle size is dominant by the amount of used molecular surfactant (Manca et al. 2008). Increasing amount of molecular surfactant are required if smaller particles are in need. However, the use of molecular surfactant has been proven to impact significantly on resulting drug release profiles. By controlling the particle size and total amount of loaded model drug, PLGA microparticles prepared by using different amount of PVA as stabilizer were applied to study its effect on drug release. As a result, PLGA microparticles contained 4% (w/v) of PVA released distinctively slower than PLGA microparticles contained 1% (w/v) of PVA. While from the perspective of controlled drug lease, the use of molecular surfactant could greatly affect the achievement of predetermined drug release profiles. Furthermore, the production of dispersed particles demand a high surfactant concentration. As a stable emulsion is a prerequisite of particle fabrication, extensive studies have been conducted on this issue and it revealed that a good emulsification process is facilitated by using over 3% (w/v) of molecular surfactants in order to yield particles with smaller size and a satisfactory polydispersity index. Therefore, the manipulation of particle size via conventional emulsion could suffer greatly on undesirable off-design of controlled drug release with the presence of molecular surfactant and inevitable dependency of the amount of surfactant for particle fabrication.

2.3.2 Rapid Expansion of a Supercritical Solution (RESS)

It is obvious that for controlled drug release, performance of polymeric systems fabricated by conventional emulsions may not be satisfactory. Different fabrication methods are developed in order to circumvent the limitations by getting rid of the utilization of additives or reactants. In this regard, the rapid expansion of supercritical solutions (RESS) technique could fulfill these requirements by precluding the use of any surfactant or even organic solvent during the preparation of particles (Byrappa et al. 2008). Generally, by pumping supercritical carbon dioxide (*sc*CO_2) continuously into the bulk machine, a rapid reduced pressure is produced within an extraction unit to induce precipitation of feed-in substrate into crystalline. Thereafter, solid particles are hence formed and they could be brought down by the expansion vessel like snow in the gas or on the glass like a frost in a precipitation unit. Besides, one of the most commonly applied supercritical fluids (SCFs) is *sc*CO_2, by using which that organic solvent can be substituted. However, such technique possesses several drawbacks in other respects of production. Although the use of surfactants and organic solvent is ridded, difficulties lay first on the harvest on collecting particles from gaseous stream. Furthermore, solubility of compounds in SCFs is usually very low and increased solubility relies on the use of co-solvents, which again introduce organic solvents. This brings a high cost for sufficient productions and its solution devastates its advantage. But above all, a fatal defect of RESS is that there is no control on the size distribution of particles. Of which

that in conventional emulsion-based method, can at least be improved by applying agitation or homogenization.

2.3.3 Pickering Emulsion Solvent Evaporation

As a potential alternative, emulsions stabilized by colloids instead of molecular surfactants, i.e. Pickering emulsions, were firstly proposed by Ramsden and Pickering (Pickering 1907). It has attracted considerable interest and recently been studied for biomedical applications, such as the fabrication of DDSs (Marto et al. 2016). In this regard, Pickering emulsions own superiorities by using colloids as stabilizers over conventional emulsions in (i) better biocompatibility, (ii) enhanced stabilized and droplet size controllability, (iii) facile process and scalable productivity. In addition, from the perspective of processing, the use of Pickering emulsions can be prevailing because it can be processed under mild and simple setup by using low-energy emulsification tools under ambient temperature (Larson-Smith et al. 2012).

The use of colloids as emulsifiers distinguishes it from conventional emulsions that are based on molecular surfactants (Chevalier et al. 2013;Li et al. 2015). Due to the absence of molecular surfactants, toxicity of emulsions is reduced and hence minimize possible adverse effects such as irritancy, cytotoxicity and hemolytic behavior (Arechabala et al. 1999;Moradi et al. 2012). Such issues raised undesirably due to the chemical residuals in DDSs fabricated from conventional emulsions by using molecular surfactants or

polymerization by using different reaction agents. Furthermore, the strongly adsorbed colloidal particles at the interface of oil-water enables Pickering emulsions exhibit distinctive physical stability by forming a compact steric barrier on the surface of droplets to prevent droplet coalescence (Melle et al. 2005). Moreover, by carrying out solvent evaporation, solidification of Pickering emulsion droplets can lead to productions of composite DDSs in solid dosage forms with walls of stabilizers as a coating simultaneously. Furthermore, versatility of the DDSs can be elevated with more flexible control on desired characteristics. Additionally, surface modifications of the prepared encapsulation systems can be performed by using functional stabilizers, which promote a more concrete control of system properties. For instance, silica nanoparticles (SiO_2) were modified into responsive hairy stabilizer by being grafted with surface-anchoring co-polymers (Liu et al. 2016). In such design, functionalized SiO_2 were demonstrated to have tunable surface wettability in response to specific changes of environmental conditions and solely these particles could emulsify not only single emulsions, but also double emulsions. Moreover, by covalently modified layered alpha-zirconium phosphate (ZrP) disks with thermosensitive polymers, poly(N-isopropylacrylamide) (pNIPAM), modified ZrP disks were demonstrated to become a thermosensitive stabilizer (Wang et al. 2017). The resulting systems were investigated and they were able to release loading in fast responses to the change of temperature. Thus, versatility of as-prepared systems to accommodate challenging situations could be incorporated by first programming stabilizers, which allow greater room for

advanced design. Pickering emulsions are hence regarded as a potential strategy for the fabrication of tailored DDSs facilely.

With the rapid development of materials, a wide range of particles have been extensively studied as stabilizers. Depending on the origins of materials, Pickering emulsions can be divided as solid particle and soft material-based emulsions.

As a replacement of molecular surfactants, stabilization of emulsions by solid particles shares a totally different mechanism (Chevalier et al. 2013). With no need for being amphiphilic, solid particles that are partially wetting of water and oil becomes the prerequisite to drive them to anchor at oil-water interfaces (Binks et al. 2000b;He et al. 2013). Otherwise, if solid particles with a highly hydrophilic surface, they would be totally wetted by water and remain dispersed in aqueous phase with no emulsions formed. Similarly, solid particles could not become emulsion stabilizers with too hydrophobic surfaces as they could only wet in oil phase.

A variety of inorganic particles including silica, hydroxyapatite, graphene and carbon nanotubes are extensively investigated for the stabilization of Pickering emulsions (Wu et al. 2016). Among all, for biomedical applications such as drug release and delivery, most of the research focus on the use of biocompatible particulate stabilizers. Since the partial wetting of solid particulate stabilizers is of great importance, pretreatments are

often required to tune their surface wetting. As one of the most extensively studied biocompatible solid particulate stabilizers, hydrophilic SiO_2 particles are preferentially stabilize O/W emulsions (Binks et al. 1999) whereas hydrophobically modified SiO_2 particles are more likely to stabilize W/O emulsions (Binks et al. 2000a). For instance, core-shell structured composite microspheres were prepared by firstly modifying SiO_2 particles with ethacryloxypropyltrimethoxysilane (MPTMS) in order to prepare W/O emulsions (Maeda et al. 2010). It was illustrated that hydrophilic model drug could be loaded within the core and the system were capable to release drug in a sustained release pattern. Aside from covalent modification, it was lately reported that by non-covalently bonding negative charged SiO_2 particles with cationic chitosan oligomers, the surface hydrophilicity of SiO_2 particles was tuned to be partially wetting for the stabilization of O/W emulsions in micron size (Alison et al. 2016). Such approach enables the prepared systems to exhibit a long-term stability.

In conventional emulsions, the control of emulsion droplets relies independently on the amount of used molecular surfactants, which has a direct relation with the size of obtained solid particles. Generally, under a certain oil mass ratio, the higher the amount of used surfactant, the smaller the resulting particle size. Situation in Pickering emulsions stabilized by solid particles is widely divergent. Particles with dual wettability in size range above 10 nm are almost inevitable anchored on the oil-water interface (Larson-Smith et al. 2012). The irreversible adsorption of anchored solid

particles can be attributed to the superb stability of Pickering emulsions because the tightly packed solid particle layer they formed lead to an insurmountable energy barrier that is typically in the order of several thousand magnitudes greater than thermal energy for droplet shrinkage (Pickering 1907). Once the coverage of droplets is complete, the size of droplets is settled. In order to obtain solid particles within desirable size ranges, strict control over the dosage as well as the size distribution of used solid particulate stabilizers need to be carefully exercised. When the amount of solid particulate stabilizers is too low, only a small interfacial area of droplets is stabilized and the resulting droplet size can be very large. On the other hand, if a non-limiting amount of solid particle with a broad size distribution is added, the amount of adsorbed particle will remain constant once the surface of emulsion droplets is fulfilled (Chevalier et al. 2013). In other words, there is a critical particle concentration that need to be defined first for the control of the resulting particle size from solid particle-stabilized emulsions.

However, fabrication of monodisperse systems based on such method usually limit by the requirement of tedious chemical formulations of solid particulate stabilizers, which brings extra difficulties of the fabrication. Moreover, most of current studies has little control on the size distribution of solid particulate stabilizers that commonly results in systems with high polydispersity (Fujii et al. 2013). Not to mention that many DDSs fabricated based on inorganic material-stabilized emulsions suffer from their poor

performance on biocompatibility and biodegradability. Novel techniques have therefore been further studied and developed in order to obtain monodisperse systems.

2.3.3.1 Microchannel Emulsification

As described, in solid particle-stabilized emulsions, the particle size and size distribution of resulting systems are dominant by the sizes of the used particulate stabilizers. For the purpose of breaking from this constraint, microchannel emulsification is developed by adopting microfluidic devices. Study has revealed that in a customized microfluidic system, the oil phase can be pumped out to the channels by using a microsyringe, where silica suspension as the aqueous phase has been already presented (Xu et al. 2005). Different from traditional emulsifications, microfluidic system allowed the formation of oil droplets first, followed by the formation of emulsion droplets when they met stabilizers in the programmed channels. By controlling the physical parameters of the channels and flowing rates of each phase, monodispersed SiO_2-coated emulsion droplets were resulted in micron size. It is noteworthy that SiO_2 particles are easily aggregated in aqueous environment and in this study, the original SiO_2 particles that were in tens of nanometers were used in a broad size distribution and SiO_2 particle-aggregates were applied as stabilizers. In addition, comparison, it was found that emulsion droplets resulting from homogenization had thinner SiO_2 outer layer, which could be ascribed to the high shear force induced during homogenization. On the same footing, SiO_2 particle-aggregates with an original SiO_2

particle size of 12 nm were applied to stabilize O/W emulsions (Priest et al. 2011). It was revealed that the presence of nanoparticle solution has no impact on the droplet formation dynamic based on microfluidic emulsification. Recently, model drug-loaded silica/chitosan colloidosomes were fabricated based on the use of a tailored microfluidic device (Su et al. 2016). Being injected into the oil phase where glutaraldehyde as crosslinker was presented, the dispersed phase containing of chitosan molecules and SiO_2 were further stabilized when the hydrophilic SiO_2 were *in situ* modified by chitosan moved from the inner dispersed phase to the oil-water interface. Chitosan molecules were interacted with not only the SiO_2 via covalent and hydrogen bonding, but also with glutaraldehyde through chemical crosslinking at the interface between the dispersed phase and SiO_2 particles. By varying the concentration of chitosan and SiO_2 particle solution and corresponding weight ratio, monodisperse microspheres and microcapsules coated by silica/chitosan were obtained. Additionally, it was also reported by using a smaller size of SiO_2 particles together with the application of a longer solidification time (8h), monodisperse colloidosome in 160 nm could be obtained.

Therefore, it can be concluded that in microfluidic systems with proper design, the fabrication of monodisperse Pickering emulsion droplets and systems with tunable size ranges can be achieved. The size of emulsion droplets can be controlled more flexibly by altering the parameters of the microfluidic chip and the injecting rates of the mobile

phase. However, it should be noticed that the fabrication of monodisperse emulsion droplets is directly bound up to the uniform channel width, which is firmly settled after the fabrication of microfluidic device. Unfortunately, current fabrication techniques for producing microfluidic devices including UV-lithography, wet chemical etching and micromatching rely heavily on the existed machines and to this day, all the presented channels are remained in micron sizes (Mark et al. 2010). Furthermore, without a strict control on the flow rate of the continuous phase, the uniformity and the monodispersity of emulsion droplets could be greatly affected. Although on micron-scale that the impact of solid particulate stabilizers on the sizes of resulting systems can be weaken, the yield of particulate systems on nano-scale is still fully dependent on the reduction of the particle size of stabilizers and at the expense of time, which are non-generalizable in many cases.

2.3.3.2 Membrane Emulsification

Aside from the try of making the use of microfluidics, membranes as another auxiliary equipment for Pickering emulsions have been recently studied. Basically, the realization of membrane emulsification is based on either pressing a dispersed phase or a coarse emulsion through a uniform microporous membrane under a certain level of pressure (Joscelyne et al. 2000). A variety of membranes have been developed for membrane emulsification with the basis of different materials, such as glass membranes,

ceramic membranes and metal membranes (Charcosset 2009). Monodisperse emulsion droplets can be regulated by changing the used membrane with different porous size.

In recent, membrane emulsification has been investigated for the preparation of particulate systems with narrow size distribution based on solid particle-stabilized emulsions (Wu et al. 2016). In this study, a traditional membrane emulsification called direct membrane emulsification (DME) was applied. DME usually requires the dispersed phase to be pressed or injected through the membrane into the continuous phase under a specific critical trans-membrane pressure. However, the preparation process is proven to be time-consuming and this technique is only suitable for materials with low viscosities, which greatly limits its applications. Thereafter, based on DME, stirred cell membrane emulsification (SCME) that is assisted by mechanical stirring at different rates has been developed with improvements. By using SCME, modified polystyrene nanoparticles (PS)-stabilized emulsions of 44-269 μm in size and colloidosome microcapsules were successfully prepared (Thompson et al. 2011). It was resulted that the application of an annular ring membrane with a mean pore diameter of 5 μm and a mean pore spacing of 200 μm could lead to the fabrication of emulsion droplets with relatively narrow size distribution. The average droplet diameter could be controlled by changing the stirring rate in a suitable range. In addition, by adding crosslinker in the oil-phase before injecting it into the continuous phase, microcapsules coated by crosslinked modified PS nanoparticles with low polydispersity were obtained.

Instead of injecting the dispersed phase into the continuous phase, premix membrane emulsification (PME) technique has been developed by pressing the premix emulsion, i.e. coarse emulsion through the membrane and was afterwards broken into uniform-sized droplets under high trans-membrane pressure. Based on PME, chitosan-coated alginate nanoparticles were used to prepare PLGA colloidosome microparticles with a mean particle size of around 10 μm (Nan et al. 2014). The yield of collidosomes was achieved by first preparing uniform alginate nanoparticles. Solid alginate nanoparticles crosslinked by Ca^{2+} were fabricated in different size ranged from 300 to 400 nm by using Shirasu Porous Glass (SPG) membranes with average pore sizes of 1.4 μm, 2.8 μm and 5.2 μm based on surfactant-stabilized emulsions via PME. Afterwards, driven by the electrostatic attraction, chitosan was coated on the alginate nanoparticles and lead to uniformed solid chitosan-coated alginate nanoparticles. Monodisperse emulsion droplets that were stabilized by chitosan-coated alginate nanoparticles were obtained via a SPG membrane with a mean pore diameter of 25.9 μm. In comparison with PVA-stabilized PLGA microspheres, relatively monodisperse PLGA microparticles coated by chitosan-coated alginate nanoparticles performed a significantly reduced burst release of loaded insulin, which indicated themselves as potential oral drug delivery carriers.

Rather than being stationary, rotational membrane emulsification (RME) rotates to induce the detachment of droplets from the membrane. Generally, it was reported that the setup of RME is conducted by using a cylindrical stainless steel membrane (Yuan et al. 2010). The membrane was mounted on an overhead stirrer and carefully positioned in the center of a stationary cylindrical container with specific design containing the continuous phase. By optimizing the rotation rate and pressing pressure, injection rate of the dispersed phase and flux rate of the continuous phase, microspheres with highly narrow size distribution were fabricated based on silica nanoparticle-stabilized emulsions via RME. Comparing with systems fabricated from conventional high-shear homogenization, the improved control over size distribution is available from the RME technique. Additionally, in order to obtain monodisperse particles from RME, kinetics of particle adsorption has to be understood. A critical droplet detachment time should be controlled for the formation of stable emulsions. Coalescence of emulsion droplets will occur once the adsorption of particles is longer than its critical detachment time. The resulting particulate systems will therefore be coalesced with a high polydispersity (Manga et al. 2012).

Comparing to conventional Pickering emulsions that mostly assisted by a high-pressure valve homogenizer, enhanced manipulation on particle monodispersity can be achieved by using either microfluidic techniques or membrane emulsification techniques. However, it is noteworthy that not only the channels of microfluidic devices, but also

the pore size of membrane emulsification devices, can be only prepared in micro-scale. Of which are inevitably restricted by the current fabrication method for such devices and the resulting particulate systems based on solid particle-stabilized emulsions are mostly within micro-sized. Moreover, as noticed, the obtain of systems with desirable particle size cannot achieved without the use of customized accessories, which could be high cost and could bring complexities to fabrications. Besides, the dependency of the particle size and particle size distribution on the used solid stabilizers is still high. The yield of smaller size of particulate systems and lower polydispersity requires the successful preparation of monodisperse solid stabilizers, which introduces further difficulties for the whole fabrication.

2.4 Challenges and Development Trend

In summary, challenges of the existing fabrication methods for particles as DDSs mainly lay on (i) the inevitable use of molecular surfactants, (ii) the dependence of bulk or customized devices for improved performance and (iii) the lack of efficient control on polydispersity of resulting systems.

Currently, Pickering emulsions are commonly regarded as a good candidate for overcoming the abovementioned challenges. Although the development of soft material-based emulsions is a late-starter in comparison with solid particle-stabilized emulsions, attention has already shifted to soft materials including polymers and

proteins as stabilizers (Bouyer et al. 2012;Li et al. 2015). One of the most important reason is that soft material-based Pickering emulsions are capable in circumventing one of the limitations, the lack of good biodegradability, of solid particle-stabilized Pickering emulsions for biomedical applications. Similar to solid particulate stabilizers, soft materials such as responsive microgel, as a new class of stabilizers, can anchor to the oil-water interface irreversibly to form stable emulsions. Many soft materials themselves can bring intrinsic functionalities such as surface responsiveness including thermo-sensitivity and pH-sensitivity as the coating of the resulting systems, which cannot be easily incorporated without chemical modifications of most solid particulate stabilizers.

One of the most well-known and widely studied examples is the case of pNIPAM microgels. As a kind of temperature-sensitive polymers, pNIPAM microgels that composed of weakly crosslinked three-dimensional network can become swollen in water below its volume phase transition temperature (VPTT = 33 ℃) but shrink under higher temperature due to a change of polymer-solvent affinity (Destribats et al. 2014a). Being as emulsion stabilizers, the emulsification ability of pNIPAM microgels were revealed to be affected by its particle size (Destribats et al. 2013). Being varied from 250 to 760 nm, it was discovered that the larger the microgels, the more bridged droplets with heterogeneous monolayer occurred due to the high deformability of larger microgel. Whereas droplets with homogeneous monolayer occurred in the use of

smaller microgels. Therefore, in microgel-stabilized emulsions, stable emulsions prefer to be prepared by using small sized microgels instead of large microgels that would easily induce flocculation. On the other hand, by keeping the particle size in constant, it was revealed that the deformability is of importance in obtaining fully-covered droplets. The use of microgels with higher deformability and elastic interfaces could results in emulsions with high stability. This leads to a fact that the emulsion stability can also be governed by the level of crosslink degree of the used microgels as stabilizers. Since deformation of highly crosslinked microgels is more difficult than microgels with lower level of crosslink degree, the stabilization efficiency is greatly reduced and droplets tend to bridge together in order to be fully covered. Aside from material properties, by remaining the original particle size and the level of crosslink degree unchanged, changes in processing parameters also impact on the formations of microgel-stabilized emulsions. As temperature-sensitive systems, pNIPAM microgels become larger when the temperature is lower than its VPTT. Sharing the same mechanism, microgels with larger particle size under low temperature tend to induce flocculation and instable emulsions. Likewise, in comparison with higher input of stirring energy is input, microgels under lower stirring energy are more likely to remain their original shape and therefore droplets are covered more completely.

As stimuli-sensitive emulsions have attracted considerable interest, core/shell poly(N-isopropylacrylamide-co-methacrylic acid) [p(NIPAM-co-MAA)] microgels that are

both temperature and pH-sensitive have been applied on the study of the influences of the presence of the charges in the emulsion formation (Schmidt et al. 2011). Systems with different structures, one was composed of P(NIPAM)-core/P(NIPAM-co-MAA)-shell (MS) and one was composed of P(NIPAM-co-MAA)-core/P(NIPAM)-core (MC), were prepared in order to study the effect of the particle charge within different position. Since MAA groups can be negative charged at pH 9 but become uncharged at pH 3, by converting the pH, the charge of the shell of MS and the core of MC can be tunable. It was illustrated that, the presence of charges in microgels was of significant importance in the successful preparation of microgel-stabilized emulsions, no matter the location of charges. Moreover, in contrast to the solid particle-stabilized emulsions, the type of formed microgel-stabilized emulsions can be determined by the presence or absence of surface charges. As reported, by using octanol as the oil phase, O/W emulsions were formed by using MS microgels at pH 9 but W/O emulsions were obtained by using MC microgels at pH 3.

To improve the ability on size control and to narrow the particle size distribution, SCME was also applied on microgel-stabilized emulsions (Sun et al. 2014). Like wise, typical SPG membranes with average pore size varied from 2.5 μm to 9.2 μm was used to fabricate monodisperse pNIPAM-co-MAA microgel-stabilized emulsion droplets with diameter in between 10.1 μm to 50.9 μm.

Despite the use of polymeric microgels, protein microgels had been prepared for Pickering emulsions as well (Destribats et al. 2014b). Nevertheless, current microgel-stabilized emulsion droplets are all in micronized as well. Comparing with solid particle-stabilized emulsions, the control of the droplet size and size distribution is even harder than in microgel-stabilized emulsions. From the perspective of material properties, in solid particle-stabilized emulsions, the droplet sizes can be controlled by manipulating the particle size and size distribution of the stabilizers. While in microgel-stabilized emulsions, to achieve the same goal, not only the size of stabilizers need to be controlled, but also their crosslink degree as well as their particle charges have to be monitored. Most importantly, the fabrication of microgels frequently require the use of molecular crosslinkers. Such process may easily introduce additives to the systems and the merits of Pickering emulsions, as surfactant free-emulsions, will be vanished.

Indeed, microgels enable the resulting system to inherent their distinct characteristics directly, which is worthy to conduct further explorations for the fabrication of advanced functional systems. On the other hand, to circumvent their limitations but keep the best of their advantages, polymeric colloids as another kind of soft materials are emerged as a potential stabilizer. Chitosan as a kind of polysaccharides have shown great potential in preparing Pickering emulsions (Liu et al. 2012;Wang et al. 2016). By simply tuning the pH, chitosan can become chitosan colloids that are insoluble in water via the aforementioned protonation-deprotonation process. Based on this fascinating property,

chitosan colloid-based Pickering emulsions were firstly reported as a pH-sensitive stabilizers for the stabilization of reversible O/W emulsions (Liu et al. 2012). Without the use of any additives, chitosan colloids were obtained by simply varying the pH over its pKa based on the addition of NaOH. After hand-shaking for 5 min, O/W emulsions based on chitosan colloids were formed but deformed by lowering the pH of the systems based on the addition of HCl. Emulsions could experience at least 5 circles of formation-deformation in responsive to the pH. It is noteworthy that chitosan itself is seldom adopted as an emulsifier (Rinaudo 2006). Lately, it has been demonstrated that CS colloids are excellent stabilizers for the fabrication of drug carriers via Pickering emulsions. Under a similar protocol, chitosan-coated PLGA microcapsules with model drug loaded as DDSs were fabricated for the first time (Wei et al. 2012b). Furthermore, degradable microspheres based on pH-responsive chitosan colloid-stabilized emulsions were obtained under homogenization via photopolymerization. On the same footing, under ultra-violet (UV) irradiation, model drug-loaded solid chitosan coated-degradable microspheres with a particle size in around several micrometers were successfully fabricated by the first time via colloid-stabilized emulsions (Liu et al. 2014). In addition, it also revealed that the pH-responsive chitosan coating enabled the resulting system to release relatively slower in low pH value compared but quicker under higher pH value.

In polymer colloid-stabilized emulsions, most limitations of solid particle-stabilized emulsions and microgel-stabilized emulsions were overcome. Benefiting from the material characteristics, chemical pretreatments that are commonly required in those emulsions are replaced as described. Moreover, biodegradability of the resulting systems can be achieved without the use of non-degradable or non-biodegradable materials. Colloid-stabilized emulsions are therefore competitive to fulfill the requirements of fabricating desirable drug delivery systems. However, as revealed, the demand for monodisperse polymeric systems composed of biodegradable polymers with tunable size ranges on the nano-scale via such an attractive strategy has remained unfulfilled. In this study, to address these problems, a modified fabrication method based on colloid stabilized-Pickering emulsion and solvent evaporation was proposed. The high-intensity ultrasonication (HIU) as a cost-effective technique has been successfully applied in O/W Pickering emulsion for the preparation of PLGA-CS core-shell DDSs in which the PLGA core was stabilized by chitosan colloids formed by simple sol-gel transition.

2.4.1 High Intensity Ultrasonication (HIU)-assisted Pickering Emulsions for Preparing Monodisperse Composite Nanospheres

In light of that, HIU-assisted Pickering emulsions were proposed to overcome the limitations of the existing fabrication methods and solid-particle stabilized Pickering emulsions, the fabrication process of the proposed method was revealed in Figure 4. By

adopting the proposed method, the use of chemical additives and complicated devices can be ridded for the fabrication of monodisperse composite nanospheres. The proposed fabrication process is mainly based on the following two aspects: (i) depolymerization of carbohydrates by using HIU and (ii) dispersion of emulsions via HIU. Moreover, due to the use of soft colloids as stabilizers, applications of different materials with pendent amino and/or other functional groups that can experience protonation and deprotonation is broadened in Pickering emulsions by being adoptable as stabilizers.

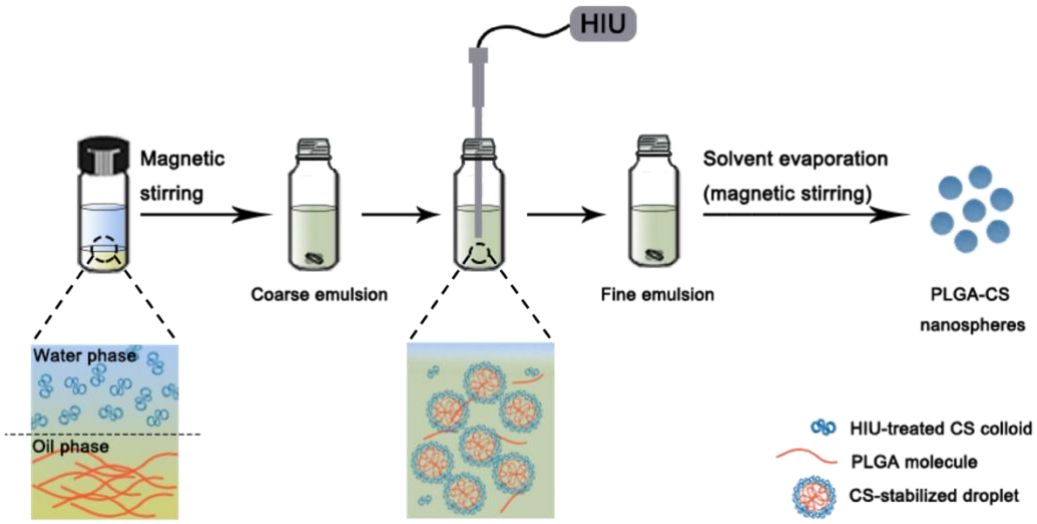

Figure 4 Schematic illustration of the preparation of PLGA-CS

2.4.1.1 Depolymerization of Carbohydrates via HIU

HIU has been widely used in various applications such as food industry to destroy microorganisms and homogenize emulsions (Roberts 1993). As a kind of mechanical waves, high intensity ultrasound is propagated by generating intensive compression and shear waves which thereafter become shock waves (Price et al. 1995). A typical and important phenomenon in HIU is cavitation. Cavitation is a process where cavities are

collapsed and lead to the changes of environmental conditions. Commonly, small cavities are induced by the rapid changes in pressure of the shock waves. Such cavities are capable in further producing turbulent flow conditions, extra-high temperature and pressure by collapsing under a positive pressure cycle (Leighton 1995). Under such phenomenon, in a very short period, it was theoretically estimated that the temperature and the pressure inside the cavitation bubbles can be raised to 5000 K and 1200 bar, respectively (Bernstein et al. 1996).

On account of the cavitation effect, HIU has been frequently applied in depolymerizing carbohydrates (Price et al. 1995). Basically, the induced high temperature can result in thermolysis and the existed mechanical forces is capable in inducing hydrolysis and cleavage of a wide range of polysaccharides (Kardos et al. 2001). For example, by applying HIU, starch with shorter molecular chains was obtained (Tomasik et al. 1995). After treated by HIU, it was found that pectin can result in less stronger gel due to the reduction of molecular chain length (Seshadri et al. 2002). Chitosan, as an important material in biomedical applications, has been reported to be depolymerized via HIU for the study of the influence of HIU on its molecular weight and degree of acetylation (DA), two governed characteristics of chitosan polymer properties (Baxter et al. 2005). By using chitosan with an initial DDA of around 81% (DA, ~19%) and an average molecular weight (M_w) of 880 kDa, acidic chitosan solution was prepared and studied by using HIU. Characterizations were carried out and it was concluded that after HIU

treatment, the DA of chitosan stayed the same while its M_w was reduced. It was therefore demonstrated that the application of HIU on chitosan is an easy and promising method to control and to tailor polymer properties.

In addition, a recent study revealed the influence of HIU on chitosan solution under base environment, in comparison with acidic environment (Wang et al. 2016). Chitosan with different DDA and dynamic viscosity were prepared into solution with different pH and HIU treatment was applied under varied durations and amplitudes. It was discovered that at pH 6.5, chitosan agglomerates were broken by HIU and self-assembled into compacted chitosan nanoparticles. As discussed in the previous session, chitosan can be protonated and therefore dissolvable when the pH of environment is lower than its pKa but deprotonated and insoluble in water when it is beyond its pKa. Aside from that, neither its surface charge nor its DDA was changed due to the application of HIU under varied conditions, which further indicated that chitosan with different morphology under different pH can be depolymerized by HIU effectively with its other properties remained unchanged. It is therefore hypothesized that insoluble HIU-treated chitosan colloids are potential candidates in preparing soft material-based Pickering emulsions with smaller droplet sizes.

2.4.1.2 Dispersion of Emulsions via HIU

Although HIU has been extensively applied in emulsion dispersions (Abbas et al. 2013), few studies have been reported by using it for the fabrication of systems without the use of molecular surfactants and crosslinkers.

Different types of oil/water emulsions stabilized by surfactants had been reported to study the influence of different conditions of HIU on emulsion formations (Gaikwad et al. 2008). Relevant studies had been conducted, such as using oil with increased density (soybean, groundnut and paraffin) and applied them as oil phases for investigations. It was observed that by increasing the ultrasonication power and duration, the size of the produced micro-droplets based on different oil phase could be effectively decreased. Additionally, in spite of the conditions of HIU, the oil phase with higher density was revealed to enable the formation of larger size of emulsion droplets. This could be contributed to its higher viscosity and interfacial tension and therefore under the same conditions of HIU, emulsion droplets with bigger size were obtained. Recently, a study on HIU assisted chitosan-based Pickering emulsions was reported (Wang et al. 2016). At different oil/water volume ratio, it was demonstrated that emulsion droplets with sizes around 2 μm could be collected under pH 6.5. In addition, under pH 6.5, the HIU-treated Pickering emulsions were capable to maintain stable for over 5 months. Although the droplet size is still on micro-scale and its size distribution is not clearly studied, in comparison with other studies based on soft material-stabilized Pickering

emulsions that are homogenized by simple hand-shaking(Chaoyang et al. 2008;Liu et al. 2012), this further indicates that the application of HIU is effective in dispersing not only conventional but also Pickering emulsions.

HIU has been currently recognized as one of homogenization techniques that could offer several desirable advantages: (i) emulsions with smaller droplet size, (ii) providing energy-efficient and facile process, (iii) low production cost and (iv) less chances of contamination (Abbas et al. 2013). Based on these previous studies, it is hypothesized that HIU can become an effective technique in assisting chitosan-based Pickering emulsions for facilely preparing biodegradable nanospheres with narrow size distribution to overcome the current limitations of DDSs.

Chapter 3 Fabrication and Characterization of PLGA-CS by HIU-assisted Pickering Emulsions

3.1 Materials

As discussed and illustrated in session 2.2.1.1 and 2.2.1.2, PLGA and chitosan are both commonly applied biodegradable polymers for not only experimental but also commercial use. Besides, as chitosan is a kind of typical polymers with pendent amino groups that can undergo reverse protonation and deprotonation process, it is therefore suitable to be chosen as the representative soft colloid as stabilizers in the demonstration of the feasibility of the proposed method.

In this study, chitosan with M_w of 50,000 and 500,000 (DDA $\geq$ 95%) and with Mw of 3000 to 6000 (DDA $\geq$ 90%) were purchased from HeFei BoMei Biotechnology (China). PLGA 50:50 (M_w = 50,000) was obtained from Jinan Daigang Bio-Technology (China), ibuprofen was purchased from Wuhan Biocar Bio-Pharmaceutical, dichloromethane (CH_2Cl_2) was purchased from Sigma Aldrich, acetic acid (AA) and sodium hydroxide (NaOH) were supplied by the International Laboratory (United States), and ethanol was purchased from Merck. All chemicals and reagents were of analytical grade and used without further purification. The water used in all experiments was purified by deionization and filtration with a Millipore purification apparatus to a resistivity greater than 18.0 MΩ cm.

3.2 Fabrication of PLGA-CS by HIU-assisted Pickering Emulsions

3.2.1 Preparation of CS Colloids

Chitosan colloids were prepared by dissolving the chitosan powder in a 1% (v/v) aqueous solution of AA to form chitosan solutions with magnetic stirring at a rate of 600 rpm for 12 hours and stored overnight to allow complete hydration and dissolution. The pH of the chitosan solutions was adjusted to 6.5 with NaOH solutions (1 M) and stirred at 600 rpm for 15 minutes. HIU-treated chitosan solutions at a pH of 6.5 were produced with a high-intensity ultrasonic processor (SKL-250W, Ningbo HaiShu Sklon Electronic Instrument Co) equipped with an ultrasonic probe (SKL-IIN, φ 6), under 40% amplitude for 8 minutes, using an ice bath to avoid overheating during the ultrasonication (Figure 6). The concentrations of the prepared chitosan colloidal solutions were 0.1% w/v. Unless otherwise specified, all chitosan colloids were prepared under the same conditions, and the HIU treatments were conducted with the same equipment as described above.

3.2.2 Preparation of CS Colloid-Stabilized Pickering Oil-in-Water (O/W) Emulsions

Oil-in-water (O/W) emulsions stabilized by HIU-treated chitosan colloidal particles were prepared by mixing 0.1% w/v chitosan colloidal solution with 2% w/w PLGA/CH_2Cl_2 at an oil volume fraction (Φ) of 0.05, with magnetic stirring for 15

minutes at 600 rpm. The prepared coarse emulsions were homogenised with a high-intensity ultrasonic processor at different amplitudes (20%, 40% and 60%) for 8 minutes in an ice bath to form fine emulsions. A schematic illustration is shown in Figure 4.

3.2.3 Fabrication of Monodisperse PLGA-CS Core-Shell Composite Nanospheres with Tunable Sizes

The prepared HIU-treated emulsions were stirred at 600 rpm for 3 hours in a 37°C water bath to remove volatile solvents. The resulting particles were collected after suspension and purification with at least three centrifugation-redispersion cycles in deionised water. Each centrifugation was conducted at 12,000 rpm for 10 minutes. Drug-loaded particles were prepared by the same method except that PLGA/CH_2Cl_2 solution containing the model drug (ibuprofen, a standard anti-inflammatory drug; 3% w/w) was used. A 1% (v/v) aqueous solution of AA was used to treat and dissolve the chitosan coating from the resulting particles. The treated particles were collected after purification with three centrifugation-redispersion cycles in deionised water and each centrifugation was conducted at 12,000 rpm for 10 minutes.

3.3 Characterizations of PLGA-CS Composite Nanospheres

3.3.1 Particle Size Determination

To understand the influence of different applied amplitude of HIU and different molecular weight of materials on the resulting particle size and size distribution, investigation of determination of particle size was conducted.

The hydrodynamic particle size and polydispersity were determined using Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The Stokes-Einstein equation was used to calculate the hydrodynamic diameters from the diffusion coefficient. Relevant analyses were conducted with software supplied by the manufacturer. The polydispersity was derived from the cumulant analysis method. Each measurement was performed in triplicate, and the obtained values were averaged to obtain a mean value.

3.3.2 Zeta Potential Measurement

To investigate the surface potential of the resulting nanosphere, zeta potential measurement was carried out in this study.

The zeta-potentials were measured at 25°C using Zetasizer Nano ZS. Three measurements were performed for each sample.

3.3.3 Fourier Transform Infrared Spectrometry (FTIR)

To confirm the compositional information of the nanoparticles, a Fourier transform infrared spectrometry (Spectrum 100T, PerkinElmer) was used.

The Fourier transform infrared spectra of the samples were measured with the KBr pellet method and taken from 4000 to 400 cm^{-1}.

3.3.4 Scanning Electron Microscopy (SEM)

To observe the size and structure of the samples obtained by adopting the proposed method, a scanning electron microscopy SEM (TESCAN VEGA3) was applied.

The specimens were prepared by first dispersing the prepared nanoparticles, dropping the aqueous suspension onto copper grids for solvent evaporation at room temperature and then being sputter-coated with gold.

3.3.5 Determination of Loading Capacity and Encapsulation Efficiency of Model Drug

In order to investigate the ability of acting as drug carrier of the resulting particles, their loading capacity and encapsulation efficiency of model drug (ibuprofen) were studied.

The supernatants of the first cycle of purification after solvent evaporation were collected, and their concentrations and volumes were used to estimate the amount of ibuprofen in PLGA-CS. The quantity of non-encapsulated ibuprofen was estimated on the basis of the fluorescence intensity at 272.5 nm of the supernatant solution as measured with an ultraviolet and visible (UV-vis) spectrophotometer (UV11, Shanghai Techcomp Instrument Ltd.). The loaded content was determined from the calibration curve, which was established from standard solutions of ibuprofen in deionised water (pH 6.5). Each experiment was repeated three times, and average values were calculated with the following formulas:

$$\text{Drug loading capacity (DL \%)} = \left(\frac{weight\ of\ drug\ in\ particles}{weight\ of\ particles}\right) \times\ 100\% \qquad (1)$$

$$\text{Encapsulation efficiency (EE \%)} = \left(\frac{weight\ of\ drug\ in\ particles}{weight\ of\ feed\ drug}\right) \times\ 100\% \qquad (2)$$

3.3.6 In vitro release

To understand the drug release behaviour of the resulting systems, studies on in vitro model drug release were carried out.

In fact, most of currently existing cancer drugs are hydrophobic (Popat et al. 2013). As one of the most commonly and frequently used standard clinical anti-inflammatory hydrophobic drugs (Harris et al. 2005), ibuprofen was selected to be the model drug. In view of its representativeness, it is therefore advantageous to use ibuprofen as a hydrophobic model drug. But since it is a kind of ionisable drugs, the dissolution of

ibuprofen in aqueous environment need the control of not only pH values but also additional sample treatments to facilitate the dissolution, such as the use of ultrasonication on samples for UV analysis (Levis et al. 2003;Hu et al. 2017). The in vitro drug release experiment was carried out by dispersing a certain amount of ibuprofen–PLGA-CS into test tubes with 20 ml of phosphate-buffered saline solution at different pH values (pH 5 or 7.4) at different temperature (37°C or 60°C) using a gas bath shaker at 100 cycles per minute. In order to simulate the normal temperature of human, 37°C was selected as one of the test temperature. In comparison, to mimic the diseased conditions in tumour therapy, the extreme temperature that could be induced by thermal therapy, such as thermal-based ablation of tumours (Chu et al. 2014) and photothermal chemotherapy using gold nanoparticles (Cheng et al. 2010), 60°C was selected as another test temperature. Therefore, the release behaviour of the resulting systems under normal and extreme therapeutic temperature can be learned. Besides, according to the temperature accuracy of the used gas bath shaker, the experimental temperature may be floatable within 0.5°C. Specifically, the pH values were selected in order to simulate the microenvironment of tumour (pH 5) and the intestinal juice of human (pH 7.4).

At a predetermined time, 3 ml of the nanoparticle suspensions were extracted and centrifuged at 12,000 rpm for 10 minutes, and the supernatants were withdrawn from each sample for immediate analysis of the drug concentration with a UV-vis

spectrophotometer at 272.5 nm. After the UV analysis, the same volume of the resuspension was poured back into the corresponding test tube. The relationship between the fluorescence intensity and the concentration of ibuprofen is linear to the calibration curve established from standard solutions of ibuprofen in phosphate-buffered saline solution.

Chapter 4 Results and Discussions

4.1 Influence of Initial Molecular weight of Chitosan Colloids on Particle Sizes and Surface potential

As discussed in session 2.2.1.2 and session 2.3.3, in conventional emulsions, chitosan itself is not a good emulsifier. Nevertheless, in Pickering emulsions, chitosan colloids formed via self-aggregation that induced by simply varying the pH value (as shown in Figure 5) is recently studied as effective stabilizers due to its tunable solubility. As illustrated in session 2.2.1.2, when the pH value is higher that its pKa, soluble chitosan will turn into chitosan colloids, which are insoluble neither in water phase or oil phase.

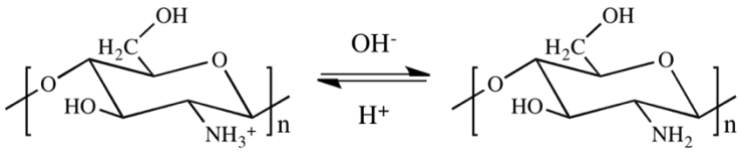

Figure 5 The reaction scheme of chitosan with the changes of pH

One of the dominant factor of the resulting system size is the size of the stabilizer in Pickering emulsions. Specifically, the application of HIU in this study is expected to conduct polymer depolymerization for reducing the size of the stabilizers, as shown in Figure 6. Therefore, in order to investigate the influence of the initial M_w of HIU-treated chitosan colloids on the resulting systems, stabilizers based on different initial M_w were prepared and results were collected for discussion.

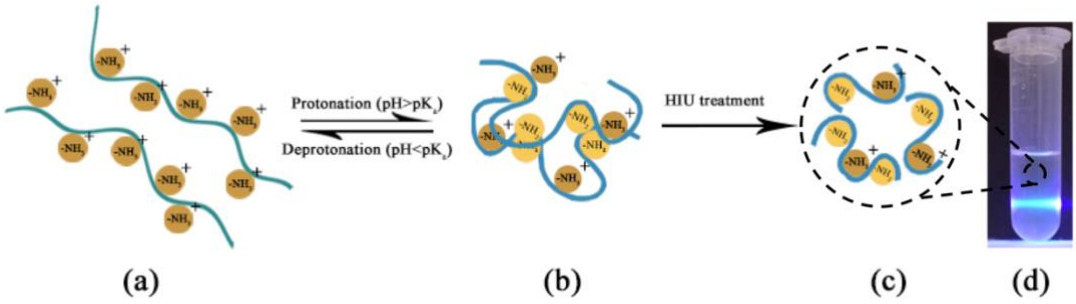

Figure 6 Schematic illustration of (a) dissolved chitosan molecules under deprotonation, (b) self-aggregated chitosan colloids obtained from protonation and (c) depolymerized chitosan colloids resulting from HIU-treatment. Photo (d) shows that the Tyndall scattering effect confirms the presence of chitosan colloids

Figure 7 illustrates the resulting particle sizes when stabilizers based on the same polymers with different initial M_w were applied under the same HIU amplitude (40%). Notably, the size of the PLGA-CS increases as the initial M_w of the chitosan increases. This result further confirms the leading role of the size of the stabilizers in the relationship between the size of the resulting systems.

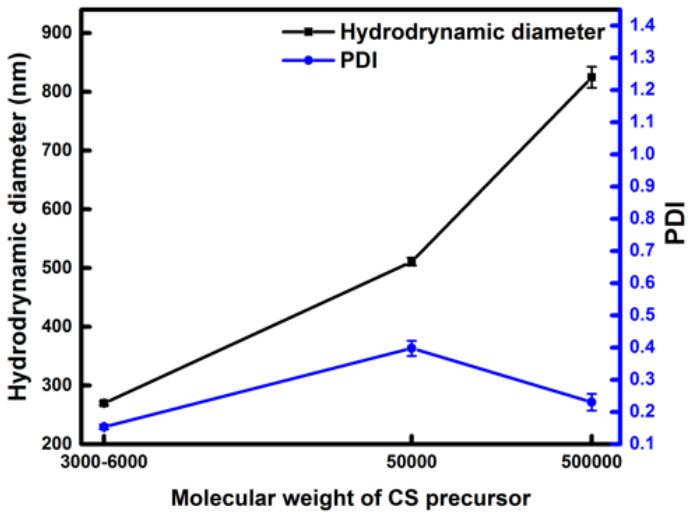

Figure 7 Hydrodynamic diameter and polydispersity of PLGA nanoparticles coated by chitosan colloids based on chitosan with different M_w

Moreover, it can be observed that the prepared particles had a high degree of monodispersity (i.e., low polydispersity), which can be attributed to the HIU treatment. HIU is a technique that generate transient cavitational thermolysis based on the propagation of high-intensity ultrasound (Portenlänger et al. 1994;Cains et al. 1998;Baxter et al. 2005;Wu et al. 2008;Wang et al. 2016). In fact, high-intensity ultrasound has proven its potential in pharmaceutical and biological applications for homogenising emulsions and depolymerising carbohydrates (Price et al. 1995;Mason et al. 1996). With such basis, the application of HIU on treating chitosan colloids enable the stabilizers to work with lower M_w. With low M_w, such stabilizer can capacitate a lower surface tension for the prepared Pickering emulsions due to their relatively lower steric hindrance, entanglements and intermolecular hydrogen-bond interaction, compare to stabilizers with high M_w (Schulz et al. 1998). More importantly, in Pickering emulsions, as the size of resulting systems is majorly dominant by the size of stabilizers, the collection of nanoscale systems can be hence attributed to the use of depolymerized stabilizers that are resulted by the use of HIU.

Besides, as the depolymerized chitosan colloids can lead to a reduction of the emulsions droplet sizes. according to Stokes' law, a reduction in the droplet size would lead to an increase in the emulsion stability since the creaming rate of an emulsion is proportional to the square of the droplet diameter (Brugger et al. 2008). As the elevation of emulsion

stability lay the foundation of good dispersibility of emulsion droplets, in combination with the size reduction of droplets, which are both induced by HIU, the particle sizes were therefore resulted in nano-scale sizes with narrow size distributions. It can hence be further controlled by altering the conditions of the applied HIU and the adopted Mw of stabilizers.

Aside from the particle size, it is also important for the systems to maintain a certain level of surface potential, as discussed in session 2.2.2.2. In this study, the surface potential of the system is mainly dominant by the one layer coating of chitosan, which is constructed by the adsorbed chitosan colloids during Pickering emulsion stabilization. Furthermore, the ζ potential of stabilizers can be altered after deprotonation as well as the depolymerisation of chitosan, in which pH plays an important role. Therefore, to investigate the influence of both the M_w and processing condition, i.e. the applied amplitude on the surface potential of the resulting particles, a comparison of ζ potential between the protonated chitosan and the surfaces of the resulting systems was conducted.

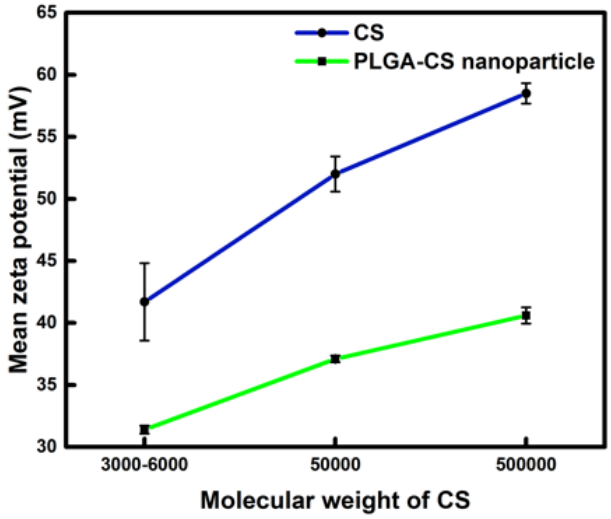

Figure 8 ζ potential of chitosan with different M_w as precursors under pH of 4.5 and PLGA-CS nanoparticles coated by chitosan colloids based on chitosan with different M_w

Figure 8 clearly shows that when chitosan was dissolved at pH 4.5, the ζ potential was highly positive (over 40 mV). On the other hand, under the same condition of HIU treatment (40% of amplitude), by using different stabilizers with corresponding initial M_w, the surface potential of the resulting PLGA-CS decreased by approximate 30 %. But still, this result indicates that the resulting surface of the systems maintained a good positive charge. The decrease of the ζ potential may be attributed to the protonation-deprotonation process of chitosan. Because the preparation of insoluble chitosan colloids is based on the self-agglomeration of chitosan molecules that are induced by the deprotonation of the soluble chitosan molecules. Besides, another reason that it is necessary to ensure a certain level of surface potential of the resulting systems is that the stability of a Pickering emulsion can be controlled by the surface charges of the stabilizers. As the same charges are repellent, the affinity of each emulsion droplet can

be therefore lowered to avoid droplet coalescence, which base the establishment of stable emulsions (Brugger et al. 2008).

Therefore, in summary, the depolymerized chitosan colloids with good positive charge enable them to become an effective biodegradable stabilizer for the preparation of stable O/W Pickering emulsions, which are suitable for the preparation of solid nano-sized particulate DDSs.

4.2 Influence of Amplitude of HIU on Particle Sizes and Monodispersity

After confirming the feasibility of the proposed fabrication method for producing nano-sized systems, compositional information of the resulting systems was thereafter collected. The Fourier transform infrared spectra of the pure chitosan, PLGA and ibuprofen are shown in Figure 9. The 3448 cm^{-1} peak is the characteristic adsorption peak of the hydroxyl groups of chitosan (Figure 9, Curve a). The representative absorbance of the carboxyl groups of PLGA can be clearly observed at 1760 cm^{-1} (Figure 9, Curve b). In addition, the peak at 1721 cm^{-1} is ascribed to the infrared absorption of the carbonyl groups of ibuprofen (Matkovic et al. 2005) (Figure 9, Curve c). Based on these results, it can be confirmed that the chitosan colloids were successfully coated on the PLGA with the loading of the model drug (ibuprofen) onto the PLGA-matrix (Figure 9, Curve d), in contrast to the pure PLGA-CS (Figure 9, Curve e).

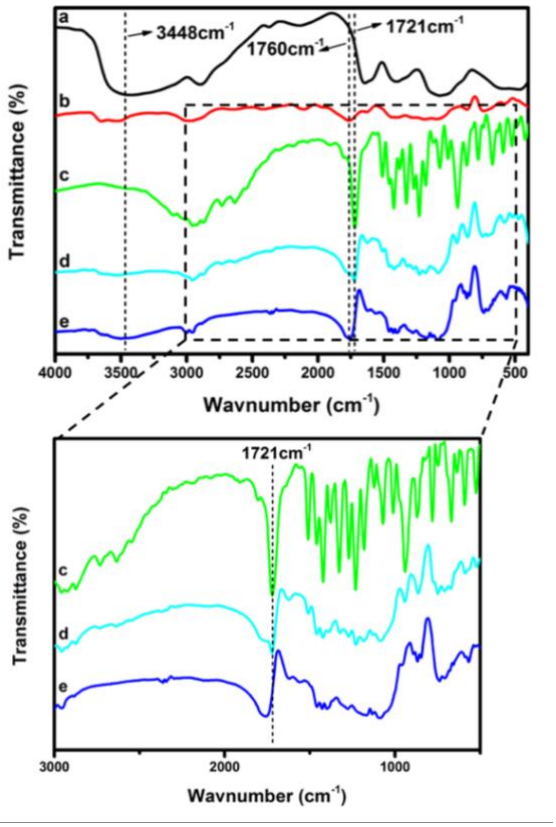

Figure 9 Fourier transform infrared spectra of (a) pure chitosan, (b) pure PLGA, (c) pure ibuprofen, (d) ibuprofen–PLGA-CS and (e) PLGA-CS

To investigate the influence of the processing condition, i.e. the amplitude of the applied HIU on the control of the resulting size of PLGA-CS, different HIU amplitudes (20%, 40% and 60%) were applied to fabricate PLGA-CS using chitosan colloids based on precursor with the same M_w of 3000 to 6000 as stabilizers. Because as revealed in session 4.1, the lower the molecular weight of the used stabilizers, the more stable the emulsions, which is beneficial for the carry out of solvent evaporation to obtain solid systems.

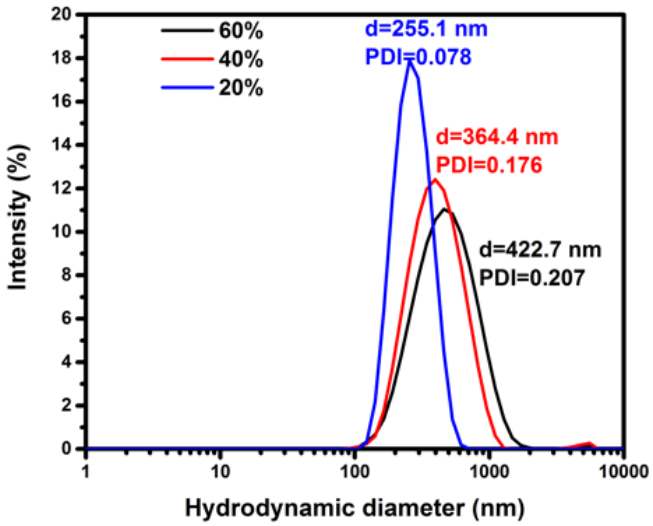

Figure 10 Hydrodynamic diameter and polydispersity of PLGA-CS nanoparticles coated by chitosan colloids based on chitosan with M_w of 3000 to 6000 as precursor under different HIU amplitudes

As shown in Figure 10, PLGA-CS with different sizes and polydispersities (i.e., 255 nm and 0.078 for 20%; 364 nm and 0.176 for 40%; and 422 nm and 0.207 for 60%) were obtained. This result further evidence that the incorporation of HIU treatment with soft colloid-stabilized Pickering emulsions can greatly reduce the resulting size of systems as compared with current existing Pickering emulsion-based method for the preparation of PLGA particles, which have a typical size range of 1 to 40 μm (Wei et al. 2012a;Liu et al. 2014;Wu et al. 2016). In fact, aside from material characteristics that we have discussed in the last chapter, one of the reason that the nano-scale systems can be obtained is that nano-sized emulsion droplets can be created by the high level of energy supplied from HIU. HIU has been demonstrated to be capable in overcoming the oil–water surface free energy and increasing the interfacial area of the two phases (Tadros et al. 2004;Ashokkumar 2011). As expressed in Equation 1, the required shear

energy, which is represented as Laplace pressure (p), for the continuous phase to break the droplets of the dispersed phase, is proportional to the interfacial tension (γ) of droplets but inversely proportional to the radius (R) of the curvature of ideally spherical droplets.

$$p = \frac{\gamma}{2R} \quad (3)$$

It has been reported that the use of soft gels as emulsion stabilisers, at a given concentration, leads to a decrease in the typical dynamic interfacial tensions (γ_t) as a function of time with the spontaneous adsorption of gels (Li et al. 2015). Therefore, according to equation 1, at a given amplitude of HIU, with the decrease of γ_t, the radius of the droplets would display a downward trend. The resulting well-dispersed nano-droplets could thereafter lead to the formation of solid particles with smaller sizes and high level of monodispersity. This is in agreement with our results.

Additionally, from Figure 10, another phenomenon can be observed is that, by using stabilizers with the same initial M_w, the lower the applied amplitude, the lower the polydispersity of the resulting solid system. In fact, despite the good stability of Pickering emulsions, mass transformation can still be induced by so-called Ostwald ripening between the droplets and the continuous phase (Binks et al. 2010;Whitby et al. 2016). As a result, when a higher input power was used, the small droplets gave rise easily to limited coalescence due to Ostwald ripening and thus lead to an increase in

droplet size (Neuhäusler et al. 1999;Dickinson 2010). When the Pickering emulsion is restored from such a ripening process and remains stable, compared to other Pickering emulsions with a higher energy input, the particles obtained with a relatively lower energy input were smaller with narrower size distribution.

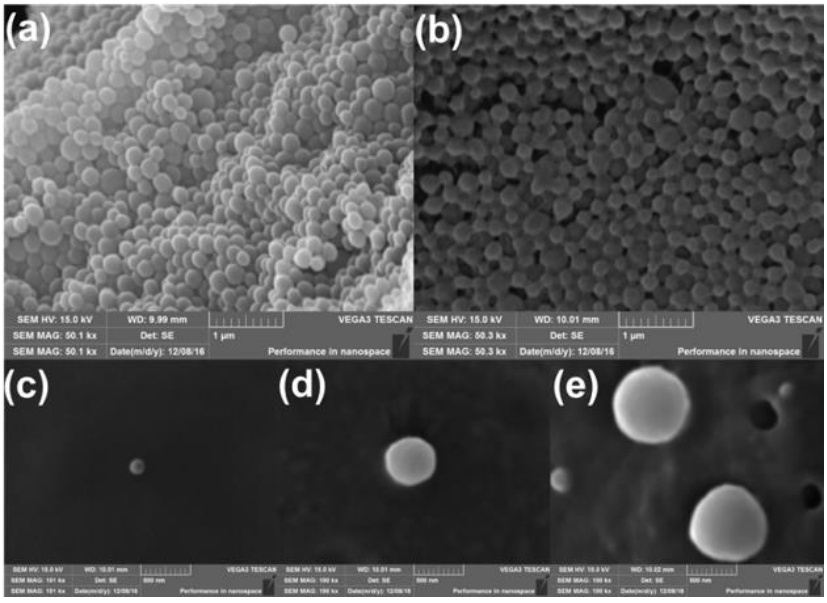

Figure 11 SEM images of PLGA nanoparticle coated by different chitosan colloids based on chitosan precursors with Mw of 3000 to 6000 (a, b, c), 50,000 (d) and 500,000 (e), where (c) was treated with 0.1% (v/v) AA solution

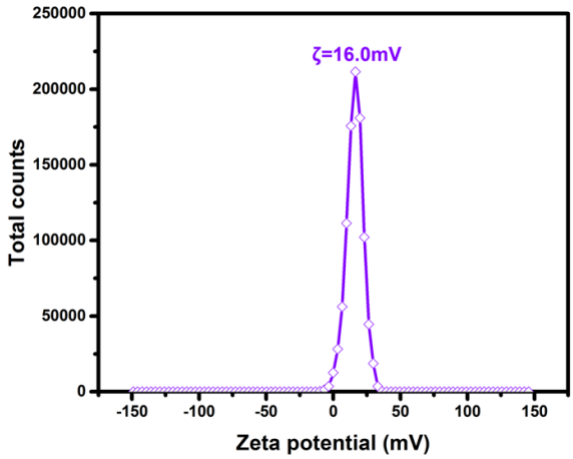

Figure 12 Zeta potential of AA-treated PLGA-CS coated by chitosan with Mw of 3000 – 6000 as precursor

The morphology of the ibuprofen-loaded PLGA-CS is shown in Figure 11. Figure 11 (a) reveals clearly the good monodispersity of the prepared particles and shows that most of the solid nanoparticles (fabricated from chitosan colloids based on chitosan with M_w from 3000 to 6000) were spherical with an average diameter of approximately 200 nm. In order to further confirm the existence of the coating chitosan layer, the obtained particles were thereafter treated with a 1% (v/v) AA solution, and the morphology of the PLGA-CS is presented in Figure 11 (b). As shown in Figure 12, the ζ potential of the surface of the treated PLGA-CS indicates the loss in the positive charge of the coating from 30 mV to 16 mV. Besides, it can be observed that the treated PLGA-CS appeared to adhere to each other due to the dissolution of chitosan. Observations of particles resulting from different chitosan precursors with M_w of 3000 to 6000, 50,000 and 500,000 are illustrated in Figure 11 (c), (d) and (e), respectively, which further supports the relevant Dynamic Light Scattering (DLS) results of the particle sizes. Furthermore, the particle size observed with SEM is smaller than the hydrodynamic particle size obtained from the DLS test because the DLS test is used to measure the hydrodynamic diameter, whereas the SEM image shows only the dry state of particles. It is worth mentioning that due to the softness of the chitosan self-aggregates prepared via protonation-deprotonation, they might not appear as spherical solid particles at the adopted interface (Liu et al. 2012;Wei et al. 2012b). Deformation and structural rearrangement of chitosan occurs due to the chain adsorption and

interactions between polymers, which therefore prevents them from being recognised by their common shape (Dickinson 2015). Hence, based on the results, the proposed strategy of a combination of Pickering emulsion and solvent evaporation with HIU is practical to produce monodisperse rigid polymeric core-shell spherical systems with tunable size on the nano-scale and without the use of undesired chemicals based on a solid hydrophobic core stabilised with walls of self-assembled colloids.

An investigation of the influence of different applied amplitudes on the surface potential of the resulting nanospheres based on chitosan colloids with the same M_w was carried out. Figure 13 shows the results of the ζ potential measurements of ibuprofen–PLGA-CS resulting from different amplitudes based on the use of HIU-treated chitosan precursors with M_w of 3000 to 6000. As observed, their surface potential remains positive around 35 mV, which indicate that different applied amplitude on the preparation of Pickering emulsions has little impact on changing the surface charge of the resulting systems. Moreover, to assess the stability of the prepared PLGA-CS nanospheres in aqueous suspensions, the size distributions and surface charges of a batch of particles (prepared under 20% amplitude by using chitosan with M_w of 3000 to 6000 as precursor) that had been stored for more than 90 days at ambient temperature, were monitored. As shown in Figure 14, the ζ potential and size are retained as compared with the data shown in Figures 8 and 10, respectively, which suggest a high colloidal stability of the prepared PLGA-CS.

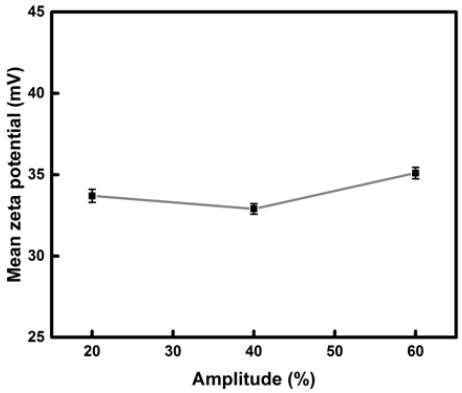

Figure 13 ζ potential of solid PLGA nanoparticle coated by chitosan with M_w of 3000 to 6000 as precursor under different amplitudes

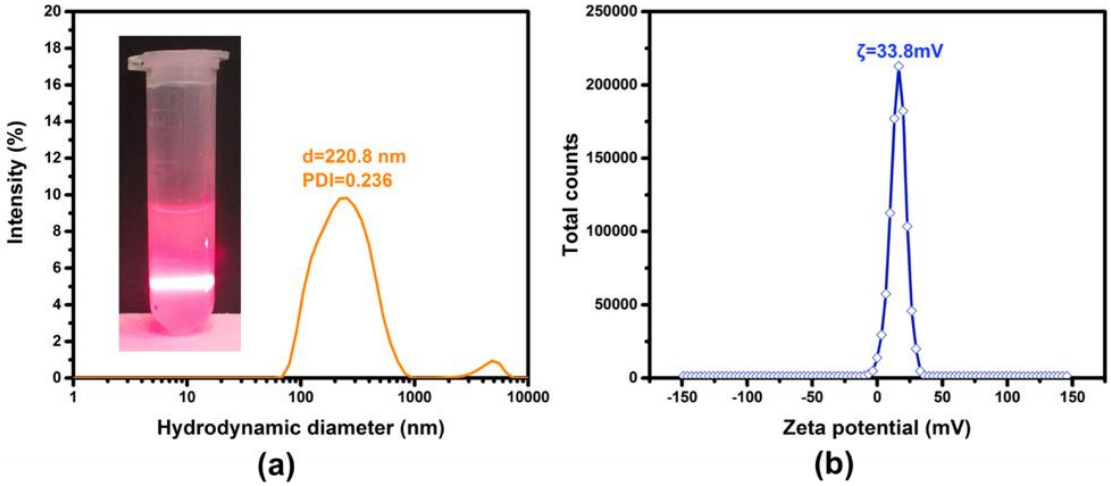

Figure 14 Size distribution (a) over 90 days of storage under ambient temperature in aqueous suspension of solid PLGA nanoparticles coated by chitosan with M_w of 3000 to 6000 as precursor with photo showing Tyndell effect by illuminating with a laser beam and its zeta potential (b)

4.3 Loading and In Vitro Drug Release Study

Ibuprofen–PLGA-CS prepared under 20% amplitude and from the use of HIU-treated chitosan with M_w of 3000 to 6000 as precursors, were used to study the drug release behaviour. Ibuprofen possesses poor water-solubility and is one of the most broadly

used standard clinical anti-inflammatory drugs. In this study, ibuprofen was selected as a model drug and loaded into the particulate systems. To determine the DL and EE of the model drug in the nanocomposites, the differences between the total amount and the supernatants before and after the washing process were measured and compared by using UV-vis spectrometry. Ibuprofen–PLGA-CS with a DL of 13.8% and an EE of 59.6%, with a DL of 28.2% and an EE of 75.1% and with a DL of 16.3% and an EE of 70.4% were used to study drug release under the conditions of pH 7.4 at 37°C and pH 5 at 37°C and 60°C.

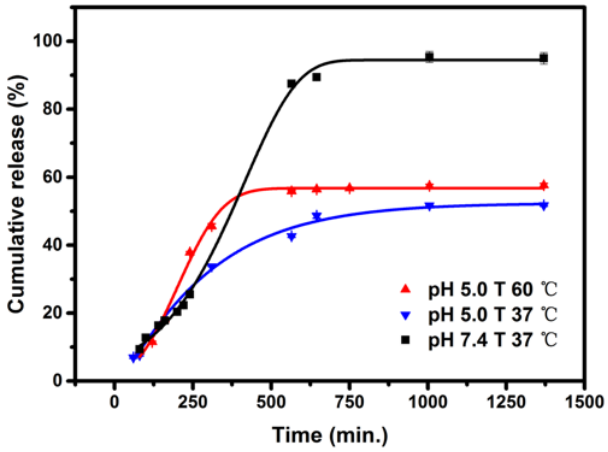

Figure 15 Cumulative model drug release from PLGA-CS nanoparticle under pH 5.0 at 60°C and 37°C and pH 7.4 at 37°C

The release characteristics of ibuprofen from the PLGA-CS at different temperature as a function of time are shown in Figure 15; each point on the graph represents the average of three tests. The release behaviour of the resulting DDS was investigated by simulating the intracellular environment (Chu et al. 2014). The diagram clearly reveals that at the same pH, ibuprofen–PLGA-CS exhibited a much higher release rate at higher

temperatures. At elevated temperatures, the rapid release of ibuprofen from the nanocomposites can be rationalised in terms of the accelerated decomposition of the consistent biodegradable polymers. At 60°C (pH 5), a rapid release in about 565 minutes occurred during the initial stage and a slow release to a platform with a maximum release of 56.4%. In contrast, the drug release at 37°C (pH 5) showed a different release tendency, and it took around 1000 minutes to reach a stable release (with a maximum release of 51.7%). Under both conditions, the model drug continued to be released from the resulting PLGA-CS nano-particulate systems for more than 1000 minutes. It therefore indicates that, the drug release behaviour of the resulting systems can be controlled under different temperature.

The release rate was slightly affected by increasing the pH from 5 to 7.4 at the same temperature (37°C). As shown in Figure 15, the release rate of ibuprofen at pH 7.4 is slightly faster than that at pH 5. It is noted that the accumulated release reached 94.9% at a higher pH because the solubility of ibuprofen is greatly affected by pH due to the protonation-deprotonation process (Wang et al. 2010). The greater solubility of ibuprofen at higher pH (i.e., 7.4) created a larger concentration gradient that resulted in faster release. The results suggest that the release mechanism of IBU could be mainly due to the bulk erosion of the PLGA core. Nevertheless, to tailor the release profile of a drug, the properties of the coated shells (e.g., thickness, pore size and degradation

rate) can play important roles in addition to offering colloidal stability to the DDS. Therefore, future study of a more sophisticated design is needed (Abend et al. 1998).

Chapter 5 Conclusions and Statement of Originality

5.1 Overall conclusions

The objectives in this study were addressed by (i) designing and proposing the modified Pickering emulsions, i.e. HIU-assisted chitosan-based Pickering emulsion and solvent evaporation, (ii) demonstrating the feasibility of the proposed method by successfully fabricating monodisperse nanoshperes (PLGA-CS) and (iii) investigating the effect of the applied material characteristics of stabilizers and the processing conditions of HIU for the resulting drug-loaded systems. It was found that the introduction of the HIU technique enabled the control of size range within nanometer-scale and achieving a narrow size distribution of the resulting core-shell–structured particulate systems. The prepared ibuprofen–PLGA-CS exhibited different release behaviours of the model drug at different temperatures and pH levels. The requirements for the design of a nano-sized particulate delivery system were met in terms of tunable sizes, enhanced solubility and good potential for controlled and targeted delivery, as demonstrated. The proposed method therefore holds promising potential for the fabrication of DDSs for controlled drug release.

5.2 Originality and contributions of the research work

To our best knowledge, this is amongst the first study to fabricate monodisperse nanospheres composed of biodegradable polymers via a modified Pickering emulsion-

based method. This method offers the main advantages of (i) eliminating the use of molecular surfactants and crosslinkers by adopting Pickering emulsions, (ii) enabling a facile fabrication of nano-scale systems and (iii) getting rid of the use of bulky or custom-made auxiliary equipment for fabricating systems with high monodispersity. It is capable of preparing systems with controllable particle size on nano-scale with narrow size distribution in the range of 255 nm to 830 nm.

Without using molecular surfactants or crosslinkers, systems produced via the proposed method may have a good potential for being applied in a variety of biomedical applications, such as the *in vivo* drug delivery for living cells or animals because all commonly used surfactants are toxic to a certain extend and systems fabricated from conventional surfactant-based methods may not be applicable.

5.3 Suggestions for future work

The release of drug from PLGA usually suffers from burst release at the initial stage. In fact, such burst release behavior cannot be eliminated by adopting the proposed method. As it has been shown that the release of model drug from the as-prepared PLGA-CS exhibits a typical two-stage release, which has been identified as a major hurdle for the present formulation approach to overcome. Besides, for the encapsulation of an environmental sensitive drug such as protein, whose nature could be affected due to the presence of high intensity ultrasonication, may be difficult by adopting the

proposed method, may be difficult by adopting the proposed method. The presence of high intensity ultrasonication does not only induce depolymerization for materials, but also generate heat during its operation. To circumvent such problem, the process conditions may need to be modified.

Therefore, future work could be concentrated on overcoming the above-mentioned limitations. By studying the mechanism of drug release from PLGA and taking the advantages of PLGA-CS, improvement of the proposed method may be made to formulate layer-coated systems for providing viable regulation of its release profile and protection of the encapsulate materials. Benefits including the realization of facile fabrication of DDSs with the ability of controlled release of a wide range of drugs may be resulted.

References

Abbas S, Hayat K, Karangwa E, Bashari M , Zhang X, 2013, "An Overview of Ultrasound-Assisted Food-Grade Nanoemulsions", *Food Engineering Reviews,* vol. 5, no. 3, pp. 139-157.

Abend S, Bonnke N, Gutschner U , Lagaly G, 1998, "Stabilization of emulsions by heterocoagulation of clay minerals and layered double hydroxides", *Colloid and Polymer Science,* vol. 276, no. 8, pp. 730-737.

Abouelmagd SA, Ku YJ , Yeo Y, 2015, "Low molecular weight chitosan-coated polymeric nanoparticles for sustained and pH-sensitive delivery of paclitaxel", *Journal of Drug Targeting,* vol. 23, no. 7-8, pp. 725-735.

Alison L, Rühs PA, Tervoort E, Teleki A, Zanini M, Isa L , Studart AR, 2016, "Pickering and Network Stabilization of Biocompatible Emulsions Using Chitosan-Modified Silica Nanoparticles", *Langmuir,* vol. 32, no. 50, pp. 13446-13457.

Allen TM , Cullis PR, 2004, "Drug Delivery Systems: Entering the Mainstream", *Science,* vol. 303, no. 5665, pp. 1818-1822.

Arechabala B, Coiffard C, Rivalland P, Coiffard LJM , De Roeck-Holtzhauer Y, 1999, "Comparison of cytotoxicity of various surfactants tested on normal human fibroblast cultures using the neutral red test, MTT assay and LDH release", *Journal of Applied Toxicology,* vol. 19, no. 3, pp. 163-165.

Arunkumar N, Deecaraman M , Rani C, 2009, "Nanosuspension technology and its applications in drug delivery", *Asian Journal of Pharmaceutics,* vol. 3, no. 3, pp. 168-173.

Ashokkumar M, 2011, "The characterization of acoustic cavitation bubbles - An overview", *Ultrasonics Sonochemistry,* vol. 18, no. 4, pp. 864-872.

Astete CE , Sabliov CM, 2006, "Synthesis and characterization of PLGA nanoparticles", *Journal of Biomaterials Science, Polymer Edition,* vol. 17, no. 3, pp. 247-289.

Bala I, Hariharan S , Kumar MNVR, 2004, "PLGA nanoparticles in drug delivery: The state of the art", *Critical Reviews in Therapeutic Drug Carrier Systems,* vol. 21, no. 5, pp. 387-422.

Basu A, Kunduru KR, Abtew E , Domb AJ, 2015, "Polysaccharide-Based Conjugates for Biomedical Applications", *Bioconjugate Chemistry,* vol. 26, no. 8, pp. 1396-1412.

Baxter S, Zivanovic S , Weiss J, 2005, "Molecular weight and degree of acetylation of high-intensity ultrasonicated chitosan", *Food Hydrocolloids,* vol. 19, no. 5, pp. 821-830.

Bernstein LS, Zakin MR, Flint EB , Suslick KS, 1996, "Cavitation thermometry using molecular and continuum sonoluminescence", *Journal of Physical Chemistry,* vol. 100, no. 16, pp. 6612-6619.

Binks BP, Fletcher PDI, Holt BL, Kuc O, Beaussoubre P , Wong K, 2010, "Compositional ripening of particle- and surfactant-stabilised emulsions: A comparison", *Physical Chemistry Chemical Physics,* vol. 12, no. 9, pp. 2219-

2226.

Binks BP , Lumsdon SO, 1999, "Stability of oil-in-water emulsions stabilised by silica particles", *Physical Chemistry Chemical Physics,* vol. 1, no. 12, pp. 3007-3016.

Binks BP , Lumsdon SO, 2000a, "Catastrophic Phase Inversion of Water-in-Oil Emulsions Stabilized by Hydrophobic Silica", *Langmuir,* vol. 16, no. 6, pp. 2539-2547.

Binks BP , Lumsdon SO, 2000b, "Influence of Particle Wettability on the Type and Stability of Surfactant-Free Emulsions", *Langmuir,* vol. 16, no. 23, pp. 8622-8631.

Bouyer E, Mekhloufi G, Rosilio V, Grossiord JL , Agnely F, 2012, "Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: Alternatives to synthetic surfactants in the pharmaceutical field?", *International Journal of Pharmaceutics,* vol. 436, no. 1-2, pp. 359-378.

Brugger B , Richtering W, 2008, "Emulsions stabilized by stimuli-sensitive poly(N-isopropylacrylamide)-co- methacrylic acid polymers: Microgels versus low molecular weight polymers", *Langmuir,* vol. 24, no. 15, pp. 7769-7777.

Byrappa K, Ohara S , Adschiri T, 2008, "Nanoparticles synthesis using supercritical fluid technology - towards biomedical applications", *Advanced Drug Delivery Reviews,* vol. 60, no. 3, pp. 299-327.

Byrne JD, Betancourt T , Brannon-Peppas L, 2008, "Active targeting schemes for nanoparticle systems in cancer therapeutics", *Advanced Drug Delivery Reviews,* vol. 60, no. 15, pp. 1615-1626.

Cains PW, Martin PD , Price CJ, 1998, "The use of ultrasound in industrial chemical synthesis and crystallization. 1. Applications to synthetic chemistry", *Organic Process Research and Development,* vol. 2, no. 1, pp. 34-48.

Caldorera-Moore M, Guimard N, Shi L , Roy K, 2010, "Designer nanoparticles: Incorporating size, shape and triggered release into nanoscale drug carriers", *Expert Opinion on Drug Delivery,* vol. 7, no. 4, pp. 479-495.

Champion JA, Katare YK , Mitragotri S, 2007, "Particle shape: A new design parameter for micro- and nanoscale drug delivery carriers", *Journal of Controlled Release,* vol. 121, no. 1-2, pp. 3-9.

Chaoyang W, Shiqu Y, Qilong S, Chengyi H, Weihua Y, Xinxing L , Zhen T, 2008, "Microcapsules for controlled release fabricated via layer-by-layer self-assembly of polyelectrolytes", *Journal of Experimental Nanoscience,* vol. 3, no. 2, pp. 133-145.

Charcosset C, 2009, "Preparation of emulsions and particles by membrane emulsification for the food processing industry", *Journal of Food Engineering,* vol. 92, no. 3, pp. 241-249.

Chen C-K, Wang Q, Jones CH, Yu Y, Zhang H, Law W-C, Lai CK, Zeng Q, Prasad PN, Pfeifer BA , Cheng C, 2014, "Synthesis of pH-Responsive Chitosan Nanocapsules for the Controlled Delivery of Doxorubicin", *Langmuir,* vol. 30, no. 14, pp. 4111-4119.

Cheng F-Y, Su C-H, Wu P-C , Yeh C-S, 2010, "Multifunctional polymeric nanoparticles for combined chemotherapeutic and near-infrared photothermal cancer therapy in vitro and in vivo", *Chemical Communications,* vol. 46, no. 18, pp. 3167-3169.

Chevalier Y , Bolzinger MA, 2013, "Emulsions stabilized with solid nanoparticles: Pickering emulsions", *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* vol. 439, no. pp. 23-34.

Chronopoulou L, Massimi M, Giardi MF, Cametti C, Devirgiliis LC, Dentini M , Palocci C, 2013, "Chitosan-coated PLGA nanoparticles: a sustained drug release strategy for cell cultures", *Colloids Surf B Biointerfaces,* vol. 103, no. pp. 310-7.

Chu KF , Dupuy DE, 2014, "Thermal ablation of tumours: biological mechanisms and advances in therapy", *Nature Reviews Cancer,* vol. 14, no. 3, pp. 199-208.

Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A , Preat V, 2012, "PLGA-based nanoparticles: an overview of biomedical applications", *Journal of Controlled Release,* vol. 161, no. 2, pp. 505-22.

De Geest BG, Sukhorukov GB , Möhwald H, 2009, "The pros and cons of polyelectrolyte capsules in drug delivery", *Expert Opinion on Drug Delivery,* vol. 6, no. 6, pp. 613-624.

De Jong WH , Borm PJA, 2008, "Drug delivery and nanoparticles: Applications and hazards", *International Journal of Nanomedicine,* vol. 3, no. 2, pp. 133-149.

Destribats M, Eyharts M, Lapeyre V, Sellier E, Varga I, Ravaine V , Schmitt V, 2014a, "Impact of pNIPAM Microgel Size on Its Ability To Stabilize Pickering Emulsions", *Langmuir,* vol. 30, no. 7, pp. 1768-1777.

Destribats M, Rouvet M, Gehin-Delval C, Schmitt C , Binks BP, 2014b, "Emulsions stabilised by whey protein microgel particles: towards food-grade Pickering emulsions", *Soft Matter,* vol. 10, no. 36, pp. 6941-6954.

Destribats M, Wolfs M, Pinaud F, Lapeyre V, Sellier E, Schmitt V , Ravaine V, 2013, "Pickering Emulsions Stabilized by Soft Microgels: Influence of the Emulsification Process on Particle Interfacial Organization and Emulsion Properties", *Langmuir,* vol. 29, no. 40, pp. 12367-12374.

Dickinson E, 2010, "Food emulsions and foams: Stabilization by particles", *Current Opinion in Colloid and Interface Science,* vol. 15, no. 1-2, pp. 40-49.

Dickinson E, 2015, "Microgels - An alternative colloidal ingredient for stabilization of food emulsions", *Trends in Food Science and Technology,* vol. 43, no. 2, pp. 178-188.

Discher DE , Ahmed F 2006. Polymersomes. *Annual Review of Biomedical Engineering.*

Doppalapudi S, Jain A, Domb AJ , Khan W, 2016, "Biodegradable polymers for targeted delivery of anti-cancer drugs", *Expert Opinion on Drug Delivery,* vol. 13, no. 6, pp. 891-909.

Fang J, Nakamura H , Maeda H, 2011, "The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of

the effect", *Advanced Drug Delivery Reviews,* vol. 63, no. 3, pp. 136-151.

Ferrari M, 2005, "Cancer nanotechnology: Opportunities and challenges", *Nature Reviews Cancer,* vol. 5, no. 3, pp. 161-171.

Fredenberg S, Wahlgren M, Reslow M , Axelsson A, 2011, "The mechanisms of drug release in poly(lactic-co-glycolic acid)-based drug delivery systems - A review", *International Journal of Pharmaceutics,* vol. 415, no. 1-2, pp. 34-52.

Fu Y , Kao WJ, 2010, "Drug Release Kinetics and Transport Mechanisms of Non-degradable and Degradable Polymeric Delivery Systems", *Expert opinion on drug delivery,* vol. 7, no. 4, pp. 429-444.

Fujii S, Okada M, Nishimura T, Sugimoto T, Maeda H, Hamasaki H, Furuzono T , Nakamura Y, 2013, "Hydroxyapatite-coated poly(ϵ-caprolactone) microspheres fabricated via a Pickering emulsion route: effect of fabrication parameters on diameter and chemical composition", *Composite Interfaces,* vol. 20, no. 1, pp. 45-56.

Gadad A, Soni A, Dandagi P , Mastiholimath V, 2011, "Nanotechnology in drug delivery: A review", *Indian Drugs,* vol. 48, no. 11, pp. 5-16.

Gaikwad SG , Pandit AB, 2008, "Ultrasound emulsification: Effect of ultrasonic and physicochemical properties on dispersed phase volume and droplet size", *Ultrasonics Sonochemistry,* vol. 15, no. 4, pp. 554-563.

Gutiérrez JM, González C, Maestro A, Solè I, Pey CM , Nolla J, 2008, "Nano-emulsions: New applications and optimization of their preparation", *Current Opinion in Colloid and Interface Science,* vol. 13, no. 4, pp. 245-251.

Harris RE, Beebe-Donk J, Doss H , Burr Doss D, 2005, "Aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs in cancer prevention: a critical review of non-selective COX-2 blockade (review)", *Oncology reports,* vol. 13, no. 4, pp. 559-583.

He Y, Wu F, Sun X, Li R, Guo Y, Li C, Zhang L, Xing F, Wang W , Gao J, 2013, "Factors that affect pickering emulsions stabilized by graphene oxide", *ACS Applied Materials and Interfaces,* vol. 5, no. 11, pp. 4843-4855.

Heller J, 1980, "Controlled release of biologically active compounds from bioerodible polymers", *Biomaterials,* vol. 1, no. 1, pp. 51-57.

Heller J, 1984, "Biodegradable polymers in controlled drug delivery", *Critical Reviews in Therapeutic Drug Carrier Systems,* vol. 1, no. 1, pp. 39-90.

Hombreiro-Pérez M, Siepmann J, Zinutti C, Lamprecht A, Ubrich N, Hoffman M, Bodmeier R , Maincent P, 2003, "Non-degradable microparticles containing a hydrophilic and/or a lipophilic drug: preparation, characterization and drug release modeling", *Journal of Controlled Release,* vol. 88, no. 3, pp. 413-428.

Hu Y, Han W, Chen Y, Zou R, Ouyang Y, Zhou W, Yang Z , Wang C, 2017, "One-Pot Fabrication of Poly(ε-Caprolactone)-Incorporated Bovine Serum Albumin/Calcium Alginate/Hydroxyapatite Nanocomposite Scaffolds by High Internal Phase Emulsion Templates", *Macromolecular Materials and Engineering,* vol. 302, no. 4, pp.

Hughes GA, 2005, "Nanostructure-mediated drug delivery", *Nanomedicine: Nanotechnology, Biology, and Medicine,* vol. 1, no. 1, pp. 22-30.

Iqbal M, Zafar N, Fessi H , Elaissari A, 2015, "Double emulsion solvent evaporation techniques used for drug encapsulation", *International Journal of Pharmaceutics,* vol. 496, no. 2, pp. 173-190.

Jain JP, Yenet Ayen W, Domb AJ , Kumar N 2011. Biodegradable Polymers in Drug Delivery. *Biodegradable Polymers in Clinical Use and Clinical Development.* John Wiley & Sons, Inc.

Joscelyne SM , Trägårdh G, 2000, "Membrane emulsification - A literature review", *Journal of Membrane Science,* vol. 169, no. 1, pp. 107-117.

Kamaly N, Yameen B, Wu J , Farokhzad OC, 2016, "Degradable Controlled-Release Polymers and Polymeric Nanoparticles: Mechanisms of Controlling Drug Release", *Chemical Reviews,* vol. 116, no. 4, pp. 2602-2663.

Kardos N , Luche JL, 2001, "Sonochemistry of carbohydrate compounds", *Carbohydrate Research,* vol. 332, no. 2, pp. 115-131.

Kim HO, Kim E, An Y, Choi J, Jang E, Choi EB, Kukreja A, Kim MH, Kang B, Kim DJ, Suh JS, Huh YM , Haam S, 2013, "A biodegradable polymersome containing Bcl-xL siRNA and doxorubicin as a dual delivery vehicle for a synergistic anticancer effect", *Macromolecular Bioscience,* vol. 13, no. 6, pp. 745-754.

Koch HP, 1991, "Controlled drug delivery systems", *Scientia Pharmaceutica,* vol. 59, no. 2, pp. 85-100.

Kumar MNVR, Muzzarelli RAA, Muzzarelli C, Sashiwa H , Domb AJ, 2004, "Chitosan chemistry and pharmaceutical perspectives", *Chemical Reviews,* vol. 104, no. 12, pp. 6017-6084.

Lammers T, Kiessling F, Hennink WE , Storm G, 2012, "Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress", *Journal of Controlled Release,* vol. 161, no. 2, pp. 175-187.

Lamprecht A, Schäfer U , Lehr CM, 2001, "Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa", *Pharmaceutical Research,* vol. 18, no. 6, pp. 788-793.

Lankalapalli S , Kolapalli VRM, 2009, "Polyelectrolyte complexes: A review of their applicability in drug delivery technology", *Indian Journal of Pharmaceutical Sciences,* vol. 71, no. 5, pp. 481-487.

Larson-Smith K , Pozzo DC, 2012, "Pickering emulsions stabilized by nanoparticle surfactants", *Langmuir,* vol. 28, no. 32, pp. 11725-32.

Lassalle V , Ferreira ML, 2007, "PLA nano- and microparticles for drug delivery: An overview of the methods of preparation", *Macromolecular Bioscience,* vol. 7, no. 6, pp. 767-783.

Leamon CP , Low PS, 2001, "Folate-mediated targeting: From diagnostics to drug and gene delivery", *Drug Discovery Today,* vol. 6, no. 1, pp. 44-51.

Lee JS , Feijen J, 2012, "Polymersomes for drug delivery: Design, formation and

characterization", *Journal of Controlled Release,* vol. 161, no. 2, pp. 473-483.
Leighton TG, 1995, "Bubble population phenomena in acoustic cavitation", *Ultrasonics - Sonochemistry,* vol. 2, no. 2, pp.
Levis KA, Lane ME , Corrigan OI, 2003, "Effect of buffer media composition on the solubility and effective permeability coefficient of ibuprofen", *International Journal of Pharmaceutics,* vol. 253, no. 1-2, pp. 49-59.
Li ZF , Ngai T 2015. Emulsions stabilized by soft microgel particles. *RSC Soft Matter.*
Liu H, Wang C, Zou S, Wei Z , Tong Z, 2012, "Simple, reversible emulsion system switched by pH on the basis of chitosan without any hydrophobic modification", *Langmuir,* vol. 28, no. 30, pp. 11017-24.
Liu H, Wei Z, Hu M, Deng Y, Tong Z , Wang C, 2014, "Fabrication of degradable polymer microspheres via pH-responsive chitosan-based Pickering emulsion photopolymerization", *RSC Advances,* vol. 4, no. 55, pp. 29344-29351.
Liu M, Chen X, Yang Z, Xu Z, Hong L , Ngai T, 2016, "Tunable Pickering Emulsions with Environmentally Responsive Hairy Silica Nanoparticles", *ACS Applied Materials and Interfaces,* vol. 8, no. 47, pp. 32250-32258.
Lü JM, Wang X, Marin-Muller C, Wang H, Lin PH, Yao Q , Chen C, 2009, "Current advances in research and clinical applications of PLGA-based nanotechnology", *Expert Review of Molecular Diagnostics,* vol. 9, no. 4, pp. 325-341.
Lu Y , Chen SC, 2004, "Micro and nano-fabrication of biodegradable polymers for drug delivery", *Advanced Drug Delivery Reviews,* vol. 56, no. 11, pp. 1621-1633.
Maeda H, Brandon M , Sano A, 2003, "Design of controlled-release formulation for ivermectin using silicone", *International Journal of Pharmaceutics,* vol. 261, no. 1-2, pp. 9-19.
Maeda H, Okada M, Fujii S, Nakamura Y , Furuzono T, 2010, "Pickering-Type Water-in-Oil-in-Water Multiple Emulsions toward Multihollow Nanocomposite Microspheres", *Langmuir,* vol. 26, no. 17, pp. 13727-13731.
Maeda H, Wu J, Sawa T, Matsumura Y , Hori K, 2000, "Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review", *Journal of Controlled Release,* vol. 65, no. 1-2, pp. 271-284.
Mahon E, Salvati A, Baldelli Bombelli F, Lynch I , Dawson KA, 2012, "Designing the nanoparticle-biomolecule interface for "targeting and therapeutic delivery"", *Journal of Controlled Release,* vol. 161, no. 2, pp. 164-174.
Malcolm RK, Woolfson AD, Toner CF, Morrow RJ , Mccullagh SD, 2005, "Long-term, controlled release of the HIV microbicide TMC120 from silicone elastomer vaginal rings", *Journal of Antimicrobial Chemotherapy,* vol. 56, no. 5, pp. 954-956.
Manca ML, Loy G, Zaru M, Fadda AM , Antimisiaris SG, 2008, "Release of rifampicin from chitosan, PLGA and chitosan-coated PLGA microparticles", *Colloids and Surfaces B: Biointerfaces,* vol. 67, no. 2, pp. 166-170.
Manga MS, Cayre OJ, Williams RA, Biggs S , York DW, 2012, "Production of solid-stabilised emulsions through rotational membrane emulsification: Influence of

particle adsorption kinetics", *Soft Matter,* vol. 8, no. 5, pp. 1532-1538.

Marcucci F , Lefoulon F, 2004, "Active targeting with particulate drug carriers in tumor therapy: Fundamentals and recent progress", *Drug Discovery Today,* vol. 9, no. 5, pp. 219-228.

Mark D, Haeberle S, Roth G, Von Stetten F , Zengerle R, 2010, "Microfluidic lab-on-a-chip platforms: Requirements, characteristics and applications", *Chemical Society Reviews,* vol. 39, no. 3, pp. 1153-1182.

Marto J, Ascenso A, Simoes S, Almeida AJ , Ribeiro HM, 2016, "Pickering emulsions: challenges and opportunities in topical delivery", *Expert Opinion on Drug Delivery,* vol. 13, no. 8, pp. 1093-1107.

Mason TJ , Cordemans ED, 1996, "Ultrasonic intensification of chemical processing and related operations: A review", *Chemical Engineering Research and Design,* vol. 74, no. 5, pp. 511-516.

Matkovic SR, Valle GM , Briand LE, 2005, "Quantitative analysis of ibuprofen in pharmaceutical formulations through FTIR spectroscopy", *Latin American Applied Research,* vol. 35, no. 3, pp. 189-195.

Melle S, Lask M , Fuller GG, 2005, "Pickering Emulsions with Controllable Stability", *Langmuir,* vol. 21, no. 6, pp. 2158-2162.

Meng F , Zhong Z, 2011, "Polymersomes spanning from nano- to microscales: Advanced vehicles for controlled drug delivery and robust vesicles for virus and cell mimicking", *Journal of Physical Chemistry Letters,* vol. 2, no. 13, pp. 1533-1539.

Moradi M , Yamini Y, 2012, "Surfactant roles in modern sample preparation techniques: A review", *Journal of Separation Science,* vol. 35, no. 18, pp. 2319-2340.

Müller M, Vörös J, Csúcs G, Walter E, Danuser G, Merkle HP, Spencer ND , Textor M, 2003, "Surface modification of PLGA microspheres", *Journal of Biomedical Materials Research - Part A,* vol. 66, no. 1, pp. 55-61.

Nafee N, Taetz S, Schneider M, Schaefer UF , Lehr CM, 2007, "Chitosan-coated PLGA nanoparticles for DNA/RNA delivery: effect of the formulation parameters on complexation and transfection of antisense oligonucleotides", *Nanomedicine: Nanotechnology, Biology, and Medicine,* vol. 3, no. 3, pp. 173-183.

Nair LS , Laurencin CT, 2007, "Biodegradable polymers as biomaterials", *Progress in Polymer Science (Oxford),* vol. 32, no. 8-9, pp. 762-798.

Nan F, Wu J, Qi F, Fan Q, Ma G , Ngai T, 2014, "Preparation of uniform-sized colloidosomes based on chitosan-coated alginate particles and its application for oral insulin delivery", *Journal of Materials Chemistry B,* vol. 2, no. 42, pp. 7403-7409.

Neuhäusler U, Abend S, Jacobsen C , Lagaly G, 1999, "Soft X-ray spectromicroscopy on solid-stabilized emulsions", *Colloid and Polymer Science,* vol. 277, no. 8, pp. 719-726.

O'neal DP, Hirsch LR, Halas NJ, Payne JD , West JL, 2004, "Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles", *Cancer Letters,*

vol. 209, no. 2, pp. 171-176.

Onaca O, Enea R, Hughes DW , Meier W, 2009, "Stimuli-responsive polymersomes as nanocarriers for drug and gene delivery", *Macromolecular Bioscience,* vol. 9, no. 2, pp. 129-139.

Palivan CG, Goers R, Najer A, Zhang X, Car A , Meier W, 2016, "Bioinspired polymer vesicles and membranes for biological and medical applications", *Chemical Society Reviews,* vol. 45, no. 2, pp. 377-411.

Parveen S, Misra R , Sahoo SK, 2012, "Nanoparticles: A boon to drug delivery, therapeutics, diagnostics and imaging", *Nanomedicine: Nanotechnology, Biology, and Medicine,* vol. 8, no. 2, pp. 147-166.

Peniche C, Argüelles-Monal W, Peniche H , Acosta N, 2003, "Chitosan: An Attractive Biocompatible Polymer for Microencapsulation", *Macromolecular Bioscience,* vol. 3, no. 10, pp. 511-520.

Pickering SU, 1907, "CXCVI.-Emulsions", *Journal of the Chemical Society, Transactions,* vol. 91, no. 0, pp. 2001-2021.

Pillai O , Panchagnula R, 2001, "Polymers in drug delivery", *Current Opinion in Chemical Biology,* vol. 5, no. 4, pp. 447-451.

Popat K, Mcqueen K , Feeley TW, 2013, "The global burden of cancer", *Best Practice and Research: Clinical Anaesthesiology,* vol. 27, no. 4, pp. 399-408.

Portenlänger G , Heusinger H, 1994, "Polymer formation from aqueous solutions of α-D-glucose by ultrasound and λ-rays", *Ultrasonics - Sonochemistry,* vol. 1, no. 2, pp.

Prajapati VD, Jani GK , Kapadia JR, 2015, "Current knowledge on biodegradable microspheres in drug delivery", *Expert Opinion on Drug Delivery,* vol. 12, no. 8, pp. 1283-1299.

Prasad Shastri V, 2003, "Non-degradable biocompatible polymers in medicine: Past, present and future", *Current Pharmaceutical Biotechnology,* vol. 4, no. 5, pp. 331-337.

Price GJ, White AJ , Clifton AA, 1995, "The effect of high-intensity ultrasound on solid polymers", *Polymer,* vol. 36, no. 26, pp. 4919-4925.

Priest C, Reid MD , Whitby CP, 2011, "Formation and stability of nanoparticle-stabilised oil-in-water emulsions in a microfluidic chip", *Journal of Colloid and Interface Science,* vol. 363, no. 1, pp. 301-306.

Ravi Kumar MNV, 2000, "A review of chitin and chitosan applications", *Reactive and Functional Polymers,* vol. 46, no. 1, pp. 1-27.

Rinaudo M, 2006, "Chitin and chitosan: Properties and applications", *Progress in Polymer Science (Oxford),* vol. 31, no. 7, pp. 603-632.

Roberts RT, 1993, "High intensity ultrasonics in food processing", *Chemistry and Industry (London),* vol. no. 4, pp. 119-121.

Saito G, Swanson JA , Lee KD, 2003, "Drug delivery strategy utilizing conjugation via reversible disulfide linkages: Role and site of cellular reducing activities", *Advanced Drug Delivery Reviews,* vol. 55, no. 2, pp. 199-215.

Schärtl W, 2010, "Current directions in core-shell nanoparticle design", *Nanoscale,* vol. 2, no. 6, pp. 829-843.

Schierholz JM, Steinhauser H, Rump AFE, Berkels R , Pulverer G, 1997, "Controlled release of antibiotics from biomedical polyurethanes: morphological and structural features", *Biomaterials,* vol. 18, no. 12, pp. 839-844.

Schmidt S, Liu T, Rütten S, Phan K-H, Möller M , Richtering W, 2011, "Influence of Microgel Architecture and Oil Polarity on Stabilization of Emulsions by Stimuli-Sensitive Core–Shell Poly(N-isopropylacrylamide-co-methacrylic acid) Microgels: Mickering versus Pickering Behavior?", *Langmuir,* vol. 27, no. 16, pp. 9801-9806.

Schulz CP, Rodríguez SM, Del Blanco FL, Pistonesi M , Agulló E, 1998, "Emulsification properties of chitosan", *Colloid and Polymer Science,* vol. 276, no. 12, pp. 1159-1165.

Seshadri R, Weiss J, Hulbert G , Mount J, 2002, "High-intensity ultrasound influences optical and rheological properties of pectin gels", *Food Hydrocolloids,* vol. 17, no. pp. 191-197.

Shah P, Bhalodia D , Shelat P, 2010, "Nanoemulsion: A pharmaceutical review", *Systematic Reviews in Pharmacy,* vol. 1, no. 1, pp. 24-32.

Solans C, Izquierdo P, Nolla J, Azemar N , Garcia-Celma MJ, 2005, "Nano-emulsions", *Current Opinion in Colloid and Interface Science,* vol. 10, no. 3-4, pp. 102-110.

Steichen SD, Caldorera-Moore M , Peppas NA, 2013, "A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics", *European Journal of Pharmaceutical Sciences,* vol. 48, no. 3, pp. 416-427.

Su Y, Zhao H, Wu J , Xu J, 2016, "One-step fabrication of silica colloidosomes with in situ drug encapsulation", *RSC Advances,* vol. 6, no. 113, pp. 112292-112299.

Sun G, Qi F, Wu J, Ma G , Ngai T, 2014, "Preparation of Uniform Particle-Stabilized Emulsions Using SPG Membrane Emulsification", *Langmuir,* vol. 30, no. 24, pp. 7052-7056.

Tadros T, Izquierdo P, Esquena J , Solans C, 2004, "Formation and stability of nano-emulsions", *Advances in Colloid and Interface Science,* vol. 108-109, no. pp. 303-318.

Thompson KL, Armes SP , York DW, 2011, "Preparation of Pickering Emulsions and Colloidosomes with Relatively Narrow Size Distributions by Stirred Cell Membrane Emulsification", *Langmuir,* vol. 27, no. 6, pp. 2357-2363.

Tian Q, Zhang C-N, Wang X-H, Wang W, Huang W, Cha R-T, Wang C-H, Yuan Z, Liu M, Wan H-Y , Tang H, 2010, "Glycyrrhetinic acid-modified chitosan/poly(ethylene glycol) nanoparticles for liver-targeted delivery", *Biomaterials,* vol. 31, no. 17, pp. 4748-4756.

Tibbitt MW, Dahlman JE , Langer R, 2016, "Emerging Frontiers in Drug Delivery", *Journal of the American Chemical Society,* vol. 138, no. 3, pp. 704-717.

Tiwari G, Tiwari R, Sriwastawa B, Bhati L, Pandey S, Pandey P , Bannerjee SK, 2012, "Drug delivery systems: An updated review", *International Journal of*

Pharmaceutical Investigation, vol. 2, no. 1, pp. 2-11.

Tomasik P , Zaranyika MF, 1995, "Nonconventional methods of modification of starch", *Advances in Carbohydrate Chemistry and Biochemistry,* vol. 51, no. pp. 243-318.

Torchilin VP, 2000, "Drug targeting", *European Journal of Pharmaceutical Sciences,* vol. 11, no. SUPPL. 2, pp.

Uhrich KE, Cannizzaro SM, Langer RS , Shakesheff KM, 1999, "Polymeric Systems for Controlled Drug Release", *Chemical Reviews,* vol. 99, no. 11, pp. 3181-3198.

Ulbrich K , Šubr V, 2004, "Polymeric anticancer drugs with pH-controlled activation", *Advanced Drug Delivery Reviews,* vol. 56, no. 7, pp. 1023-1050.

Ungaro F, D'angelo I, Miro A, La Rotonda MI , Quaglia F, 2012, "Engineered PLGA nano- and micro-carriers for pulmonary delivery: Challenges and promises", *Journal of Pharmacy and Pharmacology,* vol. 64, no. 9, pp. 1217-1235.

Vilela C, Figueiredo ARP, Silvestre AJD , Freire CSR, 2016, "Multilayered materials based on biopolymers as drug delivery systems", *Expert Opinion on Drug Delivery,* vol. no. pp. 1-12.

Wang H, Zhao Y, Wu Y, Hu Y-l, Nan K, Nie G , Chen H, 2011a, "Enhanced anti-tumor efficacy by co-delivery of doxorubicin and paclitaxel with amphiphilic methoxy PEG-PLGA copolymer nanoparticles", *Biomaterials,* vol. 32, no. 32, pp. 8281-8290.

Wang JJ, Zeng ZW, Xiao RZ, Xie T, Zhou GL, Zhan XR , Wang SL, 2011b, "Recent advances of chitosan nanoparticles as drug carriers", *International journal of nanomedicine,* vol. 6, no. pp. 765-774.

Wang S, Song Z, Wang J, Dong Y , Wu M, 2010, "Solubilities of ibuprofen in different pure solvents", *Journal of Chemical and Engineering Data,* vol. 55, no. 11, pp. 5283-5285.

Wang X, Zeng M, Yu YH, Wang H, Mannan MS , Cheng Z, 2017, "Thermosensitive ZrP-PNIPAM Pickering Emulsifier and the Controlled-Release Behavior", *ACS Applied Materials and Interfaces,* vol. 9, no. 8, pp. 7852-7858.

Wang XY , Heuzey MC, 2016, "Chitosan-Based Conventional and Pickering Emulsions with Long-Term Stability", *Langmuir,* vol. 32, no. 4, pp. 929-36.

Wei Z, Wang C, Liu H, Zou S , Tong Z, 2012a, "Facile fabrication of biocompatible PLGA drug-carrying microspheres by O/W pickering emulsions", *Colloids Surf B Biointerfaces,* vol. 91, no. pp. 97-105.

Wei Z, Wang C, Zou S, Liu H , Tong Z, 2012b, "Chitosan nanoparticles as particular emulsifier for preparation of novel pH-responsive Pickering emulsions and PLGA microcapsules", *Polymer,* vol. 53, no. 6, pp. 1229-1235.

Weiren C, Liuqun G, Wei R , Ye L, 2014, "Stimuli-responsive polymers for anticancer drugs delivery", *Materials Science and Engineering C,* vol. no. pp.

Whitby CP , Wanless EJ, 2016, "Controlling pickering emulsion destabilisation: A route to fabricating new materials by phase inversion", *Materials,* vol. 9, no. 8, pp.

Wischke C , Schwendeman SP, 2008, "Principles of encapsulating hydrophobic drugs

in PLA/PLGA microparticles", *International Journal of Pharmaceutics,* vol. 364, no. 2, pp. 298-327.

Wu J , Ma GH, 2016, "Recent Studies of Pickering Emulsions: Particles Make the Difference", *Small,* vol. no. pp. 4633-4648.

Wu T, Zivanovic S, Hayes DG , Weiss J, 2008, "Efficient reduction of chitosan molecular weight by high-intensity ultrasound: Underlying mechanism and effect of process parameters", *Journal of Agricultural and Food Chemistry,* vol. 56, no. 13, pp. 5112-5119.

Wu W, Shen J, Banerjee P , Zhou S, 2010, "Chitosan-based responsive hybrid nanogels for integration of optical pH-sensing, tumor cell imaging and controlled drug delivery", *Biomaterials,* vol. 31, no. 32, pp. 8371-8381.

Xu QY, Nakajima M , Binks BP, 2005, "Preparation of particle-stabilized oil-in-water emulsions with the microchannel emulsification method", *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* vol. 262, no. 1–3, pp. 94-100.

Xu S, Luo Y, Graeser R, Warnecke A, Kratz F, Hauff P, Licha K , Haag R, 2008, "Development of pH-responsive core-shell nanocarriers for delivery of therapeutic and diagnostic agents", *Bioorganic & Medicinal Chemistry Letters,* vol. 19, no. 3, pp.

Yuan Q, Cayre OJ, Manga M, Williams RA , Biggs S, 2010, "Preparation of particle-stabilized emulsions using membrane emulsification", *Soft Matter,* vol. 6, no. 7, pp. 1580-1588.

Yun YH, Lee BK , Park K, 2015, "Controlled Drug Delivery: Historical perspective for the next generation", *Journal of Controlled Release,* vol. 219, no. pp. 2-7.

Zdrahala RJ , Zdrahala IJ, 1999, "Biomedical applications of polyurethanes: A review of past promises, present realities, and a vibrant future", *Journal of Biomaterials Applications,* vol. 14, no. 1, pp. 67-90.

Zhou J, Romero G, Rojas E, Ma L, Moya S , Gao C, 2010, "Layer by layer chitosan/alginate coatings on poly(lactide-co-glycolide) nanoparticles for antifouling protection and Folic acid binding to achieve selective cell targeting", *Journal of Colloid and Interface Science,* vol. 345, no. 2, pp. 241-247.