Effect of polylactide based copolymers structural modifications on encapsulation and release of cytostatics

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Téma práce:	**Vliv strukturních modifikací kopolymerů polylaktidu na enkapsulaci a uvolňování cytostatik.**

Zásady pro vypracování:

I. Teoretická část
1. Describe state of the art in the field of polymers used in particulate drug delivery applications.
2. Summarize the key factors influencing the properties of polymer based nanoparticles in drug delivery systems.
3. Focus on bioresorbable polyesters including polylactic acid.
II. Praktická část
1. Synthesize polylactic acid with various morphology depending on optical isomerism of its precursor. Characterize the prepared polymers by spectroscopic and chromatographic techniques.
2. Encapsulate selected cytostatics into sub micro and nanoparticles based on prepared polymers.
3. Investigate degradation behaviour of the prepared systems under abiotic conditions and release kinetics of the cytostatic from the particles into a various environment.
4. Process the experimental data systematically into tables and figures and discuss the results with literature dealing with relevant topic.

Rozsah diplomové práce:

Rozsah příloh:

Forma zpracování diplomové práce: **tištěná/elektronická**

Seznam odborné literatury:

1)SVENSON, Sönke. Polymeric drug delivery. Washington, DC: American Chemical Society, 2006, xiii, 337 s. ISBN 0841239185.
2)SVENSON, Sönke a Robert K PRUD'HOMME. Multifunctional nanoparticles for drug delivery applications: imaging, targeting, and delivery. New York: Springer, 2012, x, 373 s. ISBN 978-1-4614-2304-1.
3)Other relevant books and journals accesibble through the Library of the Tomas bata University in Zlin.

Vedoucí diplomové práce: **prof. Ing. Vladimír Sedlařík, Ph.D.**
Centrum polymerních materiálů

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ABSTRAKT

Tato práce se zabývá bioresorbovatelnými částicovými systémy na bázi polymeru kyseliny mléčné určených pro dodávání léčiv. Těžiště výzkumu spočívá v experimentálním stanovení a popisu vlivu optické isomerie prekurzoru kyseliny polymléčné na vlastnosti jejich nanočástic zahrnující jejich morfologii, účinnost imobilizace modelové bioaktivní látky cytostatika doxorubicinu a následné kinetiky jeho uvolňování prostředí simulující tělní tekutiny. Výsledky naznačují významný vliv morfologie polymeru kyseliny mléčné na všechny sledované charakteristiky.

Klíčová slova: kyselina polymléčná, morfologie, nanočástice, doxorubicin, krystalinita, kinetika uvolňování

ABSTRACT

This work is aimed at particulate biodegradable polymer systems based on polylactic acid for drug delivery applications. The main focus of the research is based on experimental determination and description of the effect of polylactic acid precursor optical isomerism on encapsulation efficiency and release kinetics of model cytostatic, Doxodubicin, from particulate systems into the body fluids simulating environments. The results reveal a significant effect of the polylactic acid morphology on the studied characteristics.

Keywords: polylactic acid, morphology, nanoparticles, doxorubicin, crystallinity, release kinetics

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I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

CONTENTS

INTRODUCTION

The biomedical use of bioresorbable polymers has begun in the late 1960‘s with the approval of the first bioresorbable structures. Since that time, numerous applications in this field have been made. During the 1980s and 1990s, several drugs delivery systems were developed to improve the efficiency of drugs and minimalize toxic effects [1]. Generally, we can say that polymer systems can ensure target delivery of drugs, enable optimal dosage for a long time which increases the efficiency of drugs.

Nanoparticles have gained a great attraction in the field of drug delivery because they have unique properties like the ability to allocate and deliver bioactive compounds to specific intra or extracellular compartments. The increase in selectivity leads an enhancement of the therapeutic effect and a reduction in the required dosage and the related side effects. It expects more safety and more effective pharmacotherapy of cancer diseases from nanoparticles because drugs are delivered into the affected tissue or organ where will be operated right into cancer cells and adverse effect on the healthy tissue will be decreased [2, 3].

Biodegradable polyesters such as PLA (poly(lactic acid)), PLGA (poly(lactic-co-glycolic acid)) or PCL (polycaprolactone) have been used as carriers in delivery systems due to their biocompatibility [4]. Numerous efforts have focused on optimization of fabrication by blending of polymers and synthesis of copolymers with different compositions. It is a challenge to design a microsphere-controlled-release matrix based on polyesters with high molecular weights such as PLA, PLGA, and PCL because these polymers have a very slow degradation rate, especially at low temperature. It is needed to fully understand the relationship among morphology, drug distribution and release kinetics [5].

Keeping these aspects in mind, this diploma work is focused on the detailed investigation of the optical isomerism of the PLA precursor on nanoparticle preparation process, the morphology of nanoparticles and other selected characteristics such as release kinetics of the model bioactive compound.

I. THEORY

1 POLYMERS FOR DRUG DELIVERY SYSTEMS

Polymers are the most varied class of materials which have changed our lives over the past decades. Polymeric delivery systems are mainly intentional to achieve either a temporal and spatial control of drug delivery. The first synthetic polymer-based drug delivery system (DDS) led to an interest in the design and synthesis of biodegradable polymers [6]. As mentioned before, the selection and design of a polymer is a challenging task due to the diversity of structures and requires. Additionally, the selected polymer must have properties for extensive biochemical characterization and specific preclinical tests to prove its safety [8].

Surface properties, such as hydrophilicity, lubricity, smoothness and surface energy play a certain role in the interaction with living systems. The biocompatibility with tissues and blood and determination the water sorption capacity of the polymers affect hydrolytic degradation. Materials for long-term use (orthopedic, dental implants) must be water-repellent to avoid erosion process or degradation [8]. Bulk properties, which include molecular weight, adhesion, solubility, have to be considered because of controlled delivery systems. A consideration of surface and bulk properties can support the design of polymers for application in drug delivery. Biodegradable polymers are widespread use in drug delivery because they can be degraded to non-toxic monomers inside the body. Design and synthesis of new combinations of polymers will extend the scale of new drug delivery systems in the future [7].

Tab. 1 gives a list of polymers that have been considered for medical applications [7].

Tab. 1 Representative list of polymers used in drug delivery and medical applications

Classification	Polymer	Application	Reference
Natural polymers			
Protein-based polymers	Collagen	medical carriers, connective tissue, drug delivery	[13, 14]
	Gelatine	tissue engineering, gene delivery, cell culture	[15]
Polysaccharides	Chitosan	nanoparticles, pharmaceutics, cosmetics	[16, 17, 18]
	Starch	pharmaceutics, control release of drug	[19, 20]
	Hyaluronic acid	targeted drug delivery	[21]
	Pectin	controlled release, thickener, emulsifier, gelling agents, emulsifier in food, pharmaceutical	[22]
	Cyclodextrin	high-performance carrier	[23, 24]
Synthetic polymers			
Biodegradable			
Polyesters	Poly(lactic acid)	drug delivery, bone therapy,	[42, 117, 120, 121]
	Poly(glycolic acid)	fibre-forming applications, suture	[29]
	Poly(lactide-co-glycolide)	drug delivery	[30, 31]

	Polyorthoester	Insulin delivery, chemotherapy of brain cancer	[27, 33]
	Microbial polyesters - polyhydroxyalkanoates	Multifunctional NPs, drug delivery, therapeutic applications	[34]
Non-biodegradable			
Silicone		membranes, diffusion-controlled release	[35]
Polyvinyl Alcohol			
Ethyl Vinyl Acetate			
Cellulose ester derivatives		enteric coated dosage forms, pharmaceutical controlled release, micro-porous membrane filters	[36]

Because its bioresorbability and biocompatibility in the human body, PLA has been widely studied for use in medical applications [9].

Following script describes the current state of research in the field of polymer system for controlled release and targeted transport of drug. It summarizes basic natural and synthetic polymers which are used in controlled delivery transport. Moreover, it provides a brief overview of methods of preparation and characteristics of polymer nanoparticles and also loading and release mechanisms. Great attention is paid to the description of polylactic acid‘s modifications, properties and applications as a nanoparticle drug carrier in drug delivery.

1.1 Natural polymers in drug delivery systems

Natural polymers are macromolecules composed of repeating structural units, which are joined by covalent bonds. Proteins, carbohydrates, and lipids have potential drug delivery systems since they have physicochemical properties which make them suitable as

starting material for developing smart delivery systems with high selectivity and control of the release rate. Natural polymers exhibit biocompatibility and biodegradability and due to these properties well suited for this purpose.

In the last decade natural polymers have received great attention caused by their applications in the fields of environmental protection and also because of their special characteristics:

- Biodegradability – they are 100 % biodegradable.
- Lack of toxicity – they are non-toxic.
- Economy – they are inexpensive and large amount can be obtained.
- Safety – they are without any harmful side effects.
- Availability – they are widely distributed in large quantities.

There are also a few disadvantages include uncontrolled hydration rate or changes in microbial contamination [10].

Protein-based polymers

Collagen is found in the extracellular matrix of connective tissue and has a typical triple helical structure of repeating units (Figure 1) [11]. It is the main structural protein in the extracellular space in connective tissues. It is the most abundant protein in mammals, making up from 25 % to 35 % of the whole body protein content. The attractiveness of collagen as a biomaterial possess low immunogenicity and therefore it is seen by the body as a normal constituent rather than foreign matter [12]. Collagen can be processed into a variety of forms such as sheets, tubes, sponges, fleeces, powders, injectable solutions and dispersions and all of these have found use in medical practice [13].

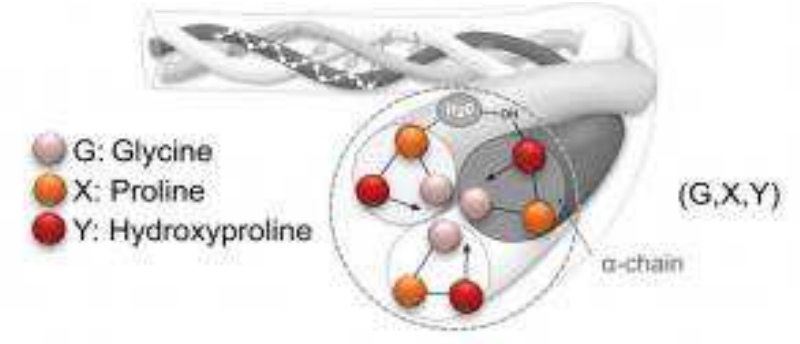

Figure 1 Collagen structure

Regardless of various applications of collagen as a drug vehicle are reported in the literature, however, only a few collagen-based drug delivery products are going into clinical trials or are marketed. The major reasons are:

- the high cost of preparation of pure type collagen;
- variability of isolated collagen (e.g. fiber size, impurities etc.);
- hydrophilicity which leads to swelling and more rapid release compared with synthetic polymers.

Successful uses of collagen for controlled drug release are shields in ophthalmology, cancer treatment or carrying antibiotics, these functions target local drug delivery and the applications profit from some of the qualities of collagen:

- appropriate biocompatibility;
- degradation into well tolerated physiological compounds;
- processing on aqueous base [14].

Gelatine is a common natural polymer which is soluble in water and is produced by denaturation of collagen. Due to its outstanding properties such as biocompatibility, biodegradability and low antigenicity it has been used in pharmaceutical and medical applications. Additionally, gelatine can be easily manipulated due to its isoelectric point that allows it to change from negative to positive charge according to an appropriate physiological environment. Gelatine found its uses as support material for gene delivery, cell culture and tissue engineering [15].

Polysaccharides

Chitosan is one of the most commonly used natural polymers in the production of nanomedicine because it shows very good absorption-enhancing, bioadhesivity as well as control release and effectiveness when formulating a nanoparticulate form. From a chemical point of view is chitosan a copolymer consisting of glucosamine and N-acyl glucosamine units (Figure 2).

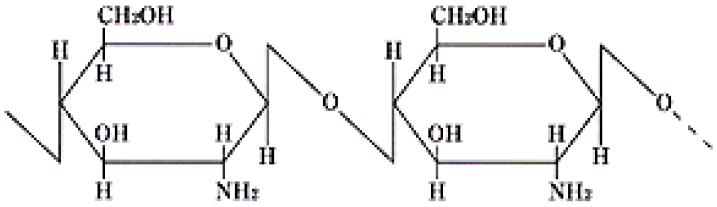

Figure 2 Chitosan structure

It is mostly obtained by deactivation of chitin derived from the exoskeleton of crustaceans. The degree of sidechain can be a source of manipulation for specific drug-delivery applications [16]. It is a cationic polymer, biodegradable, biocompatible and non-toxic. Chitosan is a good antimicrobial agent and with ovalbumin, gels are used for pharmaceutical and cosmetic applications and together with non-ionic surfactants is a good bioadhesive agent [17]. Several reviews are available reporting the most prominent applications of chitosan‘s nanoparticles, reinforcing the potential of these carriers in the biopharmaceutical and biomedical fields [18]

Starch is used by plants as a storage of energy. It is a heterogeneous polymer and is found in two forms – amylose and amylopectin. In pharmaceutical applications starch has found its use as a filler, diluent binder and is also used as a controlled release matrix [19]. Modified starch was tested for general applicability of a new pregelatinized starch product in directly compressible controlled release systems. It was prepared by enzymatic degradation of potato starch followed by precipitation, filtration, and washing with ethanol. Advantages of this material are the easy preparation of tablets, a potential of a constant release rate for an extended period of time and its ability to incorporate high percentages of drugs with different physiochemical properties [20].

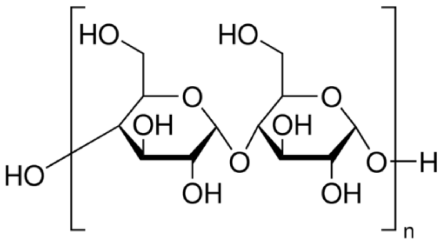

Figure 3 Starch structure

Hyaluronic acid is one of the most widely used biocompatible polymers for medical use. It is a naturally occurring polysaccharide which belongs to the group of glycosaminoglycans. Hyaluronic acid and the other glycosaminoglycans are negatively

charged heteropolysaccharide chains which have a capacity to absorb large amounts of water. Hyaluronic acid and products derived from hyaluronic acid are widely used in the biomedical field. Hyaluronic acid solutions are characteristically viscoelastic and pseudoplastic [21]. This viscoelastic property is important in its use as a biomaterial in controlled by the concentration and molecular weight of the hyaluronic acid chains.

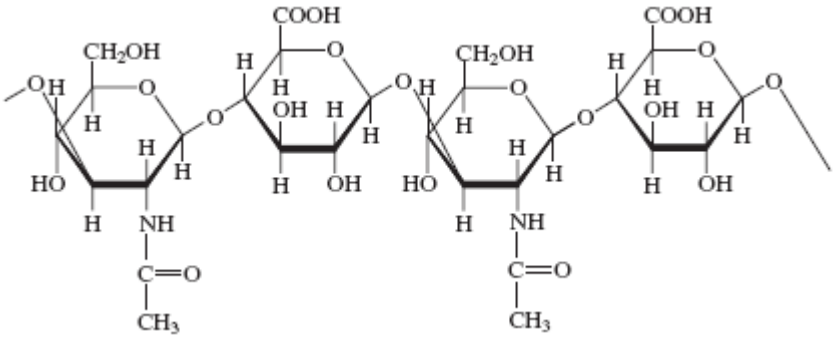

Figure 4 Hyaluronic acid structure

Pectin is linear polysaccharide occurring in the cell walls of plants. It was proven that pectin has a gastro-retentive as well as the controlled release properties [22]. Pectin is also used as thickeners, emulsifiers, gelling agents, emulsifiers in food, pharmaceutical, and other industries.

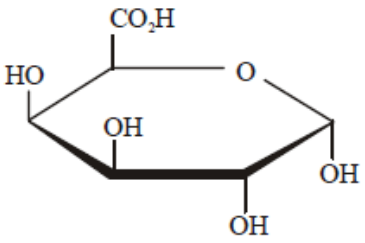

Figure 5 Pectin structure

Cyclodextrin is oligosaccharide consisting of 6 to 8 glucose units joined by α-1,4 glycosidic bonds.

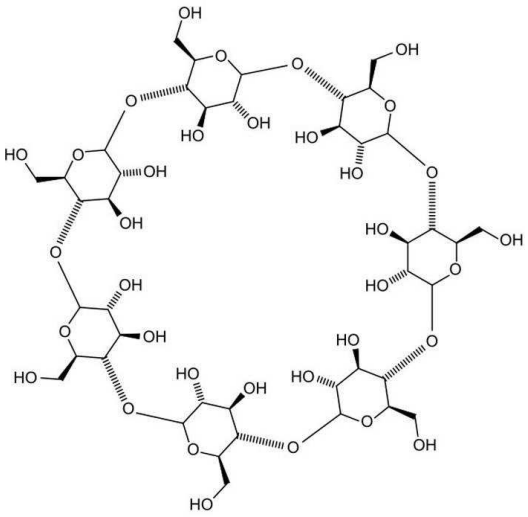

Figure 6 Cyclodextrin structure

Cyclodextrin remains intact during their passage throughout the stomach and small intestine of a digestive tract. Nevertheless, in the colon, they undergo fermentation in the presence microflora into small monosaccharides and thus can be absorbed [23]. Cyclodextrin has a potential to be high-performance carrier materials that have the ability to alter chemical, physical and biological properties of drug molecules due to the formation of inclusion complexes both in a solution and a solid state. They are known to be able to be hydrolyzed and only slightly absorbed in the passage through the stomach and small intestine, and are fermented by colonic microflora into small saccharides [24].

1.2 Synthetic polymers in drug delivery systems

Synthetic polymers can be used as a drug delivery system as a polymeric drug itself or in cooperation with a small molecule drug or with biomacromolecules (proteins, poly(nucleic acids)). In a case the polymer is not a drug itself, it is considered as a drug carrier, reducing toxicity, immunogenicity or degradation and improving a targeting function and circulation time. The polymer has to be non-toxic, water-soluble, non-immunogenic [25].

1.2.1 Biodegradable synthetic polymers

In the biomedical field, in particular, in controlled drug delivery, biodegradable polymers have found wide application. After release, the loaded drug, the polymeric carrier has to degrade into the body in non-toxic products which are easily reabsorbed or eliminate [26].

The advantages of using these systems are based to control the release rate of the loaded compound(s) leading to a decrease a dosage which results in a reduction of side effects with improving and prolonging the therapeutic effect.

A plenty of biodegradable polymers have been synthesized to deliver drugs, cells, enzymes or macromolecules. The appreciation of the wide acceptability from these polymers is from the fact that the biodegradability can be manipulated by a variety of labile groups such as ester, anhydride, carbonate, amide, and others [7]. Biodegradation can be of enzymatic, chemical or microbial origin. These can be applied either separately or simultaneously and are usually influenced by many other factors. [27].

Polyesters

A group of **polyester-based** polymers is one of the main investigated for drug delivery and belong here poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and also their copolymers poly(lactic acid-co-glycolic acid) (PLGA) [6]. PGA is the simplest linear aliphatic polyester, produced through polycondensation of glycolic acid. It shows a high melting point and high crystalline ratio and its insolubility in water. The degradation by hydrolysis, producing glycolic acid which is a natural metabolite. Due to low mechanical properties, it has limited applications

Modification of the biological, mechanical and thermal properties of PLA is possible by regulation of its stereochemistry [28]. This topic and further and more detailed information about PLA will be discussed in another chapter.

Polyglycolic acid (PGA) is obtained by ring-opening polymerization (which will be discussed later on) of the cyclic diester of glycolic acid. PGA is a crystalline polymer with excellent fiber-forming properties an was commercially introduced as the first synthetic absorbable suture under the name Dextron^{TM}. The low solubility, high melting temperature (T_m=225 °C) of PGA limits its use for drug delivery applications. Since it can not be made into films, rods, capsules or microspheres it is used as a solvent or using melt techniques [29].

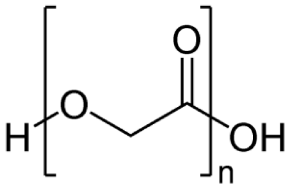

Figure 7 Polyglycolic acid structure

Extensive research has been performed in developing a full range of co-polyesters **poly(lactide-co-glycolide)** (PLGA) polymers. Both L- and DL-lactides have been used for copolymerization. The ratio of glycolide to lactide at different compositions allows control of the crystallinity of the polymers. When the crystalline PGA is copolymerized with PLA, the degree of crystallinity is reduced and this leads to increases in rates of hydration and hydrolysis. It can be concluded that the degradation time of the copolymer is related to the ratio of monomers used in synthesis. In general, the higher the content of glycolide, the quicker the rate of degradation [30]. This property is used in drug delivery applications, for example, non-steroidal anti-inflammatory drugs have been incorporated into PLGA and investigated for the treatment of rheumatoid arthritis, osteoarthritis and related diseases [31].

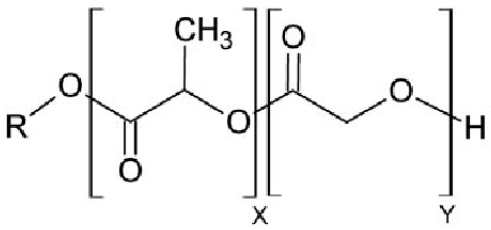

Figure 8 Poly(lactide-co-glycolide) structure

Polyorthoesters (POE) have unique characteristics among all biodegradable polymers because mechanical properties can be varied by choosing different diols in their synthesis [32]. A number of applications have been developed for polyorthoesters such as a periodontal delivery and pH-sensitive systems for insulin delivery. By a variation of monomer ratio was found first US Food and Drug Administration (FDA)-approved polymer-base chemotherapy of brain cancer [27].

The polymer has high hydrophobicity and water permeability which can cause a surface erosion degradation. POEs were developed for drug delivery applications by ALZA Corporation in the early 1970s. The most important property is the synthesis versatility that allows a simple and reproducible production of materials having the desired mechanical

and thermal characterization as well as the drug release rates that can be modified from a few days to many months [33].

Microbial polyhydroxyalkanoates (PHA) have been a subject of the recent development of novel functionalized PHAs. It has opened up new possibilities to combine the good biocompatibility of PHA-based drug delivery systems to improve drug loading and release properties, targeting or imaging functionalities. There are studies about functionalized PHAs, PHA–drug and PHA–protein conjugates, multifunctional PHA nanoparticles, and micelles as well as biosynthetic PHA particles for drug delivery. These developments in combination with the generally excellent biocompatibility of PHA materials are expected to further expand the interest in PHA materials for drug delivery and other therapeutic applications [34].

1.2.2 Non-biodegradable synthetic polymers

Non-biodegradable polymers are used in diffusion-controlled system [35]. Due to nonbiodegradable polymers, there is no initial burst release in diffusion-controlled systems. The thickness and permeability of the polymer, the solubility and the release area of the drug determine the release kinetics of the drug form the diffusion-controlled system. Silicone, cross-linked Polyvinyl Alcohol (PVA), and Ethyl Vinyl Acetate (EVA) are mostly used in drug formulations. The permeability or the impermeability of the silicones is decided by the thickness and the grade used. EVA is impermeable to many drugs, therefore, commonly used as a membrane to surround the drug core. PVA is used as controlled elution membrane in the release area because they are permeable to various lipophilic drugs. Alteration in the thickness layer helps in achieving the desired release kinetics.

Cellulose ester derivatives are widely used in pharmaceutical controlled release and are generally water-insoluble with good film-forming characteristics. The most available formulations are enteric coated dosage forms which are usually produced applying acid resistant polymeric coats containing phthalate derivatives of cellulose esters. Cellulose nitrate and cellulose acetate mixture are exploited to prepare microporous membrane filters used in pharmaceutical industry [36].

1.2.3 Advanced polymers

Stimulus-responsive or „smart“ materials undergo conformational variations with only small changes in their environment such as in pH, temperature ionic strength, and electric or magnetic fields. The characterizes of „smart“ polymers is the ability of synthetic polymers (hydrogels) to imitate the nonlinear reply of biopolymers (proteins, DNA etc.) generate by interactions between monomers [37]. Hydrogels resemble natural tissue because of their water-absorbing property. Figure 9 shows their possibility to undergo structural changes in response to biological, chemical and physical stimuli have given the concept of „stimuli“ response drug delivery systems [38].

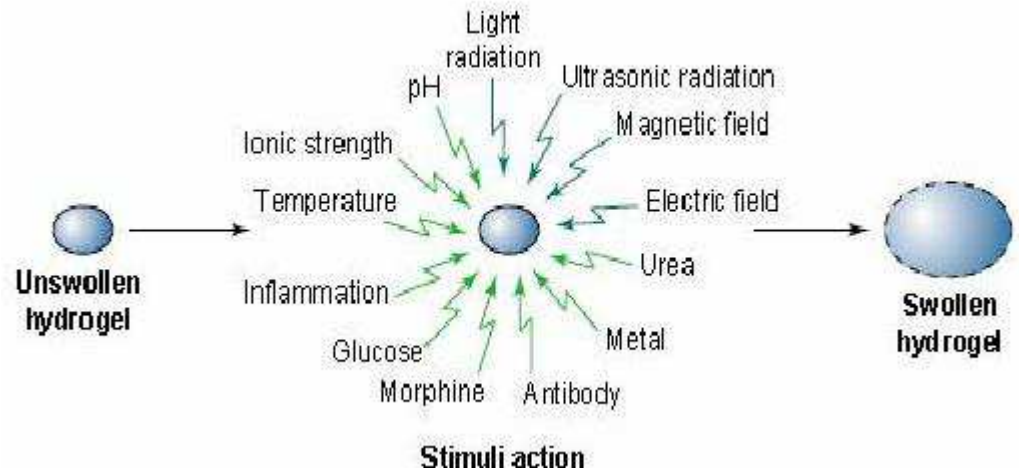

Figure 9 Stimuli response of hydrogel

When an enzyme is released into a smart hydrogel, the product of the enzymatic reaction could activate the gel's phase transition. Then it would lead to a translation of chemical signal (presence of a particular substrate) into an environmental signal (e.g., pH change) and then into a mechanical response (shrinking or swelling of the smart gel). For drug delivery would this means a system in which active components are released in response to a chemical signal, for example, insulin release in response to rising glucose concentration.

The swelling or shrinking of smart hydrogels occurs in response to pH or temperature changes and could be used to control drug release because the diffusion of a drug is dependent on the gel state [39].

Several smart systems have been commercialized and are widely used in different fields:

- Diabetics need medical applications that will sense sugar levels and deliver insulin appropriately.
- The aerospace industry could use smart airfield to control drag and turbulence.
- Architects are designing smart buildings which can control the flow or heat and sunlight by self-adjusting windows.
- In agriculture, irrigation systems will be needed to optimize the world's food supply.

Over the past years, significant interest has developed in mucoadhesive polymers because mucous membranes represent a large surface area of the body for drug delivery. Mucoadhesive polymers can increase the residence time of a delivery system by adhering to mucosal surfaces where a therapeutic is to be delivered.

The versatility and untapped potentials of smart polymeric materials make them an interesting investigated area of chemistry and biology. Because of their stimulus-responsive behavior which occurs in aqueous solutions are polymers and hydrogels becoming attractive in biotechnology and medicine [38].

2 NANOPARTICLES FOR DRUG DELIVERY

The controlled drug delivery seeks the development of suitable drug carriers that can pass on a sufficient dose of the drug. In controlled drug delivery systems is the drug transported to the target, increasing the influence on the needed tissues, minimize its side effects [40], improve efficacy, reduce toxicity and protect the drug from rapid degradation and enhance drug concentration in the target tissue, therefore lower doses of drugs are required [41].

Nanoparticles are defined as particulate distribution [41]. Beneficial in nanoparticles designing for drug delivery is controlling the size of particles and surface properties. Nanocarriers suitable for biomedical applications have to be biocompatible (no immune response or ill effect with an integration with a biological system), biodegradable and nontoxic (harmless to a biological system) itself and also the metabolites [42], ideally inexpensive. The characteristics that must be considered while designing and preparing nanocarriers are summarized in Figure 10.

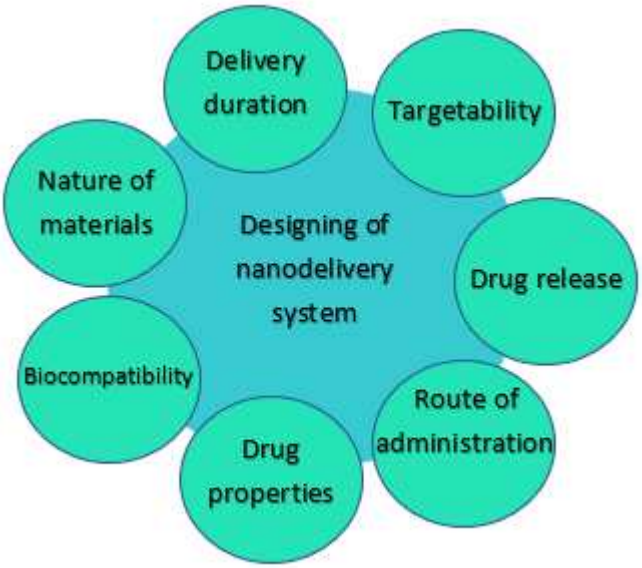

Figure 10 Properties desire for designing of nanocarriers

2.1 Preparation method

Nanoparticles can be prepared from different types of materials such as polysaccharides, proteins, and synthetic materials and the selection depends on many factors including require the size of nanoparticles, properties of the drug such as solubility,

stability, surface characteristics, biocompatibility and toxicity, degree of biodegradability, drug delivery profile desired [43,44].

Emulsion Evaporation method

This method is useful in the preparation of biodegradable and biocompatible micro and nanoparticles and it is based on the emulsification of polymer organic solution into a water phase, followed by organic solvent evaporation. The polymer and the hydrophobic drug are dissolved in organic solvents like dichloromethane, chloroform and ethyl acetate. The emulsification of this polymer-drug mixture is done by using an emulsifying agent or surfactant, which forms specific types of the emulsion such as oil in water [45,46]. After the formation of emulsification, the system evaporates the organic solvent under vacuum, which leads to polymer precipitation and nanoparticle formation [47]. The particle size of the nanostructure is influenced by several parameters like polymer concentration, type of stabilizer and operational set-up [48].

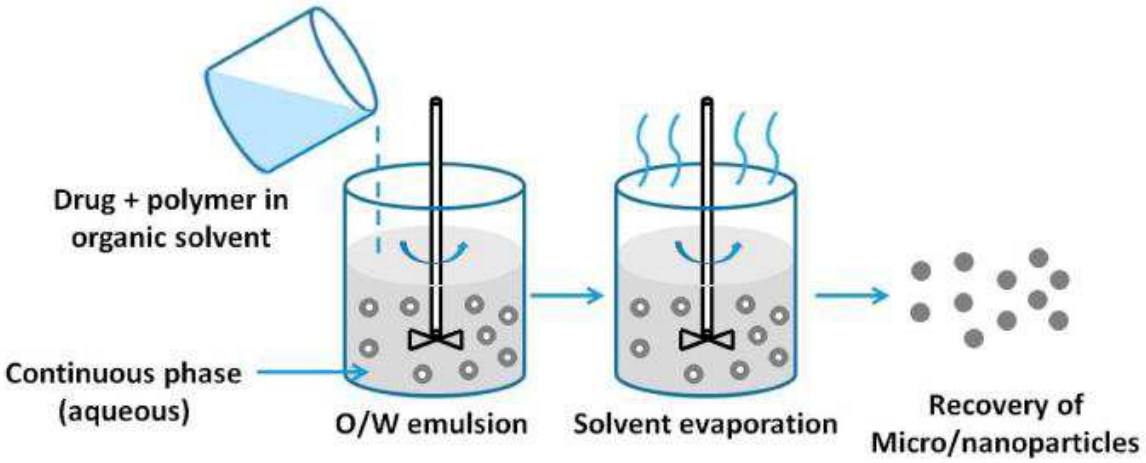

Figure 11 Preparation of NPs by solvent evaporation method [49]

Emulsion Diffusion Method

There are single emulsion and double emulsion methods. Single emulsion encapsulation method is used for the formulation of oil soluble (hydrophobic) substances [50], while double emulsion is captured by entrapment of hydrophilic chemicals [51, 52].

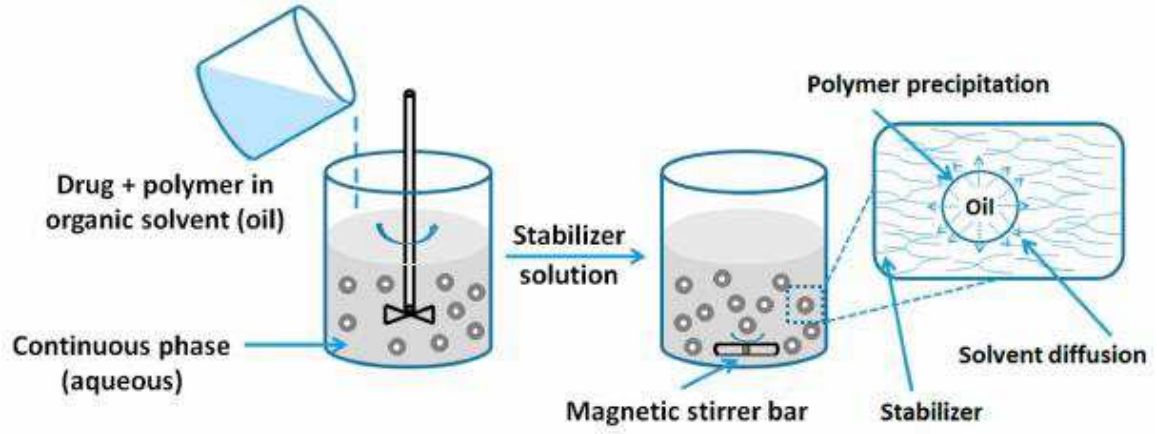

Figure 12 Preparation NPs by emulsion diffusion method

One of the key requirements of the emulsion diffusion method is the selection of an organic phase (oil phase) containing polymer solution which must be partially miscible in the aqueous phase. The most important fabrication step is solvent diffusion. In this step, the organic phase diffuses from the oil phase to outer water phase and the particles become hardened. The selection of the surfactants in the outer water phase is also important to the successful fabrication. Different kinds of surfactants, such as non-ionic surfactant polyvinyl alcohol (PVA) [53], anionic surfactant sodium dodecyl sulfate (SDS) [54] and cationic surfactant didodecyl dimethyl ammonium bromide (DMAB) [55], are commonly applied. The amount of surfactant used has an effect on the properties of the NPs. Low surfactant concentration leads to a high polydispersity and particle aggregation [56]. However, if excessive surfactants are used, the loading of the drug will decrease due to a strong interaction between the drugs and surfactants. The suitable concentration of surfactant is crucial to successful fabrication [57].

Salting-out Method

Salting out is another method for the fabrication of polymer nanoparticles. Firstly, the polymer is dissolved into the organic solutions (oil phase) - typical solvents are tetrahydrofuran (THF) and acetone. The aqueous phase consists of the surfactant and solution of electrolyte and the electrolytes should not be soluble in the organic solvent [58, 59]. The oil phase is emulsified in an aqueous phase by mechanical stirrer under strong shearing force. The difference between the emulsion diffusion method and salting out method is that there is missing solvent diffusion step. The distilled water is added to the formed O/W emulsion under magnetic stirrer (to decrease the ionic strength in the electrolyte). The hydrophilic organic solvents migrate from the oil phase to aqueous phase,

which results in the formation of the nanoparticles. In the end, the salting-out agent is eliminated by centrifugation and the samples are purified. The schematic of the salting-out processes is schematically presented in the picture below.

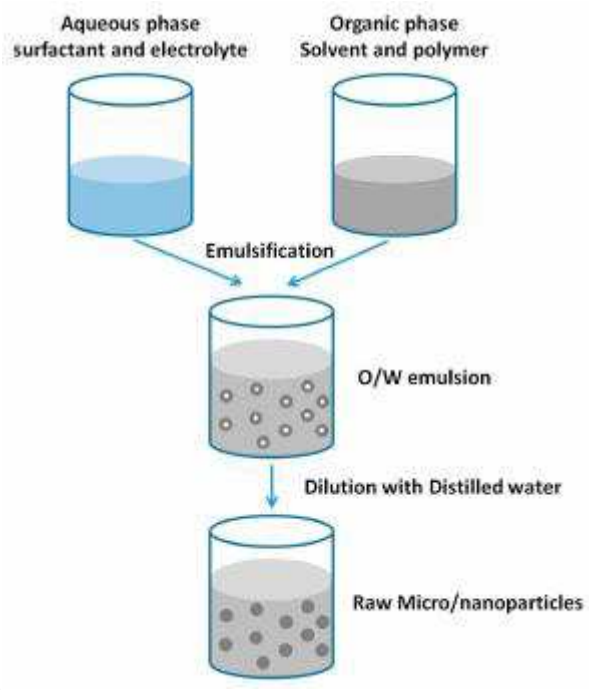

Figure 13 Preparation of NPs by salting out the method

Nanoprecipitation Method

Nanoprecipitation (also called solvent displacement or interfacial deposition method) was first developed and introduced by Fessi's group [60]. The principle of this method is known as Marangoni effect [61]. In the nanoprecipitation method, the nanoparticles are obtained in the colloidal suspension when the oil phase is slowly added to aqueous phase under gentle stirring (Figure 14). Formation of the NPs needs only one step so it has the advantage of easy operation. The key parameters of the fabrication procedure, like organic phase injection rate, aqueous phase agitation rate and the oil phase/aqueous phase ratio, have great influence on the nanoprecipitation method [52]. Because of the absence of shearing stress very narrow particle size distribution can be obtained.

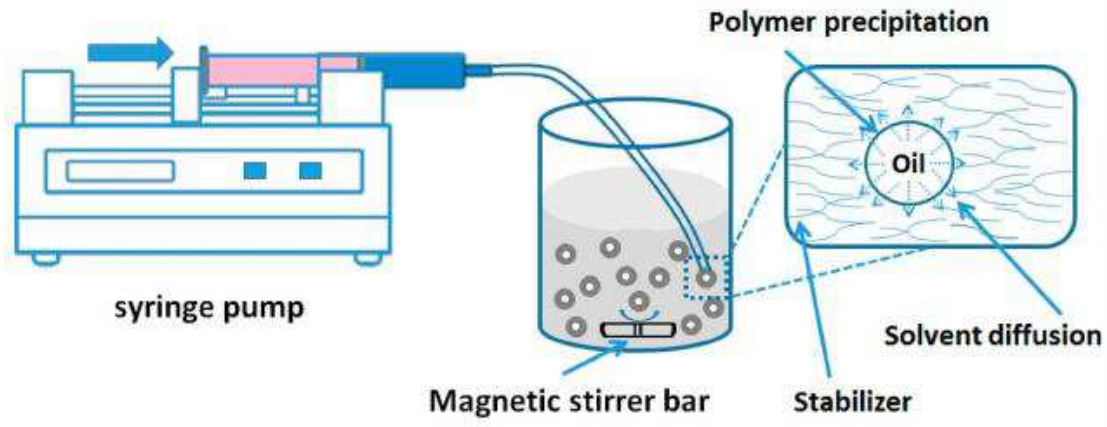

Figure 14 Preparation of NPs by nanoprecipitation method

Polymer and drug are dissolved in a water-miscible organic solvent, for example, methanol or acetone. The solution is afterward added to an aqueous solution which contains a surfactant in a dropwise manner. Because of rapid solvent diffusion, the NPs are formed immediately. Finally, the solvents are removed under reduced pressure.

Polymerization method

This method uses monomers that are polymerized to form nanoparticles in an aqueous solution. Surfactants and stabilizers which are used during polymerization are then removed from a dispersion system by ultracentrifugation or re-suspension of the particles. Their size and nanocapsule formation depend on the type and concentration of the surfactants and stabilizers used. [62]

Supercritical fluid technology

The disadvantage of using conventional methods such as solvent evaporation, solvent diffusion is that they use organic solvents. Nanoparticles are then toxic and unfit for use in the biological system. A supercritical fluid relies on control of solubility via manipulation of temperature and pressure and it is defined as a solvent above its critical temperature because under those conditions the solvent exists as a single phase regardless of pressure [63]. Hence supercritical fluid technology replaces organic solvents and involves controlled growing the particles to attain the desired morphology. The particles do not need to undergo further processing or treatment and this makes supercritical fluid technology amenable to produce biomolecules in their native pure state [64].

Self-assembled nanoparticles

Self-assembly of nanoparticles has been identified as a process where the building blocks spontaneously organize into ordered structures by thermodynamic and other constraints. However, in order to successfully profit nanoparticle self-assembly in technological applications and to provide efficient scale-up, a high level of direction and control is required [65]. It is a method in which the local interactions control the pre-existing components, especially in the disordered system to form organized structures without any external direction. Self-assembly in the aqueous environment results in the formation of a core-shell type of structure, which means outer shell comprises of hydrophilic segments and the inner core is composed of hydrophobic segments. This offers an advantage of encapsulating hydrophobic drugs which can be accommodated by hydrophobic interactions.

The self-assembly method mechanism relies on a weak non-covalent bond, such as hydrophobic interactions and van der Waals or ionic and hydrogen bonds. This method promises a low cost, high yield technique with a wide range of technological applications [66].

2.2 Methods for characteristic

When nanoparticles of various compositions are prepared by some of the above-mentioned methods, characterization of the microspheres is then followed to examine the particle size and size distribution, the colloidal stability, the drug encapsulation efficiency, the surface chemistry, the morphology, the stability and it's in vitro release behavior. Its necessary to investigate the effect of polymer types, solvents, and drug loading.

Electron microscopy

Due to a small size of nanoparticles electron microscopy (EM) is required for examination the surface morphology of the polymeric microspheres like information on the size, size distribution, and the shape of nanomaterials. On a size of the particles and size distribution in nanoparticle system depends several properties like toxicity, ability to target, *in vivo* distribution and also can influence the drug release, drug loading and stability of nanoparticles [7].

Scanning electron microscopy (SEM) can be used to obtain that information, however, TEM uses more powerful electron beams and provides, therefore, greater detail

at the atomic scale, such as information about the granularity of a sample and the crystal structure.

Many biological compounds like liposomes and proteins and engineered polymers like dendrimers are invisible to EM without heavy-metal staining procedures. The reason is that molecules like this do not deflect an electron beam sufficiently [67].

Encapsulation efficiency

The encapsulation efficiency (EE%) is defined by the concentration of the included material (such as active ingredients, drugs, proteins, pesticides, antimicrobial agents, etc.) detected in the formulation over the initial concentration used to make the formulation.

Encapsulation efficiency (EE) can be calculated by using the formula [68]:

$$EE = \frac{Amount\ of\ drug\ bound}{Total\ amount\ of\ drug\ used\ for\ nanoparticle\ production}$$

Zeta potential

Zeta potential is a potential difference between the dispersion medium and the stationary layer of fluid which is attached to a dispersed particle. The zeta potential is a measure of the magnitude of the electrostatic repulsion between particles and is one of the main parameters known to affect stability.

Typically, the higher the zeta-potential, the more stable is the colloid. Zeta potential that is less negative than −15 mV represents the beginning of agglomeration of particles. When the zeta-potential is zero the colloid will precipitate into a solid.

Zeta potential is an important value to understand the state of a nanoparticle's surface and predict the long-term stability of the nanoparticle [69].

X-ray photoelectron spectroscopy (XPS)

The surface chemistry of the microspheres is analyzed through X-ray photoelectron spectroscopy (XPS). X-ray photoelectron microscopy uses an input beam of x-rays instead of electrons and detects the kinetic energies of emitted electrons [70].

Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermal analysis which is used for characterization of the physical state inside the microspheres. The samples are sealed in aluminum pans with lids and are purged with pure dry nitrogen. A temperature rate speed is set up and the heat flow is recorded.

2.3 In vitro drug loading

A successful nanoparticle system is the one which has high loading capacity to reduce the number of carriers. The drug can be loaded during the nanoparticles preparation or post-preparation [71].

For manipulation of the timing and amount of drug from nanoparticles is needed a good understanding of release mechanisms. Five possible methods for drug release are known: (1) desorption of drug bound to the surface, (2) diffusion through the polymer wall of nanocapsules, (3) diffusion through the nanoparticle matrix, (4) nanoparticle matrix erosion, (5) a combined erosion-diffusion process [70].

The nanoparticles are classified as nanocapsule and nanosphere and the drug molecules are either adsorbed on the surface or entrapped inside [72].

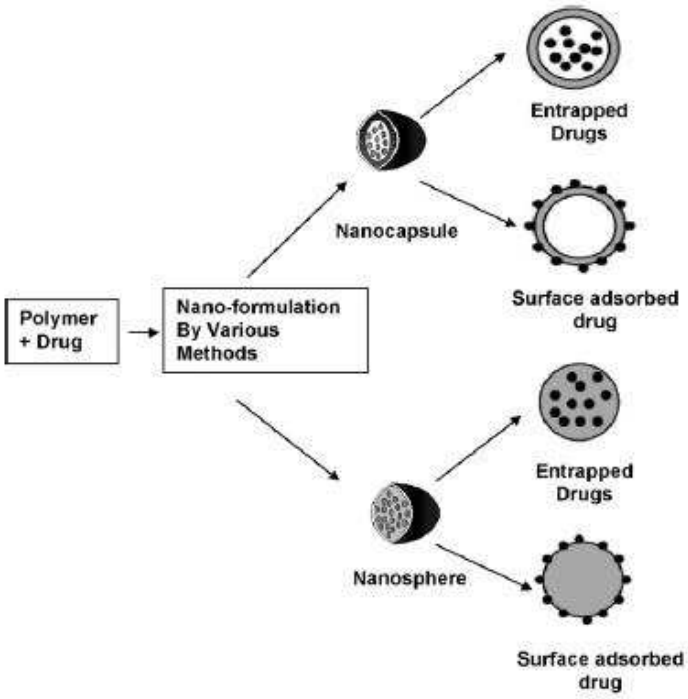

Figure 15 Structural classification of nanoparticles

2.4 Mathematical Modelling in Drug Delivery

Controlled release formulations bring engineers and pharmacists to work together with the common aim of realizing more and more effective products. For this purpose, the use of mathematical modeling turns out to be very useful. It allows the measurement of some important physical parameters, such as the drug diffusion coefficient and resorting to model fitting on experimental release data. Thus, mathematical modeling has a very important value in the process optimization of such formulation [73].

I will describe examples of those mathematical models such as first-order model and a Higuchi model, as well as a Korsmeyer-Peppas model.

First-order model

This model has been used to describe absorption and/or elimination of some drugs. The release of the drug which followed first-order kinetics can be expressed by the equation:

$$log\ Q_t = log\ Q_{max} + \frac{K_{t-t_{lag}}}{2.303},$$

where Q_t is the cumulative concentration (mg drug/mg carrier) of drug released at certain time t (h); Q_{max} is the maximum value for the concentration that can be released from the systém under certain conditions (mg drug loaded per mg of a carrier); k represents the kinetic constant (h^{-1}), which is the release rate from the particles at initial time t; K is the release constant.

Application: This model can be used to describe the drug dissolution in pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices [74].

Higuchi model

The first example of a mathematical model used for describing drug release from a matrix system was proposed by Higuchi in 1961. This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension; (iii) drug particles are much smaller than system thickness; (iv) matrix swelling and dissolution are insignificant; (v) drug diffusivity is constant; and (vi) perfect sink conditions are always achieved in the release environment.

The mathematical expression for the Higuchi model was applied by the following formula:

$$drug_{rel} = K_H t^{1/2},$$

where K_H is the Higuchi dissolution constant; $drug_{rel}$ (mg/mL) is the cumulative release of drug at time t, and t is the time of release (h).

Application: This formula can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water-soluble drugs [75, 76].

Korsmeyer-Peppas model

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first, 60 % drug release data were fitted in Korsmeyer-Peppas model [77].

The mechanism of drug release according to the Korsmeyer-Peppas model, the data of drug release are fitted with next formula:

$$R(\%) = K_H t^n,$$

where $R(\%)=M_t/M_{load}$ (the fraction of drug released at time t); M_t is amount of drug released at certain time t; M_{load} is amount of drug loaded in a carrier initially; K_H is the dissolution constant, n represents the release exponent, indicative of the drug release mechanism and it is function of t (fractional drug release) [77].

3 POLYMER STRUCTURAL FACTORS IN DRUG DELIVERY SYSTEM

Recent nanotechnology can be used in drug delivery by applying novel self-assembled materials and devices of nanoscale size. In order to design a better-controlled drug delivery system, it is crucial to understand the chemical, physical and biological properties of drug carrier. Mechanical strength, swelling behavior, capacity to undergo hydrolysis and biodegradation rate of the polymer are directly influenced by the degree of crystallinity [78].

3.1 Factors affecting drug release

The drug release depends on polymer matrices, loading efficiency of drug and also on a size of nanoparticles. Larger particles have a smaller initial burst release. In the case of the nanosphere, where the drug is constantly distributed, the release occurs by diffusion or erosion. If the matrix erosion is slower than the diffusion of a drug, the mechanism of release is controlled by a diffusion process. The rapid initial release is caused by weakly bound or adsorbed drug to the large surface of nanoparticles [79]. To control drug release of PLA based polymers can use the addition of another polymer, for example, PEG which has been polymerized into a PLA creating PLA-PEG-PLA copolymer [80].

Polymer nanocarriers are used as transport modules of drug delivery technology. It is necessary to precisely control and reproduces their synthesis on a large scale because their properties and performances are strongly dependent on their size and shape. Fundamental studies and practical applications of polymer nanocarriers are slowed by the difficulty of using the current methods to produce monodispersed nanocarriers in large quantities and with high reproducibility [81]. Polymer materials release drug by the following mechanisms:

(i) diffusion,

(ii) solvent activation, which involves either swelling of the polymer or osmotic effects,

(iii) chemical reaction, which is accomplished either by polymer degradation or chemical cleavage of the drug from polymer [82].

Diffusion drug delivery systems

The most common applicable mechanism for describing drug release is diffusion, whereby the drug migrates from its start position in the polymeric system to the outer surface of the polymer and then to the body. In diffusion based drug delivery systems is drug dissolved in a system which is not able to swell or a fully swollen matrix which does not decompose during their activation time [83]. Fick's law of diffusion with either constant or variable diffusion coefficients is commonly applied in modeling diffusion-controlled release [84].

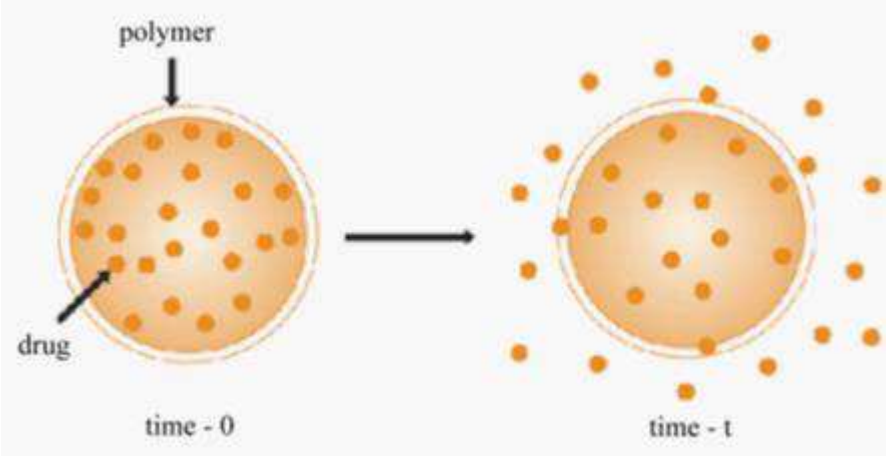

Figure 16 Diffusion-based drug delivery [84]

Solvent activated systems

Solvent activated systems for example hydrogels swell and release the drug when exposed to the aqueous environment while remaining insoluble in aqueous solutions due to chemical or physical crosslinking of individual polymer chains.

Hydrogel nanoparticles have gained attention in recent years as one of the most promising nanoparticulate drug delivery systems. It is due to their unique potentials via combining the characteristics of a hydrogel system (hydrophilicity and extremely high water content) with a nanoparticle (very small size). Swelling-controlled release occurs when diffusion of the drug is faster than swelling of hydrogel [85]. Several polymeric hydrogel nanoparticulate systems have been prepared and characterized, based on both

natural and synthetic polymers. Among the natural polymers, chitosan and alginate have been studied extensively for preparation of hydrogel nanoparticles and from the synthetic group, hydrogel nanoparticles based on poly (vinyl alcohol), poly (ethylene oxide), poly (ethyleneimine) and poly-*N*-isopropylacrylamide have been reported with different characteristics and features with respect to drug delivery.

Application: hydrogels are excellent candidates for encapsulating biomacromolecules including proteins and DNA due to their lack of hydrophobic interactions which can denature these fragile species [86].

Factors affecting degradation

After releasing the loaded drug, the polymeric carrier supposed to degrade to products which are not toxic and are easily resorbable. The main reason why using these systems are beneficial is based to improve and prolong the therapeutic effect and control the release rate of the loaded compound(s), which lead to decreasing a dosage which results in a reduction of side effects [87].

The degradation is a process of chain cleavage leading to a molecular weight reduction. Degradation by erosion takes place in devices that are prepared from soluble polymers and in such cases, the device erodes as water is absorbed into the systems causing the polymer chains to hydrate, swell, disentangle and dissolved away from the dosage form. Another way is degradation from chemical changes to the polymer including cleavage of covalent bonds, ionization, and protonation of the polymer backbone or side chains. The mechanism of polymers erosion can be described either physically or chemically [88].

To enhance the desirable properties of drug delivery systems, it is essential to understand the factors affecting the degradation and design a drug delivery device.

- Effect of Composition

The composition of the polymer is the key factor to determine the hydrophilicity and degradation rate of a delivery matrix which affects the rate of degradation. Many investigations of polymer composition with its degradation have been done [89, 90]. These results show that increase a percentage of glycolic acid in the oligomers of PLGA increases the weight loss of polymer. The amount of glycolic acid is a crucial parameter in tuning the hydrophilicity of the matrix and thus the degradation rate and release of the drug.

- Effect of Crystallinity (or T_g)

The composition of the polymer also affects important properties such as glass transition temperature and crystallinity which have indirect effects on degradation rate [91]. The crystallinity of lactic acid (PLLA) increases the degradation rate because the degradation of the semi-crystalline polymer is accelerated because of an increase in hydrophilicity [92].

- Effect of Weight Average Molecular Weight (M_w)

Polymers with higher molecular weight have usually exhibited lower degradation rates [93]. Molecular weight has a straight relation with the polymer chain size. Polymers with higher molecular weight have longer polymer chains, which need more time to degrade than short polymer chains. Nevertheless, this is opposite for PLLA due to an inversely proportional degree of crystallinity with the molecular weight [93, 94].

- Effect of Drug Type

The mechanism of polymer-drug matrix degradation and the parameters of drug release rate are changed as a function of drug type [95]. The presence of drug may change the degradation mechanism, as well as influence the rate of matrix degradation. However, efforts to correspond the release rate parameters to the drug chemistry (as defined by the density of OH groups) or hydrophilicity do not produce a strong relationship. It is clear that the effect of the chemical properties of the drug has to be understood due to the drug-release mechanisms of a particular system using biodegradable polymers [96].

3.2 Modification techniques

Although biodegradable and biocompatible materials have the potential to be used in many areas, their applications are often limited due to their lack of suitable functional groups. Therefore, a variety of experiments have been made to functionalize polymer NPs, including the improvement of hydrophilicity of polymer and the conjugation with targeting ligand for the increase of targeting efficiency [97]. Some of the modification techniques for the polymer-based drug delivery NPs are described in the following sections.

Improving Hydrophilicity

Most of the biodegradable polyesters are hydrophobic like for example poly(lactide-co-glycolide) (PLGA) and its hydrophobic index is dependent on the ratio between the amount of two monomers lactic acid (LA) and glycolic acid (GA).

One of the most important limitations of the practical drug formulations of NPs is poor hydrophilicity, especially for the hydrophilic drugs. The hydrophobic drug carriers are recognized as a foreign substance by the body. The drug carriers with the hydrophobic surface are surrounded by the mononuclear phagocytic system (MPS), which can absorb the carriers, especially in the liver. Therefore, one of the purposes for the modification of surface with hydrophilic components is to make the carriers unrecognizable by the MPS.

To enhance the hydrophilicity poly(ethylene glycol) (PEG) has been conjugated to the polymer. PEG is a biocompatible, non-toxic and water soluble polymer [97].

Chitosan Functionalization

In order to improve the functionality of PLGA surface chitosan is grafted onto PLGA via the amino groups (Figure 17).

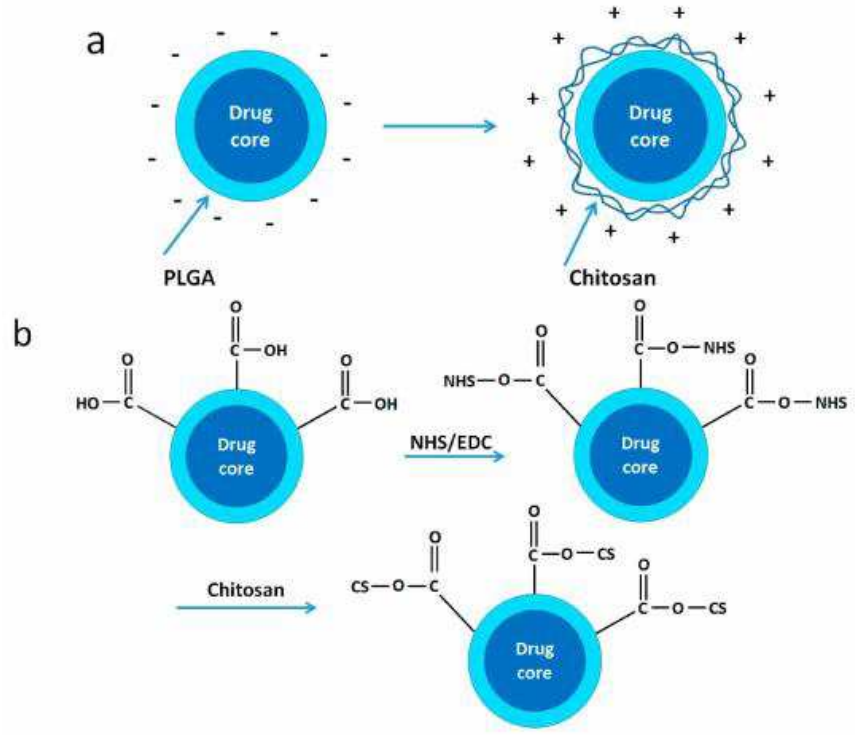

Figure 17 Schematic maps of chitosan modified PLGA NPs by (a) physical adsorption method and (b) chemical binding method [98]. Copyright Springer, 2016.

Chitosan functionalization on the PLGA surface explores the broader use of this polymer. This surface modification can also increase the particle surface zeta potential, which is beneficial for the in vitro cytotoxic effects because the NPs adheres strongly to the cells as cancer cells generally have a negative charge on their surface [99]. Chitosan is a natural cationic polymer which has a very good biocompatibility, biodegradability, and nontoxicity. Therefore, using chitosan and its derivatives to coat onto nanoparticle surface or prepare NPs has been widely investigated in recent years [100].

Targeting Functionalization

Targeting functionalization of surfaces enables the drugs to be delivered to the determined area and to specifically target the cancerous cells. The transportation of the drugs to the defined areas can be mainly achieved in two ways: active and passive targeting.

pH-Sensitive Coating

A pH-sensitive coating can protect loading of the drug from being released in the oral delivery route. As many drug delivery carriers are administered orally, it would be

beneficial if the drug carriers are pH-sensitive and delivery carriers pass through this environment. The properties of water-insolubility at low pH and water-solubility at high pH polymers have been investigated in recent years. The release rate and the timing of the drugs to be released can be controlled by the pH values of the environments. However, the drug release mechanisms from the coated NPs are not fully understood yet [101]. There are many complicated processes, for example, it was reported that the release of chemotherapeutic drug phenylpropanolamine hydrochloride from ethylcellulose coated particles was released not only by the drug dissolution but also an osmotic effect. Eudragit S100 is another popular pH-sensitive polymer and widely used for coating the PLGA NPs. It is pH sensitive and insoluble under pH 7. There are carboxyl and ester groups in the polymer structure of Eudragit S100 and the ratio is 1:2 [102, 103].

Plasma Treatment

Modification by plasma treatment can be divided into two categories, (i) one is gas plasma treatment and the other is (ii) plasma polymerization. Plasma treatment is very suitable for material surface modification. As this technology can very easily adopt the reactive groups or chains onto the material surfaces, it is commonly used for the cell affinity improvement [104].

For the gas plasma treatment are used different gases, such as oxygen, ammonia, a combination of nitrogen and hydrogen to immobilize different functional groups, including amine, hydrogen and carboxylic groups.

The modification of polymer surfaces by plasma polymerization is substrate independent. Plasma modifications will enhance the effectiveness of NPs' delivery system and cell affinity. Lee et al. investigated the PLGA surface modification by plasma treatment, in order to influence the cell affinity on the polymer surface [105]. The trials showed that cell proliferation was significantly improved. Additionally, plasma treatment can make the PLGA surface more hydrophilic. Hasirci et al. [106] applied oxygen plasma technology to modify PLGA surface and concluded that the PLGA water contact angle decreased from 67″ to 38″ after the treatment.

4 POLYLACTIC ACID

Polylactic acid (PLA) belongs to the family of aliphatic polyester and it is a biodegradable material with excellent biocompatibility and bio-absorbability and low toxicity. At present, it is one of the most promising biodegradable biopolymers and can be processed with numbers of techniques. It is relatively cheap and is commercially available (large-scale production) in a wide range of grades. The high crystallinity and low hydrophobicity of PLA reduce a degradation rate, which results in a worse soft tissue compatibility [107].

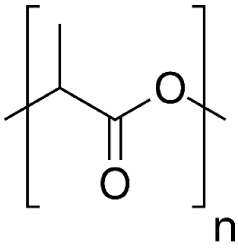

Figure 18 Polylactic acid structure

PLA is a semicrystalline polymer with thermoplastic properties and two enantiomeric forms of PLA, poly-L-lactide (PLLA) and poly-D-lactide (PDLA). PDLA is amorphous, resulting in a weaker and more rapidly degrading material [108]. PLLA is a polymer with good biodegradability and biocompatibility, perfect mechanical properties and processability. In the past 60 years, it has been studied and adopted as a biomedical material in tissue engineering and drug delivery. For practical application is essential to understand the rate and mechanism of biodegradation and those properties can affect various factors such as molecular weight, the enantiomeric composition, environment, processing methods and sterilization.

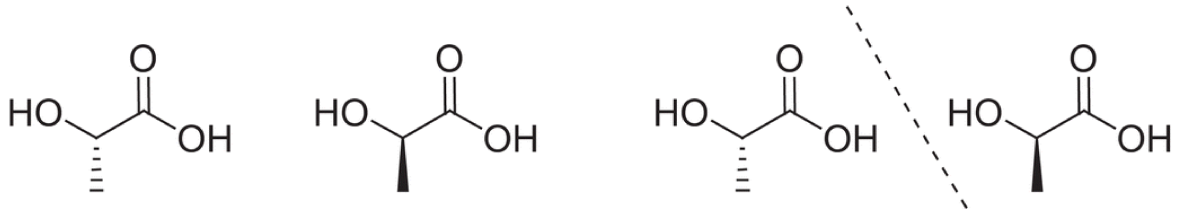

Figure 19 Structures of L-, D- and DL-lactic acid

As long as the basic monomer (lactic acid) is produced from a renewable resource (carbohydrates) by fermentation, PLA complies with the worldwide concept of sustainable development and has a classification of environmentally friendly material.

Polylactic acid contains an asymmetric α-carbon which is in stereochemical terms described as the D or L. The enantiomeric forms of the polymer PLA are poly D-lactic acid (PDLA) and poly L-lactic acid (PLLA). Because of their biodegradability and biocompatibility, PLLA and PDLLA have been investigated extensively as carriers for drug delivery. To consider this background, it is important to reveal the mechanism of drug release in order to develop new dosage form using these polymers.

For control drug release from biodegradable polymers have been identified three mechanisms: Fickian diffusion through the polymer matrix, diffusion through pores in the matrix and drug liberation by polymer erosion [109]. As was mentioned before, release mechanism varies depending on many factors: polymer molecular weight, copolymer ratio, a stereoisomer of lactic acid, physicochemical properties of drugs, the shape of the matrix and the preparation method [110].

In general, the polymer PLA can be made in semi-crystalline form (PLLA) or completely amorphous (PDLA) and crystallinity is affected by the preparation method of the matrix. It was reported that drug release is faster from the crystalline PLLA than from amorphous matrix [108].

4.1 Synthesis

The synthesis of PLA starts from the lactic acid production and ends with its polymerization. There are three possible way how to synthesize PLA, (i) lactic acid is condensation polymerized to reach a low molecular weight polymer, which is brittle and for the most part is unsuitable, (ii) an azeotropic dehydrative condensation, which can yield high molecular weight PLA, (iii) main process is ring-opening polymerization of a lactide monomer (Figure 20) [111].

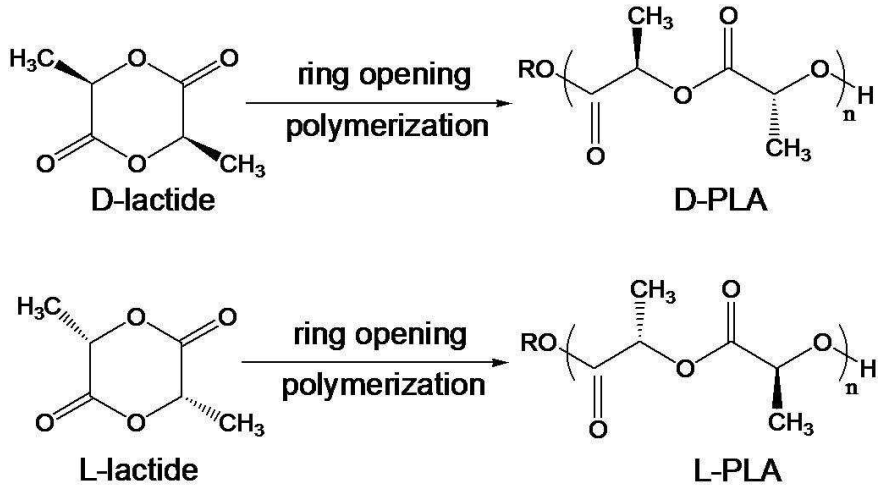

Figure 20 Ring-opening polymerization

The cyclic lactone polymerization is usually too slow for a production of high molecular weight material, however, it is possible to increase the polymerization by activation of a Zn- or Sn-based catalysts with the carboxyl ester. Anyway, the catalyst could be introduced as potentially cytotoxic materials, so only stannous octoate $Sn^{II}(CO_2CH(^nBu)(Et))_2$ is commonly used because of FDA approval as an s food stabilizer [112].

4.2 Precursors

Lactic acid can be obtained either by carbohydrate fermentation or by chemical synthesis. It is a hydroxy acid with an asymmetric carbon atom and two optically active configurations – D and L isomers. Lactic acid is prepared in large quantities (approximately 200kT per year) by the bacterial fermentation of carbohydrates.

Lactide is usually obtained by the depolymerization.

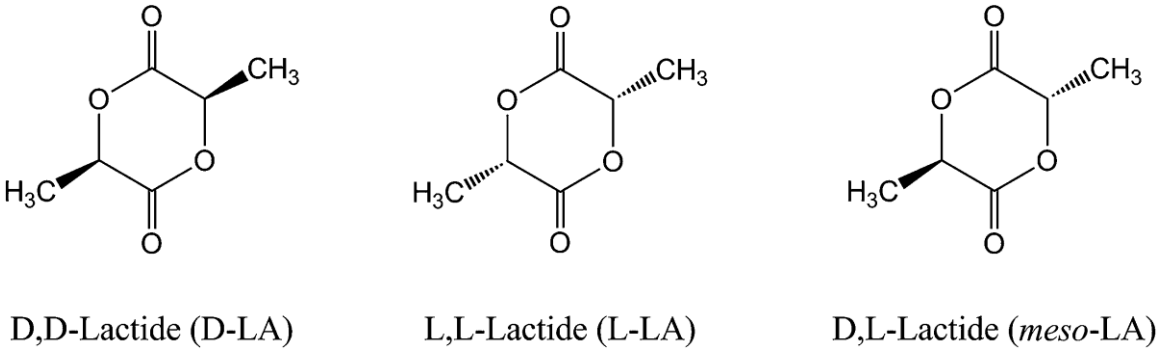

Figure 21 Structures of D-, L-, meso-Lactide

4.3 Crystallinity and Thermal Properties

Indeed as other polymers, the properties depend on its molecular characteristics, as well as on the presence of ordered structure, crystallinity, spherulite size, morphology and degree of chain orientation [113]. PLA can be produced in a completely amorphous or with up to 40 % crystalline. For example, PLA resins containing more than 93 % of L-lactic acid are semi-crystalline, however, when it contains 50-93 % of it, it is amorphous. The crystallization kinetics of PLA is rather slow in comparison to other polymers. The rate of crystallization increase with a decrease of a molecular weight and it is dependent on a structural composition [114]. The crystallization and crystallization rate is determined by the decrease in the melting point of the different copolymers. PLLA/PDLA stereo complexes are efficient nucleating agents for PLLA, with increases in both the crystallinity and also the crystallization rate, up to 60 percent [115]. For a determination of the crystallinity, levels are used differential scanning calorimetry (DSC).

Glass transition temperature (T_g) of PLA ranges from 50 °C to 80 °C, while its melting temperature (T_m) ranges from 130 °C to 180 °C. For example, enantiomerically pure PLA is semi-crystalline with thermal properties T_g of 55 °C and T_m of 180 °C. T_m increases with increasing molecular weight, however, the crystallinity decreases with increasing molecular weight. The T_g of PLA is also determined by the different types of lactide in its macromolecule. The T_m also decrease in the presence of meso-lactide units in the structure [116].

4.4 Degradation

Abiotic

The thermal degradation of lactic-acid-based polymers can follow different mechanisms, such as zipper-like depolymerization, thermohydrolysis, thermo-oxidative degradation, in the presence of catalyst residues and transesterification which give simultaneous bond making and bond breaking [117, 118].

Another option of abiotic degradation is hydrolytic degradation. PLA hydrolysis leads to chain fragmentation and this can affects parameters such as the PLA structure, molecular weight, and distribution, morphology, mechanical and thermal history [114, 117]. Aliphatic polyesters hydrolysis starts with a water intake phase, followed by a split of the ester bond in a random way. The amorphous parts of the polyesters undergo hydrolysis earlier than crystalline regions because of a higher range of water intake. This step gives more space and mobility to the remaining non-degraded chains, which leads to their reorganization and an increased crystallinity. The second stage of the hydrolytic degradation is degradation of crystalline regions of the polyesters which lead to an increased rate of mass loss and to complete resorption [119]. The explanation of this phenomenon is an autocatalytic effect due to the increasing amount of compounds which contain carboxylic end groups. These compounds are not able to permeate the outer shell and are continuously dissolved in the surrounding buffer solution [119].

Biotic

The biodegradation of lactic acid based polymers for biomedical applications has been evaluated *in vivo* and *in vitro* for PLA based surgical implants [120]. The *in vivo* studies have shown that the key role in the degradation plays pH of the solution [114]. PLA degrades in multiple steps with different mechanisms.

4.5 Applications

Because its bioresorbability and biocompatibility in the human body, PLA has been widely studied for use in medical applications [121].

PLA-based materials are developed for the production of screws and plates as the bone therapy progresses. PLA resorbs or degrades upon implantation into the body, but most of its mechanical properties are lost within a few weeks [120]. The valuable advantage of

resorbable composite implants is that they do not need to be removed with a second operative procedure, as with metallic or non-resorbable composite prostheses. To improve the mechanical properties, PLA is reinforced with a variety of non-resorbable materials, including carbon and polyamide fibers.

Micro and nanoparticles are an important category of delivery systems used in medicine, and the use of PLA is interesting due to its hydrolytic degradability and low toxicity. The most important properties of the micro and nanoparticles are the drug release rate and the matrix degradation rate which are affected by the particle designed the material properties [117].

Porous PLA scaffolds have been found to be potential reconstruction matrices for damaged tissues and organs.

PLA fibers are also used in different applications as, for example, non-woven textile for clothes. One of the first commercially available fiber formed bioresorbable medical products is based on copolymers of GA in combination with l-lactide (Vicryl) [42].

5 PLA IN DRUG DELIVERY

New drug delivery technologies are innovating the drug discovery. Development and creating R&D focussed pharmaceutical industries to increase the global advancements. In this regard, novel drug delivery systems have many benefits, which includes improved therapy by increasing efficiency and duration of drug activity, increased patient compliance through the elimination of drug dosage and improved site-specific delivery to reduce the unwanted side effects [123].

Following text describes the current state of research in the field of PLA nanoparticles in controlled release and targeted transport of drug.

Tab. 2 List of novel researches of PLA nanoparticles in drug delivery

Study	Drug	Application	Reference
PLA/Chitosan nanoparticles	nifedipine	hypertension treatment	[124]
PLGA	5-fluorouracil	chemotherapeutics	[125]
CA-(PCL-ran-PLA)	docetaxel	chemotherapeutics	[126]
PLA)-coated mesoporous silica nanoparticles	resveratrol	neurodegenerative diseases	[127]

One of a recent publication [124] presented results of zeta potential, water contact angle, atomic force microscopy image, in vitro solubility, and content of heavy metals in PLA/chitosan nanoparticles loading nifedipine. In addition, the in vivo test of the PLA/chitosan nanoparticles loading nifedipine in the mice is one of the highlights of this work. The Zeta potential result shows that the charged surface of the PLA/chitosan nanoparticles loading nifedipine depends on nifedipine content. Nifedipine plays a role in the increase of hydrophobic property, swelling degree as well as the regular surface. The PLA/chitosan/nifedipine nanoparticles are dissolved in the solutions with different pH (6,8–1,2). The in vivo test of PLA/chitosan nanoparticles loading nifedipine on mice was evaluated by the change in arterial pressure, diastolic pressure, systolic pressure and heart

rate. The obtained results confirm that the PLA/CS nanoparticles loading nifedipine are appropriate to apply in the treatment of hypertension patients lately.

Another paper [125] was investigated 5-fluorouracil (5-FU) which is a chemotherapeutic agent that has been used for the treatment of a variety of malignancies. Due to its short biological half-life, multiple dosing is required. Poly(D, L-lactide-co-glycolide) (PLGA) is biocompatible, biodegradable and widely used for drug delivery, however, issues such as insufficient loading and inappropriate burst release kinetics have affected progress into the clinic.

Current limitations of cancer therapy include the lack of effective efficiency for target delivery of chemotherapeutic drugs, and also the difficulty of achieving significant capability by a single treatment. Herein, this study [126] reported a synergistic chemo-photothermal strategy based on functionalized CA-(PCL-ran-PLA) NPs for effective delivery of docetaxel (DTX) and increased therapeutic effect. The developer NPs achieved advantages, such as (i) improved drug loading content and encapsulation efficiency initiated by star-shaped copolymer CA-(PCL-ran-PLA); (ii) effective target delivery of drugs to tumor sites; (iii) significant therapeutic efficacy caused by synergistic chemo-photothermal treatment. Therefore, with excellent bio-properties and simple preparation procedures, the DTX-loaded NPs effectively increased the local drug concentration in tumor sites, minimized side effects, and significantly eliminated tumors.

Oxidative stress acts as a trigger in the course of neurodegenerative diseases. An antioxidant-based therapy can be effective to enhance the harmful effects of oxidative stress. Resveratrol (RSV) has been shown to be effective at removing reactive oxygen species or reactive nitrogen species generation in the central nervous system, but the delivery of RSV into the brain through systemic administration is inefficient. In this research paper [127] was presented an RSV delivery vehicle based on polylactic acid (PLA)-coated mesoporous silica nanoparticles (MSNPs), conjugated with a ligand peptide of low-density lipoprotein receptor (LDLR) to enhance their transcytosis across the blood-brain barrier.

6 AIMS OF THE WORK

Although the wide number of polymers available for biomedical application, many researchers are focused on PLA-based products. Since lactide exists in three isomeric forms (l-lactide, d-lactide, and meso-lactide) various PLA homo- and stereo-copolymers with different properties can be prepared by adjusting l/d ratios in the monomer [112].

This study is focused on the encapsulation and release behavior of a hydrophilic drug, Doxorubicin (DOX), by an adjusting of low molecular weight polylactic acid (PDLLA) based nanoparticles, containing a different percentage of D-Lactate. Doxorubicin is an anthracycline widely used due to its broad range of antitumor activity. DOX acts as DNA intercalant which disrupts the replication and transcription process in cancer cells [128]. However, DOX can diffuse either in tumor and healthy cells, which causes serious side effects. Cardiotoxicity is one of the most studious problems which limits the anthracycline applications. As reported in numerous studies one of the most effective ways to limit the cardiotoxicity is through reduction of distribution DOX in cardiac tissue. This could be done by selective carriers which can accumulate the drug only in the target site [129].

The aim:

- Preparation of PDLLA with different crystallinity;
- preparation and characterization of nanoparticles;
- encapsulation and release kinetics of drug (doxorubicin) in different physiological media (simulated gastric fluid, intestinal fluid, and blood).

II. ANALYSIS

7 MATERIALS AND PREPARATION METHODS

7.1 Materials

- $C_3H_6O_3$L-lactic acid, 80% water solution was bought from Lachner Neratovice, Czech Republic.
- Sodium chloride, potassium dihydrogen phosphate, sodium carbonate and sodium hydroxide were obtained from Penta, Prague, Czech Republic.
- The C_3H_6O solvent acetone, sodium chloride, sodium hydroxide, sodium phosphate, and potassium were acquired from IPL Lukes, Uhersky Brod, Czech Republic.
- Chloroform CHCl3 (HPLC grade) and acetic acid CH3CO2H (HPLC grade), hydrochloric acid was purchased from Chromservis, Prague, Czech Republic.
- Methane sulfonic acid (MSA) by Sigma-Aldrich.
- Doxorubicin

7.2 Instruments and Equipment

- KRUSS Optronic-Germany
- Ultra Turrax IKA T18 basic
- HERAEUS Multifuge X1R – Thermo-Scientific
- Varian Cary UV 300
- Stuart orbital incubator SI5000
- NICOLET 320 FTIR
- Netzsch DSC 200 F3
- Agilent HT-GPC 220

7.3 PLA Synthesis

Different forms of polylactic acid (PDLLA) were prepared by polycondensation reaction using MSA as an initiator followed by characterization of the amount of D-lactate in PDLLA by polarimetry analysis (KRUSS Optronic-Germany). L- and D- lactic acid were added at a different weight ratio to obtain different % D in the final product. The solution was added to a double-necked flask (250 ml) followed by connection to distillation apparatus and placed into an oil bath. The dehydration step was under required conditions

at 160 °C and reduced pressure 15 kPa for 4 hours. Afterward, the initiator was added under stirring and this mixture of dehydrated L, D-lactic acid and the initiator was again connected to the vacuum source and the reaction proceeded for 24 hours at 160 °C. The product was then cooled down to room temperature and dissolved in acetone and this solution was precipitated using methanol/distilled water - 1:1 mixture. This dissolving-precipitation procedure was repeated three times. The product was dried at 45 °C for 48 hours and formulations were prepared in following ratios:

- PLLA
- PDLLA 10:90
- PDLLA 25:75
- PDLLA 40:60

7.4 Nanoparticles preparation and characterization

Double emulsion solvent evaporation technique was used for DOX nanoparticles preparation. The prepared formulations were dissolved in 5 ml chloroform and 8ml of an aqueous solution with 3% PVA (w/v) was added. DOX was dissolved in water (0,5 mg/ml; V=2ml) followed by homogenization (Ultra Turrax IKA T18 basic) at 12000 rpm for 5 minutes.

Thereafter, the obtained W/O was added to 160 ml of aqueous solution containing PVA (0,1%; w/v) and kept under stirring to remove the solvent and solidify the nanoparticles. The nanoparticles were twice washed with distilled water and ultracentrifugated (HERAEUS Multifuge X1R – Thermo-Scientific) at 12000 rpm for 20 minutes.

The DOX content in the particles was determined by UV-Vis spectrophotometer (Varian Cary UV 300) at 480 nm and the concentration obtained by a standard curve. Encapsulation efficiency (EE) was calculated according to:

$$EE\,(\%) = \left(\frac{D_t - D_f}{D_t} \right) \times 100$$

where D_t is a total amount in mg of DOX added and D_f represents the concentration after an encapsulation process (mg/ml). All studies were performed in triplicate.

7.4.1 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy is one of the spectroscopy techniques and in principle is based on the interaction of molecules with electromagnetic radiation in an infrared region ($\lambda = 0,3 – 300$ μm). Some of the infrared radiation is absorbed by the sample and some of it is transmitted. The result is a spectrum with molecular absorption and transmission which is represented by different peaks and it is unique for every molecular structure. The peaks correspond to the frequencies of vibration between atomic bonds and the size of the peaks is an indication of the amount of present material [130].

To identify the structure of PLLA and PDLLA samples were analyzed by using FTIR method (NICOLET 320 FTIR) which is equipped with attenuated total reflectance (ATR) accessory utilizing Zn-Se crystal over a range of 4000–650 cm^{-1}. The uniform resolution of 2 cm^{-1} was maintained.

7.4.2 Differential scanning calorimetry (DSC)

Differential scanning calorimetry is thermal analysis method determines the temperature and heat flow associated with material transitions as a function of time and temperature. Tested sample and a comparative standard material are kept at the same temperature and heat flow is measured. As a result of DSC measurement, we can get a graph with a dependence of the differential rate of heat heating ($J.s^{-1}$) at temperature. This method can be used to determine glass transition, melting temperature, the heat of fusion, crystallinity, crystallization kinetics and phase transitions [131].

This DSC analysis (Netzsch DSC 200 F3) was used for investigation of the PDLLA formulations and to determinate the crystalline and amorphous structure. Approximately 8 mg of the sample was placed into an aluminum pan, calibrated and samples were heated from -10 °C to 180 °C with a heating range of 10 $°C.min^{-1}$ in a nitrogen atmosphere (60 $ml.min^{-1}$).

7.5 Drug release

Simulated physiological fluids enzyme-free (intestinal, gastric, blood) were prepared according to the European Pharmacopoeia standards procedure to study a release kinetic. The drug-loaded nanoparticles were suspended in 50 ml of a media in a concentration 1 mg/ml and placed into an orbital incubator (Stuart orbital incubator SI5000) at 37 °C, 120

rpm frequency. 3 ml of the release medium was withdrawn at a determined time and replaced with an equivalent volume of fresh buffer, to maintain the total volume constant and analyzed by UV-Vis spectroscopy at 480 nm afterward.

The amount of released drug (DR) was determined by the following equation:

$$DR(\%) = \left(\frac{D_t}{D_0} \right) \times 100$$

where D_t (mg) represents the amount of drug detected in the external media at time t_t and D_0 (mg) the amount of loaded drug. All studies were performed in triplicate.

8 RESULTS AND DISCUSSION

8.1 PLA Characterization

In fact, the amount of D-lactate in PDLLA influences the biocompatibility, usability, and processability of the final products and especially the hydrolytic degradation. The content of D-lactate can be usually determined by high-performance liquid chromatography (HPLC) after hydrolysis of PDLLA or nuclear magnetic resonance (NMR) and infra red (IR) spectroscopy. To compare those methods, the HPLC analysis is time-consuming for high Mw PDLLA, while the last two methods require complicating operational procedures [132].

In our case, the molecular weight of the products was characterized by gel permeation chromatography (Agilent HT-GPC 220) equipped with a dual detection system (refractive index and viscometric detector) at 40 °C in tetrahydrofuran (THF). The flow rate 1.0 $ml.min^{-1}$ and injection volume of 100 μL. The weight average molar mass M_w, number average molar mass M_n, and molar-mass dispersity ($Đ = M_w/M_n$) were determined from the peak (Tab. 3).

The D- content was determined with polarimetry method due to the accuracy and also the simplicity of this method. The results are reported in Tab. 3.

Tab. 3 Molecular weight and content of D-lactate in the prepared PDLLA formulations

	M_w [g/mol]	M_n [g/mol]	PDI	% of D
PLLA	8000	5400	1,48	-
PDLLA 10:90	8000	5000	1,60	10
PDLLA 25:75	8200	5400	1,51	25
PDLLA 40:60	6600	4500	1,47	40

Gel permeation chromatography analysis confirmed the low molecular weight of the products and PDI in a range of 1,47-1,60. Those gained data agree with results which were measured under the same conditions [129].

Tab. 4 DSC data of PDLLA

	PLLA	PLLA 40:60	PDLLA 25:75	PDLLA 10:90
Sample weight [mg]	1,7500	2,4500	3,0600	3,7600
T_g [°C]	56,06	36,89	37,62	40,23
T_m [°C]	152,01	-	-	-

The temperature range in DSC analysis was set up from -10 °C to 180 °C with a heating rate 10 °C/min. This data are comparable with literature data [133]. The glass transition temperature (T_g) for PLLA was approximately 56 °C and in the literature was reported 54 °C – 64 °C. Crystallinity is an important factor which affects the glass transition dynamics and enthalpy behavior. As it is evident from measurement, the presence of D form causes a decrease of Tg from around 56 °C to 40 °C and this is also related to lost of crystallinity. The melting point (T_m) demonstrates the shift from a crystalline form (PLLA) to an amorphous form as well.

Figure 22 displays the FTIR-ATR analysis and spectra of PLLA and PDLLA formulations in a 2200 – 500 cm^{-1} range.

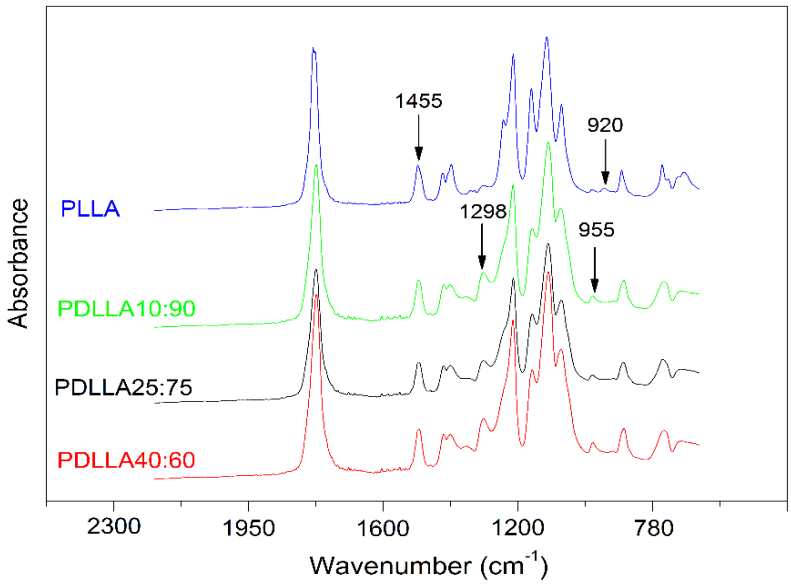

Figure 22 FTIR-ATR spectra of all PLLA and PDLLA formulations

Spectra were normalized according to the intensity of the CH_3 band at 1450 cm^{-1} [134]. The intensity of the band at 920 cm^{-1} is associated with a coupling of C-C backbone stretching. For amorphous samples (PDLLA) lack of 920 cm^{-1} wavelengths is shown. The intensity of the band at 920 cm^{-1} increase with a crystallinity of sample, while in the amorphous sample is demonstrated an increase at 955 cm^{-1}. Crystallinity differences are presented in a region 1000-800 cm^{-1}.

8.2 Nanoparticles Characterization

The effect of the amount of D-lactic acid and polymer concentration on nanoparticles size is summarized in Figure 23.

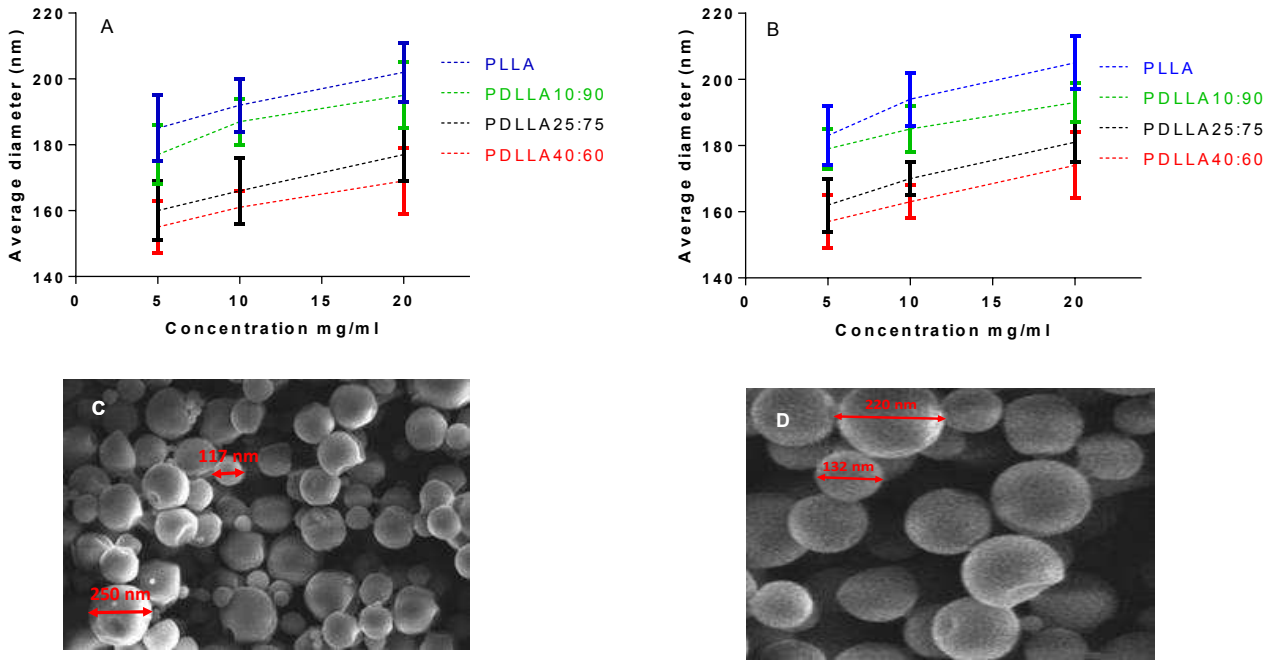

Figure 23 Dimension of all nanoparticles formulations A) unloaded; B) loaded with DOX; C) SEM micrograph of PLLA; D) SEM micrograph of PDLLA nanoparticles in dried form.

Figure 23 A, B: The average value of all formulations are in the range 150-200 nm. No significant differences are presented between the loaded and unloaded formulations at the same concentration. It indicates that DOX does not impact on the nanoparticles size, especially at high polymer concentration (20mg/ml). When the concentration of polymer is reduced from 20 mg/ml to 5 mg/ml, the average nanoparticles dimension reports a downward trend.

Figure 23 C, D: SEM analysis proved a spherical shape of the particles with a slight diameter increase compared to size in solutions. On the contrary to the definition of nanoparticle's size for drug delivery applications (less than 100 nm), depending on the target site, larger particles (100 < diameter < 500 nm) could be more suitable due to their ability to load higher amount of drug [135, 136].

The ζ-potential of all formulation was found close to zero (range from -2 to +3 mV) because of the absence of a notable number of ionic groups in the system. Polymer concentrations did not cause any significant changes in the ζ-potential values.

ζ-potential could be a prediction of the nanoparticles stability in solution. Although the low ζ-potential, obtained nanoparticles have satisfactory colloidal stability.

8.3 Encapsulation Efficiency

Due to the hydrophobicity of DOX, the encapsulation efficiency is influenced by the PDLLA structure and concentration in the initial solution. Encapsulation efficiency and relationship with polymer concentration and amount of D-lactic acid are shown in Figure 24.

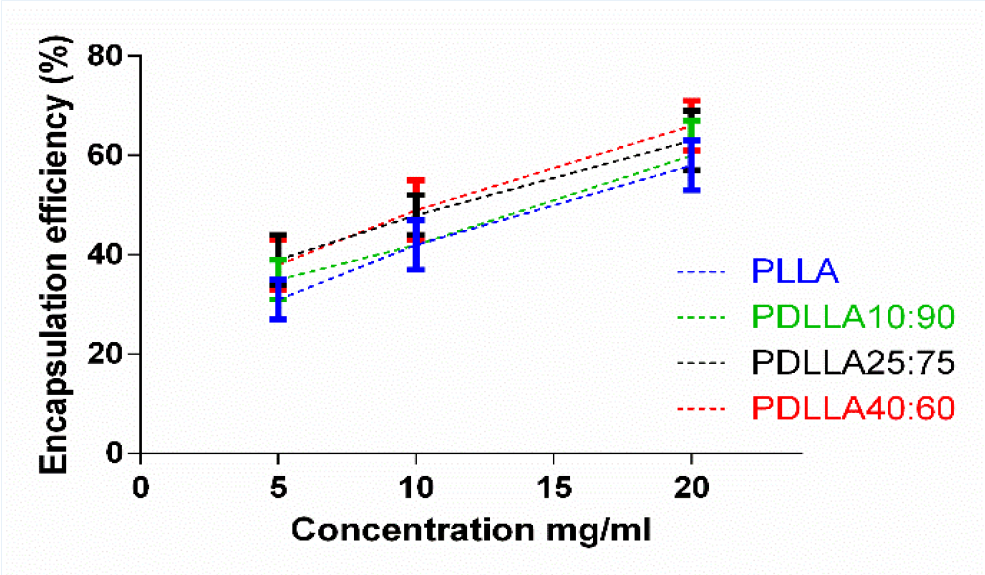

Figure 24 Relationship between DOX encapsulation efficiency, polymer concentration and polymer composition

Considering the hydrophobicity of DOX the encapsulation efficiency is influenced by the PDLA structure and its concentration in the initial solution. Increasing the amount of D-lactic acid, the polymer is amorphous and increase in the encapsulation efficiency is occurred. Contrariwise, the presence of crystalline structure reduces the encapsulation efficiency. This effect is related to how the media diffuse into the polymer carrier favoring the penetration of DOX.

In comparison with results in a previous study [137], a direct relationship between polymer concentration and encapsulation efficiency is presented. Increasing the concentration from 5 to 20 mg/ml encapsulation efficiency increased.

8.4 Release Kinetic

Release tests were done in three simulated media to simulate the conditions in three main body compartments where the drug are absorbed or have to transit through [138, 139, 140]:

a) gastric fluid,

b) intestinal fluid,

c) blood.

All the media are enzymes free and their composition is in agreement with the European Pharmacopoeia standards.

a) Release kinetic in a simulated gastric fluid with different DOX concentrations

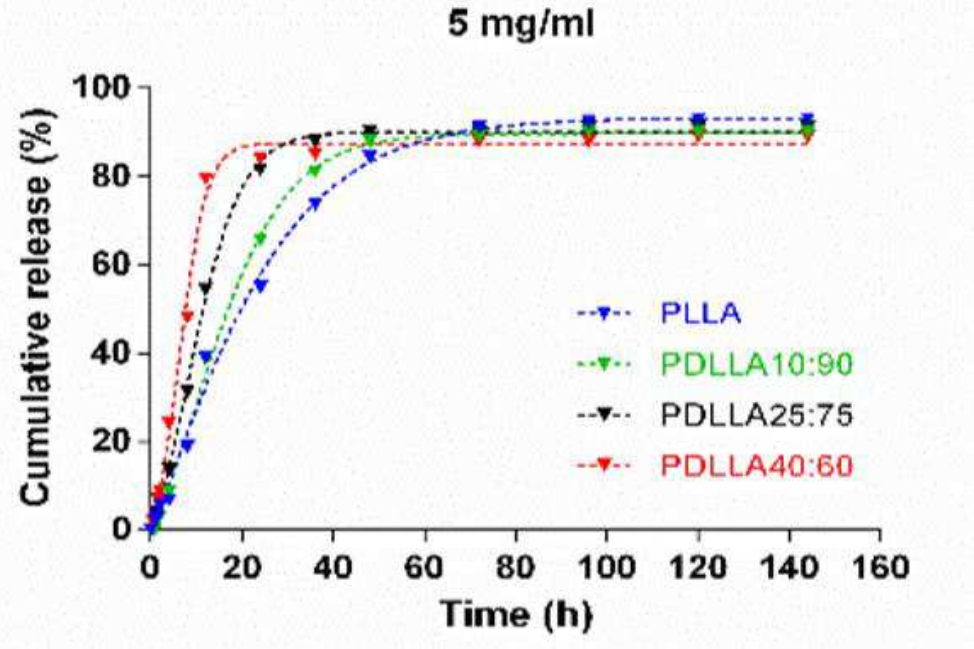

Figure *25 Release kinetic of 10 mg of DOX from NPs formulation in simulated gastric fluid*

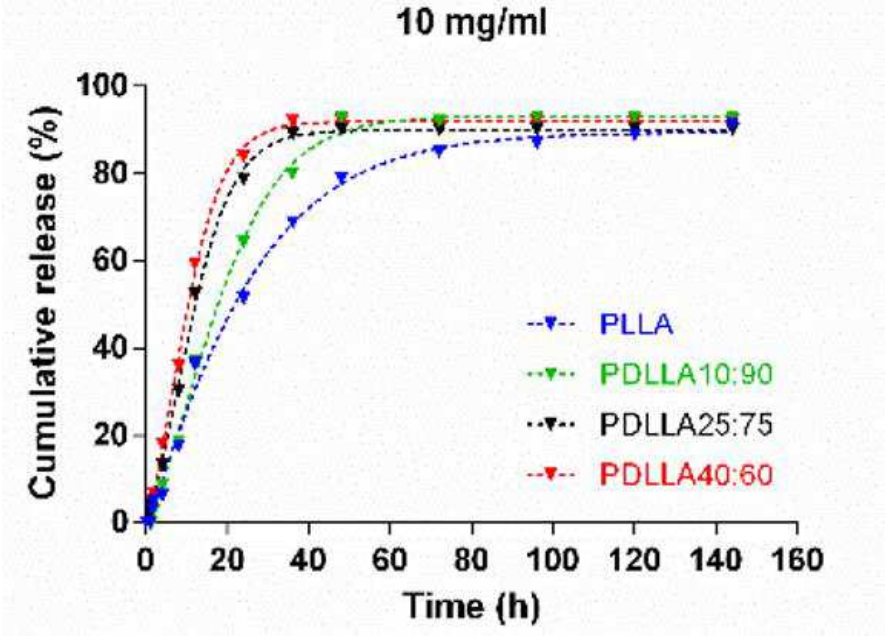

Figure 26 Release kinetic of 10 mg of DOX from NPs formulation in simulated gastric fluid

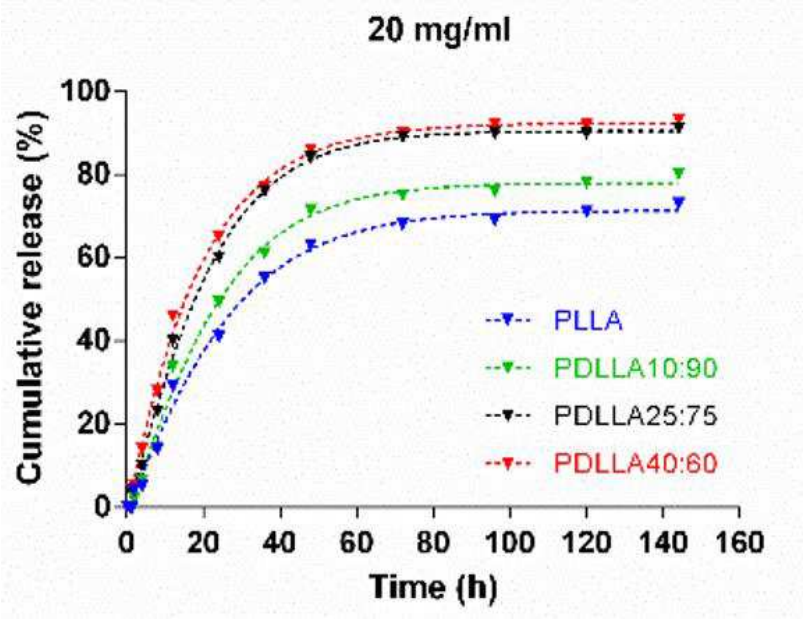

Figure 27 Release kinetic of 20 mg of DOX from NPs formulation in simulated gastric fluid

b) Release kinetic in a simulated intestinal fluid with different DOX concentrations:

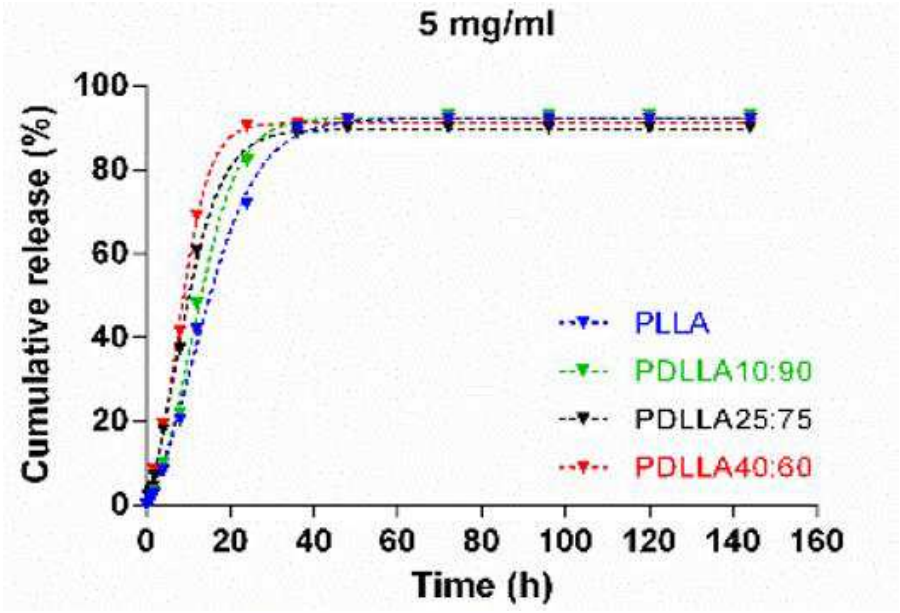

Figure 28 Release kinetic of 5 mg of DOX from NPs formulation in simulated intestinal fluid

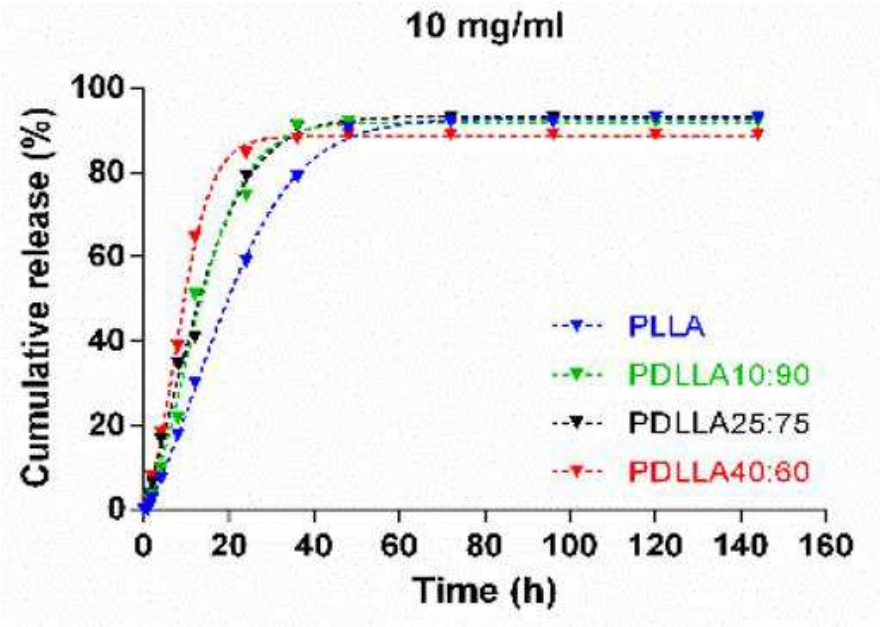

Figure 29 Release kinetic of 10 mg of DOX from NPs formulation in simulated intestinal fluid

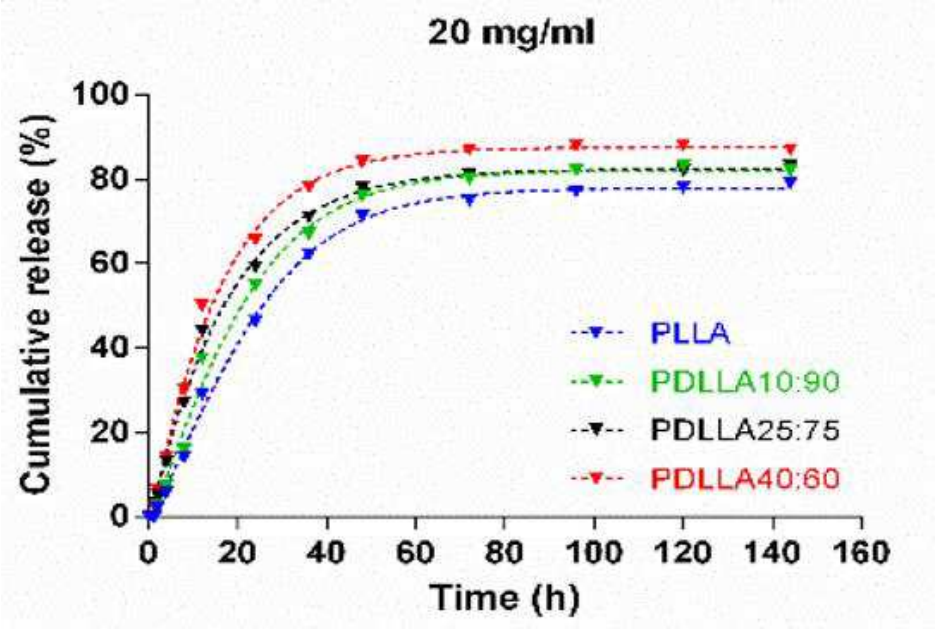

Figure 30 Release kinetic of 20 mg of DOX from NPs formulation in simulated intestinal fluid

c) Release kinetic in simulated blood with different DOX concentrations:

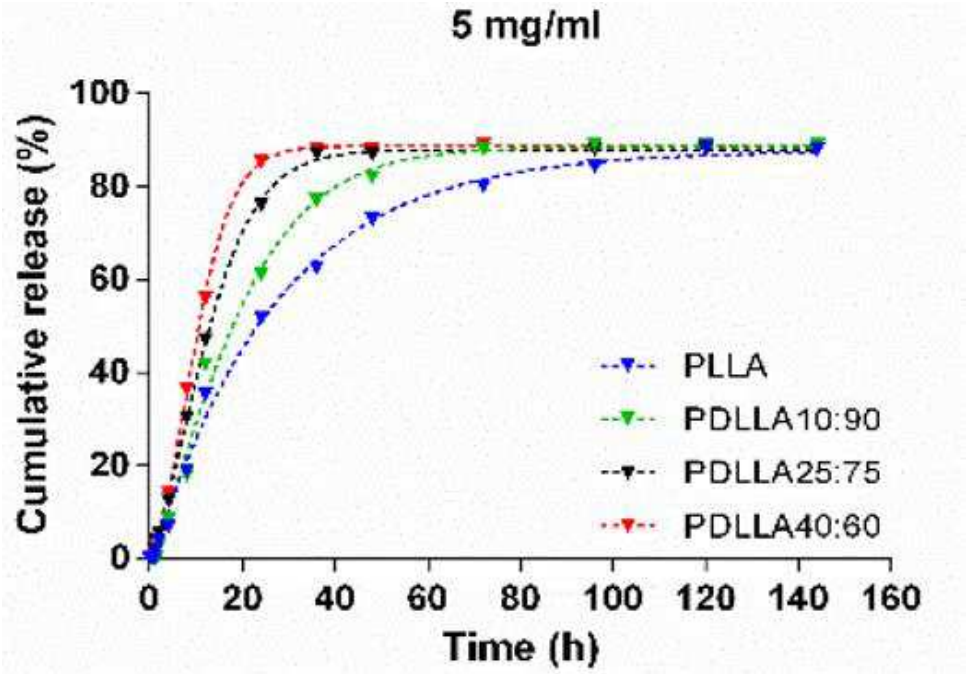

Figure 31 Release kinetic of 5 mg of DOX from NPs formulation in simulated blood

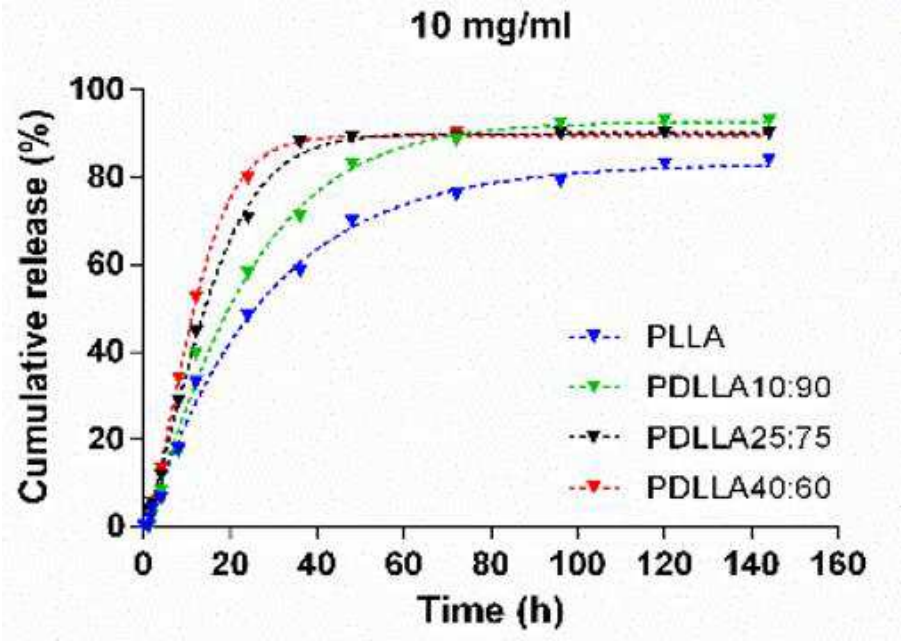

Figure 32 Release kinetic of 10 mg of DOX from NPs formulation in simulated blood

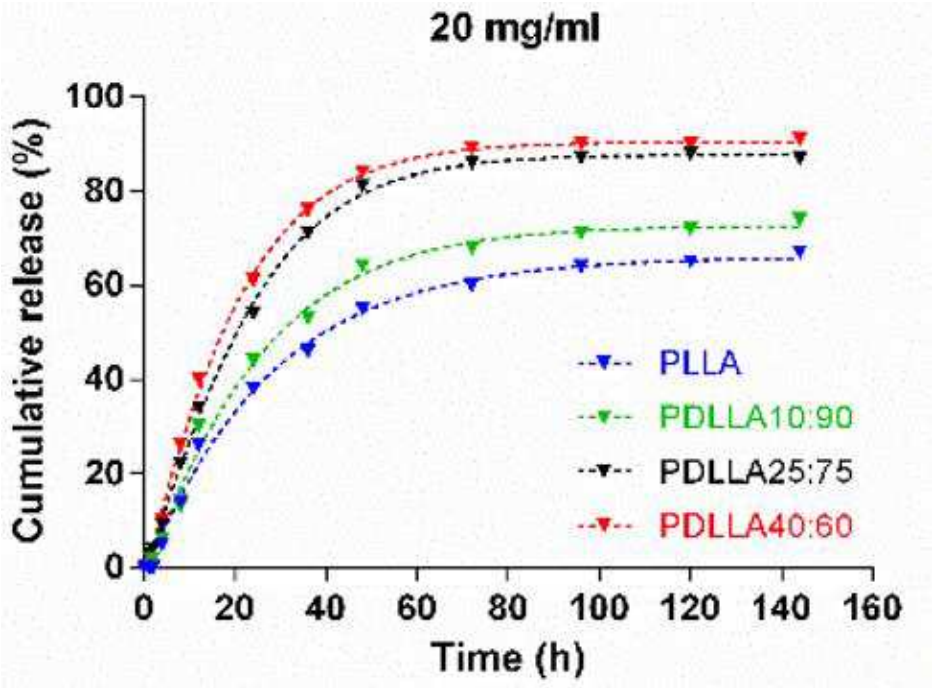

Figure 33 Release kinetic of 20 mg of DOX from NPs formulation in simulated blood

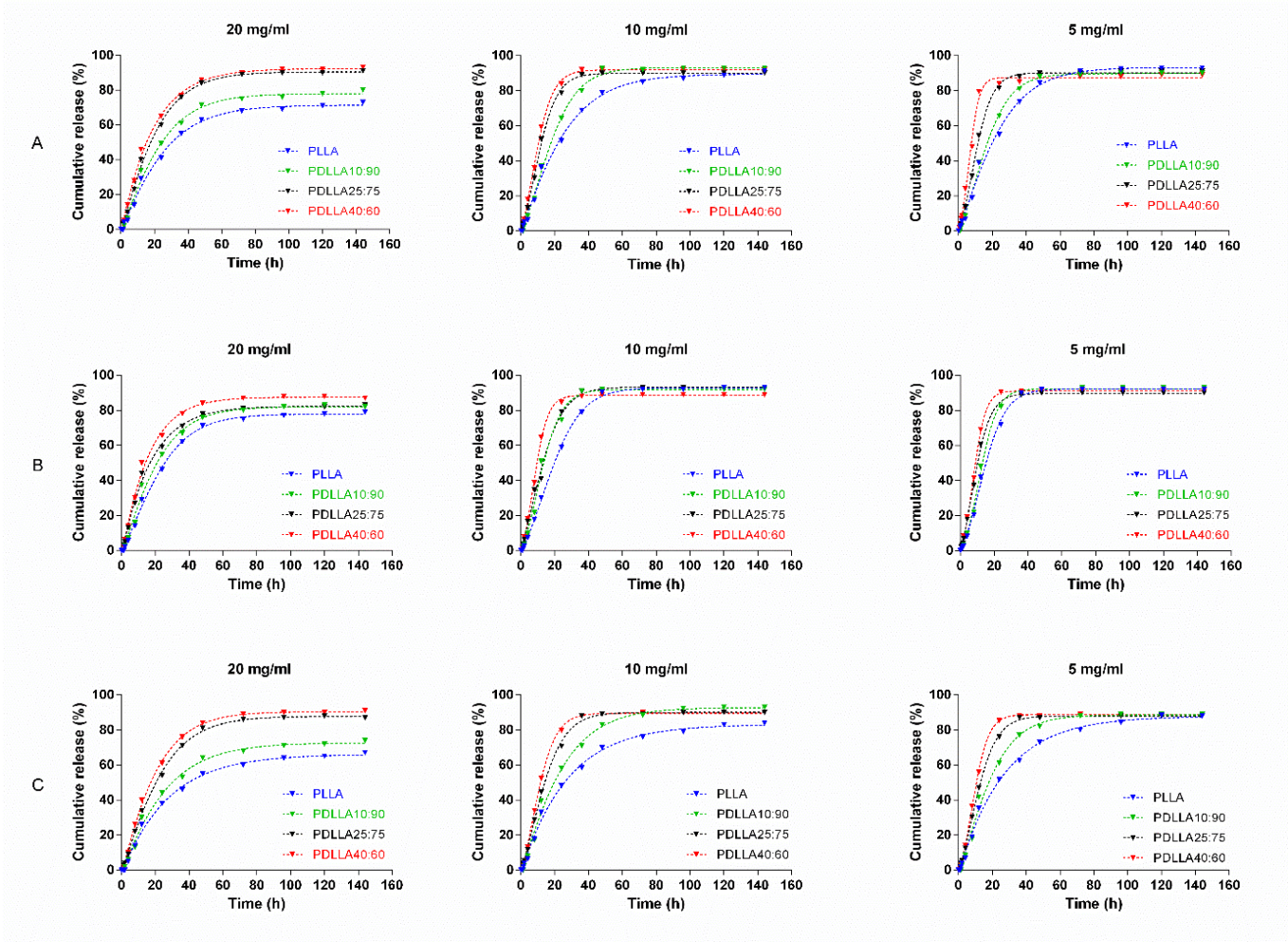

Figure 34 An overview of DOX release kinetic from all NPs formulations at different concentration in A) simulated gastric fluid, B) simulated intestinal fluid and C) Simulated blood at 37 °C

In these cases, the forces which drive the release are based on the diffusion of the drug following the penetration of the media in the system, as well as by the degradation and polymer erosion [141]. As reported, PLA can degrade in its monomer lactic acid which is soluble in water and can decrease the pH of the media, which causes an autocatalytic effect [142]. However, in the simulated blood and intestinal fluids are presented hydroxide ions which diffuse into the carrier and can neutralize the produced hydrogen ions, which inhibit the autocatalytic process [142]. PLA is a bulk-erosion polymer and because of this is the degradation slower than water penetration into the matrix, meaning that the entire polymer is hydrated and the cleavage of the chains occurs throughout the device [137].

Degradation time of PLLA and PDLLA whit comparison with their release rates prove that the mechanism of release of the drug through the polymer matrix is done mainly by the diffusion. The presence of crystalline or amorphous phases play a significant role as influence the diffusion of the media into the system and consequently the release rate.

As presented in figures 25-34, a certain amount of drug (> 15 % of total loaded) is released at the initial phase. To allow the DOX release, the diffusion path has to be filled by the media [143]. In this phase, the crystalline or amorphous phase and hydrophobic properties of PLA play a significant role.

Comparing the release rate to the amount of % of D-lactate is the diffusion of the media and the drug through the crystalline structure slower than the others PDLLA formulations. It causes a longer release time, mainly in simulated blood where approximately 60 % of DOX was released from PLLA compared to almost 90 % in the PDLLA based formulations.

The drug which is encapsulated in polymer produces a zero order release when the initial drug concentration exceeds the drug solubility inside the system [137]. A system that follows zero-order kinetics release a consistent amount of drug per unit time and this is excellent for achieving prolonged pharmacological effect [144]. The prepared PLLA and PDLLA formulations displayed DOX persistent release even after 120 hours. PLLA is characterised by slower drug release due to its stronger hydrophobic interactions between hydrophobic domain and the drug [142].

CONCLUSION

The study was focused on optical activity isomerism of the PLA precursor on nanoparticle preparation process, the morphology of nanoparticles and other selected characteristics such as the evaluation of DOX release from nanoparticles.

Nanoparticles were made by a set of low molecular weight PDLLA, containing various amount of D-Lactate (from 0 to 40 %), which was determined with polarimetry method. PDLLA were prepared by polycondensation reaction using MSA as initiator followed with nanoparticles preparation by double emulsion technique varying the polymer concentration in the range 5 to 20 mg/ml.

The molecular weight of prepared products was characterized by gel permeation chromatography and concluded that with increasing of D- content the M_w (M_n) decrease. DSC analysis was created to evaluate a crystallinity which is related to the glass transition temperature (T_g) and melting point (T_m) of the products. The presence of D- form causes a decrease of T_g and this leads also to lost of crystallinity which is evident at T_m. Apparently, the D- content shift a structure from crystalline form to an amorphous form. FTIR-ATR analysis confirmed this statement with showing lack of 920 cm^{-1} wavelengths which is linked to a coupling of C-C backbone stretching typical for a crystalline structure. Considering amorphous samples, there is increase 955 cm^{-1}.

A direct relationship was detected between polymer concentration and nanoparticles dimension which goes from a minimum of 120 nm at 5 mg/ml concentration to over 200 nm at 20 mg/ml.

Encapsulation efficiency and the release rate are also influenced by polymer concentration. Results displayed that increasing the polymer concentration together with the amount of D-Lactate a preferable encapsulation of DOX occurred reaching more than 60 % in encapsulation efficiency. However, increasing the amount of D-lactate cause a faster release of the drug in all tested media, up to 30 % faster than in pure PLLA, indicating the influence of the microstructure in the release phenomena.

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LIST OF ABBREVITATIONS

PLA	Poly(lactic acid)
PGA	Poly(glycolic acid)
PLGA	Poly(lactic-co-glycolic acid)
PCL	Polycaprolactone
DDS	Drug Delivery System
POE	Polyorthoester
FDA	US Food and Drug Administration
PHA	Microbial Polyhydroxyalkanoates
PVA	Polyvinyl Alcohol
EVA	Ethyl Vinyl Acetate
NPs	Nanoparticles
DMAB	Dimethyl Ammonium Bromide
SDS	Sodium Dodecyl Sulfate
THF	Tetrahydrofuran
O/W	Oil in Water
EM	Electron Microscopy
EE	Encapsulation Efficiency
XPS	X-ray Photoelectron Spectroscopy
DSC	Differential Scanning Calorimetry
MPS	Mononuclear phagocytic system
PEG	Poly(ethylene glycol)
CS	Chitosan
RSV	Resveratrol
LDLR	Low-Density Lipoprotein Receptor
DOX	Doxorubicin

HPLC	High-Performance Liquid Chromatography
NMR	Nuclear Magnetic Resonance
IR	Infra Red

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