**Palestine Polytechnic University**

**Faculty of Engineering**

**Electrical Engineering Department**

# Preparation of Polycaprolactone Nanoparticles Using Nanoprecipitation Technique

**Group student names:**

**Sajida Ahmro**

**Salam Abu Farah**

**Supervisor: Hassan Sawalha Manasrah**

**Submitted to the College of Engineering**
**in partial fulfillment of the requirements for the degree of**
**Bachelor degree in Biomedical Engineering**

**May, 2016**

**Palestine polytechnic University**

**Hebron – Palestine**

**College of Engineering**

**Electrical Engineering Department**

# Preparation of Polycaprolactone Nanoparticles Using Nanoprecipitation Technique

**Project Team:**

**Sajida Ahmaro** **Salam Abu Farrah**

By the guidance of our supervisor, and by the acceptance of all members in the testing committee, this project is delivered to department of electrical engineering in the college of engineering and technology, to be as a partial fulfillment of the requirement of the department for the degree of B.sc

**Supervisor signature**

**----------**

**Testing committee signature**

**---------- ---------- ----------**

**The head of department signature**

**----------**

**جامعة بوليتكنك فلسطين**

**الخليل – فلسطين**

**كلية الهندسة**

**دائرة الهندسة الكهربائية**

**تحضير جزيئات Polycaprolactone النانونية باستخدام خاصية الترسيب**

**فريق المشروع**

**ساجدة احمرو سلام أبو فارة**

**بناء على نظام كلية الهندسة واشراف ومتابعة المشرف المباشر على المشروع وموافقة اعضاء اللجنة المناقشة , تم تقديم هذا العمل الى دائرة الهندسة الكهربائية.**

**وذلك للوفاء بمتطلبات درجة البكالوريوس في هندسة االجهزة الطبية.**

**توقيع المشرف**

**----------**

**توقيع اللجنة المناقشة**

**---------- ----------**

**----------**

**توقيع رئيس الدائرة**

**----------**

## Acknowledgments

First of all, thank our god for giving us the will and patience to undertake this study and completion to our introduction of graduate project

We also indebted and deeply grateful to supervisor Dr. Hassan Sawalha for his support, encouragement and his help us to take advantages of his experience and knowledge

We wish to express special thanks to Dr.Ramzi Qwasmi, Eng.Ali Amr, Eng.Nancy Sia'ra for their helping and supporting

Very special thanks to Maryam Feroun and Riham Mozafer from Al-Quds University for their helping to get AFM analysis.

We wish to express our deep gratitude to our family, our fathers, our sisters, our brothers, and special gratitude to our mothers for their encouragement and help.

We also wish to express our gratitude to our colleges to their encouragement.

# الإهداء

- أشكر الله العلي القدير الذي أنعم عليَّ بنعمة العقل والدين....القائل في محكم التنزيل "وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ" صدق الله العظيم .
- إلى من بلغ الرسالة وأدى الأمانة....ونصح الأمة....إلى نبي الرحمة ونور العالمين.....سيدنا محمد صلى الله عليه و سلم
- الى من ضحوا بأرواحهم لنعيش بكرامة..الى من أنارو شعلة الحرية بدماءهم.....شهداء فلسطين
- إلى من جرع الكأس فارغاً ليسقيني قطرة حب ... إلى من كلّت أنامله ليقدم لنا لحظة سعادة.....إلى من حصد الأشواك عن دربي ليمهد لي طريق العلم.....والدي العزيز
- الى ...من اثقلت الجفون سهرا ... وحملت الفؤاد هما ... وجاهدت الايام صبرا ... وشغلت البال فكرا ... ورفعت الايادي دعاءا ... وايقنت بالله املا..... امي الغالية
- إلى كل من أضاء بعلمه عقل غيره فأظهر بسماحته تواضع العلماء وبرحابته سماحة العارفين..... مشرف المشروع د.حسن صوالحة مناصرة
- إلى من أناروا لنا طريق العلم والمعرفه .....الى من لم يتهانى يوما عن تقديم النصح و الارشاد.....الى من أضاء بعلمه عقل غيره..... أساتذتنا الأفاضل
- إلى من تحمل في عيونهاذكريات طفولتي وشبابي.....الى من رافقتني في السراء والضراء.....الى أصدق الأصحاب.....أختي العزيزة
- إلى من أظهروا لي ما هو أجمل من الحياة و كانوا و لا يزالو سندا لي في هذه الحياة.....إخوتي
- إلى الأخوات و الإخوة الذين لم تلدهم أمي .. إلى من تحلو بالإخاء وتميزوا بالوفاء والعطاء إلى من كانوا معي على طريق النجاح والخير.....أحبائي و أصدقائي

# Abstract:

Polymer nanoparticles are defined as particles with diameter less than 100 nm in size, are increasingly used in different application including drug delivery system as a result of their controlled and sustained release properties, subcellular size and biocompatibility with tissue and cells.

This study aims at preparing of biodegradable Polycaprolactone nanoparticles with uniform size and uniform dose drug loading efficient by using nanoprecipitation technique.

The polymer is dissolved Acetic acid, and added stepwise to a continues phase consists of non-solvent (i.e. water, Acetone, Methanol, Ethanol, Tetrahydrofuran and Acetonitrile) and surfactant (Polyvinyl alcohol, Sodium lauryl sulfate) and mixed at 1500 rpm for 30 minutes. The effect of the type of surfactant and non-solvent on the size and size distribution of the polymer nanoparticles was studied using Atomic force microscopy and scanning electron microscopy techniques.

The results showed that Polycaprolactone nanoparticles with average size of 100 nm-400 nm could be prepared. The particles prepared with Sodium laureth sulfate were smaller in size and more spherical than those prepared with Polyvinyl alcohol. Furthermore, the addition of organic non-solvents reduces the size of the particles

**الملخص:**

تعرف الجزيئات النانوبولمرية على انها جزيئات لا يتعدى قطرها 100 نانومتر , و يتم استخدامها في تطبيقات عديدة من ضمنها التطبيقات الطبية مثل تحميل الدواء .

الجزيئات النانوبوليمرية تستخدم في نظام توصيل الدواء و ذلك لانها تتميز بحجم صغير يسمح لها بالمرور بين الخلايا حيث ان هذه الجزيئات تحسن من امتصاص الدواء, تقلل الجرعات و تسمح للدواء بالوصول للاماكن المستهدفة بكفاءة عالية.

الهدف من هذا المشروع تحضير جزيئات ونانوبوليمرية محملة بدواء كارفيدايول بأحجام متجانسة و كفاءة عالية في تحميل الدواء داخل الجزيئات, و من ثم مراقبة خروج الدواء من هذه الجزيئات.

تم استخدام Polycaprolactone وSodium lauryl sulfate و Polyvinyl Alcohol كمواد أساسية لتصنيع الجزيئات.

تم فحص قابلية البوليمر للتحلل في عدد من المذيبات و هي Acetone, Acetonitrile, Acetic Acid, Dimethylformamide, Methylene chloride . وجدنا ان Acetic Acid افضل واسرع لاذابة لبوليمر .

باستخدام تقنية الترسيب تم خلط البوليمر مع الدواء مع Acetic Acid و اضافتها الى عينات مختلفة من المحلول المكون من وSodium lauryl sulfate and non-solvents وعينات مختلفة من المحلول المكون من Polyvinyl Alcohol and non-solvents باستخدام الظروف المناسبة تم انتاج الجزيئات المحملة بالدواء.

تم دراسة تأثير non-solvents و Surfactants على حجم الجزيئات و توزيع الدواء بداخلها من خلال جهاز Atomic Force Microscope.

# List of contents

**Chapter six: Results and Analysis**

# List of Figures

# List of Tables

# List of Abbreviations

| | |
|---|---|
| NPs | Nanoparticles |
| MNPs | Magnetic Nanoparticles |
| MWNTs | Multi walled Nanotubes |
| PCL | Polycaprolactone |
| PVA | Polyvinyl alcohol |
| PLA | Poly Lactide |
| QD | Quantum Dot |
| SPIONs | Super Paramagnetic Iron Oxide Nanoparticles |
| SR | Sustained release |
| SWNTs | Single walled Nanotubes |
| TG | Glass Transition temperature |
| Nm | Nanometer |
| AFM | Atomic force microscopy |
| RESS | Rapid expansion of Super critical fluid technology |
| SAS | Super critical anti –solvent. |
| DMF | Dimethylformamide |
| SEM | Scanning electron microscope |
| UV spectrum | Ultra Violet spectruim |
| ROP | ring-opening polymerization |
| PHB | Poly hydroxyl butyrate |
| SLS | Sodium laureth sulfate |
| THF | Tetrahydrofuran |

# Chapter One: Introduction

## 1.1 Project overview:

Polymer form a very important class of materials that are used for many application including rubber, plastic clothes, adhesive taps, pipes tanks and biomedical applications. Various polymer materials were used for biomaterial applications like tissue engineering and drug delivery system. Polymer nanoparticles can be applied as drug delivery system improve stability and to target drug to specific site. Nanoparticles are solid particles with size of (10-1000 nm) prepared from biocompatible and biodegradable polymer use in drug delivery system as carrier. Nanoparticles have advantages like limiting fluctuation with therapeutic period, reducing side effects and improving patient compliance.

Nanoparticles can be prepared by different techniques including emulsification solvent evaporation method, supercritical fluid technology and nanoprecipitation. In the nanoprecipitation the polymer is dissolved in a proper solvent and is added in stepwise to a continues phase consist of non-solvent and surfactant. Thenon-solvent should be selected to be miscible with the solvent,the mixture is mixed for sufficient time and nanoparticles are formed. Various types biodegradable polymer used in preparing nanoparticles include poly lactic acid(PLA), polyglycolide (PGA) andPolycaprolactone (PCL).

This study aims at preparing (PCL) nanoparticles to be used as drug delivery vehicles. PCL nanoparticles is prepared by nanoprecipitation technique and carvedilol will be encapsulated in the PCL NPs.

By preparing biodegradable carvedilol Polycaprolactone nanoparticles using nanoprecipitation technique by dissolving Polycaprolactone and Carvedilol in organic solvent and add the solution to the aqueous phase containing surfactant.

The effect of non-solvent, type of surfactant on the size and size distribution of the NPs and drug release process will be investigated.

## 1.2 Project motivation:

Preparing nanoparticles by using traditional method like emulsification technique and supercritical fluid produced particles with nonuniform size and nonuniform drug distribution in the particles.

## 1.3 Project objectives:

**The Main:** Preparing Polycaprolactone nanoparticles with uniform size and the uniform disruption of drug in the particles with high drug loading efficiency by using Nanoprecipitation technique**.**

**Sub objectives:**

1. To investigate the effect of none solvent type on the size and morphology of the nanoparticles.
2. To study the drug release process of drug model.
3. To test the effect of non -solvent on the drug release characteristics.
4. The effect of non-solvent, type of surfactant on the size and size distribution of the NPs.

## 1.4 Literature Review:

polymer nanoparticles were found in the literature reading to carvedilol to improve its pharmacokinetic properties.

In 2009 Selvakumar Kalimuthu found that nanoprecipitation technique is suitable for preparation polymer nanoparticles for loading carvedilol by using Eudragit 100 as polymer and methanol as an organic solvent and the result was that polymer concentration play important role in size and loading. [1]

In 2013, Pal et al used emulsification by evaporation method to prepper biodegradable nanoparticles of carvedilol using poly (lactied –co- glycolide) (PLGA) as biodegradable polymer. Theyobserved higher initial drug loading in the obtained nanoparticles. [2]

Ankarao , A . prepered and evaluate the oral sustained release nanoparticles of carvedilol. Theses polymer nanoparticles prepared by coaservetion method. the result showed that size of the obtained particles was big with rang (500 nm to 1000 nm). [3]

In 2015 Bani-Odeh and coworkers used nanoprecipitation technique to prepare carvedilol Polymer nanoparticles with high loading efficiency by using polylactice acid as polymer and water miscible organic solvent (acetone). the result showed the range of the nanoparticles size (100-970) nm.[4]

## 1.5 Time Plan:

The Table 1.1 shows the activities that done in the project, and the time of each one.

**Table 1.1:**Activities Planning .

| Weeks / Activities | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Obtaining required components | | | | | | | | | | | | | | | | |
| Laboratory work | | | | | | | | | | | | | | | | |
| Recording Results | | | | | | | | | | | | | | | | |
| Results analysis and conclusion | | | | | | | | | | | | | | | | |
| Documentation | | | | | | | | | | | | | | | | |

## 1.6 Project Cost:

**Table 1.2: Project cost**

| Component and material | Cost JD |
|---|---|
| Acetonitrile, acetic acid, micropipette tips, acetone, 3 bottles | 56 JD |
| SEM image | 400 JD |
| Total | 456 JD |

.

# Chapter Two :Polymer Science

## 2.1 Polymer:

Polymer derive from classical Greek “poly mean many, meres mean units”. Polymers is large molecules constructed from many small repeated units covalently bonded in very long chain with (5-1000s) of monomers and don’t have empirical formula. [5, 6, 7]

Polymer form a very important class of material without it life become different. polymer use every day in rubber, plastic clothes, and adhesive taps. Most of the polymers are organic compound contains carbon, however they can be inorganic like silicon. [7]

## 2.2 Chemical bonds:

1. Primary bonds: ionic, covalent, and metallic.

2. Secondary bonds:dipole, hydrogen, induction,van der waals.

## 2.3 Polymer classification:

Polymer can be classified in several different ways according to their origin, structure and chemical and physical properties.

### 2.3.1Classification of polymer according to Structure and types:

- Molecular structure

Polymer molecules can be single long chain or highly branched structure, the length of polymer chain or its branch depend on the conditions of polymer production.[6]

- Linear polymer: consist of along linear chain of monomer. See Figure 2.1

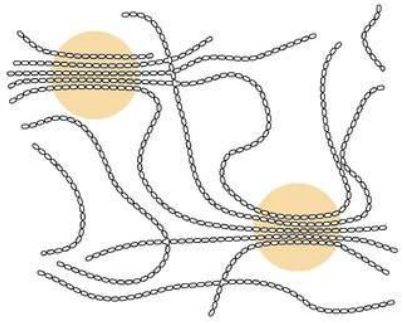


**Figure 2.1**: A polymer molecule built as a single linear chain. [6]

- Branched polymer :contains along backbone chain with several short side chain branches covalently bonded. See Figure 2.2

**Figure 2.2**: A polymer molecule with branching. [6]

- Cross-linked polymer: have monomer of one chain covalently bonded with anotherchain, the result is three dimensional molecular network. See Figure 2.3

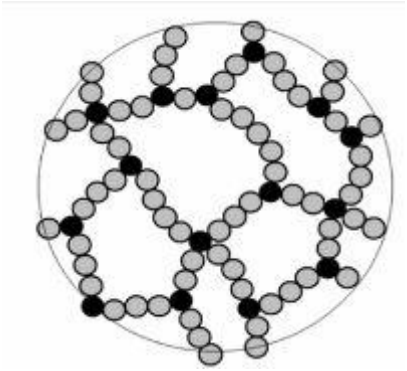


**Figure 2.3**: 3D-moluclar network. [6]

### 2.3.2 Classification of polymer according to chemistry of polymers molecules:

Another classification of polymers depends on the chemical type of monomers:

- Homopolymer: polymer contains the same type of monomers.
- Bifunctional: when polymer unit have tow active bonds to connect with other unit
- Trifunctional: when polymer unit have three bonds to connect with other units.
- Copolymer: polymer contains different type of monomers, depending on the arrangement of monomers types copolymers classifiedto:

1. Random: different repeating units are distributed randomly. [6, 8]

-AABBABABBAAABAABBA- .

2. Alternating: polymer contains alternating sequences of different monomers. [7, 9]

-ABABABABABAB- .

3. Block polymer: long sequences of one monomers type are followed by long sequence of another type. [6, 8]

AAAAA—BBBBBBBB—AAAAA—BBBB--.

4. Graft: consist of chain made from one type of monomer with branches of another type. [7]

**2.3.3Classification of polymer according toorigin**:

Polymers may be either naturally occurring or purely synthetic.

**2.3.3.1Natural polymer:** are involving in animals and plants as building material or storage substance and playing important role in biochemical reaction. natural polymers found in animal horns, tortoise shell, asphalt and tar. Enzymes, nucleic acids and proteins are polymers of biological organs. Starch cellulose and rubber are polymer with plants origin.Gelatin, Collagen, Alginate, Starch, Cellulose, and Chitosan are examples of natural polymer. [5,8]

**2.3.3.2Synthetic Polymer**: processing of polymer mainly involves preparing particular polymer by synthesis of row material which usually derived from coal and petroleum product.Poly lactic acid (PLA), Poly glycolide (PGA), Poly hydroxyl butyrate (PHP), Poly (L, D lactic-co-glycolide(PLGA)) and Polycaprolactone (PCL) are examples of synthetic polymer used in medical device. Depending on the response to heat polymerclassified to:

1. Thermoplastic: soften when heated and harden when cooled so that are totally repeated and reshaped, it easy to fabricate by application of heat and pressure. Thermoplastic linear polymer without any crosslink in chemical structure and have secondary bonds between chains. Common thermoplasts are acrylics, PVC, nylons, polypropylene.

2. Thermoset polymer: become hard during their formation. Don't soft up heating, it is network with large density of covalent crosslink between molecules chains so it become harder and stronger than thermoplastics and cannot be recycled.

Advantages of thermoset for engineering application include one or more of the flowing:

a. High thermal and dimensional stability.

b. Low cost.

c. High rigidity.

d. God electrical and thermal insulating.

e. Resistance to deformation under load. [5]

3. Elastomer (Rubber): polymer that undergo large elongation under load at room temperature and return to its original structure after load released. This because the curled polymer chain stretch during deformation, but hindered from sliding past each other by crosslinks between the molecules and after load is released most molecules recoil. [5,9]

Some synthetic polymer that is used in medical field:

a. Poly lactic acid (PLA).
b. Poly glycolide (PGA)
c. Poly(L, D lactic-co-glycolide(PLGA))
d. Poly hydroxyl butyrate (PHP)
e. Polycaprolactone (PCL).

## 2.4 Polymerization:

The reaction by which the one or more types of monomers combine to form polymer with or without elevation of anything like water, heat and any other solvent Dimers, trimmers and tetramers called low molecular polymers (oligomers). [3, 5]

Polymer don't have fixed molecular Wight .it specified by degree of polymerization (PD), which mean the number of repeat unit in the chain or ratio of average molecular Wight of polymer to Wight of repeat units. High degree of polymerization is required to develop useful properties e.g. polystyrene with PD of 7is viscous liquid while grade commercial polystyrene is solid and have PD excess 1000. [5, 8]

*Molecular weight of polymer =PD*molecular weight of repeat unit.....Equation (1)*

Polymers are classified according to the reaction by which they formed to:

1. **Addition:(chainreaction)**

   Polymer formed by reaction that link monomers together throw multiple bonds, polyethylene. poly (methyl methacrylate are examples of polymer formed by addition polymerization. There are three steps for addition reaction:

   a. Chain imitation: involves acquisition of an active site, this occurs spontaneously by the absorption of heat, light or energy. See figure 2.4 [6, 8]


**Figure 2.4:** Chain imitation. [6]

   b. Chain propagation: during propagation, initiated monomers adds another monomer and this contains addition of free radical to double bound of monomer. propagationcontinue until chain radical growing become deactivated by chain - termination. see figure 2.5 [6, 8]

**Figure 2.5:**Chain propagation[6]

c. Chain termination: the activity of radical chain growing is destroyed by reaction with another free radical chain. See figure 2.6 [6, 8]

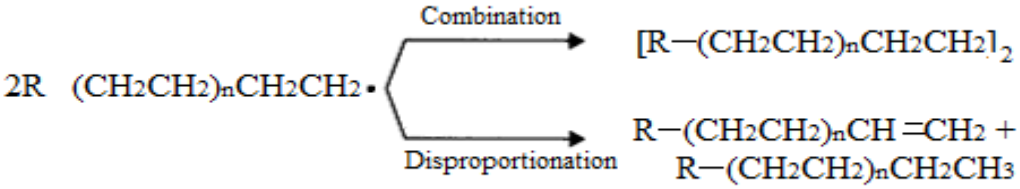



**Figure 2.6:**Chain termination. [6]

d. Condensation: (step reaction)

In condensation polymerization, the stepwise reaction occurs between active groups on the reacting units. In this reaction some of atoms of the monomers are spilt off reaction as water, alcohol, amino or carbon dioxide. Polyamide and polyester are examples of polymers formed by step reaction polymerization.[6, 8]

## 2.5 Polymer properties:

In the recent years polymers are widely in many applications due to their properties include:

1. Stress –strain
2. Viscoelasticity
3. Crestanility in polymer
4. Glass Transition
5. Melting point
6. Fracture of polymer

### 2.5.1 Stress –strain behavior:

Stress strain behavior can explain by stress strain curve that consist mainly two region elastic and plastic region.

In the Elastic region stress is proportional to strain and the deformation is completely reversible, but in the plastic region the deformation is not reversible. See figure 2.5

The Stress/Strain behavior of solid polymers can be categorized into several classes of behavior:

1. Brittle Fractureoccurs and the load rises to the breaking point at low strains such as seen in inorganic glasses.

2. Yield Behavior- characterized by a maximum in the stress/strain curve followed by yielding deformation which is usually associated with crazing or shear banding and usually ductile failure. Ductile failure exhibits a high extent of deformation on the failure surface. Yield behavior can result in necking which exhibits a close to constant load regime and a terminal increase in the stress.

3. Rubber-Like Behavior- characterized by the absence of a yield point maximum but exhibiting a plateau in an engineering stress/strain curve. Often rubber-like behavior exhibits a terminal increase in the stress followed by failure which results in a tear with little permanent deformation exhibited in the failure surface, e.g. Jell-O. See Figure 2.7.

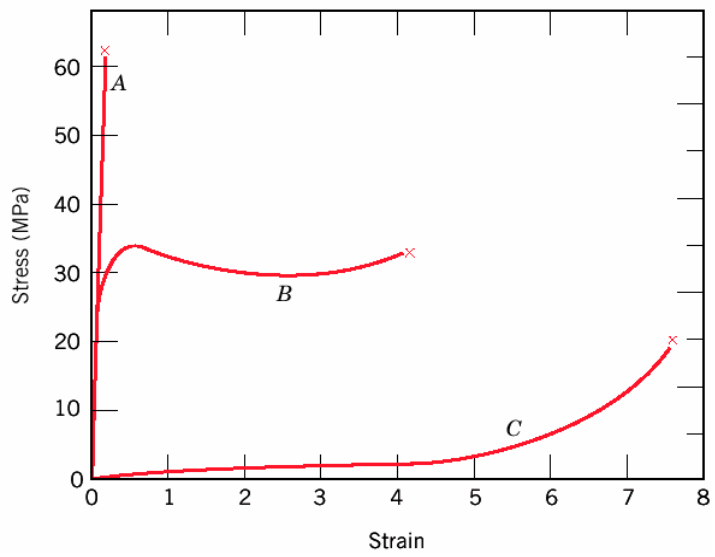



**Figure 2.7:** Stress-strain curve of different polymersA. brittle polymer,B. Highly elastic polymer, C. elastomer (rubber). [8]

- Characteristics of stress _strain behavior:

1.Modulus of stress: (10 MP -4Gp) the ratio of stress to strain.

2.Ductility: is a solid material's ability to deform under tensile stress; this is often characterized by the material ability to be stretched into a wire.

3. Yield strength: the maximum point at the curve justafter. elasticregion.

4. Tensile strength: is defined at the fracture point and can be lower than yield strength (10MP-100M P). See Figure 2.8

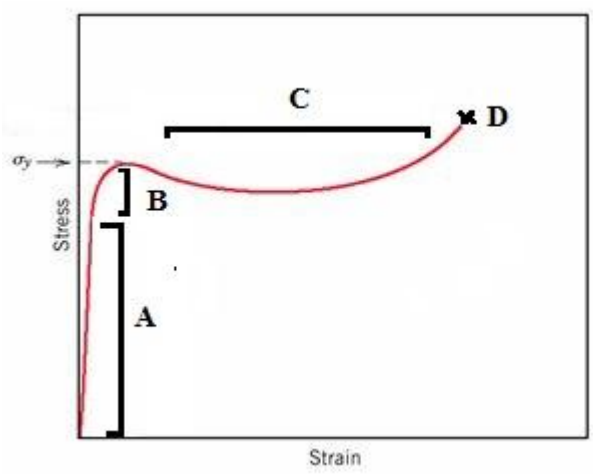



**Figure 2.8:** stress-strain curve, (A) Elastic region, (B) Yield stress, (C) plastic region, (D) plastic failure

Temperature increase lead to:

1. Decrease in elastic modules.
2. Reduction in tensile strength.
3. Increase in ductility. See Figure 2.9

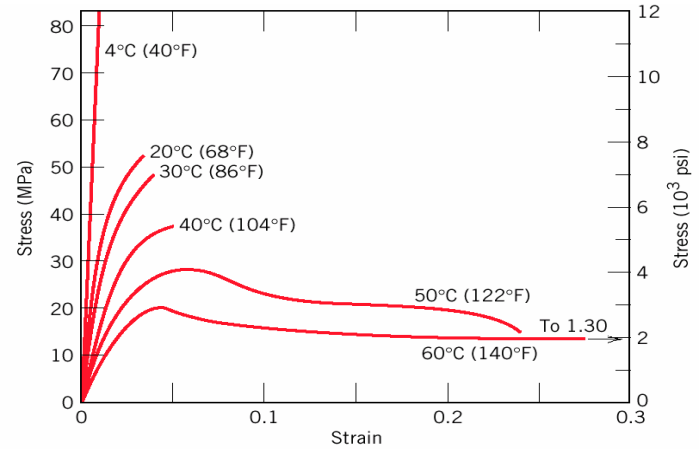



**Figure 2.9:** stress-strain behavior of PMMA with temperature increase [10]

### 2.5.2Viscoelasticity:

A polymer at specific temperature behave as liquid or solid depending on the speed of deformation. This behavior between solid and liquid state is refereed as the viscoelastic response

1. Linear viscoelasticity: which valid for polymer undergoing small or slow deformation.

2. Nonlinear viscoelasticity: which required when large rapid deformation.

### 2.5.3 Crestanility in polymer:

Most of polymer production process involves forming of melt, followed by the solidification through cooling the melt. polymer classified depending in crystallizing process:

1. Semi-crystalline polymer: the molecules arranged in regular order and pattern.

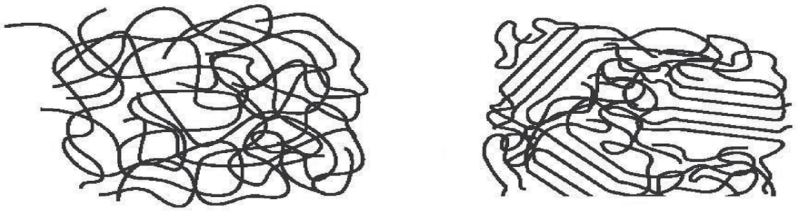
Ex: Polypropylene, Nylon. [11]

2. Amorphous polymer: have chains are not able of order arrangement. This

type characterized by short –range order of repeating units. See Figure 2.10

[8]

Ex: Polyisoprene. [11]



(A) (B)

**Figure 2.10:**(A) amorphous polymer, (B)Semi-crystalline polymer [7]

Crestanility influence many polymer properties:

1. Hardness.

2. Modulus.

3. Tensile.

4. Stiffness.

5. Melting point.

### 2.5.4 GlassTransition (Tg):

Decreasing the temperature on a polymer which is in its molten state, the polymer reaches the glass transition temperature (Tg). At this point mechanicalproperties of polymer change from soft rubber material to glass material. below Tg polymer motion are limited but above it more motion accessible. [12]

Plasticity is the ability of material to undergo plastic. this can be achieved by addition of low –molecular weight organic compounds. Plastizers are none polymers, organicliquid of high boiling point. plastizersdirectly reduce Tg.

### 2.5.5Melting point:

At the melting temperature (Tm) the chains are allowed to move around freely, thus they do not possess an ordered arrangement. Upon meltingpoint, the polymers absorb heat, thus melting is an endothermic transition. The melting is a first order transition since when the melting temperature is reached; the polymer's temperature does not rise until all the crystals have completely melted.See Figure 2.11

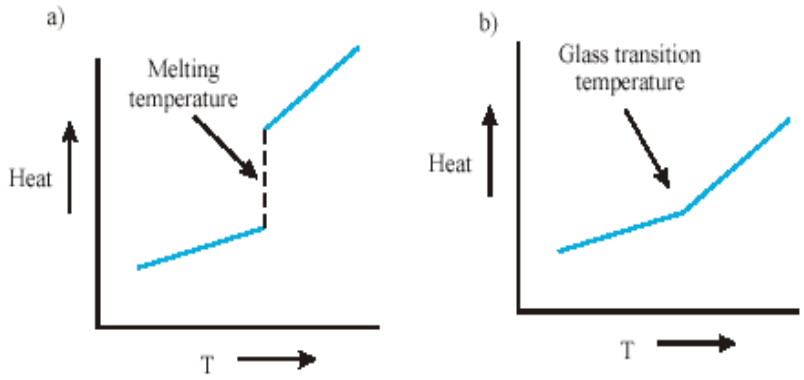



**Figure 2.11:** a. melting point,b. glass transition temperature.[12]

### 2.5.6 Fracture of polymer:

Fracture behavior of polymer is strongly affected by addition of rigid particles. Several characteristics of particles influence in the degree of fracture include:

1. Shape and size.
2. Chemical nature.
3. Surface nature.
4. Orientation.

Fracture strength of polymer are low compared to metal and ceramic. fracture initiated at stress points. brittle fracture occurs in the thermosetting polymer, while both brittle and ductile are possible in thermoplastic. [9]

## 2.6Application:

Polymer existed in natural from since life began such as DNA and protein. Polymer form basis of life, all living things are composed of polymers. man uses polymers as material for clothing, shelter, writing material and other requirements.[8]

At ninth century modification of the natural polymer started like blending rubber with additives.at middle of $20^{th}$ century plastic development startedand recognized with synthesis of cellulose nitrate which derived from natural polymer (cellulose). The first man-mad plastic comes in 1990 when Dr. Leo. Henrik developed phenol–form aldehyde (phenolic), other polymers are followed. See Table 2.1

**Table 2.1:** polymers application in life.

| **Date /Material** | **Typical use** |
|---|---|
| 1868 Cellulose nitrate | Eyeglass frames |
| 1909 Phenol–formaldehyde | Telephone handsets, knobs |
| 1919 Casein | Knitting needles |
| 1926 Alkyds | Electrical insulators |
| 1927 Cellulose acetate | Toothbrushes, packaging |
| 1927 Poly(vinyl chloride) | Raincoats, flooring |
| 1929 Urea–formaldehyde | Lighting fixtures, electrical switches |
| 1935 Ethyl cellulose | Flashlight cases |
| 1936 Polyacrylonitrile | Brush backs, displays |
| 1936 Poly(vinyl acetate) | Flashbulb lining, adhesives |
| 1938 Cellulose acetate butyrate | Irrigation pipe |
| 1938 Polystyrene | Kitchenware, toys |
| 1938 Nylon (polyamide) | Gears, fibers, films |
| 1938 Poly(vinyl acetal) | Safety glass interlayer |
| 1939 Poly(vinylidene chloride | Auto seat covers, films, paper, coatings |
| 1939 Melamine–formaldehyde | Tableware |

| | |
|---|---|
| 1942 Polyester (cross-linkable) | Boat hulls |
| 1942 Polyethylene (low density) | Squeezable bottles |
| 1943 Fluoropolymers | Industrial gaskets, slip coatings |
| 1943 Silicone Rubber goods | |
| 1945 Cellulose propionate | Automatic pens and pencils |
| 1947 Epoxies | Tools and jigs |
| 1949 Allelic Electrical connectors | |
| 1954 Polyurethane | Foam cushions |
| 1956 Acetal resin | Automotive parts |
| 1957 Polypropylene | Safety helmets, car pet fiber |
| 1957 Polycaprolactone | Appliance parts |
| 1959 Chlorinated polyether | Valves and fittings |
| 1962 Phenoxy | Resin Adhesives, coatings |
| 1962 Polyallomer | Typewriter cases |
| 1964 Ionomer | Resins Skin packages, moldings |
| 1964 Polyimide | Bearings, high temperature films |
| 1964 Ethylene–vinyl acetate | Heavy gauge flexible sheeting |
| 1965 Polybutene | Films |
| 1965 Polysulfone | Electrical/electronic parts |
| 1970 Thermoplastic polyester | Electrical/electronic parts |
| 1971 Hydroxy acrylates | Contact lenses |
| 1973 Polybutylene | Piping |
| 1974 Aromatic polyamide | High-strength tire cord |
| 1975 Nitrile barrier resins | Containers |

## 2.7 Degradable polymers application:

Degradable polymer is specific type of polymer that break down to its monomers after short period of time and it used in many applications.

### 2.7.1 Application ofdegradable polymers inPackaging:

Packaging is important area where biodegradable polymers are used, to reduce the volume of waste, besides their biodegradability, biopolymers have other characteristics as air permeability, low temperature salability and so on. Biodegradable polymers used in packaging require different physical characteristics, depending on the product to be packaged and the store conditions.

### 2.7.2 Application of degradable polymers in Agriculture:

Plastic films were first introduced for greenhouse coverings fumigation and mulching in the 1930s. The main actions of biodegradable cover films are to conserve the moisture, to increase soil temperature and to reduce weeds in order to improve the rate of growth in plants. At the end of the season, the film can be left into the soil, where it is biodegradables.

### 2.7.3 Application of degradable polymers in Automotive:

The automotive sector aims to prepare lighter cars by use bioplastic and biocomposits. Natural fibers can replace glass fibers asreinforcement's materials in plastic can parts.

### 2.7.4 Application of degradable polymers in Electronics:

PLA and kenaf are used as composite in electronics application. Compact disks based on PLA.

### 2.7.5 Application of degradable polymers in Biotechnologies:

Biotechnology is the manipulation of living organism or their components to produce useful commercial products that improve our life and environment.

### 2.7.6Application of degradable polymers in Biomedical:

A biomaterial is essentially a material that is used and adapted for a medical application. Biomaterials can have a benign function; such as being used for a heart valve. [13]

# Chapter Three: Drug Delivery System

## 3.1 Biomedical application of polymers

There is a lot of application of polymer in biomedical filed, some of them are:

### 3.1.1 Tissue Engineering:

The field of tissue engineering an interdisciplinary field that applies the principles of engineering and the life science toward the development of biological substitutes that restore, maintain or improve tissue functions.[14, 15]

A wide range of polymeric scaffolds have been intensively studied for use as implantable and temporal devices in tissue engineering Biodegradable and biocompatible scaffolds having a highly open porous structure and good mechanical strength are needed to provide an optimal microenvironment for cell proliferation, migration, and differentiation, and guidance for cellular in-growth from host tissue. A variety of natural and synthetic polymeric scaffolds can be fabricated in the form of a solid foam, Nano fibrous matrix, microsphere, or hydrogel. Biodegradable porous scaffolds can be surface engineered to provide an extracellular matrix mimicking environment for better cell adhesion and tissue in-growth. Furthermore, scaffolds can be designed to release bioactive molecules, such as growth factors, DNA, or drugs, in a sustained manner to facilitate.[16]

### 3.1.2 Drug Delivery:

Drug delivery refer to approach, formulations and technologies for transporting a pharmaceutics compound in the in the body to achieve its therapeutic effect in safe method.

Polymer science advances have led to the development of a lot of novel drug-delivery systems. Consideration of surface and bulk erosion properties can aid in the designing of polymers for various drug-delivery applications. [4]

Biodegradable polymers mainly used in drug delivery as they can be degraded to non-toxic monomers inside the body. Novel supramolecular structures based on polyethylene oxide copolymers and dendrimers are being intensively researched for delivery of genes and macromolecules. Hydrogels that can respond to a variety of physical, chemical and biological stimuli hold enormous potential for design of closed-loop drug-delivery systems.[17]

#### 3.1.2.1 Pharmacotherapy

Pharmacotherapy Can be defined as the treatment and prevention of diseases by using drugs of chemical or biological origin. using of modern chemical synthetic method

like combinatorial chemistry production of large number of new drugs in shorter time than ever before. [18]

In short time, new drug delivery system impact on every branch of medicine including cardiology,ophthalmology, endocrinology and pain management. [19]

Drug delivery system can control the pharmacological action control of rate of drug release, site and duration of drug. Sustained release forms are designed to release a drug at predetermined rate by maintaining constant level of drug for specific period with minimum side effects.

The earliest work in the area of sustained drug delivery dosage was by Israel Lipowsik, this work involves coated pallets for prolong release which follow by development of coated particles to sustained drug delivery in $19^{th}$ century.

Designing sustained or controlled delivery have many advantages:

1. Reduce frequency of dosage.
2. Increase the effectiveness of drug by localization at site of action.
3. Providing uniform drug delivery.
4. Maximum utilization of drug.
5. Reduction in health care cost through short treatment period.
6. Increased safety margin of period potent drug

Disadvantages of sustained release drug delivery system:

1. Increased cost.
2. Toxicity due to dose dumping.
3. Unpredictable and often poor in vitro-in vivo correlation.
4. Risk of side effects or toxicity upon fast release of contained drug.

### 3.1.2.2 Classification of drug delivery systems:

1. Drug Delivery System classified according to physical state to gaseous (i.e. anesthetics), liquid, suspension, an emulsion, semisolid (i.e. ceramic, gels and past). and solid (i.e. powder, granules, tablets and capsule). Solution is one phase, while suspension is tow – phase system containing a continuous liquid phase and dispersed solid phase. An emulsion is tow –phase's system containing tow liquid phase one dispersed and one continuous phase.[18]

2. Drug delivery system can have classified according to the route of administration to Intravenous injection and Extra vascular routs. Extra vascular routs contain oral drug delivery, intramuscular injection, subcutaneous injection and pulmonary Characteristics make drug suitable for extended release:

a. Biological half-life: drug must enter the circulation at approximately the rate at which it is eliminated. generally therapeutic compounds with short half-life (2-8 hr.) are excellent candidate for sustained release.

b. Absorption: rate of release must be much slower than the rate of absorption
c. Metabolism: drug those are metabolized before absorption either in the lumen or tissue ofintestine canshow decreasedbioavailability from slower releasing dosage form. [20]

### 3.1.2.3 Polymers that are used in drug delivery system:

1. Poly lactic Acid (PLA):

Poly lactic acid (PLA) is a biodegradable, Biocompatible, high melting point (160c), high glass transition temperature (60c). PLA has disadvantage of brittleness and poor thermal stability.

Aliphatic polyester industry obtained from renewable resources such as corn or sugar beets, the monomer lactic acid (LA) having achiral carbon, exhibits tow isomeric forms L&D. [14,21]

Major attention has been devoted to strategies aimed to improve the mechanical, optical and radiological properties which improve using it in many applications such as suture thread, bone fixation device and micro –Nanocapsules. [21]

Polymer based on lactic acid very important in field of medical application such as carrier and device of drug delivery because the polyester degreed in human body by hydrolyses to non-harmful and nontoxic compound.

There many Traditional routes for PLA production include Bulk melt (MP) polycondensation of lactic acid and Ring –opening polymerization of lactide (ROP) (cyclic dimer of PLA).

PLA with low molecular weight produced by direct polycondensation, while polymer with high molecular weight is produced by ring opening polymerization.

Combination of the tow route into a multistep process is the most popular industry strategy for PLA production, LA is first polymerized to low molecular Wight polymer by polycondensation and then depolymerized and converted to the cyclic dimer in a catalytic step carried out at high temperature and low pressure. Finally, lactide undergoes ROP after suitable purification, leading to high molecular weight polymer. [21]

Various techniques used for determining molecular weight of PLA:

a. GPC: gel permeation–chromatography many applied to high molecular weight PLA polymer.
b. Nuclear magnetic resonance (NMR) spectroscopy and aqueous titration of the carboxylic acid end group are good to low molecular weight polymers
c. (HPLC) high performance liquid chromatography represent analytical technique for investigating the entire chain length distributed of the PLA low molecular weight.

The degradation process of polymeric material is the result of the interplay between degradation kinetics and diffusion phenomena of water, which control the erosion mechanism and there aretwo erosion paths:

a. Bulk erosion: occurs when water diffusion faster than polymer degradation, leading to homogenous degradation.
b. Surface erosion: when erosion starting from external surface and extending toward the interior.

For small size particles such as Nan –micro particles the characteristic dimension of the small particles is larger than the outer diffusion layer and since the diffusion, degradation is not limited, degradation occurs through bulk erosion. [21]

2. Polycaprolactone (PCL):

During 1970s and 1980s PCL was used extensively in biomaterials field and a number of drug delivery devices. It has long- term degradation (up to 3-4 years and greater than 24 months in other study) and intracellular resorption pathways. The PCL easy to manufacture and manipulate into a large range of implant and devices. [22]

PCL is an attractive polymer to use based on its elastomeric properties and high elongation. [22]

There is some physic – mechanical properties of PCL. See Table 3.1

### 3.2.3 Mechanism of drug release

Matrix technology opened prospects for controlled release (CR) fields. simple matrix tablets production in 1950s.in 1952 capsule design introduced to the release drugs at steady rate over period of hours (SR capsule) which open search for other dosage form.

Drug delivery dosage forms are classified to tow major classes according to their release mechanism:

A. Immediate release:

Drug is released directly after administration of the dosage form. This type allows the drug to dissolve in the gastrointestinal contents, with no delaying or prolonging the dissolution or absorption of the drug.

B. Modified release:

Drug releaseonly occurs sometime after administration at controlled rate in order to keep plasma concentration constant. Modified release have several advantages:

a. Reduce blood level fluctuation.
b. Reduce the frequency of dosing.
c. Enhance patient compliance.

d. Reduce side effect and toxicity.

Modified release dosage forms are divided into:

1. Delayed release: system use repetitive, intermittent dosing of adrug from one or more immediate release units incorporated in single dosage form.Drug is released at some point after the administration and release possible to control time and site of delivery in order to protect them from any degradation especially that are sensitive to PH.

2. Sustained release: include any drug –delivery that achieves slow release of drug over an extended period after administration of single dose. Sustained release system achieves steady state blood level that is therapeutically effective,nontoxic for an extended period of time, less frequency dose intake and increase in patient compliant.

3. Site –specific targeting: refer to targeting of drug directly to certainbiological location.

4. Receptor targeting: the target is the particular receptor for drug within organ or tissue (considered to be controlled).

5. Controlled release (CR): CR have the characteristics that are used in a variety of administration routes, includingoral, transdermal and vaginal administration in contrast to sustained release which are used for oral dosage forms.[4, 19, 24]

The drug concentration should be above minimal effective concentration (MEC) and below the minimal toxic concentration usually the drug concentration in the body is determined in plasma, because plasma easy to access and can be measured using many techniques like high performance liquid chromatography (HPLC). [18]

Getting the desired concentration of drug depend on:

a. Frequency of dosing.
b. Clearance rate.
c. Rout of administration. [18]

## 3.2Oral Delivery System:

The oral rout is the most popular route used for administration of drugs, because it is most natural, uncomplicated, cost –effective manufacturing, patient acceptance and the ease of administration and gastrointestinal physiology offer more flexibility in dosage form design than most other routes. [23, 24]

Oral controlled release drug delivery system provides continuous release of drug at predictable period throughout gastrointestinal (GI) transient andtarget the delivery of drug to specific region. [23, 24]

All the pharmaceutical product formulated for oral delivery system must developed within the intrinsic characteristics of GI physiology.

Limitation of oral conventional dosage form:

1. Frequent administration is necessary.
2. The unavoidable fluctuation of drug concentration may lead to under or over medication in narrow therapeutic index drug.
3. A typical–peak plasma concentration time profile is obtained which makes attainment of steady state condition impossible. [26]

- Classification of oral controlled release systems:

**1. Diffusion controlled system:**

Diffusion process shows the movement of drug molecules from higher concentration to one of lower concentration region.

This type divided into two categories:

A. Reservoir system: water insoluble polymeric coated material covers the core of drug. drug will partition into membrane and exchange with fluid surrounding the particles. The active agent is released by diffusion process.
B. Matrix system: is defined as a mixed composite of one or more drugs with gelling agent used widely for prolongs and control release of the drug that is dissolved or dispersed. Diffusion occurs when drug passes from polymer matrix into the external environment .in this type the active agent has a progressively longer distance to travel and there for requires a longer diffusion time. See Figure 3.1

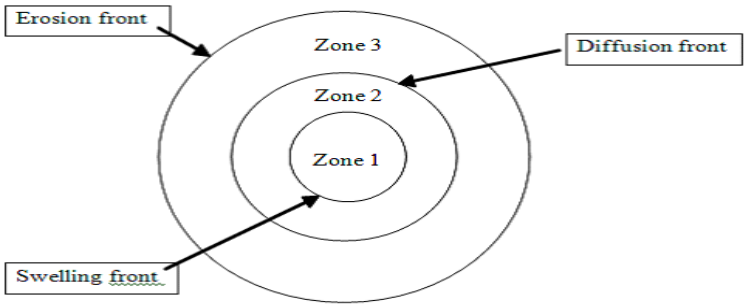



**Fig.3.1:**Matrix diffusion controlled drug delivery system [23]

**2. Dissolution controlled system:**

These systems are employed most commonly in the production of enteric dosage forms, coating material dissolved in natural or alkaline media. Thickness and composition of the coat material control the rate of drug release. This type of CS divides into two categories:

a. Matrix dissolution control: the drug in this system composed a slow dissolving carrier, the rate of drug release is controlled by dissolution fluid penetration rate into the matrix.

b. Encapsulation**:** these system containing coating of individual particles or granules of drug with dissolving material.

**3. Erosion system:**

This system using biodegradable polymer mixed with water .as the result of natural biological process especially by hydrolysis into smaller compound that are easily to be disposed from the body .as polymer degrades drug will release at constant rate.

**4. Diffusion and dissolution control system**:

The drug is homogenously dispersed in a matrix and it release either by swelling controlled mechanism or by hydrolysis or by enzymatic attack

Sustained release matrix tablet: least complicated approach to manufacture of sustained release drug forms by direct compression of drug, release retardant and additive to form tablets in which drug is embedded in matrix core of retardant. [4,23,25]

The design of oral sustained release delivery system depends on different variables:

1. The disease being treated.
2. The patient.
3. The length of therapy.
4. Properties of drug. [23]

## 3.3Targeted drug delivery:

Targeted drug delivery is advanced method of delivering drugs to the patient, aims to control the distribution and concentration of drug within the body organs, tissues and cells, which enhance the activity and specificity of the drug and reduce its toxicity and side effects.

Drug delivery system required chemists, biologist and engineers to optimize this system which depend in this criteria:

1. Drug properties
2. Side effect of the drug
3. Targeted side
4. The disease
5. Route taken for the delivery drug [26]

- Types of targeting drug delivery:

1. Passivetargeting: it refers to accumulation of drug or drug carrier system at specific site e.g. anticancer. Drug release or drug action are limited to selective site within the body such as tumor, but not the liver.

2. Active targeting: a specific legend receptor interaction for intracellular localization which occurs only after blood circulation and extravasations, the active targeting can be classified in three different levels:
   a. First order targeting: refer to restricted distribution of a predetermined target site, organ or tissue e.g. lymphatic, peritoneal cavity, plural cavity,joint... etc.
   b. Second order targeting: refer to selective delivery of drug to specific cell types e.g. tumor cell and suffer cells in liver.
   c. Third order targeting: refer to selective delivery of drug to intracellular site of target cell e.g. receptor based ligand mediated entry of drug complex in to a cell by endocytosis. [27]

Figure 3.2 show the type of targeting drug delivery in which part A show the passive targeting that the drug accumulates in particular region, part B show the active tumor targeting and part C show active vascular targeting.

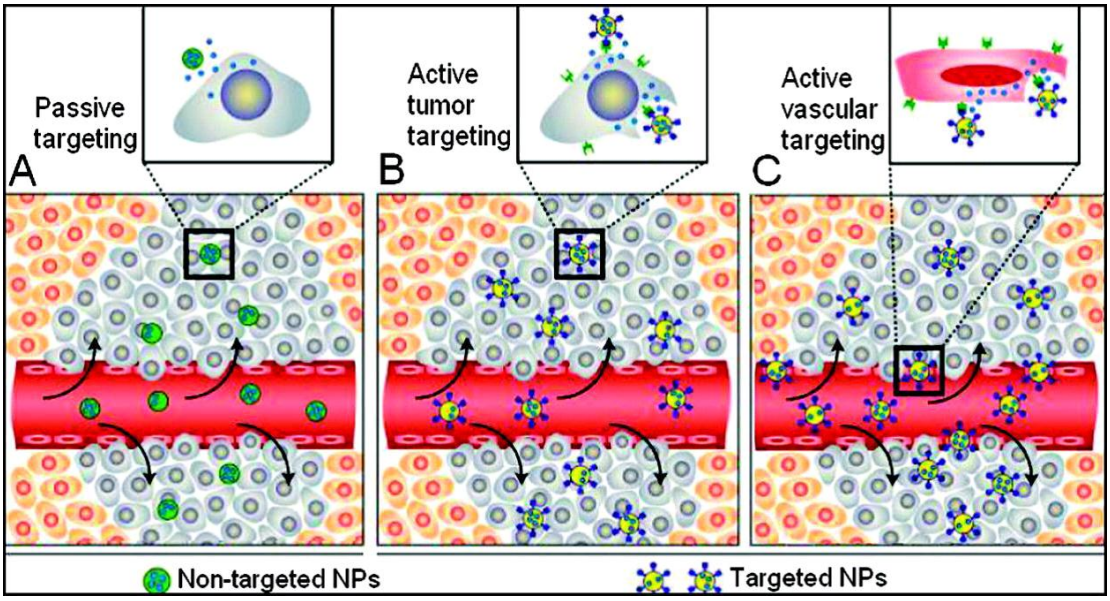



**Figure3.2**: Passive*vs* active targeting [27]

# Chapter Four: Nanoparticles Material and Preparation Technique

## 4.1 Nanotechnology and Nanomedicine:

**Nanotechnology** is a broad interdisciplinary area of research, it is multidisciplinary grouping of physical, chemical, biological, engineering and electronic process. [14]

The term of Nanotechnology was first used in 1997by Japanese ScantiestNori Tamigch, but the origin of nanotechnology has been attributed to lecture presented by Richard Feynman. See Figure 4.1

Nanoscience and nanotechnology are the study and application of extremely small things.

Nanotechnology began as since in 1982 with discovery of the scanning tunneling microscope, which allows studying and manipulating molecules at atomic scale. [4]

A main challenge in nanotechnology is to incorporate different functions
Into small size devices to improve their properties and obtain multifunctional particles characterized by high versatility and applicability. [21]

**Nanomedicine** is the science and technology of diagnosis, treating and prevention disease, injury, relieving pain and improving human health using molecular tools and molecular knowledge of the human body. [16]

Polymer nanoparticles are carriers of drug of natural, semi synthetic and synthetic polymer nature at nanoscale to micro s. [28]

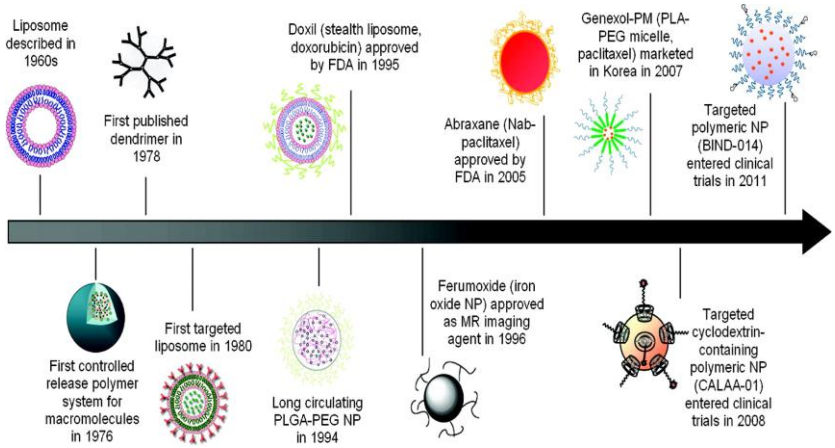



**Figure4.1:** Historical timeline of clinical-stage nanoparticle technologies [28]

In the recent decade's polymer are widely used as biomaterial due to their desired properties:

1. Good biocompatibility.
2. A verity of structure.
3. Easy design and preparation.
4. Polymeric material proved the effectiveness in stabilizing and protecting the drug molecules.[28]

The smart drug delivery system has been successfully made by development in polymer science in the bio- Nanotechnology filed.

## 4.2Types of Nanomaterial

Many types of nanomaterial prepared to use in medicine such as: see figure 4.2

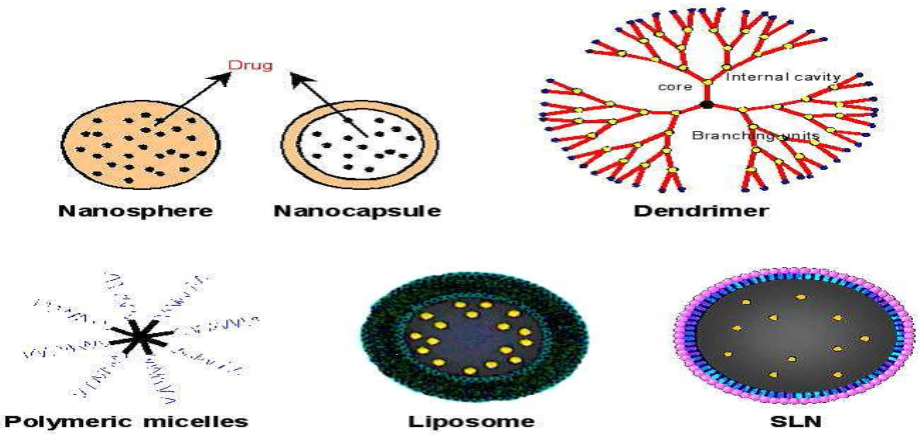



**Figure 4.2:** Types of nanoparticles[28]

### 4.2.1 Quantum dots(QDS):

Nano crystal of semiconductor material include cadmium as core and zinc as a shell, these crystal glow when exited by a light such as leaser, gives different optical colors. This character use in medicine asbiosensor, proteins tracking microscopic detection, live cell imaging and targeting to cells, tissues and tumors. [29]

### 4.2.2 Liposome:

Are small artificially designed vesicles composed of phospholipid bilayers surrounding with size ranging from 20 – 10000 nm

Many of liposome formulation are rapidly taken up by macrophages and this can be exploited either for macrophages-specific delivery of drugs or for passive drug targeting which allow slow release of drug over time from these cells into the general circulation

It was the first Nanomedicine placed on the market under trade name Doily that loaded with doxorubicin bythe way cardio toxicity of doxorubicin decreased. [4]

### 4.2.3 Micelles:

Are surfactant molecules that arrange theme in spherical shape when placed in aqueous solution.

### 4.2.4 Dendrimers:

Greek word," Dendron" means tree while" mere" means branch. dendrimers are a 3D nanoscale material, constructed from core and shell to form what is called generation by controlled steps that increase the number of small branching around a central core molecule.

They have a precise architecture, size and shape. they also have high purity, uniformity, loading capacity and low toxicity and immunogenicity.See Figure 4.3

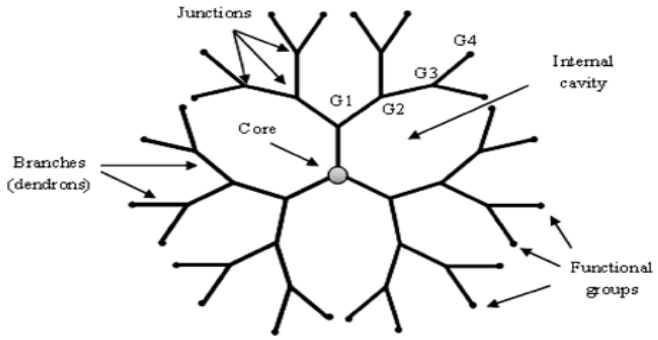



**Figure 4.3**: Schematic description of dendrimer structure [29]

### 4.2.5 Fullerene:

Fullerene (Buckyballs shape) is a kind of spherical shape constructed from 60 carbon atoms, which form spherical hollow structure with 1nm in diameter. Harold. W.korto discovered fullerene in 1985.

Fullerenes represented as 20,40,60,70 or 84 carbons but 60 carbonsare most common.See Figure 4.4

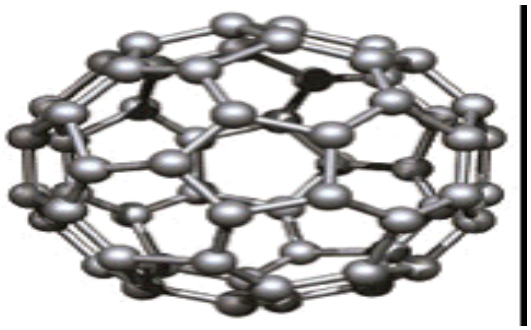


**Figure 4.4:**Atomic structure of a spherical fullerene [15]

### 4.2.6 Carbon Nanotubes:

CNTs long thin cylinder of carbon,which discovered in 1991 by Semi Iijimal. CNTs can be considered as sheet of graphene rolled into cylinder.See Figure 4.5

There is tow type of CNTs:

a. Single walled carbon nanotubes (SWCNTs).
b. Multi-walled carbon nanotubes (MWCNTs).

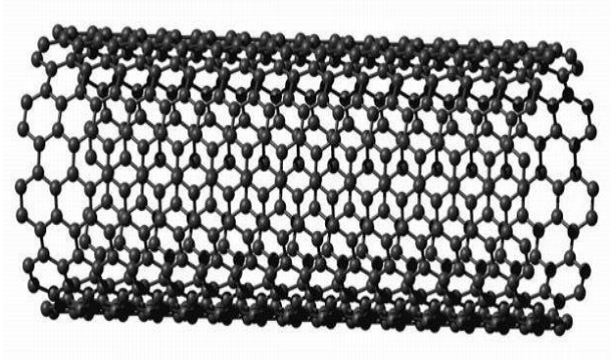


**Figure4.5:** carbon nanotube [30]

### 4.2.7 Graphene:

Graphene is planner monolayer, 2Dhexagonal carbon atoms with a carbon –carbon bond length of 0.142nm discovered in 2004. See Figure 4.6

Graphene commonly used in the nanomedicine application due to:

1. High specific surface area.
2. High thermal conductivity.
3. Excellent mechanical stiffness.
4. God biocompatibility.
5. Fast electron transportation.

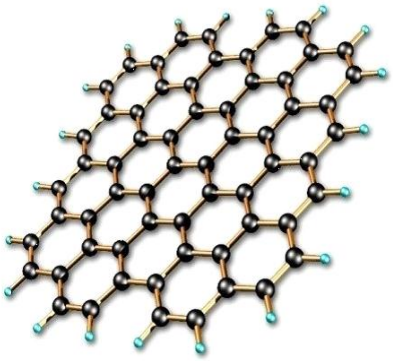


**Figure4.6:** Graphene structure [30]

### 4.2.8Magnetic Nanoparticles (MNPs):

MNPs are classed of nanoparticles with (5-500) nm, which can be manipulated using magnetic fieldformed by magnetic element such as iron, nickel and their chemical compound.

MNPs can bind to drugs, antibodies, proteins, and enzymes, also can be directed to an organ, tissue or tumor by using an external magnetic field or can be hated in alternating magnetic field for use of hyperthermia.Super paramagnetic iron oxide nanoparticles (SPIONs) is an example of MNPs.

### 4.2.9 Microsphere and nanoparticles:

Microsphere and nanoparticles consist of biocompatible polymers and belong either to the soluble or the particle type carriers.

Formation of drug into nanoparticles can occur at the surface of the particles and in nucleus, depending on the physicochemical characteristic of the drug.

The site of drug in corporation affects its release rate from the particle.

After systematic administration or transportation, they quickly distribute to the target site and subsequently become internalized by the cells of the phagocytic system. Besides microsphere s and nanoparticles which are mostly used for call selective delivery ofdrug, they have more recently been studied for their application in the oral delivery of peptides and peptidmimetics. [26]

## 4.3 Methods for PNPs preparation:

Nemours method had been available to fabricate nanoparticles depend on the physical and chemical properties of polymer, these techniques are mainly top-down and bottom up process.

The biodegradable polymeric Nano-micro particles are commonly prepared by different technique:

### 4.3.1 Emulsification and solvent evaporation method:

Particles prepared by dissolving the drug and polymer into water-immisble organic solvent and producing aNano-emulsion, the organic solvent is removed by elevating temperature or reduced pressure.See Figure 4.7

This technique is applied to lipophilic agents. The type and amount of dispersing agent, stringing rate, viscosity andtemperature of two phases determine the size of the formed particles. [4, 19]

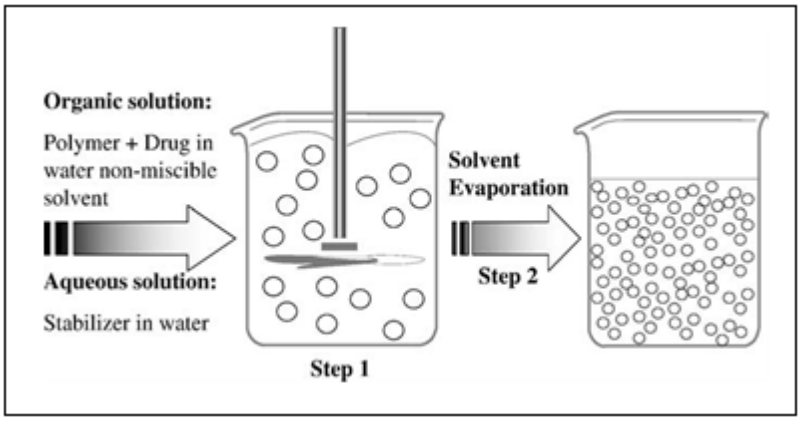



**Figure 4.7:** Schematic representation of the emulsification/solvent diffusion technique[31]

### 4.3.2Modified emulsion and solvent diffusionmethod:

Based on the separation of water miscible solvent from aqueous solution through the salting out effect .is done by dissolving electrolyte like magnesiumacetate, calcium chloride in aqueous in order to salt water miscible solvent out.

Polymer and drug in organic phase emulsified to aqueous phase which contains the stabilizer (like PVP) and hydroxyl ethyl cellulose and salting out electrolytes, then solvent and salting out agent are removed by cross filtration technique. See Figure 4.8

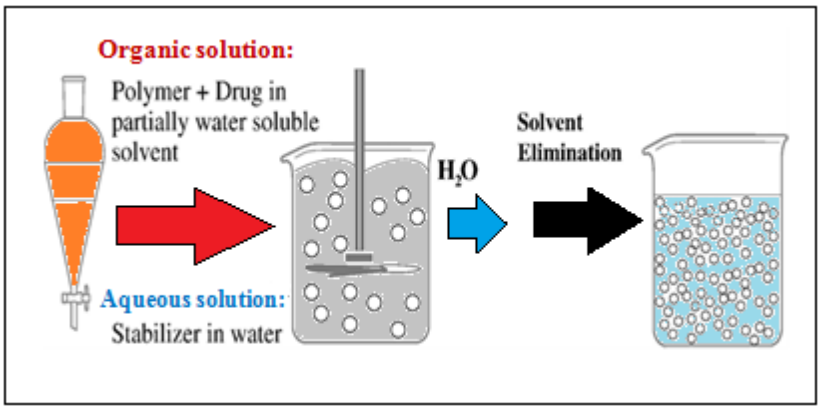



**Figure 4.8:** Schematic diagram of solvent emulsification technique for the Preparation of drug loaded micro/nanoparticles. [31]

### 4.3.3 Supercritical fluid technology:

This method become the attractive alternative method because:

1. Friendly solvent to environment.
2. Profitably method because of the particles which is produced in high purity.
3. Without any trace of organic solvent

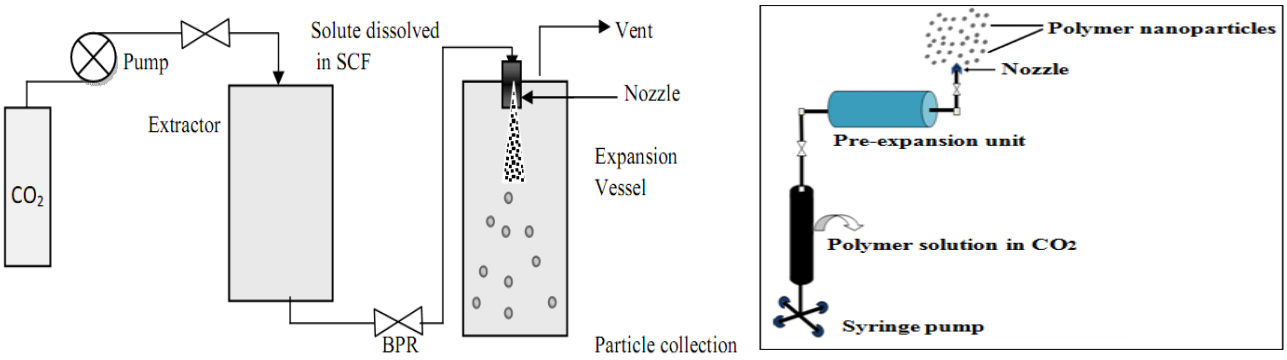
In this technique the drug and polymer are solubilized in a supercritical fluid such as CO2 instead of organic solvent, and the solution is expanding throughthe nozzle. Evaporation the supercritical fluid done by the spraying process and the particles eventually participate. [4]

There is tow classification of the Supercritical fluid technology:

a. Rapid expansion of Super critical fluid technology(RESS):

The solute of interest is solubilized in super critical fluid and the solution expand through the nozzle. the solvent power of super critical fluid dramatically decreases and the solute eventually predicated.[32]

b. Supercritical anti –solvent (SAS):In this method the solution is charged with the super critical fluid in the precipitation vessel containing in an organic solvent .at high pressure enough anti-solvent power will be lower and solute precipitation. See Figure 4.9




1 2

**Figure 4.9:**1. SAS equipment concept,2.RESS equipment concept. [31, 33]

### 4.3.4 Nanoprecipitation or solvent displacement method:

This method based on interfacial deposition phenomenon flowing the displacement of solvent miscible with water from lipophilic solution .It done by slowly addition of organic solvent which miscible with water such as acetate other contains hydrophilic and hydrophobic polymer or amphiphilic drug to aqueous phase containing surfactant ,then evaporate the organic solution by rot vapor and centrifuge. See Figure 4.10

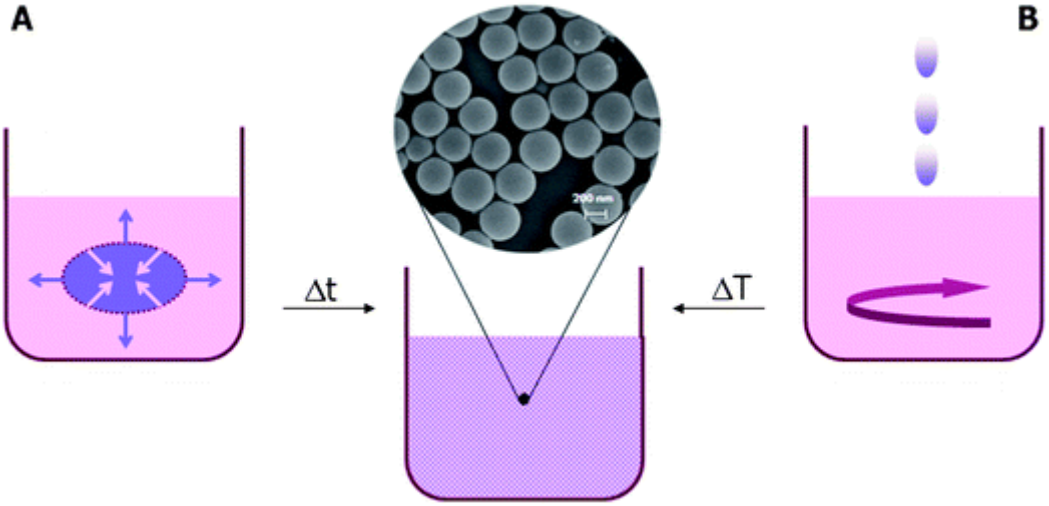



**Figure 4.10:** A schematic representation of the Nanoprecipitation methods Applying dialysis in a membrane. [33]

Figure 4.11 show SEM images of polymeric nanoparticles of some polymers that is using in preparation on PNPs:

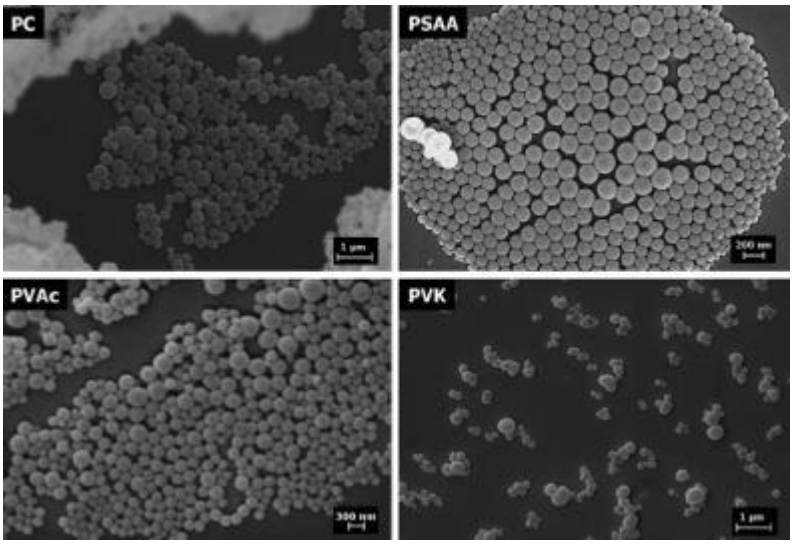



**Figure 4.11:**SEM images of polymeric nanoparticles of poly (carbonate)(PC0 Poly (styrene-co-acrylic acid) (PS-co-AA), poly (vinyl acetate) (PVA) and Poly (vinyl carbazide) (PVK) prepared by dialysis of the polymers

## 4.4 Carvedilol:

Carvedilol sold under the brand name Coreg, Coreg CR, Carvil, Dilated and Carvedilol was discovered by FritzWiedemann**.** at Boehringer Mannheim and was initially approved in the U.S. in 1995[4]

Carvedilol antihypertensive drug and nonselective β-adrenergic agent with a 1 blocking activity. Carvedilol is used to treat congestive heart failure, hypertension (high blood pressure), angina pectoris and cardiac arrhythmias. It is also used after a heart attack that has caused your heart not to pump as well. [4]

As carvedilol is β blocker, it has many advantages that make it distinct in hypertension treatment, the additional a blockage will decrease blood pressure and lower heart rate. carvedilol also has the ability to decrease lipids and glucose elevation. [4]

**4.4.1 Chemical Structure** Carvedilol is chemically known as 1-(9H-carbazol-4yloxy)-3-[[2-(2-methoxyphenoxy)-ethyl] amino]-propan-2-ol and its molecular formula (C24H26N2O4) Carvedilol chemically belongs to the class of organic compounds which are known as Carbazolecharacterized by three ring system which consist of apyrrolering fused on either side to a benzenering as seen in figure 4.12. [30]

I

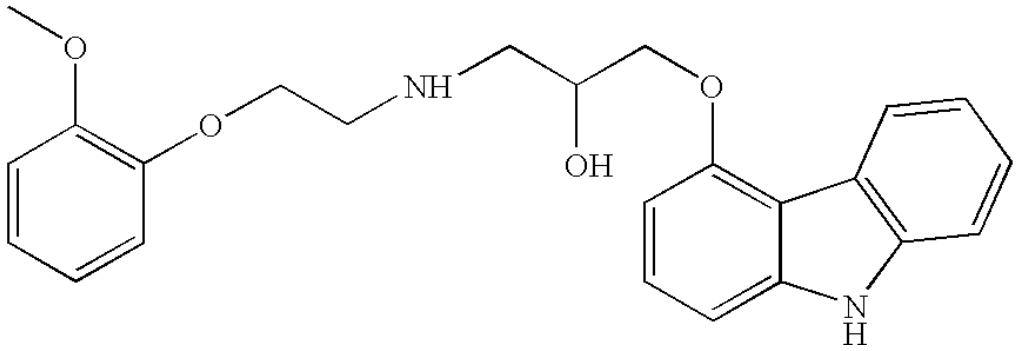



**Figure 4.12**: chemical structure of carvedilol. [30]

- **Physiochemical properties:**

1. Carvedilol is white to off white powder.
2. molecular weight 406.5.
3. molecular formula C2H26O4.
4. Formal charge 0.
5. Melting point 113-117.
6. It is freely soluble in methylene chloride and methanol, slightly soluble in ethyl ether and particularly soluble in water and gastric fluids.
7. Tablet for oral administration: COREG (carvedilol) is a white, oval, film-coated tablet containing3.125 mg, 6.25 mg, 12.5 mg, or 25 mg of carvedilol.

- **Pharmacokinetic properties:**

1. the poor water solubility of carvedilol affects its dissolution and accordingly its bioavailability not exceed 30%.
2. Half-life of carvedilol generally ranges from 7 to 10 hours.
3. Carvedilol is more than 90% bound to portion plasma, primarily with albumin.
4. Carvedilol has two enantiomers: R (+)-carvedilol and S (-)-carvedilol. R (+)-carvedilol undergoes preferential selection for metabolism, so the mean half-life of the enantiomer is about 5 to 9 hours compared with 7 to 11 hours for the S (-)-enantiomer.

5. Both the R (+) and the S (-) enantiomers of carvedilol were metabolized in human liver by CYP3A4, 2C19 and 2E1.

### 4.4.2 Formulation:

Based in our knowledge carvedilol available only as solid oral pharmaceutical dosage forms including:

- Immediate-release tablets, Oral.
- Capsule Extended Release 24 Hour, Oral.

# Chapter Five: Experimental Work

## 5.1 Materials and Method:

In this study polymer was dissolved in organic solvent with addition surfactants and different type of non-solvent as drop wise by using nanoprecipitation technique.

### 5.1.2 Materials:

In this study, ester terminated Polycaprolactone polymer (Molecular weight 70,000-90,000 g/mol) was supplied by Sigma Aldrich, Acetic Acid (purity 99%). Acetonitrile (purity 99% and molecular weight 41.05 g/mol), Acetone (99.9% and molecular weight 58.08 g/mol), Tetrahydrofuran (98.45% and molecular weight 72.11 g/mol), Ethanol (the 95% and molecular weight 46.06844 g/mol),and Methanol(the 95% and molecular weight 32.04 g/mol) were used as non solvent, Polyvinyl alcohol and Sodium dodecyl sulfate were used as surfactants, the pervouis matrials were supplied by Labtich Supply company (Qalqelia-Palestain) .

Carvedilol was obtain from Bet Jala companey(Bethlehem- Palestain ). EDTA was provided by Alfa Aesar, A Johnson Matthey companey. Material for phosphate buffer saline,NACL, KCL, $Na_2HPO_4$, and $KHPO_4$ were obtained from Al-Shams Companey (Nablus-Palestine).

Figure 5.1show block diagram that summarizes work destination .

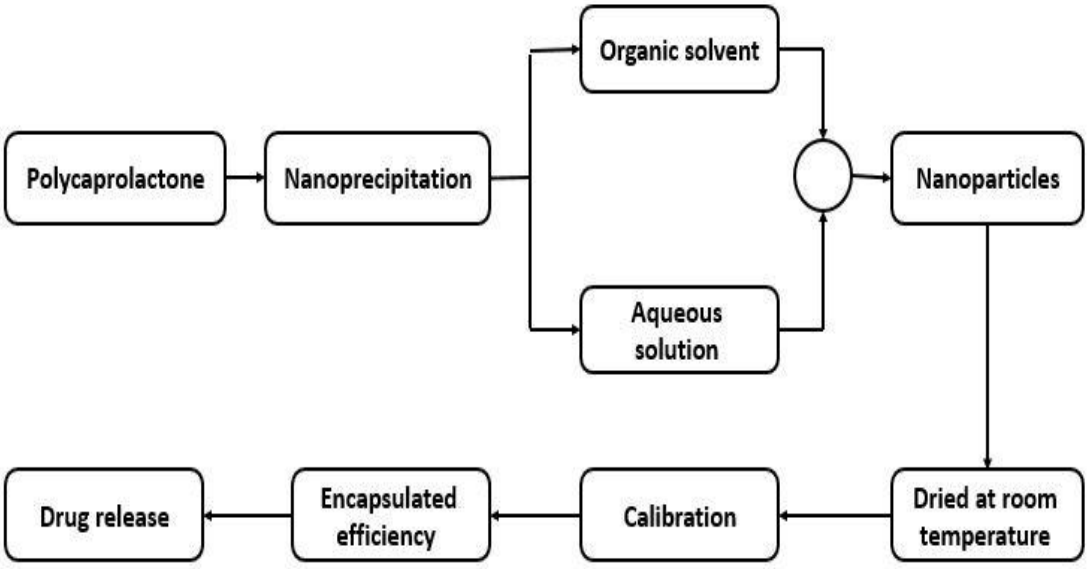



**Figure 5.1** Block Diagram

## 5.2 Method:

PCL nanoparticles were prepared by nanoprecipitation technique and visualized by using atomic force microscope and Scanning electron microscope.

### 5.2.1 Preparation of Polycaprolactone particles:

PCL NPs were prepared using nanoprecipitation technique. In this procedure the organic phase was prepared using specific quantity of PCL polymer to obtain 1%(PCL/Acetic acid). With mild stirring 0,7ml of PCL solution was added in step wise mode to 13 ml of non-solvents contains 10ml 5% (w/w) PVA or SLS aqueous solution and 3ml ofdifferent non-solvents as show in the table 5.5.

The formed milky emulsion was left 30 minute under mild stirring for evaporation thesolvents. Centrifugation was done at 14000 rpm for 40 minute to precipitate the particles and wash with distal water. the Centrifugation and washing step was repeated threetimes.

**Table 5.3:** Formulas of PCL nanoparticles preparation.

| | **Formulas and Quantities** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Materials (ml)** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** |
| **PVA 5%** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **PCL 1%** | 3 | 3 | 3 | 3 | 3 | 3 | 3 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
| **SLS 5%** | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Acetone** | 7 | ---- | ---- | ---- | ---- | ---- | ---- | 7 | ---- | ---- | ---- | ---- | ---- | ---- |
| **Acetonitrile** | ---- | 7 | ---- | ---- | ---- | ---- | ---- | ---- | 7 | ---- | ---- | ---- | ---- | ---- |
| **THF** | ---- | ---- | 7 | ---- | ---- | ---- | ---- | ---- | ---- | 7 | ---- | ---- | ---- | ---- |
| **Methanol** | ---- | ---- | ---- | 7 | ---- | ---- | ---- | ---- | ---- | ----- | 7 | ---- | ---- | ---- |
| **Ethanol** | ---- | ---- | ---- | ---- | 7 | ---- | ---- | ---- | ---- | ----- | ---- | 7 | ---- | ---- |
| **Distal Water** | ---- | ---- | ---- | ---- | ---- | 7 | ---- | ---- | ---- | ----- | ---- | ---- | 7 | ---- |

### 5.2.2 Characterization of NPs:

The morphology of the nanoparticles was visualized using AFM. The particles size and size distribution were observed. The samples have been deposited on a substrate (5 × 5 $mm^2$**)** of mica and let the samples became dried at room temperature. The AFM images were treated with WS×M 5.0 develop 6.5 software. The size and size distribution were determined by taking the average of 100 counted particles. [4]

However, the resolution of the AFM images of the obtained nanoparticles was poor, Scanning electron microscope (SEM) was used to visualized the particles with high resolution.

# Chapter Six: Result and analysis

Polycaprolactone NPs were prepared using nanoprecipitation technique which is known as solvent displacement method. This method is characterized by:

- Its facial and rapid particles formation.
- Controlled particle size.
- Using low toxic solvent.

## 6.1 Effect of type of surfactantand nonsolvent on the morphology and average of PCL NPs:

The morphology of PCL NPs prepared with two different types of surfactant (PVA and SLS) and different types of non-solvents including Acetone, Ethanol, THF, Acetonitrile, Methanol, and Ethanol were visualized using SEM. In general, when PVA used as surfactant a spherical and small NPs were obtained whereas SLS didn't produce PNs for most of the formulations as shown in the figures 6.1 and 6.2.PVA is seem to be compatible with PCL and could cover the whole surface of the PCL droplets during emulsification allowing for the production of spherical and rather small particles. However, the surface affinity between PCL and SLS seem to be rather weak and coverage of the surfactant during emulsification was not complete allowing coalescence of the droplets and resulting in film-like structure rather than particles.

The average size of NPs was measured from the SEM images [see Appendix A] and the results are shown in Table 6.1. The results showed that the size of the NPs is strongly dependent on the type of nosolvent used. NPs prepared with acetonitrile and water as a nonsolvent gave the smallest particles (~ 250 nm) whereas the particles prepared with THF were the largest (~550 nm). The difference in the average size might be ascribed to the difference in the mutual interaction between solvent, nonsolvent and the polymer and the difference in the interfacial tension during emulsification.

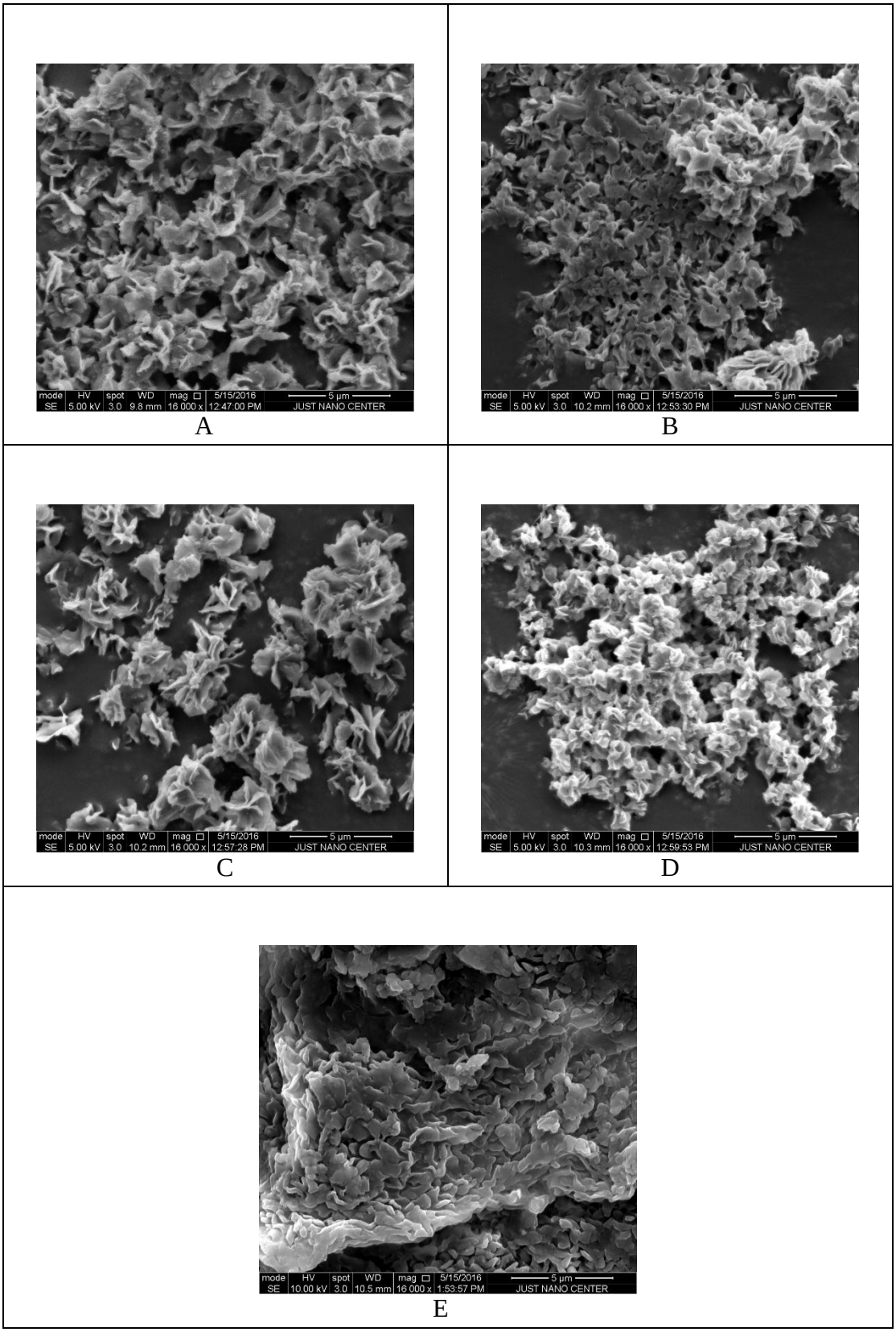
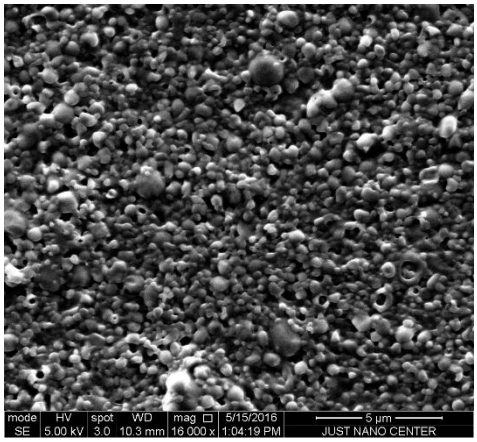
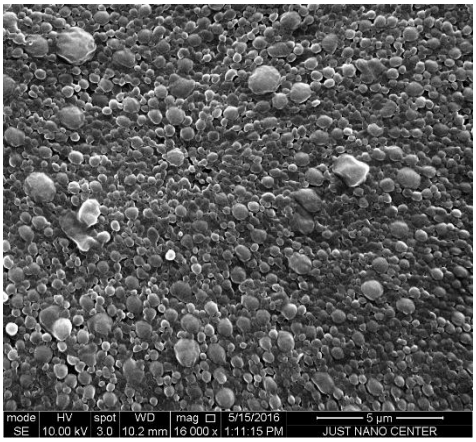


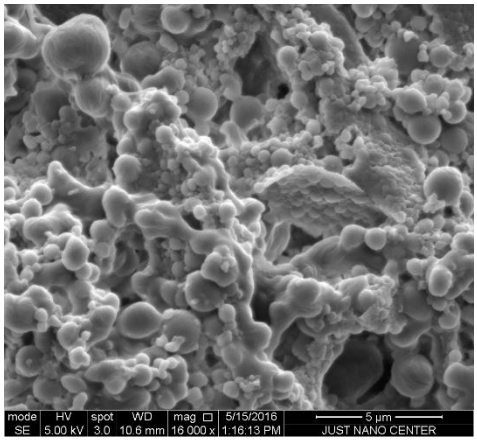

**Figure 6.1:** SEM image of different formulation with SLS as a surfactant and different non-solvent (A) Methanol(B) Distilled Water. (C) Ethanol. (D) Additional Amount of distilled water. (E) THF.


mode HV spot WD mag 5/15/2016 5 µm
SE 5.00 kV 3.0 10.3 mm 16 000 x 1:04:19 PM JUST NANO CENTER

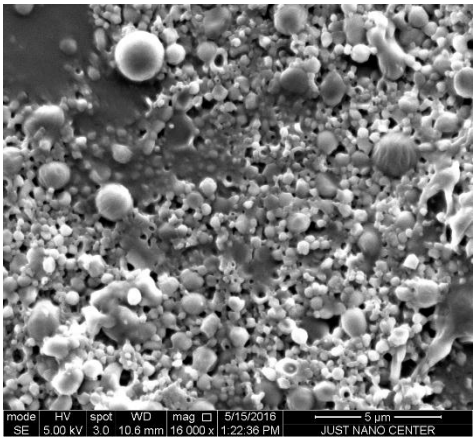
A


mode HV spot WD mag 5/15/2016 5 µm
SE 10.00 kV 3.0 10.2 mm 16 000 x 1:11:15 PM JUST NANO CENTER

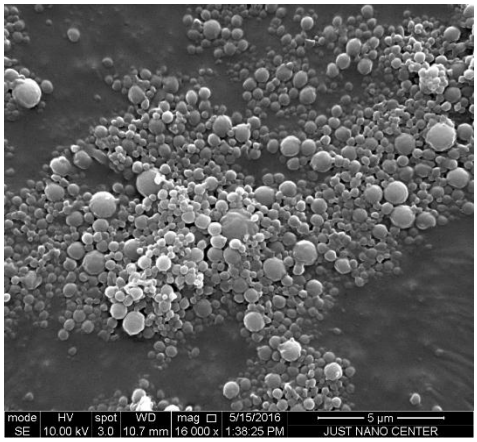
B


mode HV spot WD mag 5/15/2016 5 µm
SE 5.00 kV 3.0 10.6 mm 16 000 x 1:16:13 PM JUST NANO CENTER

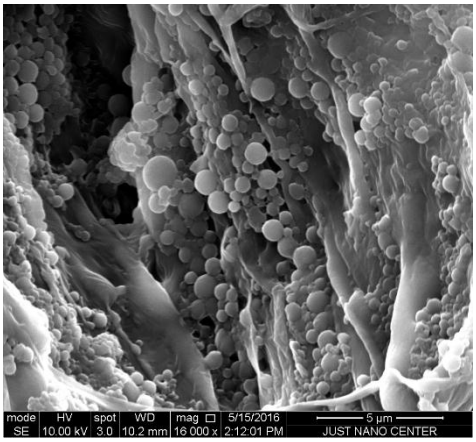
C


mode HV spot WD mag 5/15/2016 5 µm
SE 5.00 kV 3.0 10.6 mm 16 000 x 1:22:36 PM JUST NANO CENTER

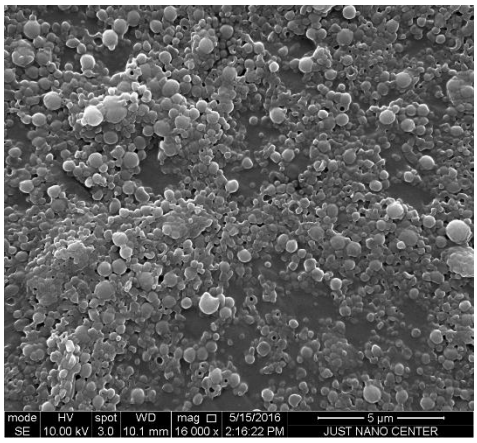
D


mode HV spot WD mag 5/15/2016 5 µm
SE 10.00 kV 3.0 10.7 mm 16 000 x 1:38:25 PM JUST NANO CENTER

E


mode HV spot WD mag 5/15/2016 5 µm
SE 10.00 kV 3.0 10.2 mm 16 000 x 2:12:01 PM JUST NANO CENTER

F




G

**Figure 6.2:** SEM images of PCL NPs prepared with PVA as surfactant and different non-solvent (A) Methanol(B) Additional Amount of Distilled Water. (C) Ethanol. (D) Distilled Water. (E) Acetonitrile. (F) THF. (G) Acetone.

**Table 6.1:** Average size of PCL NPs prepared with different types of surfactants and non-solvents

| **PVA** | | **SLS** | |
|---|---|---|---|
| **Non-solvent** | **Average size** | **Non-solvent** | **Average size** |
| Methanol | 490.98 | Acetonitrile | 183.18 |
| Water | 243.68 | | |
| Additional amount of water | 373.22 | | |
| Ethanol | 422.96 | | |
| Acetonitrile | 278.50 | Acetone | 373.22 |
| THF | 549.82 | | |
| Acetone | 397.08 | | |

## 6.2 Conclusion:

- Polycaprolactone can used as polymer that used in preparation of nanoparticles.
- Nanoprecipitation technique is easy, rapid and effective technique for preparation of nanoparticles, this method has been applied to various polymeric materials such as PLA, PCL.
- Nanoparticles is more efficient to use in inside the body, because its size is small enough to pass through the cell of tissue, so it can load drug and deliver it to the cell.
- The smallest and narrowest size distribution of PNPs was obtain when acetic acid was used as organic solvent.
- Using PVA as surfactant gave smaller size particles compere to the particles size using SLS as surfactant.
- Using Q-water as non-solvent gave the smallest nanoparticles size than the other non-solvents (Acetone, Ethanol, THF, Acetonitrile, Methanol, and Ethanol). while using ethanol gave the largest size particles camper to the particles were prepared by other non-solvents.
- The results showed that PCL nanoparticles with average size of 100 nm-400 nm could be prepared. The particles prepared with SLS were smaller in size and more spherical than those prepared with PVA. Furthermore, the addition of organic non-solvents reduces the size of the particles

## 6.3 Challenges:

- This study is the first orientation toward biomaterial science in the biomedical engineering in the university.
- Lack of the equipment related to the nanotechnology so experimental work was completed in al Al-Quds university Nano laboratory.
- Lack of materials needed in this project due to desired properties (purity, molecular weight).

## 6.4 References:


[1] kalimthua .S ;formulation and evaluation of carvedilol loaded Eudragit.E1000 nanoparticles .Journal of Materials Chemistry,2009.16 (4).

[2] Sadr, M ; synthesis and identification of carvedilol by ultrasound method. Journal of nanostructure in chemistry, 2009

[3] Ankora.A, Pharmacytes: An Ideal Vehicle for Targeted Drug Delivery. Journal Nanoscience and Nanotechnology, 2006 .6 (8).

[4] Bani-Odeh.M; preparation of nanoparticles loaded by drug; 2013.

[5] Jayadev. S, Polymer Science.*Indian Institute of Science, Bangalore*, 2001.

[6] Jeff. A; General Chemistry Laboratory Revision 1.6: The Structure of Polymeric Substances. Prentice Hall, 2013.

[7] Vasileios. K, *Polymeric materials.* Christopher Hall School of Engineering, 2009.

[8] Ebewele .R, *Polymer Science and Technology* .CRC Press LLC, 2000.

[9] Osswald. T, *Understanding Polymer Processing Processes and Governing Equations*. Carl Hanser, 2010.

[10] Brian. S, *Introduction to Materials and Engineering science*, John Wiley & Sons, 2004.

[11] Gaetano.L, Peters G, *Crstallinity and Linear Rheological Properties.* Carl Hanser Verlage, 2013.

[12] P. Makromolekülen, *Differential Scanning Calorimetry Investigation of Polymers.* Humboldt-Universität Zu Berlin, 2009.

[13] Vroman. I, lan. T; Biodegradable Polymers, Journal of Materials, 2009.2(7).

[14] Creely. K, *Nanoparticles: An occupational hygiene review.The Health and Safety Executive.*Report 217, 2004

[15] Duncan. R, *Nanomidicin Consensus Conference*. An ESF – European Medical Research Councils (EMRC) report, 2004.

[16] Heinze. T, Becer. R, *Synthetic polymeric nanoparticles by Nanoprecipitation*. Journal of Materials Chemistry,2009.19(4).

[17] Howard. J,*Nanomaterial Production and Downstream Handling Processes .Journal of National Institute for Occupational Safety and Health,* 2013. 102(21).

[18] Bhowmik.D,Gopinath .H, *Controlled Release Drug Delivery Systems*. Journal the Pharma Innovation, 2012.1(10).

[19] Abhilash .M,*Potential applications of Nanoparticles*. Journal of Pharma and Bio Sciences, 2010 .1(1).

[20] Arjun.Singh, Ritica .*Sharma; Sustained Release drug delivery system*. Journal of International research of pharmacy, 2012.3(9)

[21] Codari. F, *Poly (Lactic Acid) Polycondensation and Degradation and Nanoparticles synthesis*. Politecnico di Milano, 2011.

[22] Woodruff .M, Hutmacher. D, *The return of a forgotten polymer—Polycaprolactone in the 21st century*. Journal Elsevier, 2010 .4(2).

[23] Dixit. N, Maura. S, *Sustained Release Drug Delivery System*. Indian Journal of Research in Pharmacy and Biotechnology, 2013. 1(3).

[24] Dutta .A, Paswan, *Recent Trends in Scope and Opportunities of Control Release Oral Drug Delivery Systems*. Journal Pharmaceutical Sciences, 2012.1 (1)

[25] Kidamb.S, Gubta .R, *Particulate carrier system.* Mocsha Publishing House, 2012.3(11).

[26] Kushal. Modi, Pragna. Shelat, Oral *controlled release drug delivery system* .Moksha Publishing House, 2013.4(3).

[27] Rani. Kirti, Paliwal .Saurabh; *Review on Targeted Drug Delivery: it's Entire Focus on Advanced Therapeutics and Diagnostics*. Journal of Applied Medical Sciences. 2014, 2(331).

[28] Sawalha. H, *Polylactide microcapsules and films: preparation and properties*.Journal of membrane science, 2008. 325(2).

[29] Kubiak. M, *Dendrimers – fascinating nanoparticles in the application in medicine*. Science Technique, 2014. 68 (2).

[30] Wang. L, *Introduction to Nanomaterial and Nanotechnology*, Graduate Seminar 730, 2011.

[31] Nagavarma. B, Hemant. S, *Different Techniques for Preparation of Polymeric Nanoparticles*.Asian Journal of Pharmaceutical and Clinical Research, 2012.5(3).

[32] Aminabhavi.T, Anandrao. R, Biodegradable polymeric nanoparticles as drug delivery devices Control Release. Journal of Controlled Release, 2001. 70 (1–20).

[33] Parhi. R, Suresh. P, *Supercritical Fluid Technology*.Journal of Advanced Pharmaceutical Science and Technology, 2013. 1(1).

[34] Delaney. J,Schubert. U, *Nanoprecipitation and Nanoformulation of Polymers: From History to Powerful Possibilities Beyond Poly (Lactic Acid)*. Journal of Soft Matter, 2010.7 (81).

[35] Arencón. D, José. V, *Fracture Toughness of Polypropylene-Based Particulate Composites*. Journal of Materials, 2009. *2*(4).

[36] Murakami. H, Kobayashi. M; Preparation of poly (DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent
Diffusion method. International Journal of Pharmaceutics, 1999.187 (143).

[37] Bashar .Seamed. F, *Applications and Drug Release in Microcapsule*. Journal of Chemical and Pharmaceutical Sciences Fundamentals of Manufacturing, 2012.5(1).

[38] Misirlis .D, *Development of a Novel Drug Delivery System Based on Polymeric, Thermoresponsive*, Hydrogel Nanoparticles. Journal of Infoscience(EPFL), 2005.12(2).

[39] Parashar. T, Patel .C, *Novel Oral Sustained Release Technology. Journal of International Research and Development in Pharmacy and Life Sciences*, 2013. 2(2).

[40] Langer .R, Drug *delivery and targetin*g. Journal of Clinical Oncology 1998.392 (30).

[41] Robert. Lenz, *Biodegradable Polymers and Plastics in Japan: Research, Development, and Applications.* .Springer Berlin Heidelberg, 1995 .107(65).

[42] Bennet. D, Kim. S,*Polymer Nanoparticles for Smart Drug Delivery*. Lex Innvova, 2014.

# Appendix A

| | PVA | | | | | | | SLS | |
|---|---|---|---|---|---|---|---|---|---|
| Number | Methanol | Additional Water | Ethanol | Distilled Water | Acetonitrile | THF | Acetic | Acetonitrile | Acetone |
| 1 | 556 | 882 | 235 | 253 | 800 | 1290 | 411 | 300 | 500 |
| 2 | 444 | 352 | 294 | 591 | 199 | 823 | 529 | 285 | 500 |
| 3 | 556 | 352 | 647 | 718 | 285 | 588 | 529 | 267 | 400 |
| 4 | 222 | 352 | 294 | 380 | 285 | 882 | 352 | 171 | 600 |
| 5 | 556 | 235 | 294 | 507 | 228 | 882 | 411 | 143 | 600 |
| 6 | 333 | 882 | 294 | 507 | 342 | 764 | 294 | 142 | 400 |
| 7 | 444 | 352 | 705 | 169 | 313 | 647 | 705 | 171 | 400 |
| 8 | 556 | 529 | 411 | 253 | 399 | 764 | 529 | 114 | 400 |
| 9 | 444 | 294 | 705 | 338 | 285 | 764 | 1647 | 142 | 300 |
| 10 | 1440 | 470 | 705 | 253 | 256 | 588 | 411 | 142 | 400 |
| 11 | 444 | 352 | 411 | 211 | 256 | 705 | 294 | 114 | 400 |
| 12 | 556 | 294 | 352 | 253 | 285 | 705 | 352 | 142 | 300 |
| 13 | 333 | 294 | 294 | 253 | 228 | 588 | 705 | 171 | 400 |
| 14 | 667 | 294 | 411 | 296 | 199 | 647 | 294 | 142 | 400 |
| 15 | 444 | 294 | 705 | 211 | 342 | 1000 | 294 | 171 | 600 |
| 16 | 556 | 764 | 529 | 169 | 199 | 705 | 705 | 142 | 500 |
| 17 | 889 | 352 | 470 | 169 | 342 | 352 | 470 | 142 | 400 |
| 18 | 777 | 529 | 529 | 256 | 199 | 470 | 352 | 114 | 500 |
| 19 | 556 | 352 | 705 | 169 | 313 | 470 | 411 | 114 | 400 |
| 20 | 556 | 411 | 705 | 84.4 | 199 | 411 | 470 | 114 | 500 |
| 21 | 444 | 411 | 529 | 296 | 228 | 705 | 294 | 142 | 400 |
| 22 | 556 | 294 | 235 | 296 | 342 | 470 | 352 | 171 | 400 |
| 23 | 444 | 352 | 352 | 211 | 513 | 294 | 352 | 142 | 500 |
| 24 | 444 | 294 | 411 | 296 | 313 | 352 | 352 | 142 | 400 |
| 25 | 222 | 117 | 529 | 422 | 199 | 294 | 352 | 171 | 300 |
| 26 | 556 | 529 | 470 | 253 | 256 | 352 | 705 | 142 | 500 |
| 27 | 667 | 294 | 705 | 169 | 313 | 294 | 235 | 142 | 400 |
| 28 | 667 | 294 | 352 | 169 | 256 | 352 | 235 | 171 | 400 |
| 29 | 333 | 529 | 235 | 127 | 313 | 529 | 352 | 142 | 300 |
| 30 | 556 | 352 | 529 | 169 | 256 | 529 | 235 | 142 | 500 |
| 31 | 444 | 294 | 176 | 296 | 199 | 705 | 176 | 200 | 400 |
| 32 | 444 | 411 | 352 | 296 | 171 | 1350 | 705 | 171 | 400 |
| 33 | 556 | 232 | 352 | 169 | 228 | 705 | 352 | 171 | 400 |
| 34 | 556 | 352 | 352 | 169 | 513 | 235 | 470 | 171 | 400 |
| 35 | 667 | 352 | 352 | 296 | 199 | 529 | 529 | 171 | 400 |
| 36 | 557 | 352 | 529 | 169 | 484 | 294 | 176 | 114 | 600 |
| 37 | 444 | 352 | 294 | 296 | 256 | 294 | 352 | 142 | 500 |
| 38 | 444 | 294 | 235 | 169 | 342 | 411 | 176 | 142 | 400 |
| 39 | 333 | 352 | 411 | 127 | 256 | 529 | 352 | 542 | 500 |
| 40 | 222 | 232 | 352 | 169 | 199 | 529 | 352 | 480 | 500 |
| 41 | 333 | 232 | 352 | 169 | 171 | 352 | 294 | 200 | 400 |
| 42 | 333 | 232 | 176 | 169 | 171 | 352 | 235 | 85 | 300 |
| 43 | 444 | 232 | 176 | 127 | 199 | 411 | 176 | 142 | 400 |
| 44 | 333 | 232 | 235 | 127 | 313 | 294 | 294 | 114 | 400 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 45 | 444 | 470 | 235 | 127 | 228 | 823 | 294 | 171 | 400 |
| 46 | 556 | 411 | 352 | 169 | 199 | 232 | 176 | 114 | 600 |
| 47 | 222 | 352 | 411 | 169 | 228 | 232 | 352 | 480 | 400 |
| 48 | 444 | 235 | 411 | 127 | 199 | 117 | 294 | 171 | 400 |
| 49 | 222 | 235 | 647 | 269 | 228 | 411 | 235 | 514 | 500 |
| 50 | 333 | 705 | 706 | 127 | 199 | 470 | 235 | 114 | 300 |
| **Average** | 490.98 | 373.22 | 422.96 | 243.6889 | 278.5 | 549.82 | 397.08 | 183.18 | 432 |