An-Najah National University
Faculty of Graduate Studies

Preparation and Characterization of Carvedilol-Loaded Poly (D, L) Lactide Nanoparticles/Microparticles as a Sustained Release System

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Dedication

This thesis is dedicated with gratitude

To:

My Beloved Husband for his patience and

Encouragement with Love and Respect

To:

My honey daughter

To:

Anyone who reads and appreciates this work

Acknowledgments

First of all I heartily thank my God for giving me the will and patience to undertake this study and completion to my Master's degree.

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I would like to express my special, sincere thanks and gratitude to my husband family for their continuous support and efforts.

الإقرار

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

Preparation and Characterization of Carvedilol-Loaded Poly (D, L) Lactide Nanoparticles/ Microparticles as a Sustained Release System

تحضير وتوصيف الجزيئات البوليمرية بحجم النانو والمايكرو المحملة بدواء الكارفيدايلول كنظام تحرير دوائي مستدام

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Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work، and has not been submitted elsewhere for any other degree or qualification.

Student's Name: محمد سليمان محمود بني عودة **اسم الطالبة:**

Signature: **التوقيع:**

Date: 29-09-2015 **التاريخ:**

List of Abbreviations

AFM	Atomic Force Microscopy
AIC	A kaike Information criteria
APIs	Active pharmaceutical ingredients
AUC	Area Under the Curve
BMI	Body Mass Index
C_2H_3N	Acetonitrile
CHF	Congestive Heart Failure
CNTs	Carbon Nanotubes
CR	Controlled Release
D	Dimension
DCM	Dicloromethane
EP	Europe
EtOH	Ethanol
F	Formula
FDA	Food and Drug Administration
G	Generation
g	Gram
HEtC	Hydroxyl Ethyl Alcohol
HTN	Hypertension
mg	Milligram
ml	Millilitre
MNPs	Magnetic nanoparticles
MWNTs	Multi walled Nanotubes
nm	Nanometer
NNI	National Nanotechnology Initiative
NPs	Nanoparticles
O/W	Oil in Water
P.O.E.C.E	Poly Oxy Ethylene Cetyl Ether

PA	Poly Acrylates
PCL	Poly caprolactone
PDLA	Poly (D-lactide)
PDLLA	Poly(D,L-lactide)
PEG	Poly Ethylene Glycol
PGA	Poly Glycolide
PHB	poly (hydroxyl butyrate)
PLA	Poly Lactide
PLGA	Poly(lactide-co-glycolide)
PLLA	Poly(L-lactide)
PNPs	Polymeric nanoparticles
PVA	poly Vinyl Alcohol
PVP	Poly Vinyl Pyrrolidone
QD	Quantum Dot
SPIONs	Super paramagnetic iron oxide nanoparticles
SR	Sustained Release
SWNTs	Single walled Nanotubes
T*g*	Glass Transition Temperature
THF	Tetrahydrofuran
US	United State
W/O	Water in Oil
W/O/W	Water in Oil in Water
α	Alpha
β	Beta
μm	Micrometer

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Preparation and Characterization of Carvedilol-Loaded Poly (D, L) Lactide Nanoparticles/Microparticles as a Sustained Release System

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Abstract

Background: Polymeric nano and micro particles are promising delivery systems for the enhancement of the bioavailability of highly lipophilic drugs prone to first pass metabolism.

Purpose: This study aims at preparing carvedilol polymeric nanoparticles and microparticles with high loading efficiency. Other objective was to study carvedilol release profile from the obtained particles at room and body temperatures.

Method: Carvedilol PDLLA nanoparticles and microparticles were prepared using nano-precipitation method. PVA was used as emulsifying agent. The effect of the solvents (acetone, tetrahydrofuran, acetonitrile, ethanol and dichloromethane) and the polymer amount on the size, size distribution and morphology of the formed particles were studied using atomic force microscope (AFM).

Results: Spherical polymeric particles were obtained in all solvents used. The used method is easy, rapid, reproducible, effective (high loading capacity of carvedilol) and consequently can be used as new strategy for the development of carvedilol controlled release dosage forms. The *in vitro*

release profile of carvedilol has shown a sustained release pattern with a little rapid release rate at body temperature in comparison to that at room temperature.

Conclusion: The carvedilol loaded PDLLA nanoparticles have been successfully prepared with high loading efficacy and small particle size when acetone was used as the organic solvent and 12.5 mg of PDLLA polymer was used. Microparticles obtained when dichloromethane was used. The Korsmeyer Peppas with T lag model was the best one to explain the sustained release behavior.Chapter One

Chapter One

Introduction

Chapter One
Introduction

1.1 Nanotechnology and Nanomedicine

Despite its youth and still being as an emerging science, nanotechnology can be no longer regarded as a simple vision, as it has been until the late twentieth century, but it deals with a reality that its benefits are present amongst us. Although the term "nanotechnology" was first used in 1974 by the Japanese scientist Norio Tamiguchi [1], but the origin of nanotechnology has been attributed to the lecture presented by Richard Feynman, Nobel Prize winner in Physics in 1965, at the California Institute of Technology (CalTech) in 1959 entitled “There is plenty of room at the bottom”, predicting that soon or later we could manipulate atoms in an individual manner [2-4]. Nanotechnology as a science began in 1982 with the development of the scanning tunneling microscope by Heinrich Rohrer and Gerd Binnig, which allows studying and manipulating molecules at the atomic scale, and they received the Nobel Prize in Physics in 1986 for this development [5].

Therefore, in 2000 the US government established the National Nanotechnology Initiative (NNI) to deal with all activities related to nanotechnology and defined Nanotechnology as the science, engineering, and technology that conducted at the nanoscale, which is about 1-100 nm. Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering [6].

One of the attractive applications of nanotechnology is in the field of medicine and biology (that was defined later as nanomedicine) that aims to monitor, control, identify, construct, repair, defense and improve all human biological systems. Applications of nanotechnology to medicine have a significant and attractive event in the diagnosis, treatment and prevention of disease [7, 8]. Nanomedicine includes nanoscale structured materials and devices that hold a promise for advanced diagnostics biosensors, smart drugs, and targeted drug delivery [9, 10]. Molecular machine systems such as nanorobots are also one of the important and promising tool of nanomedicine that could be used for the diagnosis and destruction of pathology cause, cell surgery, chromosome replacement, and physiological functions improvement [10].

1.2 Types of Nanomaterials

Many types of nanomaterials were designed and prepared in order to be applied to medicine such as:

1.2.1 Quantum Dots (QDs)

QDs are nano-crystals of semiconductor material such as cadmium as a core and zinc as a shell, these crystals glow when excited by a light source such as a laser, and different sizes of QDs give different emission and different optical colors [11], figure 1. This character gave it an important use in medicine as biosensors, proteins tracking, microscopic detection, live cell imaging, and *in vivo* targeting to cells, tissues, and tumors [12].

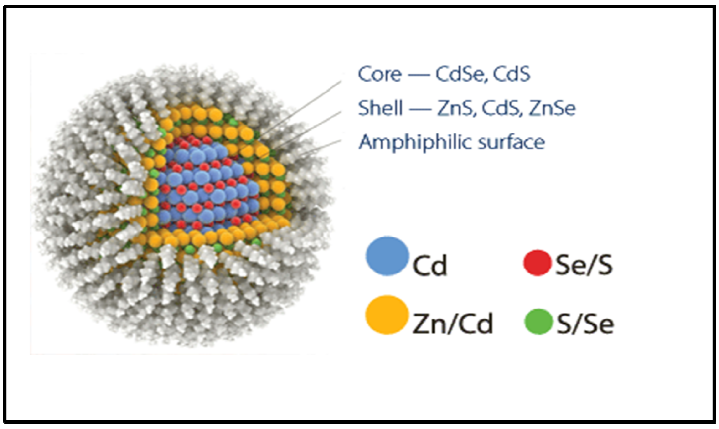

Figure (1): Quantum dot
(http://www.en.rusnano.com/press-centre/news/88604)

1.2.2 Liposomes

Liposomes are artificial and nano-sized spherical vesicles synthesized from natural surfactants such as phospholipids and cholesterol derivatives, figure 2. It was the first nanomedicine placed on the market under the trade name Doxil® that loaded with doxorubicin. Cardiotoxicity of doxorubicin decreased when it was prepared as liposome [13].

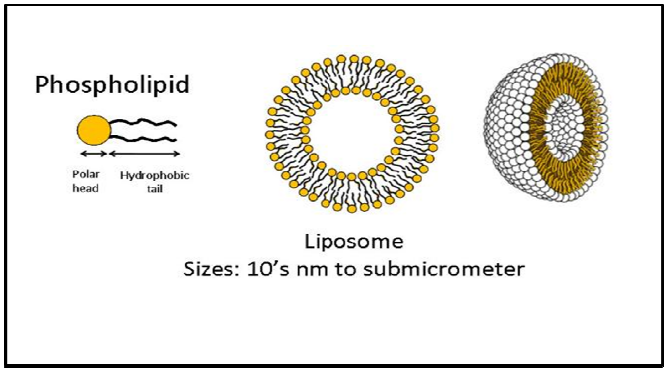

Figure (2): Liposome
(http://www.reemazeineldin.com/Liposome.html).

1.2.3 Micelles

Micelles are surfactant molecules that arrange themselves in a spherical shape once they placed in aqueous solutions. A typical micelle in

aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with surrounding water, sequestering the hydrophobic single-tail regions in the micelle centre to form the normal micelle that is oil in water micelle, figure 3. Micelles have the head groups at the centre with extending tails out called inverse micelles [14, 15].

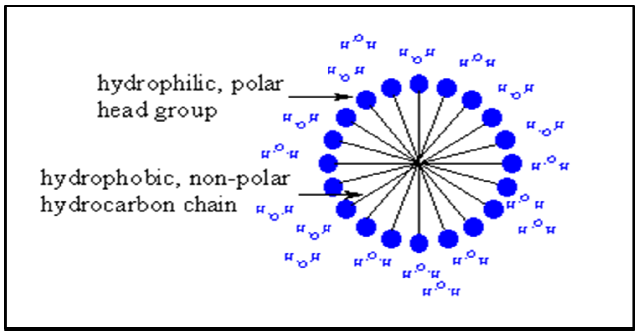

Figure (3): Micelle structure
(http://www.chem.ucalgary.ca/courses/350/Carey5th/Ch26/ch26-1-2.html)

1.2.4 Dendrimers

The name comes from the Greek word dendron which means tree, while mer stands for branch. Dendrimers are a novel 3D nanoscale materials, precisely constructed core–shell structures to form what is called generations by a controlled steps that increase the number of small branching molecules around a central core molecule [16-18]. Maximum ten generations can be found in a single dendrimer molecule, figure 4. Dendrimers have polyvalent surface. They have a precise architecture, size and controlled shape and size [17, 18]. Also they have high purity, uniformity, loading capacity, and low toxicity and immunogenicity [19].

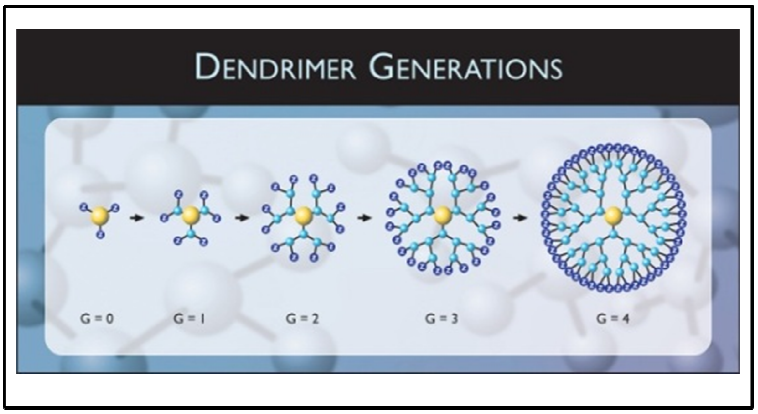

Figure (4): Dendrimers structure and generations.

(http://www.chemheritage.org/discover/online-resources/chemistry-in-history/themes/microelectronics-and-nanotechnology/tomalia.aspx)

1.2.5 Fullerene

Fullerene or buckyballs shape is a kind of spherical shape composed of 60 carbon atoms molecule that form spherical hollow structure with 1 nm in diameter. They werediscovered in 1985 by Harold W. Kroto [20]. Fullerenes can be represented as 20, 40, 60, 70, or 84 carbons. C_{60} is the most common one, figure 5.

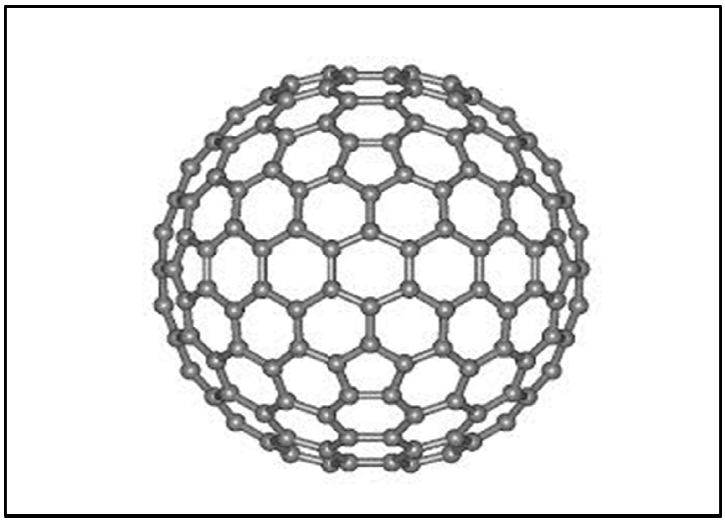

Figure (5): Fullerene structure.

(http://www.nanotube.msu.edu/fullerene/fullerene-isomers.html)

1.2.6 Carbon Nanotubes (CNTs)

CNTs are long and thin cylinders of carbon. They were discovered in 1991by Sumio Iijima [21]. CNTs can be considered as a sheet of graphene (a hexagonal lattice of carbon) rolled into a cylinder. Two types of CNTs were available; single walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) [22], figure 6. CNTs have mechanical, electrical, magnetic, and thermal properties like High capacity to transmit heat, low density, high resistance, strength, and stiffness [23, 24].

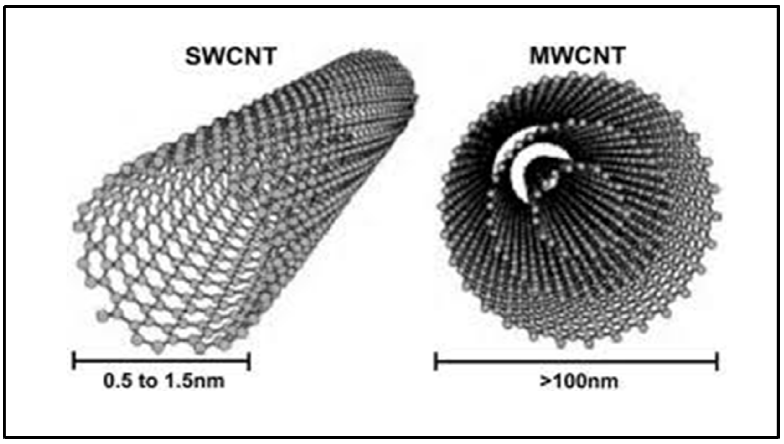

Figure (6): Single and multi walled carbon nanotubes.
(http://pixgood.com/multi-walled-carbon-nanotubes.html)

1.2.7 Graphene

Graphene is a planner monolayer, 2D hexagonal carbon atoms discovered in 2004 with a carbon–carbon bond length of 0.142 nm [25], figure 7. Graphene has specific properties such as high specific surface area, high thermal conductivity, excellent mechanical stiffness, good

biocompatibility, and fast electron transportation which results in promising and interesting applications in the nano medicinal fields [25-28].

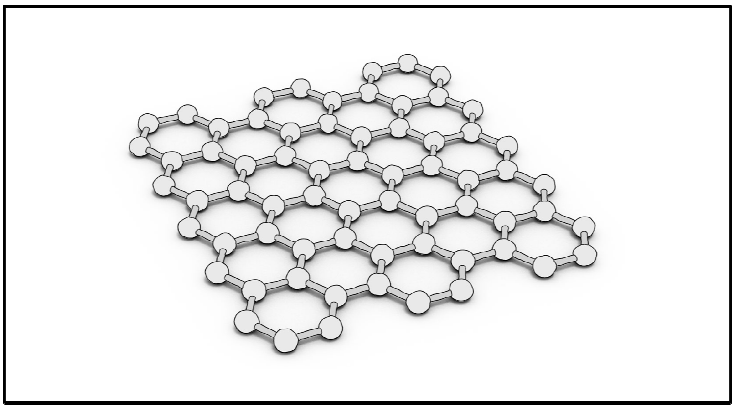

Figure (7): Graphene structure.
(http://www.jameshedberg.com/scienceGraphics.php?id=graphene-simple)

1.2.8 Magnetic Nanoparticles (MNPs)

MNPs are class of nanoparticles with 5–500 nm in diameter which can be manipulated using magnetic field formed by magnetic elements such as iron, nickel, cobalt and their chemical compounds. MNPs can bind to drugs, proteins, enzymes, antibodies, or nucleotides and can be directed to an organ, tissue, or tumor using an external magnetic field or can be heated in alternating magnetic fields for use in hyperthermia [29]. Super paramagnetic iron oxide nanoparticles (SPIONs) is an example of the most used MNPs [30].

1.2.9 Biodegradable Polymeric Nanoparticles (PNPs)

PNPs are colloidal dispersions composed of biocompatible and biodegradable polymers where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Recently, biodegradable

PNPs have attracted huge attention as potential drug delivery systems in view of their applications in the controlled release of drugs. PNPs could be amorphous or crystalline with core–shell structure and size range from 10-1000 nm [31-33].

PNPs have several advantages such as:

(i) Encapsulating hydrophobic drugs.

(ii) Target antibodies, peptides or aptamers to the site of interest [34].

(iii) Increasing drug stability with a control release properties and decrease the toxicity that may occur from the drug in the traditional Pharmaceutical forms [32, 33].

(iv) Improving water solubility of low water soluble drugs.

Traditionally, PNPs can be prepared mainly by two methods:

(i) Dispersion of the preformed polymers.

(ii) Polymerization of monomers.

Depending on the used method in nanoparticles preparation, nano spheres or nanocapsules can be obtained, figure 8. Nanocapsules are vesicular cavity contains the drug surrounded with a polymeric membrane, while in nanospheres the drug physically and uniformly dispersed in a polymeric matrix [35]. Many investigators designed different PNPs loaded with different agents like drugs especially in cancer therapy, antibodies,

proteins, nucleosides, contrast agents to increase imaging resolution and diagnostic agents [36, 37]. NPs are distinctive in that their surface can be modified with polyethylene glycol, polyethylene oxide, poloxamer, polysorbate or poloxamine. Pegylated NPs could increase the bioavailability and reduce recognition of immune system [38, 39]. The formed polymeric NPs will differ in size, size distribution, charge and morphology according to the polymer and the method used. Several techniques followed in PNPs preparation according to what it will be used for, target achieved, and agents to be entrapped.

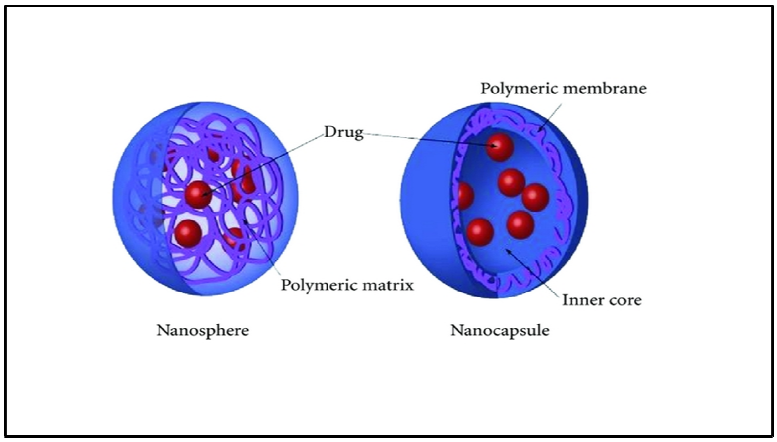

Figure (8): PNPs; nanospheres and nanocapsules.
(http://www.hindawi.com/journals/mi/2012/126463/fig3/)

1.3 Methods for PNPs Preparation

1.3.1 Emulsification and Solvent Evaporation Method

This method based on two parts; firstly Oil in water emulsion formation and then solvent evaporation. O/W emulsion formed by dispersion of organic phase contains the active agent and the polymer in the aqueous phase that contains stabilizing agent such as polyvinyl alcohol

(PVA). Then, organic solvent is evaporated under mild stirring. NPs size is obtained using a dispersing agent and high-energy homogenization. Filtration with a membrane syringe filter before solvent evaporation could be useful in reducing size distribution organic solvent evaporated under stirring to about one hour or more [40], as shown in figure 9. The type and amount of dispersing agent, stirring rate, viscosity and temperature of the two phases all of these parameters determine the size of the formed particles. Emulsification and solvent evaporation method applied to lipophilic agents.

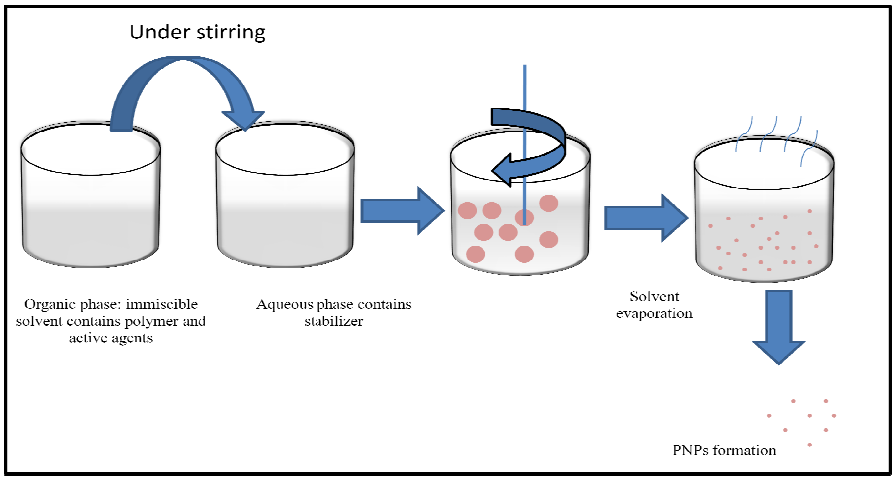

Figure (9): Emulsification and Solvent Evaporation Method.

1.3.2 Modified Emulsion and Solvent Diffusion Method

Emulsion modification based on the salting out effect that is done by dissolving electrolytes like magnesium acetate, calcium chloride or sucrose in aqueous phase in order to salt water miscible solvents out. The organic phase containing the polymer and the drug emulsified to the aqueous phase that contains the stabilizer like poly vinyl pyrrolidone (PVP) and hydroxyl ethyl cellulose and the salting out electrolytes. The solvent and the salting

out agent are then removed by cross flow filtration technique [41, 42], as shown in figure 10.

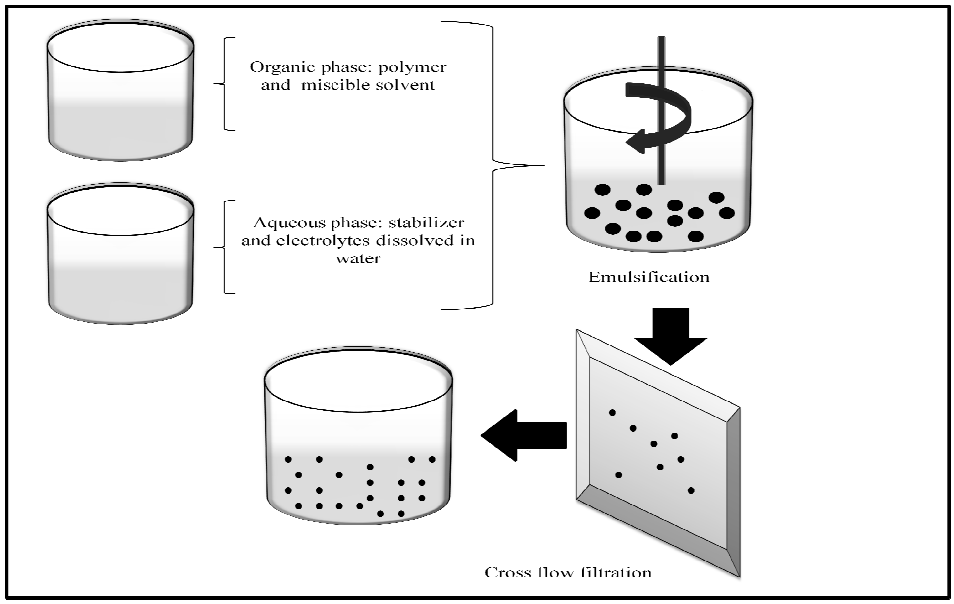

Figure (10): Modified Emulsion and Solvent Diffusion Method.

1.3.3 Double Emulsions Method

Two emulsification steps are done in this method; the organic phase contains the polymer added to aqueous phase that the active ingredient and stabilizer are dissolved in, this addition form primary emulsion o/w. Then, the primary emulsion added slowly to aqueous phase that contains stabilizer like PVA to form w/o/w emulsion. This method is very important to hydrophilic drugs since we could load active pharmaceutical ingredients (APIs) in higher amounts [43, 44].

1.3.4 Nano-Precipitation or Solvent Displacement Method

This method is based on the interfacial deposition phenomenon of polymers following the displacement of solvent miscible with water from a

lipophilic solution. Nano-precipitation is done by slowly addition of organic solvent which is water miscible such as acetone, acetonitrile and others that contains the hydrophobic polymer and hydrophobic or amphiphilic drug to aqueous phase containing surfactant and let it under stirrer to about 30 mins, then evaporate the organic solvent by rotavapor and by centrifuge precipitate the NPs and collect them after washing 3 times with water to remove the residual surfactant [45, 46], figure 11. The advantages of this method are that the size obtained is small and narrow distributed and it is very easy to remove all of the toxic organic solvent.

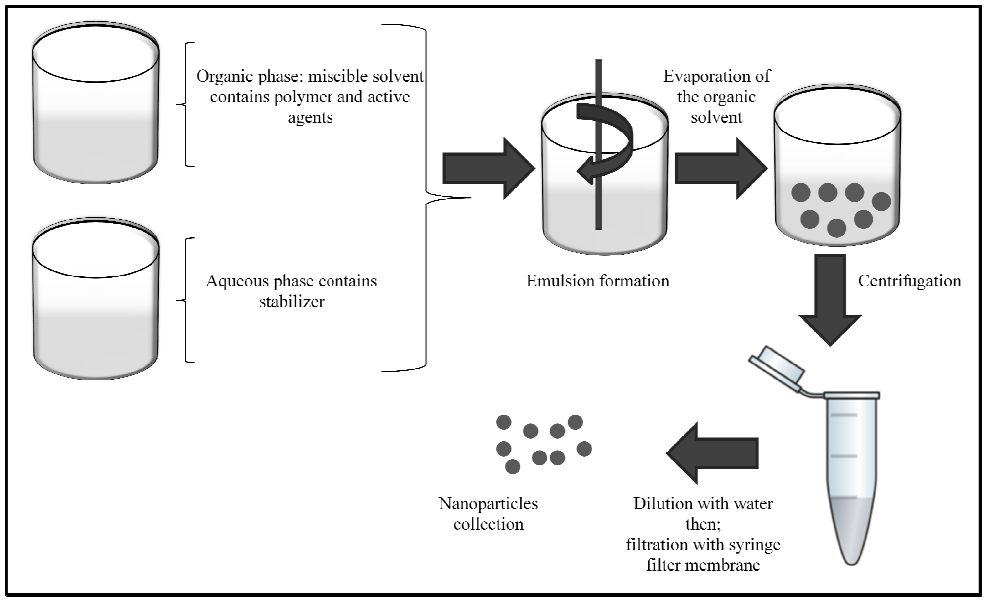

Figure (11): Nanoprecipitation Method.

1.3.5 Supercritical Fluid Method

In this technique the drug and the polymer are solubilized in a supercritical fluid such as CO_2 instead of organic solvent, and the solution is expanded through a nozzle. Spraying process used to evaporate the supercritical fluid, and the particles eventually precipitate. No solvent used

here and so it is clean method, figure 12. It has the advantage of protein encapsulated especially for insulin. Other advantage is that this method is suitable for scaling up production with high quality particles [47, 48].

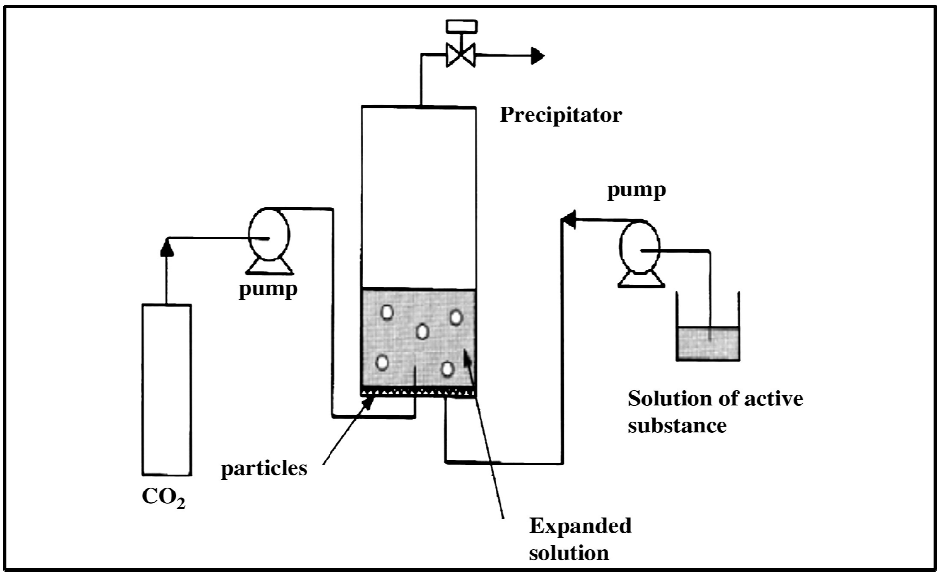

Figure (12): Supercritical Fluid Method [49].

1.4 Types of Polymers

Several types of biodegradable polymers are used in PNPs design. These polymers can be classified into natural and synthetic polymers. Natural polymers include: serum albumin, gelatin, collagen, alginate, starch, cellulose, and chitosan [50]. Synthetic polymers include: poly lactide (PLA), poly glycolide (PGA), poly (D, L, lactide-co-glycolide) (PLGA), poly caprolactone (PCL), poly acrylates (PA), poly (hydroxyl butyrate) (PHB), and poly dioxanone or their copolymers were used [50-52]. Natural polymers have limitations of higher cost and less purity. Synthetic polymers have fewer limitations than that associated with natural polymers and also have the ability to release drug slowly and need several

days to complete release. In contrast to natural polymers, synthetic polymers have good mechanical strength and degradation rate that can be easily modified, but their surfaces are hydrophobic and lack of cell-recognition signals but naturally derived polymers have the advantage of biological recognition ability [50]. Many pharmaceutical researches were done on biodegradable polymers in order to be applied and used for medicine especially for control release applications [53, 54]. Polymer biodegradability engineered into polymers by the addition of chemical linkages such as anhydride, amide, or ester bonds in order to form heteroatom -containing polymer backbones; as heteroatom easily degrade counter to C-C bond. Degradation occurs usually by hydrolysis or enzymatic cleavage of the labile heteroatom to result in natural byproducts such as gases (CO_2, N_2), water, biomass, and inorganic salts [54, 55]. Polymers must not only biodegradable but also they should be biocompatible, inert, non toxic, tensile strength, mechanical strength, and controlled degradation rate [56]. The understanding of the physical, chemical, and biological properties of the polymer is helpful, before formulating any drug delivery system. Molecular mass of the polymer or block copolymers play a role in the degradation rate and also in rate of drug release [54, 55, 57, 58].

1.5 Polylactides

Polylactides (PLA) are aliphatic, thermoplastic, biocompatible and biodegradable polyesters that can be produced from renewable resources

[59-61]. The basic building block of PLA which is lactic acid formed by food stocks fermentation like starch, corn, and sugar, (figure 13) [62]. Lactic acid can be then converted into the cyclic di-ester lactide by a combined process of oligomerization and cyclization. By ring opening the closed lactide is then polymerized into Polylactide [63, 64]. Other method used for PLA production is a direct condensation route. The disadvantage of this method is its difficulties of removing trace amounts of water in the late stages of polymerization that limits the ultimate molecular weight achievable as this route is based on equilibrium reaction [65].

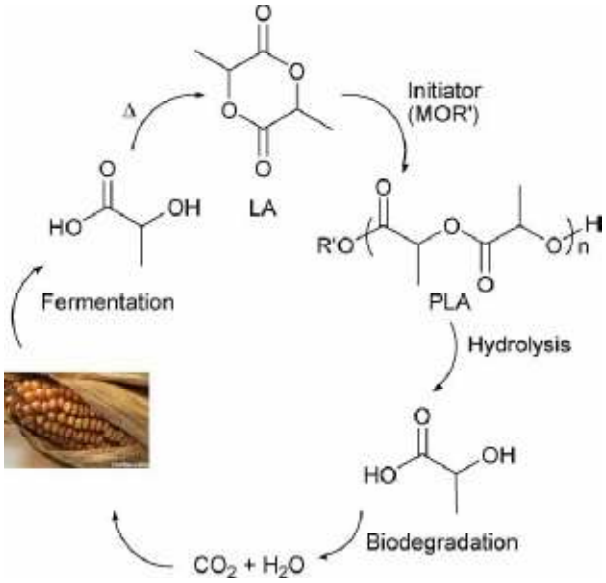

Figure (13): The life cycle of polylactide (PLA) [62].

There are two optical enantiomers of lactide; L and D enantiomers. L isomeric polymer is called PLLA, D polymer is called PDLA; both of these polymers are crystalline, PLLA has a glass transition ($\boldsymbol{T}_g$) temperature of 55–60 ºC. The copolymer of both isomers D and L is called PDLLA which is completely amorphous due to irregularities in its polymer chain structure [66], figure 14.Molecular weight and optical purity are both affect T_g. PLA

has higher T_g values when it consists of L-lactide more than D-lactide and when molecular weight of polymer increases the T_g will increase [64].

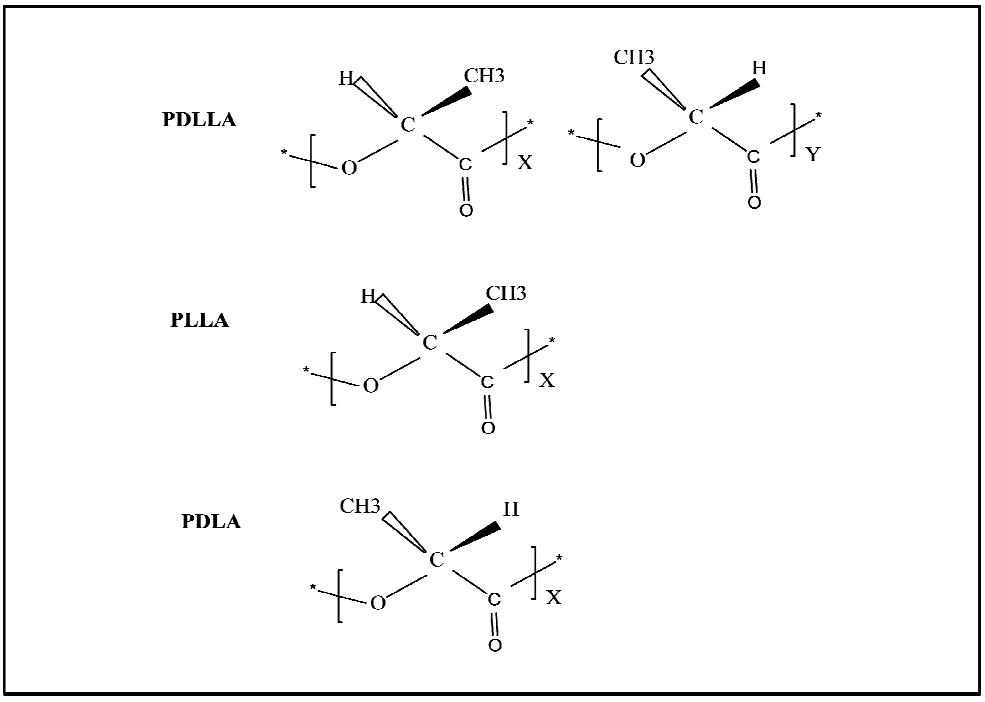

Figure (14): Chemical structures of PDLLA, PLLA and PDLA [63].

Using D, L-PLA is preferred over other Polylactide polymers as it enables more homogeneous dispersion of the drug in the polymer matrix. Polyester polymers are rapidly degraded in the environment and the by-products have a very low toxicity as they are eventually converted to carbon dioxide and water as shown in figure 13 [67]. Other than biocompatibility and biodegradability and targeting properties; Polylactide polymers have very valuable mechanical and thermal properties such as stiffness, fragility, flexibility, and lack of solidity [68, 69]. Flexibility of polylactides can be improved using different methods such as copolymerization, plasticization and blending with other low molecular weight compounds and polymers. All these positive properties played an

important role in their pharmaceutical and biomedical applications and use [67, 70-72]. Polylactide polymers were the most extensively investigated polymers for drug delivery. These polymers are hydrolyzed upon entering the body, giving lactic acid that is biocompatible moiety and eventually removed from the body by the citric acid cycle [73]. Polymer biodegradation formulations do not affect the normal cell functions, and do not have any undesirable or toxic effect according to many animal tests that have been done [37]. Polylactide polymers are approved for human use by the US FDA [74], and the drug is released in a sustained way through drug diffusion and degradation of the polymer matrix [70].

Degradation of the polymer depends on the copolymer block composition and on molecular weight of the used polymer [75]. These factors alter drug release from days to months. Due to their excellent biocompatibility and biodegradability polymers have been widely used in biomedical applications, including surgical sutures, bone fixation devices, vascular grafts, artificial skin, drug delivery systems, gene delivery systems, diagnostic applications and tissue engineering [61, 63, 76].

Nowadays, different polymeric carriers were developed to get rid of some problems associated with many drugs and to develop new dosage forms [63, 64, 77-79].

PVA is the most commonly used emulsifier that affects cellular uptake of PNPs as it remains associated at the nanoparticle surface despite of multiple washing due to the ability of this emulsifier to form

interconnected links with the polymer surface especially in PLA and PLGA NP formulations [44, 80, 81]. The amount of PVA used should be suitable and the residual amount should be removed as it may affect NPs properties such as particle size, zeta potential, polydispersity index, surface hydrophobicity, drug loading and also it could influence the *in vitro* release of the encapsulated drug. Moreover, the cellular uptake could be decreased since excess PVA would increase the hydrophilic properties of NPs surface [73, 82].

Degradation of carriers depends on size and shape. Size affects water penetration inside these PNPs and so affects the degradation and drug release. Water penetration affects electrostatic and Van Der Waals forces of carrier's surface [83]. PNPs size also affects loading efficacy; sub-micron size necessary for effective loading. PNPs shape has an effect in distribution and pharmacokinetic *in vivo* [84-86]. The spherical shape is the most favorable shape to give the best distribution, internalization, and other biological processes [87].

Accordingly, it is very important to control the size and shape of PNPs in order to obtain the best effective results. PNPs have the problem of that they could be uptaken by immune system and reticuloendothelial system (RES) especially mononuclear phagocyte by opsonization and phagocytosis [39, 88, 89].

In opsonization and phagocytosis, PNPs become covered with opsonin proteins, thereby making them more visible to phagocytic cells

which engulf and destruct or remove these materials from the blood stream. Surface modification with polyethylene glycol (PEG) like poly oxy ethyl cetyl ether (P.O.E.C.E) protects PNPs from opsonization and phagocytosis [90, 91].

There is a correlation between PNPs surface and opsonisation [92]. It has been described that opsonization could be prevented or decreased by neutralization of the PNPs surface charge or surface modification with shielding groups addition to prevent any hydrophobic or electrostatic interactions [39]. Some examples of hydrophilic polymers or surfactants that have been used as a shielding block include: (i) PVA, (ii) PVP, (iii) polysaccharides, and (iv) PEG. The block co-polymers of Poly oxy ethyl cetyl ether-PDLLA could have favorable pharmacokinetic properties especially half-lives prolongation. Furthermore, the P.O.E.C.E is hydrophilic while the PDLLA block gives hydrophobicity to the co-polymer and enables the biodegradability of P.O.E.C.E–PDLLA co-polymers with hydrolytically cleavable ester linkages. The degradation rate of P.O.E.C.E –PDLLA co-polymers can be adjusted by changing the PDLLA and P.O.E.C.E compositions [93].

1.6 Modified and Immediate Release Dosage Forms

Matrix technology opened prospects for controlled release (CR) fields. Simple matrix tablets production began in 1950s [94, 95]. In 1952, Smith Kline & French introduced the spansule, a capsule designed to release drugs at a steady rate over a period of hours (sustained release

capsule), that fired a widespread search for other dosage forms [96]. Drug delivery dosage forms are classified to two major classes according to their release mechanism:

1.6.1 Immediate Release (IR)

The drug is released immediately after administration of the dosage form. These types of dosage forms are designed to give a fast onset of drug action [97]. This type of dosage form allows the drug to dissolve in the gastrointestinal contents, with no intention of delaying or prolonging the dissolution or absorption of the drug.

1.6.2 Modified Release (MR)

MR dosage forms are designed and developed in order to release the drug in a modified way. The term MR drug product is used to describe products that alter the timing and/or the rate of release of the drug substance. MR dosage forms are systems in which the drug-release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms [98]. MR systems have several advantages include:

(i) Reduce drug blood level fluctuations

(ii) Reduce the frequency of dosing

(iii) Enhance patient compliance

(iv) Reduce the incidence of adverse side effects and toxicity and,

(v) Reduce the overall healthcare costs [98].

Conventionally MR dosage forms are divided into delayed and extended release:

1.6.2.1 Delayed Release (DR)

Drug is released at some point after the initial administration. In this kind of release it is possible to control the time and site of drug delivery in order to:

1. Protect drug from any degradation especially drugs that are sensitive to pH.
2. Protect the stomach from the irritant effect of a drug.
3. Release the drug at the desired site of action [99].

Enteric coated is one of DR dosage forms.

Enteric Coated (E.C):

E.C is an oral dosage form coated with a polymer or material that prevents or minimizes dissolution in the stomach but allows it in the small intestine and the release occurs immediately after gastric emptying [100]. These Coating polymers usually have acidic functional groups and thus only readily soluble above their pKa values which would be typically in the upper small intestine [100,101]. Enteric coated dosage form is an effective tool for oral site-specific delivery including targeting of the small intestine and the colon [102].

1.6.2.2 Extended Release (ER)

These products are formulated to make the drug available over an extended period after ingestion. This allows a reduction in dosing frequency compared to a drug presented as a conventional or immediate release formulations. ER can be classified as controlled and sustained release [103].

1.6.2.2.1 Sustained Release (SR)

This delivery system is designed to achieve prolonged therapeutic effect by continuously releasing drug over an extended period of time after administration of a single dose. SR systems achieve extended drug blood level that is therapeutically effective, non toxic for an extended period of time. Less frequency dose of intake is needed and increase in patient compliance is achieved [104].

1.6.2.2.2 Controlled Release (CR)

CR systems also offer a sustained-release property but, in contrast to sustained-release forms, controlled-release systems were designed to have constant plasma concentrations [105]. Controlled systems have the characteristics that allowed them to be used in a variety of administration routes, including transdermal, oral and vaginal administration in contrast to the sustained release which are used almost only for oral dosage forms [105].

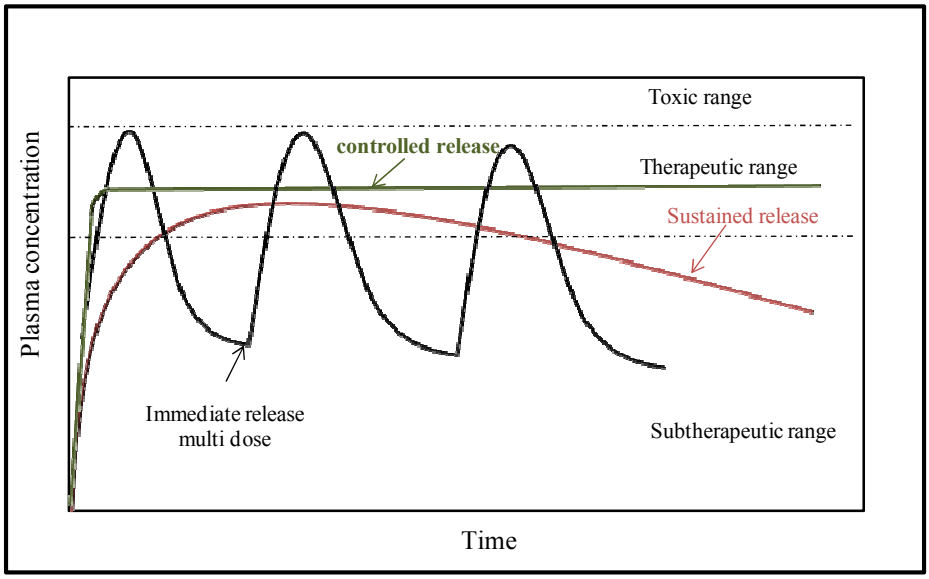

Figure (15): Plasma profiles for controlled, sustained and immediate release.

Many advantages are obtained from using sustained and controlled release formulations especially for chronic diseases that need many doses every day [103]. These advantages include:

1. Reduction in drug plasma level fluctuation and maintenance of a steady plasma level of the drug over a prolonged period of time.

2. Improvement of tolerability and reduction of adverse side effects or any toxicity that could be achieved. This will be achieved as drug plasma concentrations are maintained within a narrow window with no sharp peaks and with AUC of plasma concentration versus time curve comparable with total AUC from multiple dosing with immediate release formulations.

3. Increasing in patient compliance as sustained release reduces dosing frequency.

4. Improvement in bioavailability of some drugs.

5. Economical to the health care providers and the patient. The total cost of therapy of the controlled release product could be lower than the immediate release product with reduction in side effects. The overall expense in disease management would also be reduced.

According to the mechanism of drug release, controlled delivery systems can be classified into five classes:

1.6.2.2.2.1 Diffusion-Controlled Systems [106, 107].

Insoluble polymers are used to control the flow of water and the subsequent release of dissolved drug from those dosage forms. Diffusion occurs when a drug passes through the polymer that forms the CR system and this can occur through pores in the polymer matrix or by passing between polymer chains. There are two categories of this system:

a. Reservoir Systems

In this system a water insoluble polymer covers a core of drug. Drug will partition into the membrane and exchange with the fluid surrounding the particles. The active agent is released to the surrounding environment by diffusion process through the rate limiting membrane which is fairly constant.

b. Matrix Systems

The drug is dispersed in polymer matrix to form a homogeneous system called matrix system. Diffusion occurs when the drug passes from

the polymer matrix into the external environment. In this type of system, as the release continues, its rate normally decreases, since the active agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release.

1.6.2.2.2.2 Dissolution-Controlled Systems [108].

In these systems, the rate of dissolution of the drug is controlled by slowly soluble polymers or by micro encapsulation. When the coating is dissolved, the drug becomes available for dissolution. Thicknesses and composition of the coat control the rate of drug release. This type of controlled systems is divided into two categories:

a. Encapsulation Dissolution Control

This system involves coating of individual particles or granules of drug with a slow dissolving material.

b. Matrix Dissolution Control

In this system the drug is compressed with a slowly dissolving carrier. The rate of drug release is controlled by the rate of penetration of the dissolution fluid into the matrix, porosity, any hydrophobic additives present and the wet ability of the system and particles surface.

1.6.2.2.2.3 Erosion Systems [6].

In this system drug is mixed with biodegradable polymers. These polymers degrade within the body as a result of natural biological processes

especially by hydrolysis into biologically acceptable and smaller compounds that are easily disposed from the body. As the polymer degrades, drug release will occur and this release will be at a constant rate. The release of drug from these systems is controlled by the erosion rate of a carrier matrix. The rate of release is determined by the rate of erosion.

1.6.2.2.2.4 Osmotic Pump Systems [109].

The osmotic pump is similar to a reservoir system but contains an osmotic agent (e.g., the active agent in salt form) which acts to absorb water from the surrounding medium via a semi-permeable membrane. Pressure is generated within the system which forces the active agent out via a hole. The advantage of this type of product is that the constant release is unaltered by the environment of the gastrointestinal tract and relies simply on the passage of water into the dosage form. The rate of release can be modified by altering the osmotic agent and the size of the hole.

1.6.2.2.2.5 Ion Exchange Resins [110].

The drug is bound to the resin and released by exchanging with appropriately charged ions in contact with the ion exchange groups. This technique is applicable to certain drugs which have particular characteristics in terms of their relatively affinity for the polymers being used.

1.7 Carvedilol

1.7.1 Carvedilol Overview

Carvedilol is a nonselective β -adrenergic blocking agent with α_1 blocking activity. It is used in the treatment of mild to severe congestive heart failure (CHF), high blood pressure (BP), cardiac arrhythmias and angina pectoris [111-114]. As carvedilol is α and β blocker, it has many advantages that makes it distinct in hypertension (HTN) treatment; the addittional α blockage will vasodilate blood vesseles and Subsequently decrease BP. Carvedilol also has the ability to decrease lipids and glucose elevation and reduces the risk of death as well as the risk of hospitalization in patients with heart failure and elevated BP [59, 115].

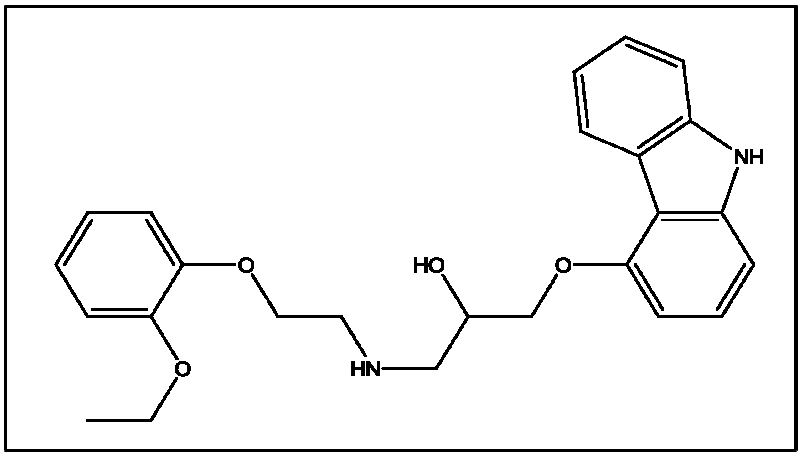

Figure (16): Chemical Structure of Carvedilol.

1.7.2 Physicochemical Properties:

Chemically Carvedilol is a ((±)-1-(carbazol-4-yloxy)-3-[[2-(o-ethoxyphenoxy) ethyl] amino]-2-propanol).

Table 1 summarizes physicochemical properties of carvedilol.

Table (1): Physicochemical properties of carvedilol [116-118].

Molecular Formula	$C_{24}H_{26}N_2O_4$
Molecular Weight	406.47424 g/mol
Hydrogen Bond Donor Count	3
Hydrogen Bond Acceptor Count	5
Rotatable Bond Count	10
Formal Charge	0
Topological Polar Surface Area	75.74 Å²
LogP	4.19
pKa (Strongest Acidic)	14.03
pKa (Strongest Basic)	8.74
Isotope Atom Count	0
Defined Atom Sterocenter Count	0
Undefined Atom Sterocenter Count	1
Defined Bond Sterocenter Count	0
Undefined Atom Sterocenter Count	0
Covalenty- Bonded Unit Count	1
Melting Point	113-117°C
Solubility	• Practically insoluble in water (0.583 mg/L). • pH-dependent solubility: ➢ less than 1 mg/L above pH 9.0 ➢ 23 mg/L at pH 7 ➢ 100 mg/L at pH 5 at room temperature

1.7.3 Pharmacokinetic Properties

The poor water solubility of carvedilol affects its dissolution and accordingly its pharmacokinetic parameters especially its bioavailability which does not exceed 30% [119]. Therefore, many attempts were carried out in order to improve carvedilol dissolution and pharmacokinetic properties. Among these attempts, solid dispersion, cyclodextrin inclusion, micro and nano carriers [120]. Both the R(+) and the S(−) enantiomers of carvedilol were metabolized in human liver microsomes by CYP2D6 and

CYP2C9 and to a lesser extent by CYP3A4, 2C19, 1A2, and 2E1, and it is primarily metabolized to 4′- (4OHC) and 5′- (5OHC) hydroxyphenyl, 8-hydroxy carbazolyl (8OHC) and *O*-desmethyl (ODMC) derivatives. The S (−) enantiomer was metabolized faster than the R (+) enantiomer although the same P450 enzymes seemed to be involved in metabolism [121, 122]. Less than 2% of the dose is excreted renally as unchanged drug [122]. Carvedilol is more than 98% bound to plasma proteins, primarily with albumin [123].

1.7.4 Formulations

Based on our knowledge carvedilol is available only as solid oral pharmaceutical dosage forms, including:

- IR tablets (3.125 mg, 6.25 mg, 12.5 mg, and 25 mg).
- CR hard gelatin capsules (10 mg, 20 mg, 40 mg, and 80 mg).

1.8 Literature Review and Similar Studies

PNPs were found in the literature regarding to carvedilol to improve its pharmacokinetic properties.

In 2009 Kalimuthu *et al.* suggested that nanoprecipitation technique is suitable for PNPs preparation of carvedilol using Eudragit 100 as a polymer and methanol as an organic solvent, and they found that polymer concentration plays a role in PNPs size and loading. In the same year Jawahar *et al* have prepared PNPs of carvedilol using PLGA. Both attempts ended with low loading efficiency [124, 125].

Ankarao *et al* have prepared and evaluated the oral SR nanoparticles of carvedilol containing egg albumin. These PNPs were prepared by coaservation method using gluteraldehyde and ethanol as the cross linking agent. The results showed that this method is reproducible, very easy and led to the efficient entrapment of drug, however the size of the obtained particles was big compared to other studies with a wide range of distribution from 500 nm to 1000 nm [126].

Carvedilol lipids NPs were also designed in 2012 by Venishetty *et al*; they have used homogenization followed by ultrasonication to prepare N-carboxymethyl chitosan solid lipid nanoparticles (MCC-SLN) loaded with carvedilol. These SLNs are useful for an effective intestinal lymphatic uptake upon oral administration. MCC-SLN may also be effective for oral delivery of protein/peptide therapeutics as they increase their stability [127].

In 2013, Pal *et al.* used the emulsification by sonication-evaporation method to design biodegradable NPs of carvedilol using PLGA as a biodegradable polymer. They observed higher initial drug loading PNPs resulting in faster drug release. Almost (but not all) of PLGA NPs of carvedilol in this work were spherical in shape [128]. In the same year, Sadr *et al*. found that ultrasonic power and time affect NPs of carvedilol in size and shape [129].

In 2014, Varshosaz *et al.* designed and developed lectin-modified poly (ethylene-co-vinyl acetate) mucoadhesive NPs of carvedilol by an

O/W solvent evaporation method. He was succeeded in preparing NPs loaded with carvedilol but there is a problem in the loading efficiency and the shape which was not spherical in all prepared NPs [130].

As it mentioned in the previous studies there is a deficiency in carvedilol loading and there is a wide size distribution the particles obtained. This study aims to optimize nanoprecipitation method by optimizing the solvents and the polymers used, and also to study the *in-vitro* release profile of the obtained particles.

1.9 Objectives and Strategy of the Study

Biodegradable PNPs and PMPs are one of the most promising delivery systems for the enhancement of bioavailability of highly lipophilic drugs as carvedilol those prone to the First pass metabolism. Moreover, the size and morphology of these particles play a critical role in determine the fate and the kinetic of developed particles.

The main objective of this study is to develop PDLLA carvedilol NPs and PDLLA carvedilol microparticles with high loading capacity.

1.9.1 Specific Objectives

1. To optimize the conditions of PDLLA NPs and microparticles preparation.
2. To determine the effect of the solvent type and the quantity of the polymer on the size, size distribution, and morphology of the prepared PNPs.

3. To characterize the formed PDLLA particles by atomic force microscopy (AFM).

4. To determine the loading & encapsulation efficiency of carvedilol in the optimized PDLLA particles.

5. To establish the *in vitro* release profile of carvedilol and compare it with carvedilol alone at different conditions.

1.9.2 Strategy

- The effect of solvents and polymer amount on the size and morphology of the obtained PDLLA NPs and microparticles were assessed by analysing their size and size distribution using AFM.

- P.O.E.C.E was used to increase the biocompatibility of the obtained particles. In fact this reagent can decrease NPS and MPs degradation since it masks them from macrophages (figure 17).

- Size, morphology, loading capacity and efficacy of the optimized PNPs were also assessed.

- Finally, an *in vitro* release profile was conducted and compared with in vitro release of the carvedilol from powder.

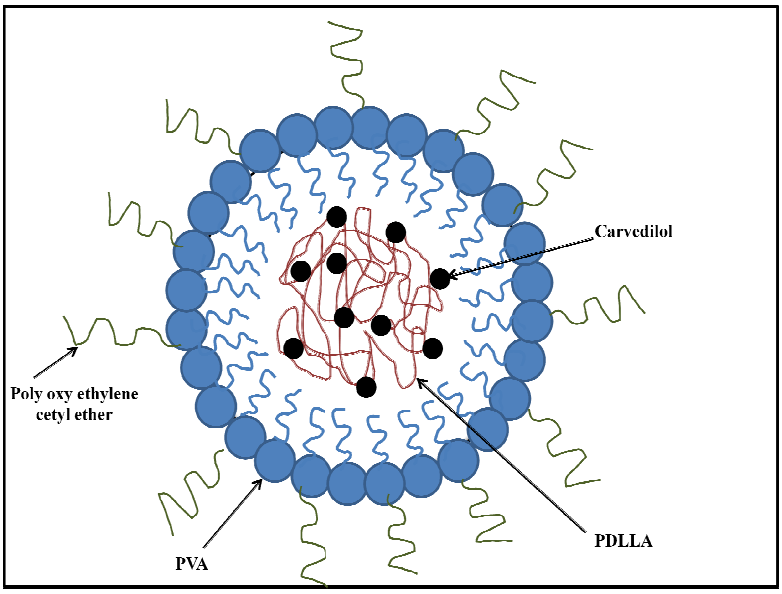

Figure (17): Schematic Presentation of Carvedilol-Loaded PDLLA NPs.

Chapter Two
Experimental Part

Chapter Two
Experimental Part

2.1 Materials, Reagents and Devices

In this project, ester terminated PDLLA polymer (intrinsic viscosity of 0.25-0.35 dl/g and molecular weight of 18,000-28,000) was purchased from Sigma-Aldrich (Sigma-Aldrich Israel Ltd.). Other two polymers from Sigma-Aldrich also were tried firstly to be used to prepare PNPs; PLLA (Mwt= 10,000) and PDLLA polymer (Mwt= 24,000-48,000). Carvedilol was provided from Bait Jala pharmaceutical company. In order to obtain milli Q water Elga purelab flex device was used. Poly oxy ethylene cetyl ether and polyvinyl alcohol surfactants were also utilized. Different organic solvents with HPLC grade were used (ethanol, methanol, acetone, tetrahydrofuran (THF), acetonitrile (C_2H_3N), and Dichloromethane (DCM)). Acrodisc GF syringe filter of 200 nm and 1000 nm size were used. Two different pH (6.8 and 7.4) Phosphate buffer was prepared. Jenway 7315 Spectrophotometer for UV/Visible spectra measurements using quartz cuvettes was used. Universal 320 Centrifuge from Hettich was utilized. Atomic Force Microscopy (AFM) using a tapping mode-AFM system (Alquds University, Abu Dees) with WSxM software designed by Nanotec Electronica (Madrid, Spain) was used image analysis. Rectangular commercial Si_3N_4cantilevers (NSG 10, NT MDT Co., Ltd.) with spring constants of 5.5–22.5 $Nm^{-1}$and resonance frequencies in the range 190 to 325 kHz were used. Other used instruments and devices were Heidolph2000 rotavapor, mill rock freeze dryer and dialysis membrane purchased from spectrum lab.

2.2 Preparation of PDLLA Particles

PDLLA NPs were prepared using nano-precipitation technique. In this procedure the organic phase was prepared using various quantities of PDLLA polymer and poly oxy ethylene cetyl ether (P.O.E.C.E). These components were dissolved in various quantities of an organic solvent (Table 2). The obtained organic phase was added drop wise to the water phase that contained 3 g of 1% PVA and 7 ml of milli Q water under mild stirring. The formed milky emulsion was left 30 minutes under mild stirring. After that, organic solvent was evaporated using rotary evaporator. The obtained system was filtrated using a 200 nm pore size membrane syringe filter; in the case of DCM the particles have been filtered by 1 µm pore size syringe filter. Centrifugation was done at 15000 rpm for 10 min to precipitate the particles and wash with milli Q water 3 times under centrifuge to remove residual PVA. NPs were collected and dispersed in 2 ml water and left in a refrigerator to be lyophilized by freeze dryer in the next day (figure 18).

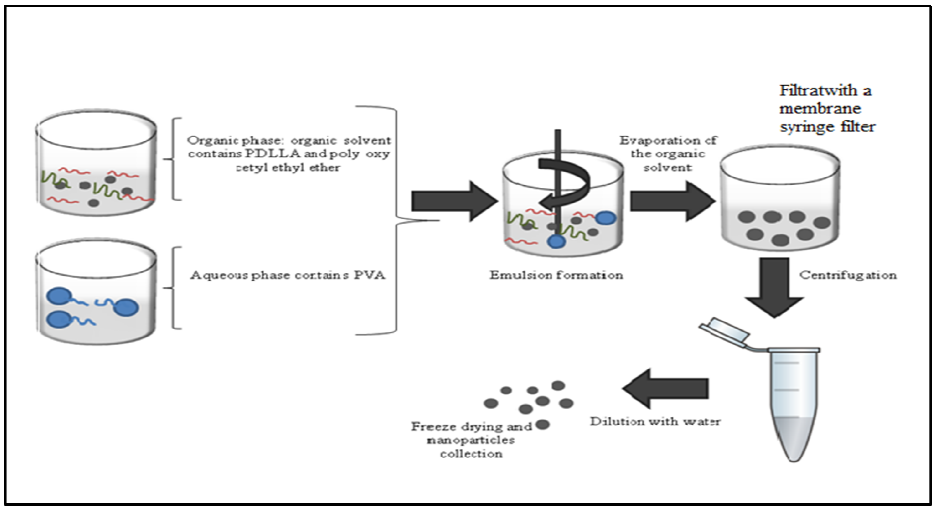

Figure (18): Schematic Representation of Nanoprecipitation Method

In order to optimize the conditions of the prepared PNPs, various formulas were prepared (table1). Organic solvent and the amount of PDLLA were varied. Size, morphology and the size distribution of the obtained particles were assessed using AFM.

Table (2): Formulas of PDLLA NPs preparation.

Materials	Formulas and Quantities									
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
PDLLA (mg)	50	50	50	50	50	50	50	50	25	12.5
PVA 1% (g)	3	3	3	3	3	3	3	3	3	3
P.O.E.C.E (mg)	5	5	5	5	5	5	5	5	5	5
Acetone (ml)	5	0	0	0	2.5	2.5	0	0	5	5
THF (ml)	0	5	0	0	2.5	0	2.5	0	0	0
C_2H_3N (ml)	0	0	5	0	0	2.5	2.5	0	0	0
EtOH (ml)	0	0	0	5	0	0	0	0	0	0
DCM (ml)	0	0	0	0	0	0	0	5	0	0
Milli Q water (ml)	7	7	7	7	7	7	7	7	7	7

2.3 Preparation of AFM Samples

The samples have been deposited on a substrate (5×5 mm^2) of mica and let to dry at room temperature. The AFM images were treated with WSxM 5.0 Develop 6.5 software [131]. The size and size distribution were determined by taking the average of 100 counted particles.

2.4 Preparation of Carvedilol-Loaded PDLLA Nano/Micro particles

Once the conditions have been optimized, a 2 mg of carvedilol has been added to the organic phase and the same procedure of preparation of PDLLA particles has been followed as reported in section 2.2.

2.5 Calibration Curve of Carvedilol by UV Spectrophotometer

A simple UV analytical method was developed in order to detect the amount of carvedilol in the prepared particles. This method was also used in order to evaluate the release of carvedilol from PNPs. λ_{max} for carvedilol was detected by dissolving known amount of the drug in known volume of methanol to obtain stock solution and UV spectrum was done , carvedilol has highest absorbance at 240 nm. Serial concentrations of carvedilol were also prepared from previous stock solution and their absorbance was registered. A calibration curve for carvedilol was constructed from these diluted concentrations in order to measure the amount of carvedilol that has been loaded inside the particles by measuring the absorbance of dispersed PNPs loaded with carvedilol compared with PNPs free carvedilol at $\lambda_{max.}$

2.6 Encapsulation Efficiency of NPs:

The total amount of carvedilol that has been loaded was determined after dissolving known amount of the obtained PNPs powder in a volume of milli Q water and measuring the absorbance by sspectrophotometer at λ_{max} = 240 nm. Unloaded carvedilol was also determined after centrifugation of the particles at 15000 rpm for 10 minutes and encapsulation efficiency calculated.

$$\boldsymbol{Encapsulation\ efficiency} = \frac{(\text{Total drug used - Free dissolved drug})}{\text{Total amount used}} * 100\%$$

….Equation 1

2.7 *In Vitro* Drug Release Profile

In vitro release profile for carvedilol was obtained using bag dialysis membrane. The Loaded PNPs or PMPs were dispersed in phosphate buffer (pH 7.4 and 6.8) and injected inside the dialysis membrane by a syringe. After that, the dialysis membrane has been immersed in 900 ml phosphate buffer (with pH of 7.4 and 6.8) at room and body temperature under mild stirring. The samples were withdrawn regularly, replaced with fresh media and then analyzed by UV spectrophotometer to calculate the amount of drug released according to the absorbance obtained at λ_{max} (240 nm).

The percentage of drug release was calculated according to equation 2:

$$\%\, release\ of\ drug\ = \frac{Amount\ of\ dug\ released\ at\ time\ t}{Total\ amount\ of\ nanocapsulated\ drug\langle mg\rangle} * 100\%$$

.....Equation 2

Drug release kinetic

The kinetic of a drug release from a sustained release system is assumed to reflect the release mechanism from the designed and produced system. Accordingly, seven kinetic models were used in order to analyze the data of carvedilol release from the obtained mixture of pure carvedilol powder, carvedilol loaded PDLLA nanoparticles and microparticles. The used models were zero order, zero order with T lag, first order, first order with T lag, Korsmeyer-Peppas, Korsemeyer_Peppas with T lag and Higuchi model (table 3). The linear egression (R^2) and Akaike Information Criterion (AIC) were calculated [132-138].

Chapter Three

Results and Discussion

Chapter Three
Results and Discussion

PDLLA NPs were prepared using a nanoprecipitation technique, also known as the solvent displacement method. This method is characterized by: (i) facile and rapid particle formation, (ii) controlled particle size, and (iii) the use of solvents with minimal toxicity. Different parameters were controlled and varied in order to find the optimum conditions to obtain the best PNPs. One of the objectives of the project was the formation of homogenous, small PNPs with a narrow size distribution. Moreover, the PDLLA polymer has received huge interest in various scientific fields, especially in the pharmaceutical area[139]. PDLLA has been approved by the FDA for human clinical use. It is an amorphous and biodegradable polymer that degrades into lactic acid (a normal human metabolite), which is later converted into carbon dioxide and water through the citric acid cycle [55].

NPs should have an effective time in the blood circulation to increase their bioavailability and the duration of action of the encapsulated drug. Accordingly, the most effective way to improve the time of circulation is to cover the surface of NPs with flexible and biocompatible water soluble polymer chains. The polymer acts as a protective mask against opsonization of the drug transporter and subsequently prevents phagocytosis by the mononuclear phagocyte system (MPS). Likewise, the polymer should prevent direct contact between NPs and phagocytic cells of the immune system [140].

PEG polymers have emerged as one of the leading families used as masking agents. "PEGylated" NPs exhibit lifetimes 50 times longer than other polymers, with greater stability and low immunogenicity. PEGylated vectors behave as invisible bodies and can be considered as second generation nano vectors [141]. Therefore, we have incorporated PVA and a derivative of PEG, poly oxy ethyl cetyl ether (P.O.E.C.E), which acts as emulsifiers. P.O.E.C.E masks the surface of NPs and increases their stability against the immune system. PVA contributes to the physical stability of the obtained emulsion [93].

A successful nano drug delivery system should have a suitable particle size, shape, and narrow size distribution. This can be achieved by selecting the most suitable formulation conditions, including the polymer and organic solvent.

Accordingly, three different types of PLA polymers were used in the production of PNPs. PLLA and PDLLA (Mwt=24000-48000) failed to produce nanoemulsions. PDLLA (Mwt= 18000-28000) gave the most suitable and best nanoemulsions. Subsequently, two formulation parameters were optimized since they had a major impact on the size, shape, and size distribution of PNPs. These two parameters were the quantity of the polymer and the type of organic solvent. Accordingly, the temperature and the stirring velocity were kept constant (room temperature and mild stirring at300 rpm).

3.1 Effect of the Organic Solvent on the Obtained Particles

Four different water miscible organic solvents (acetone, ethanol, THF and C_2H_3N) were used in order to select the best one to give the desired particles size, shape, and size distribution. Moreover, mixtures of these solvents in 1:1 ratio (acetone: THF, acetone: C_2H_3N and THF: C_2H_3N) were also tested for the same purpose. To evaluate the effect of water immiscibility on the particles, DCM was selected as the organic solvent according to Sawalha, et al [142]. Eight formulas were prepared and analyzed by AFM in order to determine the size, morphology and size distribution of the particles (Table3).

All used solvents provided the same spherical shape. However, a difference in the obtained particles size was observed (Table 3). In fact, acetone alone produced the smallest polymeric particles with the narrowest size distribution as can be observed in figure 19 and table 3.

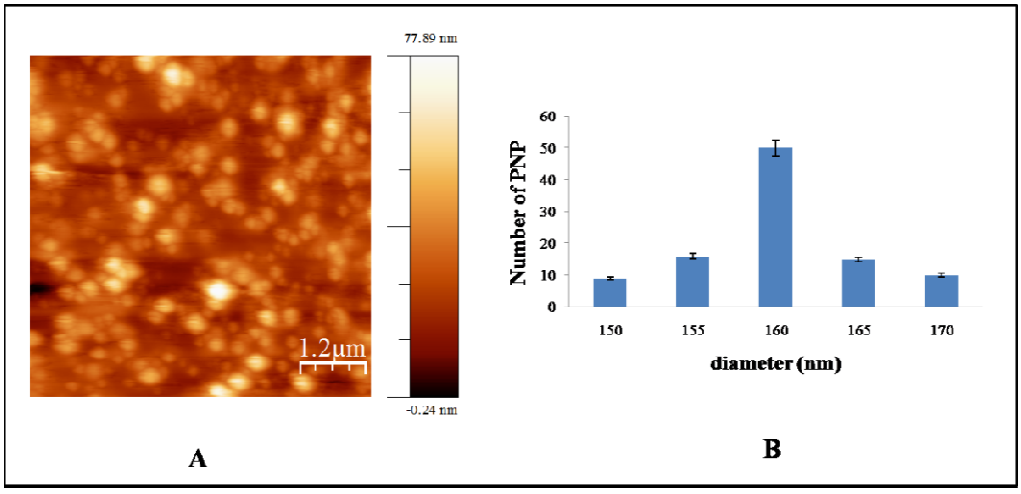

Figure (19): A) AFM image of PDLLA NPs (acetone as the solvent); B) Histogram obtained from AFM analysis.

Ethanol gave the largest particles when compared with the other miscible solvents (THF, C_2H_3N, and mixtures) (Figure 22, Table 3). Mixture of the miscible organic solvents made a difference in term of particle size and size distribution. C_2H_3N and THF mixture gave larger particles size compared with the other two mixtures (figure 25, Table 3). This indicates that a mixture of solvents decreases the interfacial tension with the aqueous phase. The addition of acetone to another organic solvent positively affected the interfacial tension of the system, since it gave smaller PNPs when compared to the other solvent mixtures (Table 3). This is in accordance with published studies showing that the addition of a water miscible solvent decreases the interfacial tension and so the removal rate from the particle increases, which solidifies the particles much more quickly and therefore decreases the particle size [142, 143]. Indeed, organic-aqueous phase interactions play a major role during the diffusion process in the formation of particles. On the other hand, the water immiscible solvent DCM produced PMPs instead of PNPs. This can be explained since the removal of DCM from the aqueous phase was slow due to the low water miscibility, which affected the coacervation of the polymer. In fact, this process took much more time, which allowed the liquid to aggregate and form larger particles. This could be logically acceptable as Sawalha, et al [144], who prepared MPs using DCM in the organic phase. The size of these particles decreased upon the addition of a miscible solvent like ethanol.

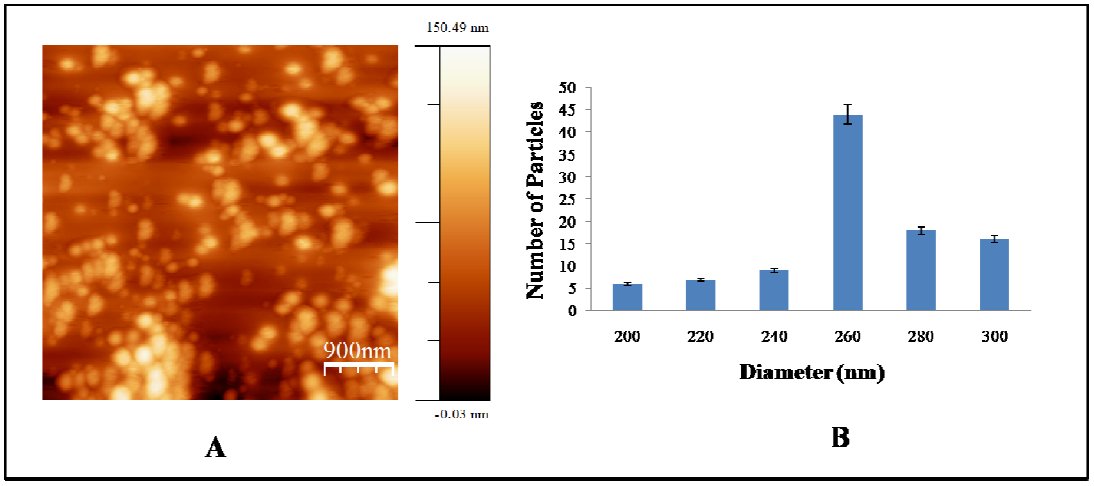

Figure (20): A) AFM image of PDLLA NPs (THF as the solvent); B) Histogram obtained from AFM analysis.

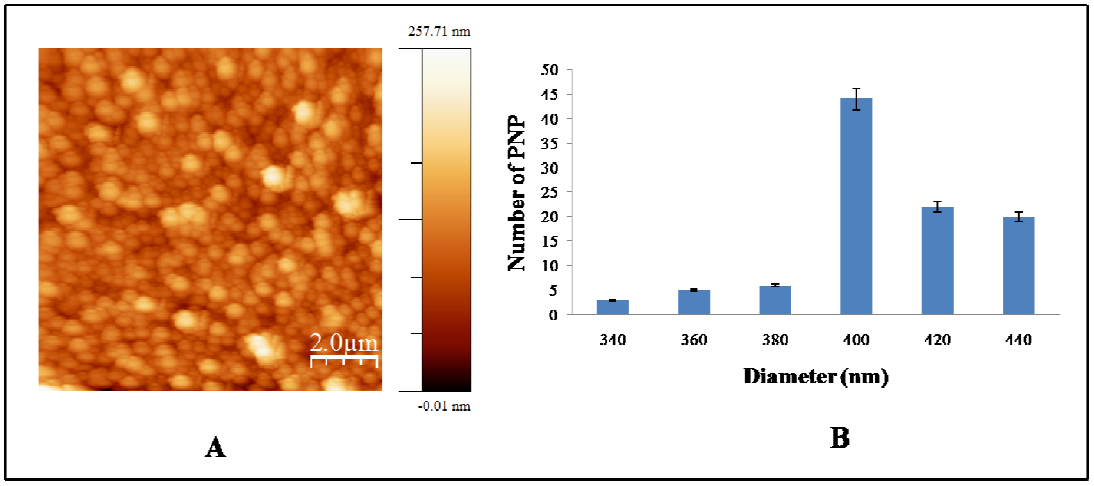

Figure (21): A) AFM image of PDLLA NPs (C_2H_3N as the solvent); B) Histogram obtained from AFM analysis.

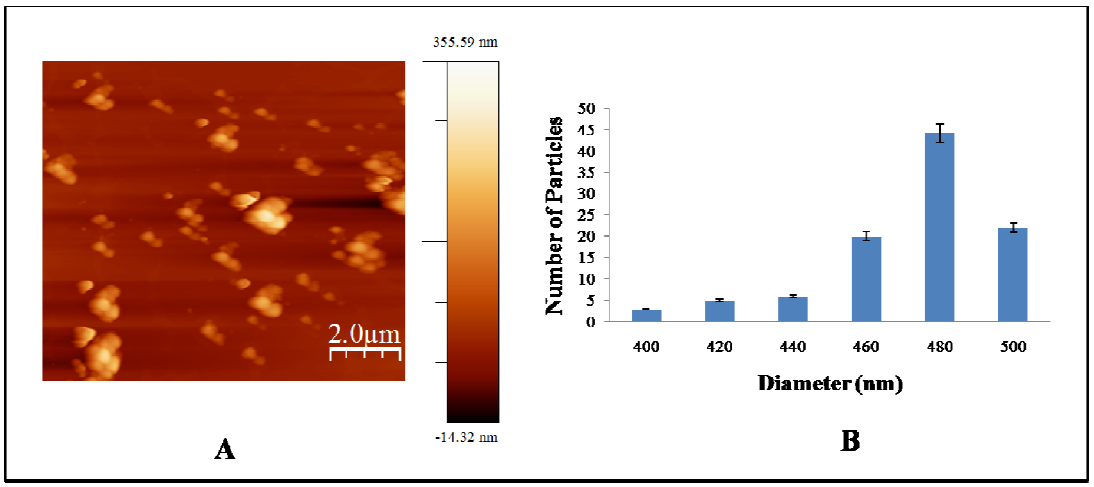

Figure (22): A) AFM image of PDLLA NPs (EtOH as the solvent); B) Histogram obtained from AFM analysis.

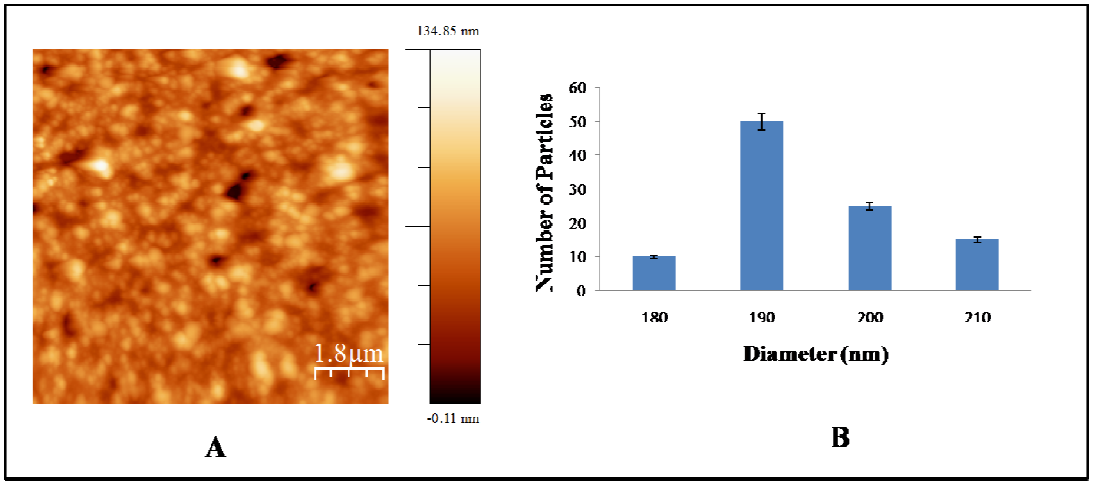

Figure (23): A) AFM image of PDLLA NPs (C_2H_3N: Acetone "1:1" as the solvent); B) Histogram obtained from AFM analysis.

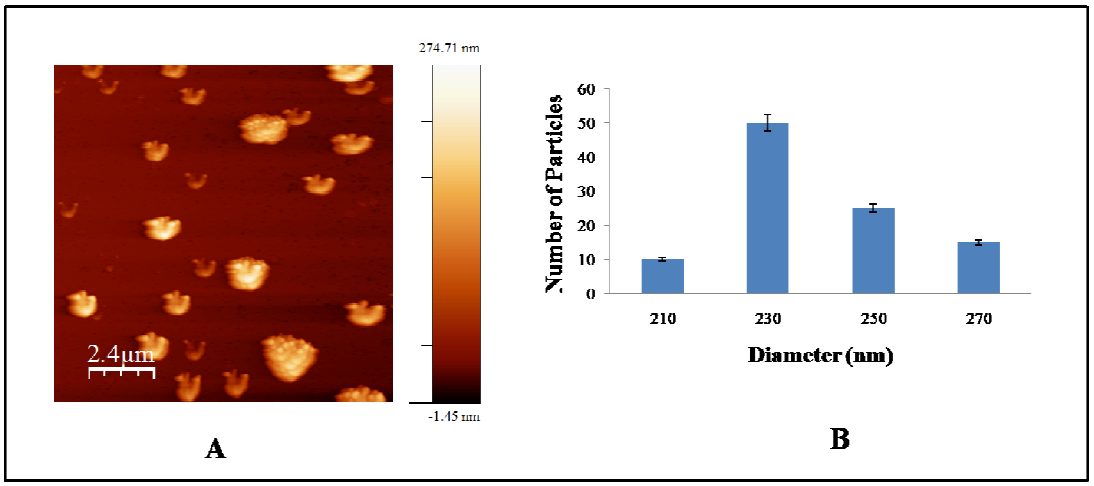

Figure (24): A) AFM image of PDLLA NPs (THF: Acetone "1:1" as the solvent); B) Histogram obtained from AFM analysis.

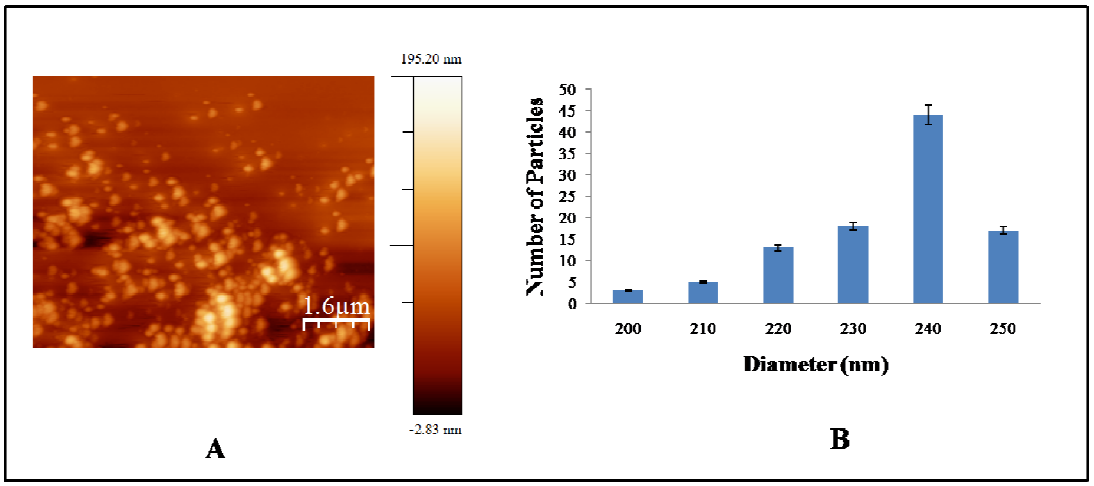

Figure (25): A) AFM image of PDLLA NPs (THF: C_2H_3N "1:1" as the solvent); B) Histogram obtained from AFM analysis.

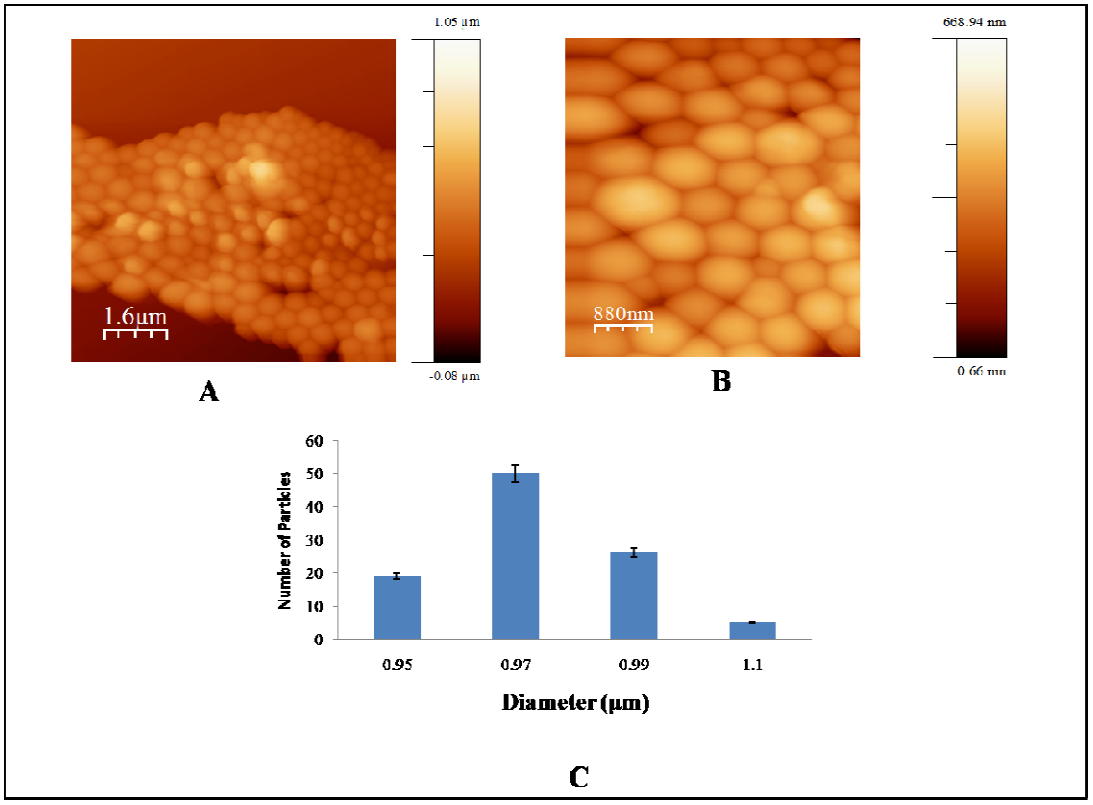

Figure (26): A) AFM image of PDLLA particles (DCM as the solvent); B) Zoom image of the formed particles; C) Histogram obtained from AFM analysis.

3.2 Effect of the Amount of PDLLA on the Size, Morphology, and Size Distribution of Polymeric Particles

Once the most suitable solvent was determined, the effect of the amount of the polymer on particle size, shape, and size distribution was assessed. Herein, the amount of the polymer used in PNP formation was changed in order to estimate the effect of this parameter on the size, morphology, and size distribution of the obtained particles. Accordingly, all other parameters were fixed and acetone was used, which had been found to be the most suitable solvent that gave the smallest PNPs. For this purpose, three different factorial amounts of PDLLA were studied (12.5 mg, 25 mg, and 50 mg). The use of 50 mg of PDLLA gave the largest particle size (Figure 19, Table 3). When 12.5mg of PDLLA was used, the

smallest and narrowest PNP size was obtained; an average size of 100 nm and a size distribution between 90-120 nm was observed (Figure 27, Table 3), while 25mg of PDLLA gave an average size of 125 nm and a size distribution of 120-140 nm (Figure 28, Table 3).In all tried factorial amount of PDLLA that used spherical shape was obtained.

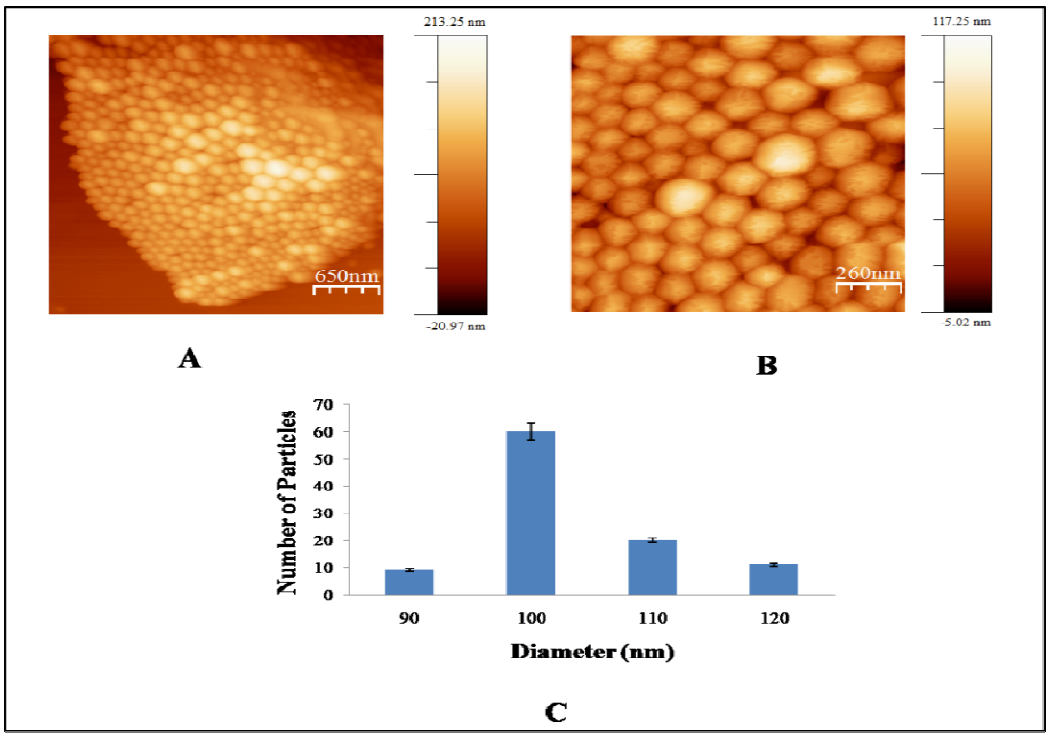

Figure (27): A) AFM image of PDLLA NPs (with 12.5 mg of PDLLA); B) Zoom image of the formed particles; C) Histogram obtained from AFM analysis.

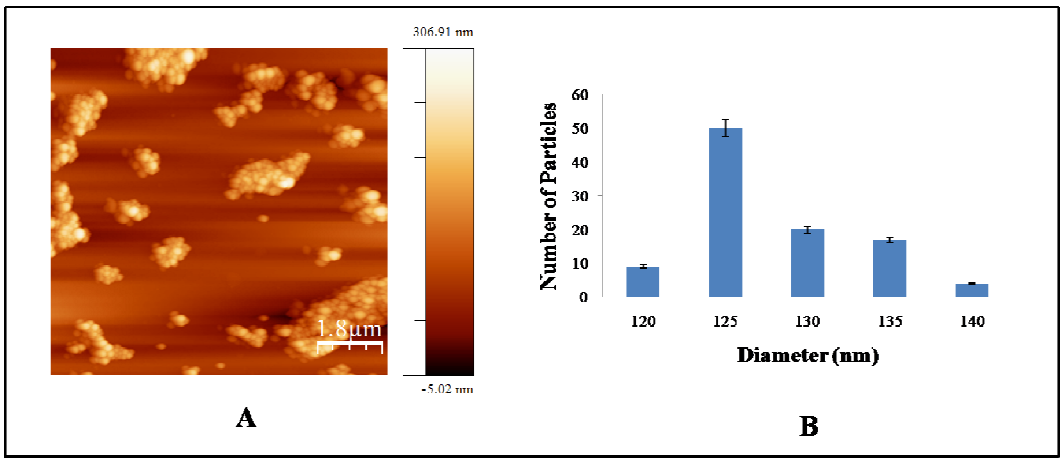

Figure (28): A) AFM image of PDLLA NPs (with 25 mg of PDLLA); B) Histogram obtained from AFM analysis.

Table (3): Average size and size distribution of the different formulas.

Formula	Average size (nm)	Size distribution (nm)
F1	160	150-170
F2	260	200-300
F3	400	340-440
F4	480	400-500
F5	190	180-210
F6	230	210-270
F7	240	200-250
F8	970	950-1100
F9	100	90-120
F10	125	120-140

It was observed that any increase in the polymer amount increased the average particle size. This effect occurs due to two reasons: i) an increase in the polymer chain per volume ratio, i.e. more polymer chain interactions and therefore more polymer aggregates will diffuse into the aqueous phase and thus form larger particles. On the other hand, ii) an increase in polymer concentration will affect the viscosity of the organic phase; a higher viscosity will in turn hold back the shear forces of the emulsion, as has been discussed by other researchers [145, 146].

3.3 Carvedilol Loaded PDLLA NPs and MPs

Once the method of preparation has been optimized, both PNPs (according to F9) and PMPs (according to F8) have been loaded with carvedilol. The morphology, size, and size distribution were evaluated using AFM. According to AFM analysis, maintenance of the spherical shape was observed, while the average size of both drug-loaded PNPs and PMPs increased. In fact, the average size of PNPs and PMPs was increased up to 30% and 23%, respectively. This increase may indicate the successful

encapsulation of carvedilol inside the particles. The loading capacity of PNPs and PMPs was estimated using a UV spectrophotometer. A calibration curve of carvedilol at λ_{max} (240nm) was established in order to measure the carvedilol concentration that had been loaded inside the particles (Figure 31).

The absorbance of PNPs and PMPs at 240 nm was measured using UV spectrophotometer by taking 5mg of each particles dispersed in 3ml milli Q water compared with particles free from carvedilol as a reference, and the encapsulation efficacy was calculated according to equation 1.

Both PNPs and PMPs showed comparable loading efficiency. In fact, 57% and 55%loading capacity was found in PNPs and PMPs, respectively. The observed encapsulation capacity is considered satisfactory compared with other published studies that used PLGA polymers where the amount of loaded carvedilol was 33% [125].

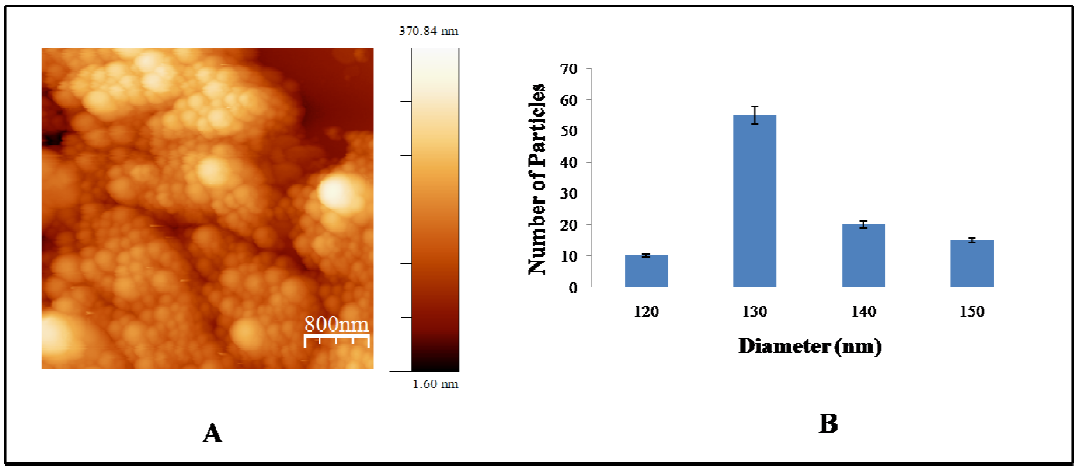

Figure (29): A) AFM image of carvedilol-PDLLA NPs; B) Histogram obtained from AFM analysis.

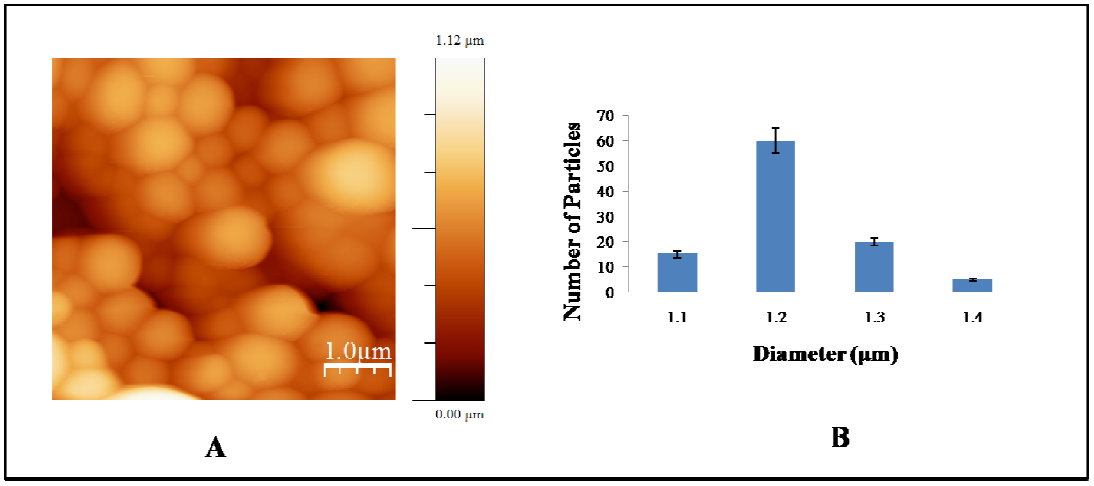

Figure (30): A) AFM image of carvedilol-PDLLA MPs; B) Histogram obtained from AFM analysis.

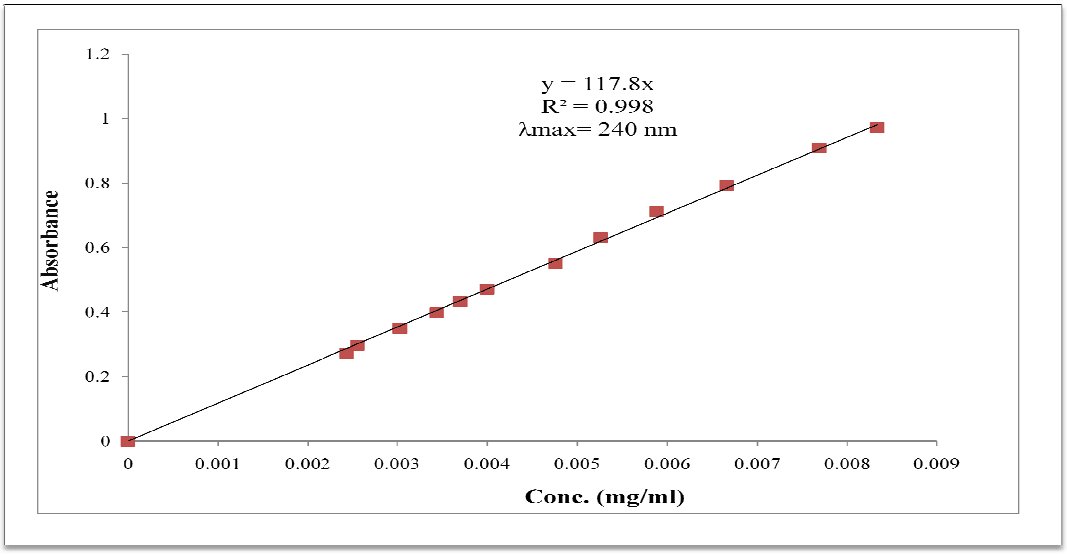

Figure (31): Calibration Curve of Carvedilol at λmax = 240 nm

3.4 *In vitro* Carvedilol Powder, PNPs, and PMPs Release Study

In vitro drug release was conducted at two different temperatures (25ºC and at 37ºC) and at two different pH phosphate buffer (6.8 and 7.4) using dialysis membrane bags.

Despite the temperature and pH changes, both PNPs and PMPs showed SR behavior when compared to the release of the carvedilol pure

drug (Figure 32, 33, and 34), also almost all the loaded quantity of carvedilol was released. However, carvedilol release from PMPs was a little slower than that from PNPs. In fact, PMPs showed complete release (97% of the loaded carvedilol) within 30 hrs while PNPs released carvedilol (98% of the loaded carvedilol) within 25h (Figure 32 and Figure 33).

This difference in drug release between PNPs and PMPs was expected since PNPs have a higher total specific surface area than PMPs. In fact, the larger surface area ratio to the volume consequently increases the degradation of the polymer which results in release of the loaded drug. [147, 148].

Regarding the effect of temperature on the release of carvedilol, greater drug release was observed at body temperature than at room temperature. The effect of temperature on drug release was also seen despite the pH used (figure 32, 33, 34, 35). This may be due to the higher permeability of the polymer membrane at body temperature than at room temperature as reported by Zhang et al [149].

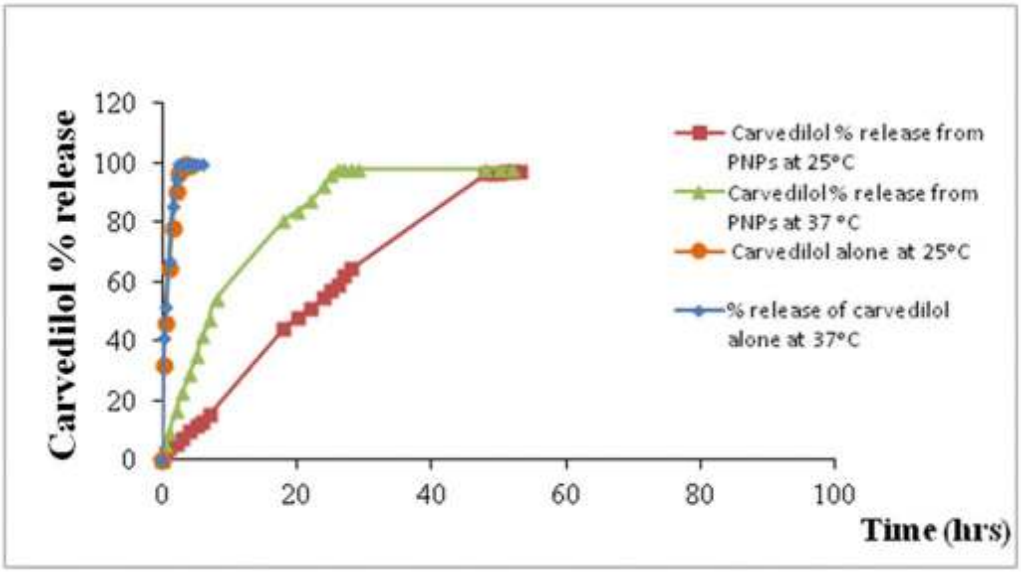

Figure (32): Release of carvedilol from PNPs at two different temperatures using phosphate buffer at pH 6.8.

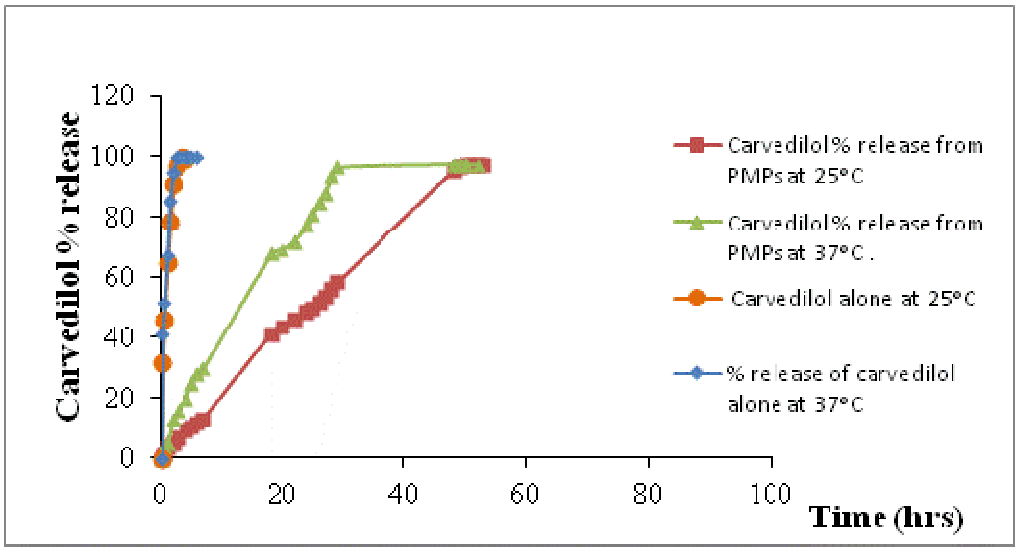

Figure (33): Release of carvedilol from PMPs at two different temperatures using phosphate buffer at pH 6.8.

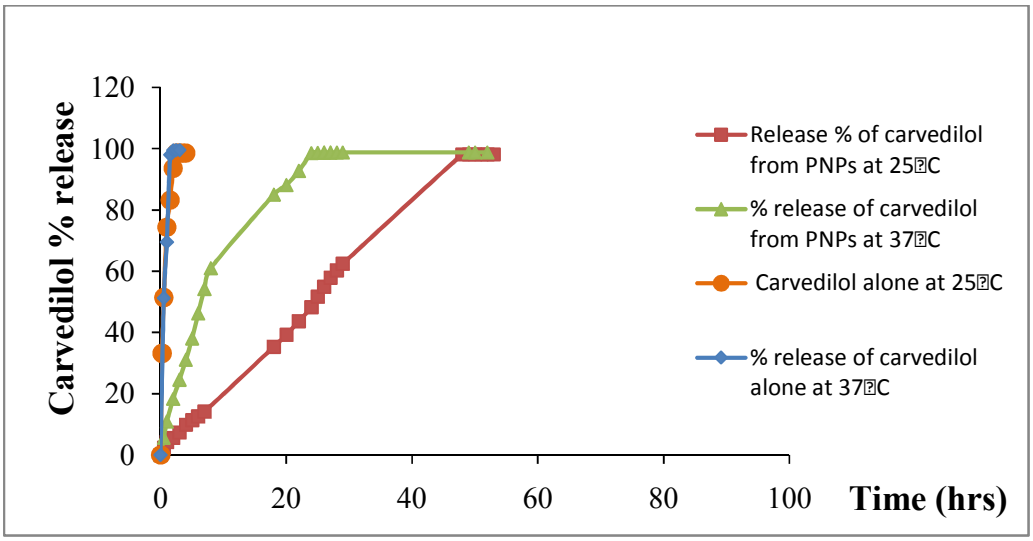

Figure (34): Release of carvedilol from PNPs at two different temperatures using phosphate buffers at pH 7.4.

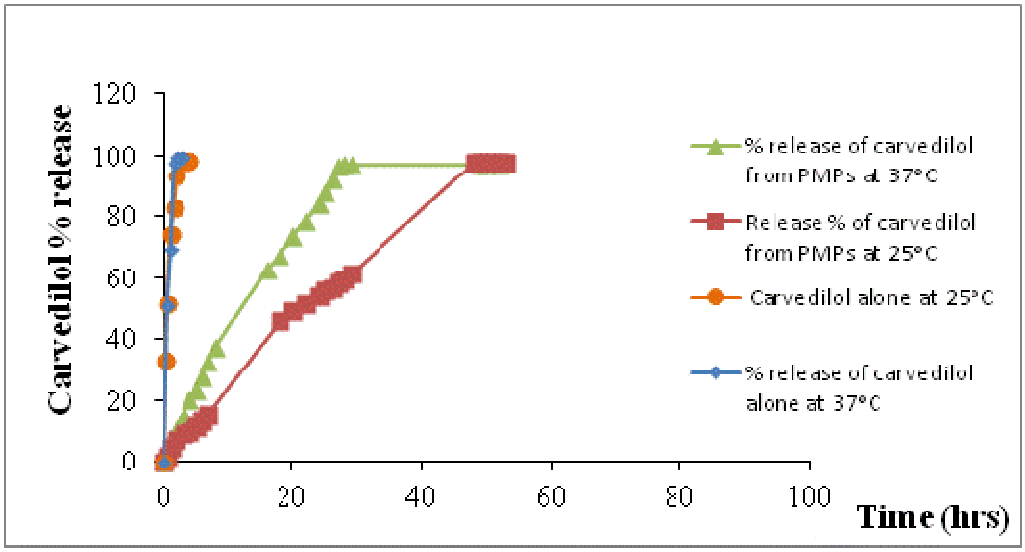

Figure (35): Release of carvedilol from PMPs at two different temperatures using phosphate buffers at pH 7.4.

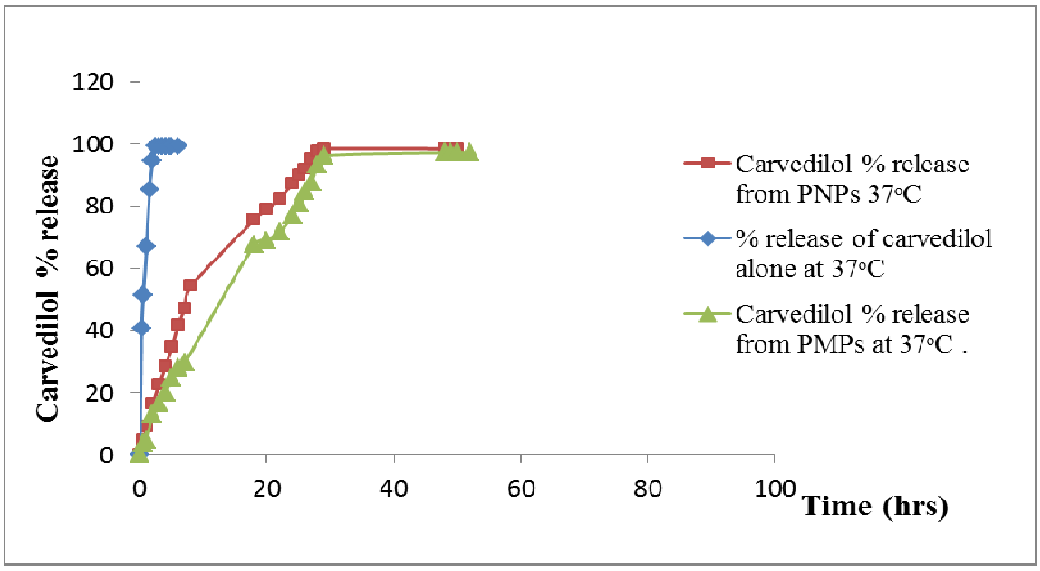

Figure (36): Carvedilol % release from PNPs, PMPs and carvedilol powder at pH 6.8 at body temperature.

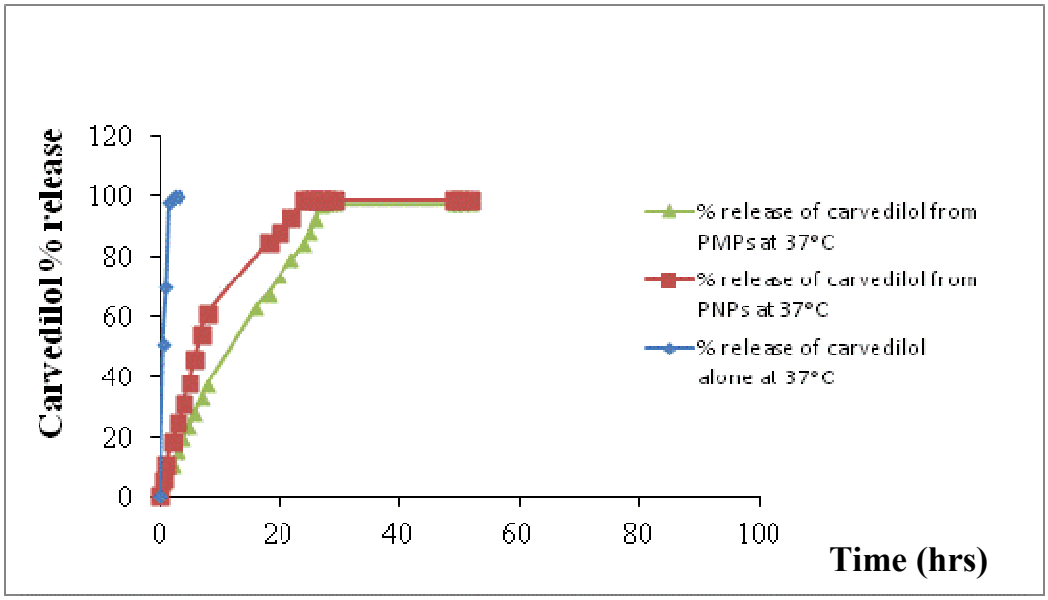

Figure (37): Carvedilol % release from PNPs, PMPs and carvedilol powder at pH 7.4 at body temperature.

Regarding the effect of PH on the release pattern of carvedilol, a decrease in the rate of drug release was observed when the pH was changed from pH 6.8 to 7.4. This pH dependent release may be due to the kinetic of hydrolysis of PLA which has been reported to be pH dependent [150].

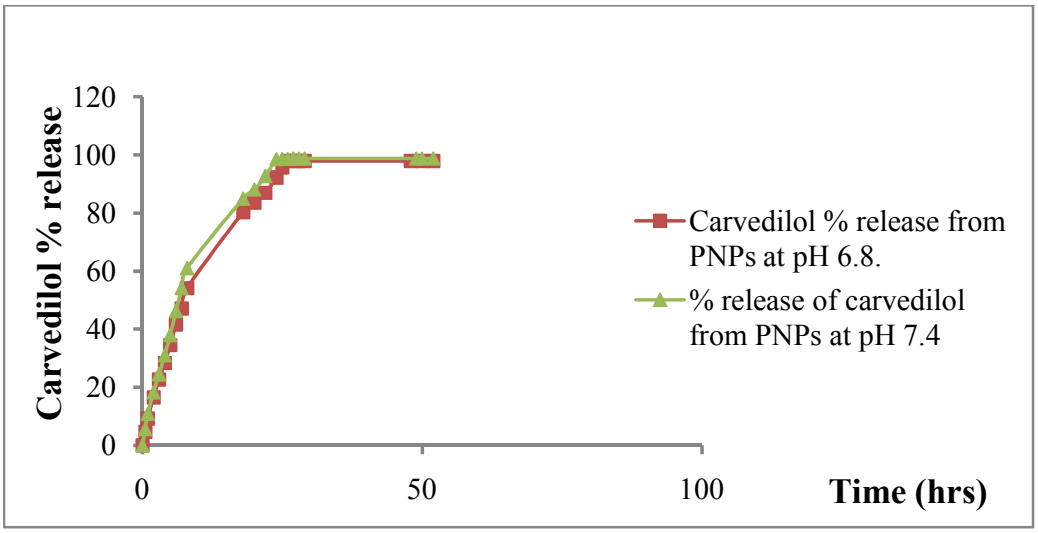

Figure (38): Carvedilol % release from PNPs at pH 6.8 and at 7.4 at body temperature.

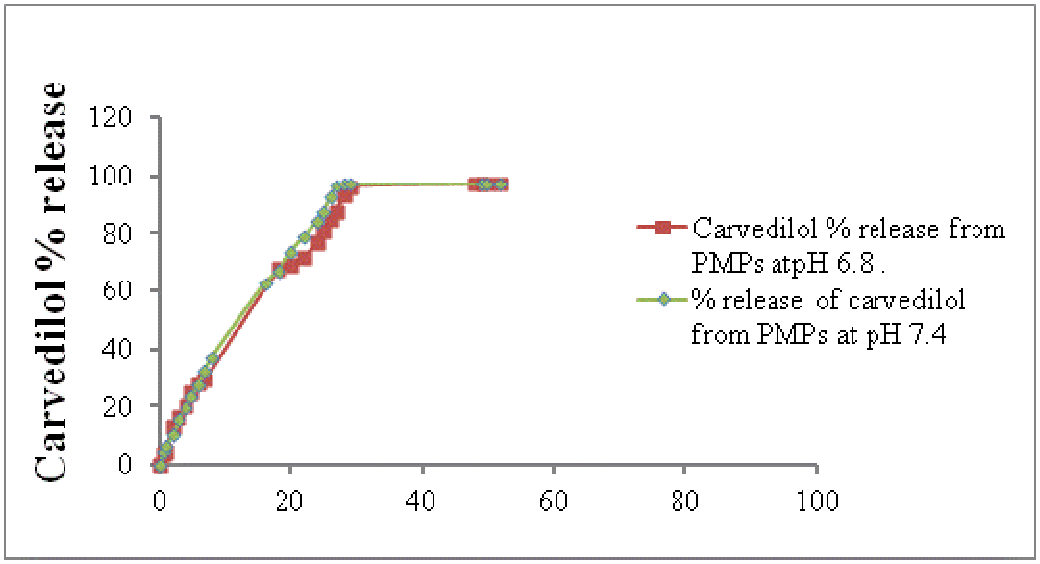

Figure (39): Carvedilol % release from PMPs at pH 6.8 and at 7.4 at body temperature.

In vitro release results suggest that a mixture of PNPs, PMPs and carvedilol pure drug could result in a sustained release formulation (figures 40 and 41). Accordingly, the release pattern of carvedilol from a mixture of the pure drug, PNPs, and PMPs was evaluated at body temperature using the above phosphate buffers. The release profiles were assessed using

different kinetic models in order to find the best sustained release model fit the data.

As shown in Table 4 the best linearity was observed with the Korsmeyer Peppas with T lag models for sustained release since it showed the highest R^2 and the lowest AIC.

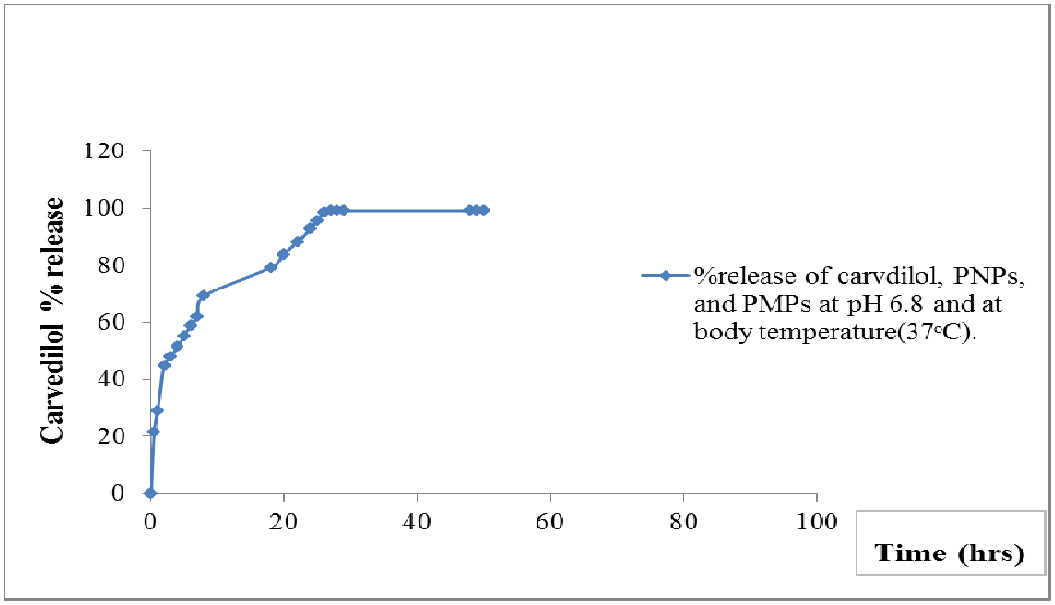

Figure (40): % release of carvedilol from mixture of PNPs, PMPs and carvedilol powder at pH 6.8 at body temperature.

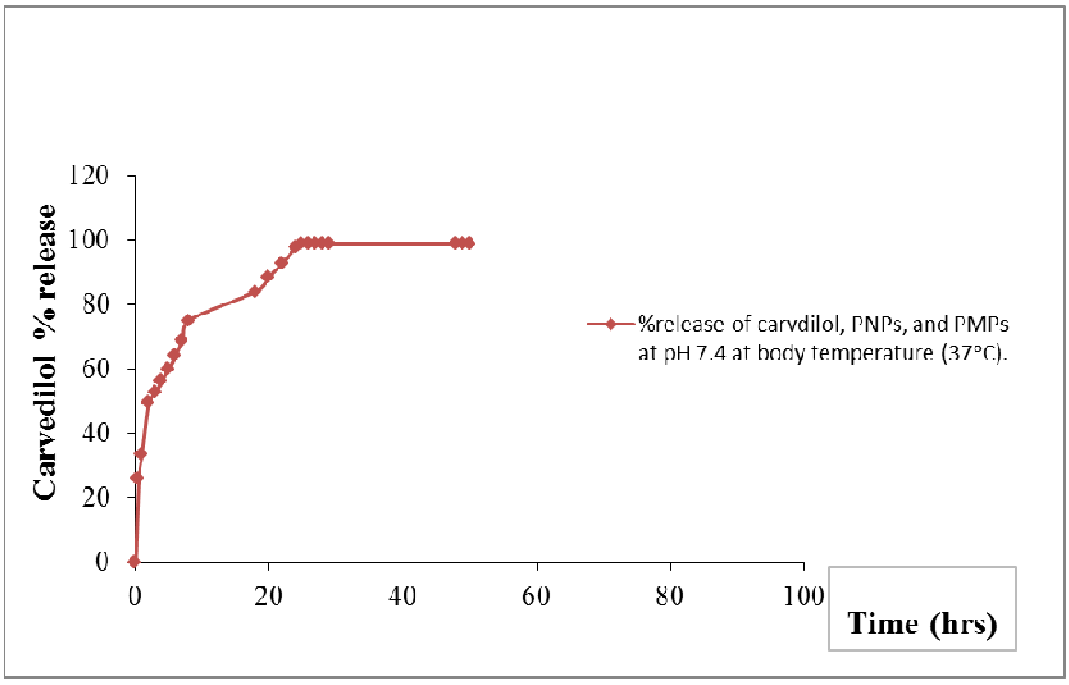

Figure (41): % release of carvedilol from mixture of PNPs, PMPs and carvedilol powder at pH 7.4 at body temperature.

Table (4): Dissolution data modelling for a mixture of PNPs, PMPs and carvedilol powder at pH 6.8 and 7.4 and body temperature.

Dissolution model	pH 6.8 at 37°C		pH 7.4 at 37°C	
	R^2	AIC	R^2	AIC
Zero order	-0.2549	223.1151	-0.6237	227.325
Zero-order with T lag	0.7027	193.4302	0.64932	195.608
First-order	0.9286	160.0641	0.92738	158.965
First-order with T lag	0.9622	148.0503	0.96134	147.095
Korsmeyer-Peppas	0.9639	147.0444	0.96105	147.262
Korsmeyer-Peppas with T lag	0.972	143.4211	0.97101	142.761
Higuchi Model	0.7606	186.6694	0.63273	194.625

Analysis of release profile can provide important information regarding the mechanisms involved in the release of carvedilol from nanoparticles and microparticles. In fact, several release mechanisms were suggested. These include desorption of the drug from the surface of the polymeric matrix, diffusion through the pores or wall of the matrix,

disintegration of the MPs with subsequent release, and dissolution and erosion of the matrix or the polymeric wall [151, 152]. The release profiles were assessed using different kinetic models in order to find the best sustained release model to fit the data.

Model selection was based on the measure of fit of linear regression (R^2) and the value of Akaike Information criterion (AIC), a measure of goodness of fit based on maximum plausibility. For a given data set, the most suitable model should show the lowest AIC and R^2 close to 1. As can be seen in table 3, the best fit linear regression line (0.972) and the lowest AIC (143.4211) for Carvedilol release at body temperature and pH 6.8 was observed using Korsmeyer-Peppas with T lag model. Same results were observed at body temperature and pH 7.4 with R2 and AIC equal to 0.971 and 142.76 respectively.

These findings are in accordance with literature, since the selected model should demonstrate similarity between observed and predicted release [153]. In fact, Korsmeyer-Peppas with T lag model is often used to describe the drug release behavior from polymeric systems when the mechanism of drug release is not well-known or when more than one kind of release mechanism is involved [154-156].

In fact, the mechanism involved is diffusion when the release exponent (n) is equal to 0.43. When the value of n is in the range <0.43 n $<$ 0.85, the suggested release is an anomalous transport that does not obey Fick's Law. Values of n less than 0.43 indicates a porous systems in which

transport occurs by a combination of diffusion through the polymeric matrix and diffusion through the pores. In our study the calculated n value was less than 0.43. This indicates a transport mechanism of Carvedilol by a combination of diffusion through the polymeric matrix and diffusion through the pores.

These results open the door toward a novel carvedilol sustained release formulation for both oral and parenteral routes. In fact, the release profile at pH 6.8 simulates a sustained pattern for the production of capsules or tablets that contain multiunit systems composed of the pure carvedilol powder, PNPs and PMPs. This would release the drug at proper time intervals, where carvedilol powder gives the initial dose while PNPs and PMPs give the maintenance doses. This type of multiple unit system showed increasing interest since it has greater advantages over single unit systems.

In fact, carvedilol S.R capsules are available as single unit system and these units show some disadvantages including safety and efficacy of the dosage form [157]. These disadvantages could be overcome by the use of the above multiple unit system.

Regarding parenteral sustained release systems, the release pattern of carvedilol, PNPs and PMPs at pH 7.4 and at 37°C showed an ideal controlled release that also was fitting with Korsmeyer-Peppas since R^2 was also close to 1 and AIC was the lowest among all models as can be shown in table 4.

According to these findings, our mixture could be produced as powder for injection. This formulation will have pH close to the blood pH. In fact, this would be of great interest in this field, especially for carvedilol, since it is not available as injection due to its poor solubility. In fact, Gruber and woog tried to formulate carvedilol immediate release injection using suitable organic solvents [158].

In fact, Gruber reported that despite the fact that parenteral and oral dosage forms of carvedilol are proposed in Europe, only oral dosage forms have been developed successfully and accordingly are commercially available [159]. Again Gurber claims that a series of experiments to incorporate carvedilol into conventional injectable preparations were carried out but all these attempts have surprisingly come to nothing. In fact many of them were not tolerated by veins due to the pH of the formulation or to the used organic solvents in order to increase the solubility of the final drug. Accordingly, these negative results exclude the use of carvedilol for injection purposes. Although, the formulation proposed by Gruber in order to produce carvedilol tolerable injection, these formulation are only for immediate release and not for sustained release purposes.

Chapter Four
Conclusion

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The production of PNPs with high encapsulation efficiency for carvedilol is possible using the nanoprecipitation technique. The smallest and narrowest size distribution of PNPs was obtained when acetone was used as the organic solvent and 12.5 mg of PDLLA polymer was used. In vitro release showed sustained release behavior for many hours at room and body temperature at two different pH levels (6.8 and 7.4). Carvedilol release was slower at room temperature and at pH 6.8. Carvedilol release was sustained at the two pHs at body temperature. Accordingly, this release could be useful for both oral and parenteral dosage forms. The Korsmeyer Peppas with T lag model was the best one to explain the sustained release behavior of these PNPs and PMPs.

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كلية الدراسات العليا

جامعة النجاح الوطنية

تحضير وتوصيف الجزيئات البوليمرية بحجم النانو والمايكرو المحملة بدواء الكارفيدايلول كنظام تحرير دوائي مستدام

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قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في العلوم الصيدلانية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

2015م

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الملخص

خلفية: الجزيئات النانو والمايكروبوليمرية هي من الأنظمة الواعدة في تسليم الأدوية بالجسم وتحسين توافرها الحيوي خصوصا الأدوية المحبة للدهون وشديدة العرضة للاستقلاب.

الهدف: الهدف من الاطروحة هو تحضير جزيئات بوليمرية بحجم النانو وحجم المايكرو وتحميلها بدواء الكارفيدايلول بكفاءة عالية. والهدف الاخر ايضا هو دراسة خروج الدواء من الجزيئات بظروف مختلفة (على درجة حرارة الغرفة ودرجة حرارة الجسم).

الطريقة: تم استخدام طريقة الترسيب في تحضير جزيئات النانو والمايكرو. تم استخدام نوع من البوليمر يدعى PDLLA. وتم استخدام بولي فينل الكحول للحصول على المستحلب لتحضير هذه الجزيئات. تم دراسة تأثير المذيبات (الأسيتون، رباعي هيدروفيوران، الأسيتونتريل، والإيثانول وثنائي كلورو ميثان) وكمية البوليمر على حجم وشكل الجزيئات المصنعة وتوزيعها الحجمي باستخدام جهاز AFM.

النتائج: تم الحصول على جزيئات كروية الشكل في جميع المذيبات المستخدمة. الطريقة المستخدمة سهلة وسريعة وفعالة (سعة عالية التحميل من الكارفيديلول) وبالتالي يمكن استخدام استراتيجية جديدة لتطوير كارفيديلول بأشكال صيدلانية جديدة. وقد أظهرت التجربة المخبرية أن خروج الكارفيدايلول من الجزيئات على درجة حرارة الجسم اسرع مقارنة بخروجه على درجة حرارة الغرفة.

الخلاصة: لقد تم تحضير جزيئات بحجم النانو والمايكرو باستخدام بـــوليمر PDLLA بكفـــاءة تحميل عالية، تم الحصول على أصغر الجزيئات باستخدام الاسيتون كمذيب عضوي وباستخدام 12.5 مليغرام من البوليمر المذكور. باستخدام ثنائي كلورو ميثان تم الحصول علـــى جزيئـــات بحجم المايكرو. وظهر أن النمط المناسب لتفسير خروج الدواء المستدام مـــن الجزيئـــات هـــو Korsmeyer with T lag.