

**PREPARATION AND INVESTIGATION OF
PH-SENSITIVE HYDROGELS/NANOPARTICLES FOR
DRUG DELIVERY**

**PH-DEĞİŞİMİNE DUYARLI
HİDROJELLERİN/NANOPARTİKUL HAZIRLANMASI
VE İLAÇ TAŞINIMI İÇİN KULLANILABİLİRLİĞİNİN
ARAŞTIRILMASI**

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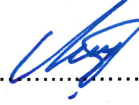
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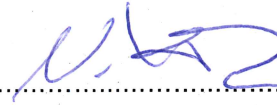
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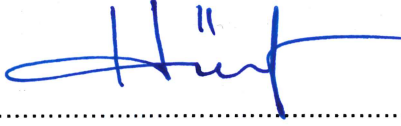
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Arwa ELAMIN

ABSTRACT

PREPARATION AND INVESTIGATION OF PH-SENSITIVE HYDROGELS/NANOPARTICLES FOR DRUG DELIVERY

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Oral administration of the drugs is considered as one of the most convenient and comfortable routes. However, an effective oral delivery of insulin still remains a challenging and elusive goal, due to bioavailability problem. The aim of our work is to develop a pH sensitive system for the oral delivery of insulin based on a unique polymeric combination of a poly (ϵ -caprolactone) (PCL) core coated with a layers of chitosan (CS) and alginate (ALG). The particles were prepared based on the double emulsion (water/oil/water) solvent evaporation method. The effect of blending hydrophilic Pluronic127 (F127) into the hydrophobic PCL matrix to improve its water permeability properties were investigated. The results showed that blending F127 into the PCL matrix improved its water permeability by creating porosity in the nanoparticles.

ALG have been chosen to provide the needed pH-sensitivity to the system. However, to coat the PCL surface with this anionic ALG layer a cationic CS layer have been added. The effect of each of the CS and ALG layers on the nanoparticle's physiochemical properties (size, encapsulation efficiency and surface charge) were investigated. The results showed that CS and ALG layer addition increased the stability of the nanoparticles. The in-vitro release studies using two different pH environment (1.2 and 7.4) to simulate the physiological conditions in gastrointestinal tract (GIT) showed that, the particles have pH-responsive release patterns. The kinetics studies of the particles release mechanism showed that, the insulin release from all the particles formulations described by the Korsmeyer- Peppas kinetic model and obeying the Fickian diffusion mechanism.

Keywords: controlled drug delivery, insulin oral delivery, polymeric particles, pH sensitive hydrogels.

ÖZET

PH DEĞİŞİMİNE DUYARLI HİDROJELLERİN/NANOPARTİKUL HAZIRLANMASI VE İLAÇ TAŞINIMI İÇİN KULLANILABİLİRLİĞİNİN ARAŞTIRILMASI

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Vücuda ilaç alım yollarından olan oral ilaç uygulaması, genel olarak en uygun ve konforlu yollardan biri olarak düşünülmektedir. Ancak, insülinin etkili bir şekilde oral olarak verilmesi biyoyararlanım probleminden ötürü oldukça zordur. Son yıllarda diyabet tedavisinde daha etkili bir oral insülin sistemi geliştirmek için yoğun bir çaba vardır. PH'a duyarlı polimerik nanopartikül sistemleri, insülinin oral yoldan verilmesi için en önemli ve umut verici stratejilerden biridir. Bu stratejide, nanopartiküllerden insülinin salım hızı pH değerlerinden etkilenmektedir. Bu şekilde midenin asidik ortamından insülin için bir koruma sağlanabilmektedir.

Bu çalışmanın amacı, kitosan (CS) ve alginat (ALG) katmanları ile kaplı poli (ϵ -kaprolakton) (PCL) çekirdeğe dayanan, insülinin oral yoldan iletimi için bir pH'a duyarlı polimerik sistem geliştirmektir. Bu amaçla, nanopartikül çift emülsiyon (su / yağ / su) çözücü buharlaştırma metodu temel alınarak hazırlanmıştır. Sisteme istenen pH'a duyarlı özellikleri sağlamak için ALG seçilmiştir. Bununla birlikte, anyonik ALG'yi PCL çekirdeğine bağlamak için katyonik olan CS tabakası PCL nanopartikül yapılarına ilave edilmiştir.

İstenilen insülin salım davranışlarını elde etmek için atlatılması gereken ilk zorluk PCL çekirdeğinin hidrofobik yapısıdır. Bu yüzden, ıslanabilirliğinin artırılması için hidrofilik Pluronic127'nin (F127) hidrofobik PCL matrisi içine eklenmesinin etkisi araştırılmıştır. Elde edilen sonuçlar F127'nin PCL matrisine eklenmesinin nanopartiküllerin fizyokimyasal özelliklerini etkilediğini ve salım davranışını geliştirdiğini göstermiştir.

Sonuçlara göre, tüm nanopartiküllerin küresel bir şekle sahip olup, homojen olmayan boyut dağılımı ile ortalama boyutları yaklaşık olarak 275.6 nm'dir. Ayrıca, CS ve ALG katmanlarının PCL matris yapısına eklenmesinin PCL nanopartiküllerinin farklı fizyokimyasal özelliklerini etkilediği görülmüştür. PCL nanopartiküllerinin ortalama boyutunun, CS eklendiğinde yaklaşık 10 μ m'ye kadar ve ALG ile kaplandığında 50 μ m'ye kadar önemli ölçüde arttığı görülmüştür.

In-vitro salım çalışmaları, nanopartiküllerin pH'a duyarlı salım modellerine sahip olduğunu göstermiştir. Kitosan ve alginat katmanları arasındaki polielektrolit kompleksleşmesi, farklı pH ortamlarında (1.2 ve 7.4) nanopartiküllerin insülin salım davranışını geliştirmiştir ve ilaç salımının, tek başına alginat ya da kitosan ile kaplama sonrası salımından daha etkili olduğu görülmüştür.

Nanopartik llerin in vitro salım davranışının kinetik alıřması, Korsmeyer-Peppas kinetik modeli ile uyumlu olduėunu ve Fickian dif zyon mekanizmasına g re ins lin salımının meydana geldiėini g stermiřtir.

Anahtar Kelimeler: kontroll  ila tařınım, insulin oral yoldan verilmesi, polimerik nanopartik ller, pH'ya duyarlı hidrojeller.



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LIST OF ABBREVIATIONS

IU	Insulin Unit
GIT	Gastrointestinal Tract
PCL	Polycaprolactone
PLGA	Poly Lactic-co-Glycolic Acid
CS	Chitosan
ALG	Alginate
F127	Pluronic127
PVA	Polyvinyl Alcohol
DCM	Dichloromethane
W/O/W Emulsion	Water-in-Oil-in-Water Emulsion
PBS	Phosphate buffer saline
NPs	Nanoparticles
EE	Encapsulation Efficiency
SEM	Scanning Electron Microscope
OM	Optic Microscope
FM	Fluorescent Microscope
DLS	Dynamic Light Scattering
FDA	Food and Drug Administration
ISF	Intestinal Simulated Fluid
GSF	Gastric Simulated Fluid
MDT	Mean Dissolution Time

1. INTRODUCTION

Diabetes is a universal health emergency of this century. The number of diabetic patients increases annually [1]. Insulin considered the main treatment of diabetes. It is a hormone produced in the pancreases by the beta cells, its main role is to control the blood sugar levels. Due to bioavailability problem, subcutaneous injections is the most common route for administering therapeutic proteins like, insulin. However, this route of administration is painful and often results in poor patient compliance [2]. Moreover, it leads to many problems including resistance, edema, lipodystrophy and may cause hypoglycemia [3].

Since the insulin discovery in 1922 and until now many attempts have been made to develop a noninvasive system for insulin delivery to overcome the subcutaneous routine problems. Attention is focused mainly onto the oral route as it is the most widely accepted means of administration [3]. The oral administration of the drugs is a convenient and comfortable route due to the ease and simplicity of taking medications that eliminates the discomfort caused by repeated injection of drugs [4]. But, even with the large use of the proteins as therapeutics, their effective oral delivery is still remain a challenging and elusive goal [5].

The main barrier facing the design of an oral system for protein delivery is the protein instabilities. The acidic degradation in the stomach, the changes of pH across the gastrointestinal tract (GIT) and the poor absorption of the proteins across GI mucosa into the bloodstream all of these presents a barriers for deliver insulin orally [6]. Thus to obtain a robust insulin oral delivery, the system should offer a protection for the insulin from the acidic stomach environment. In addition, the pH variations through the different parts of the GIT should be considered [7].

Recently, many attempts have been done to obtain an efficient oral insulin delivery. Encapsulation the insulin into a nanoparticles present a promising strategy to enhance insulin oral bioavailability. Different techniques developed to encapsulate the insulin [8]. PH-sensitive polymers have been introduced as an effective strategy for the insulin oral delivery. In this strategy the insulin release from the nanoparticle is influenced by the pH values [9].

In 1999, the use of pH-sensitive, polymers for insulin oral delivery was suggested for the first time by Lowman et al. [10] which investigated the poly (meth acrylic-g-ethylene glycol) hydrogels as a pH-sensitive vehicles to deliver the insulin. The system showed that the nanoparticles delivered the insulin by an efficient way into the intestine. This opened the door to investigate a various pH sensitive nanoparticles to deliver insulin orally.

The aim of our study is to develop a novel pH-responsive polymeric system for a controlled insulin oral delivery based on a unique polymeric combination consist of polycaprolactone (PCL) core coated with chitosan (CS) and the alginate (ALG) layers. In order to achieve the desired system performance, the first challenge to overcome was the PCL core which affected the release behavior of the nanoparticles. As it is known, binding a hydrophilic agents into the hydrophobic polymers matrices improve their water permeability properties. Thus, the effect of blending the hydrophobic PCL with a hydrophilic Pluronic127 (F127) to improve its wettability- which in turn would enhance the PCL nanoparticles release profiles- were investigated.

Alginate have been chosen to provide the system with the needed pH-sensitivity properties. However, alginate cannot be coated onto the PCL core surface without the existence of a charge oppose its negative charge.

Therefore, chitosan was chosen to provide the system with the positive charge needed. The effect of each the CS and ALG layers on the nanoparticles physiochemical properties were studied.

Thus the system construction passed through three stages. The first stage was the PCL core and the attempts to improve its releasing profiles. The second stage was the addition of the CS into the outer layer of the PCL nanoparticles, to obtain the desired positive charge needed to bind with the ALG. Finally, the obtained nanoparticles were coated with the alginate layer to form the aimed pH sensitive system to deliver insulin orally. Thus three formulations of nanoparticles (PCL, PCL/CS and PCL/CS/ALG) have been synthesized. All the nanoparticles formulations were prepared based on the double emulsion technique/ solvent extraction.

All the nanoparticles formulations were characterized with respect to different physiochemical properties using different measuring tools. Size, morphological properties and the surface topography of the nanoparticles investigated using scanning electron microscopy (SEM), atomic force microscopy (AFM) and optical microscope techniques (OM). The particle size distribution determined by means of dynamic light scattering (DLS) and the nanoparticles surface charge were measured by zeta potential measurements. Furthermore, the encapsulation efficiency and the in-vitro release behaviors of the nanoparticles were studied with the aid of the spectrophotometer technique.

The pH sensitive properties of the system were examined using two different pH environments (1.2 and 7.4) to simulate the physiological conditions in GIT. Furthermore the release kinetic for all the nanoparticles formulations were characterized.

2. GENERAL INFORMATION

2.1. Diabetes

Diabetes is one of the most severe endocrine diseases [11]. The International Diabetic Federation reported that in 2015, 415 million people were affected by diabetes. It has been reported that, diabetes is the third most common cause of death after cardiovascular diseases and cancer [1]. There are two types of diabetes, namely type 1 and type 2. When the secreted amount of insulin (the blood glucose regulating hormone) is either very little or non-existent, this is regarded as type 1 diabetes. Type 2 occurs when the body fails to use the insulin it produces in an efficient manner [12, 13].

2.1.1. Type 1 Diabetes

In type 1 diabetes is an auto-immune disease. During which, the body's immune system attacks and destroys its own beta cells. Of course, the immune system normally identifies and destroys any potentially harmful foreign substances in order to protect the body. However, in such type diseases, the immune system attacks the body's own cells. In diabetes type 1, beta cells may be destroyed over a years. However, diabetes symptoms show in a small time period. Generally, it develops in the young people but it also can appear at different ages [14]. Those patients need to take insulin every day in order to regulate their blood glucose. If they do not have access to insulin, they cannot survive [13].

2.1.2. Type 2 Diabetes

Diabetes type 2 is a result of ineffective use of insulin, and it is the most common type [12]. It can arise when the produced insulin is not enough to compensate the insulin use. The signs develop in a gradual and subtle manner. Therefore, some patients remain undiagnosed for years. Type 2 diabetes widely spread among young and obese people. Thus, hereditary and ecological factors stimulate this type of diabetes [14].

2.2. Diabetes Complications and Economic Impacts

The eventual consequence of either type is the increase in blood glucose level, which causes complications in many parts of the body such as nerve damage (neuropathy), kidney damage (nephropathy), blindness, cardiac failure, stroke and amputations [15]. Diabetes rise the risk of dying prematurely. The complications of poorly controlled diabetes in pregnancy can lead to the risk of fetal death. Diabetes and its complications present a real economic trouble to the patients as well as health systems and the whole national economies [12].

2.3. Treatment of Diabetes

Until the 1920s, Diabetes was considered one of the fatal diseases. There were no any effective pharmaceutical for its management. This changed dramatically in 1921 with the discovery of insulin. Insulin was discovered by Frederick Banting and Charles Best at the University of Toronto, Canada [16]. Insulin discovery regarded a truly miraculous in the history of medicine [17].

2.4. Insulin

Insulin is a hormone produce by the β cells of pancreas [18]. It is a hormone consist of two polypeptide chains (Figure 2.1.)[19]. Its molecular formula is $C_{257}H_{387}N_{65}O_{77}S_6$ and have a molecular weight of 5808 g/mol [13].

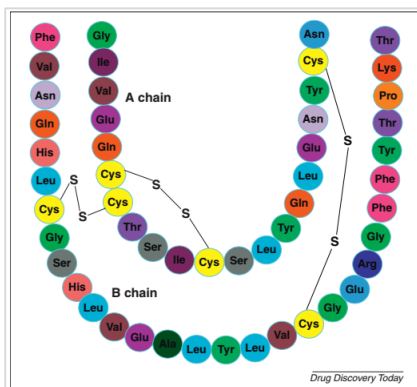


Figure 2.1. Insulin Structure [20].

2.5. Physiological Insulin Secretion and its Action Mechanism

Insulin's main role is controlling the level of blood glucose and maintain a normal blood glucose levels. Insulin produced in the pancreas. Then enters the portal vein and then delivered to liver. Normally, after food intake, the rate of secretion increases by 5- to 10- fold. Insulin secretion is regarded to be pulsatile with major peaks observed every 1.5 to 2 hours. The B cells secretion is regulated by many feedback systems, the most important system is the elevated glucose concentration, which is dose-related [21] .

Blood glucose level is controlled by regulating glucose production versus storage, thus, maintaining glucose homeostasis [13]. A spike in blood sugar level would cause increasing insulin secretion. This is because insulin acting as a "key" to open up body cells and allowing the glucose to be used for energy. In the case of hyperglycemia (i.e. excess glucose in the bloodstream) insulin encourage the glucose storing in some body parts. As a result of this, blood insulin level is depressed, and normal glucose levels are restored. (Figure 2.2) demonstrates the mechanism of insulin action.

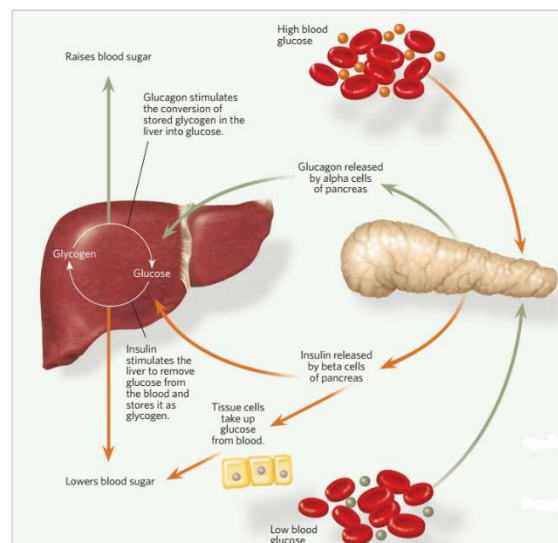


Figure 2.2. Insulin action mechanism [22].

2.6. Insulin Evolution

In 1921 and after their insulin discovery Banting, and Best, conducted their first attempt to examine the effect of insulin in hyperglycemia in diabetic animals. They saved the life of a dog dying of diabetes for 2.5 months by giving it a canine pancreas extract. On January 1922, Banting and Best produced a developed insulin extract and administrated it to Leonard Thompson, a severely diabetic 14-year-old boy.

Thompson became the first patient with diabetes to be treated with insulin injections. Within a day, Leonard's dangerously high blood glucose decreased to normal. Since insulin discovery, medical innovations continued to improve the lives of diabetics. In 1930, new animal sources of insulin (primarily beef and pork) were produced [23].

In 1936, the first long acting insulin, PZI (protamine zinc insulin) created. In 1946, the Nordisk Laboratory started to market the 2nd long acting insulin form NPH (neutral protamine Hagedorn). This action period of insulin was less than PZI and could be used together with regular insulin. Then in 1956, the Lente insulin series which was Lente, semi- Lente and ultra-Lente was released. These different forms synthesized by changing the zinc concentrations [16].

Until 1983 all the prepared forms of insulin was obtained from animals (especially beef and pork). The recombinant human insulin was presented to public use, initially in 1983. Humulin (rapid and intermediate action insulin) produced by Eli Lilly was the first 'human' insulin using gene technology approved by FDA for the US market. Then the human insulins Actrapid and Monotard had been produced by Novo produced. These species of the recombinant human insulins match the actual human insulin form, so they represent the ideal forms of the insulin therapy.

In August of 1996 the Lispro which was the first rapid-acting form of insulin, was released under the brand name Humalog. Throughout the past 15 years a various insulin analogues that characterized by different pharmacokinetics have been introduced worldwide. Nowadays, NovoRapid (Novo Nordisk), Humalog (Lilly), Levemir, Lantus and Apidra (Aventis) are the most common commercially available insulin types. Also a large number of extra insulin analogues are now under investigation [24]. Along with these works to improve a more efficient forms of the insulins, also many progressions in the area of insulin administration routes have been done.

2.7. Insulin Administration Routes

Until recently, injections were regarded as the widely preferred method for delivery of therapeutic proteins and peptides. Patients' compliance with such drug administration methods include is usually poor and limits the therapeutic value of the drug, especially for disease such as diabetes [25]. The conventional method for insulin administration is subcutaneous injections, which have a relatively short action time so, a diabetic patient would need multiple injections daily [18, 26]. This reduces patient compliance, due to pain, common infections at injection site and stress resulting from prolonged insulin therapy [27]. Moreover, the subcutaneous administration has a low efficiency for insulin targeting to the liver, only a small amount (about 20%)of the insulin reaches the liver after injection [28].

Therefore, attention has been turned to drug delivery systems, in order to develop non-invasive routes for insulin administration, to reduce the suffering of diabetic patients. Different research studies have been done for the discovery of noninvasive modes of insulin administration that will eliminate the requirement for parenteral administration.

There are several non-invasive methods which have been tested, including: oral, nasal, ocular, buccal, uterine, rectal, colonic, pulmonary, and transdermal routes (Figure 2.3.) [21]. The most convenient way remains to be the oral polypeptide route. There are many advantages of oral administration of therapeutic agents. Among them oral polypeptide delivery is superior to other drug delivery alternatives.

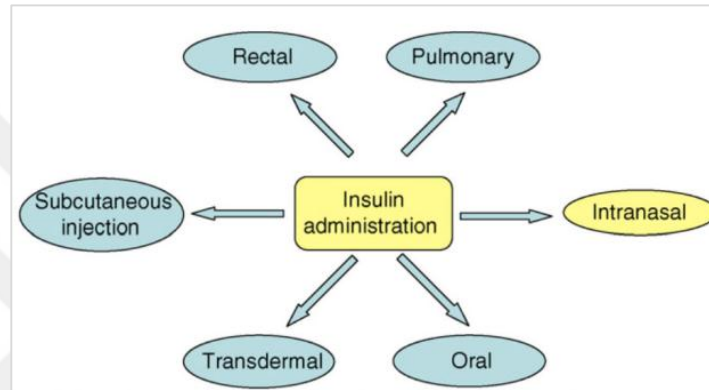


Figure 2.3. The main administration routes for insulin delivery [21].

2.8. Insulin Oral Administration

For the treatment of chronic diseases such as diabetes, that needs life-long therapy, oral administration of medications are more advantageous. Oral drug delivery is regarded as painless, comfortable and free from needle-related infections [29]. It is also cost-effective, since there is neither a need of special tools (i.e. needles) nor specialized personnel for its administration, which reduces the number of visits to the hospital [30]. In addition, the oral delivery route is more physiological.

Normally, insulin is secreted into the portal vein, and through it, delivered to liver. It is not possible to mimic glucose homeostasis, as observed in normal subjects, using subcutaneous injection of insulin.

The reason is that the subcutaneous route delivers the insulin to the peripheral blood circulation, instead of portal one (where it goes into liver);

which is the ordinary way in healthy subjects (Figure 2.4) [21, 31]. On the other hand, oral insulin intake mimics the normal route, as it delivered to the gastrointestinal tract, into portal vein, and to the liver. Furthermore, oral insulin has extra advantages with clinical consequences including; prevention of weight gain and reduction of hypoglycemia risk [17].

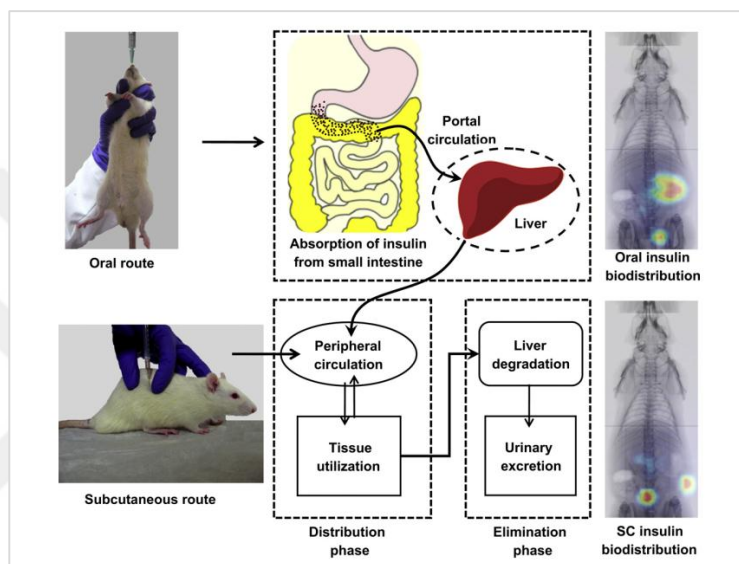


Figure 2.4. Insulin absorption in oral vs subcutaneous administration [31].

Even though oral administration considered a convenient route, designing and formulating oral route for protein drugs has been a persistent challenge due to many gastrointestinal barriers [32]. These barriers represent the greatest obstacle in the way to reach an optimal oral insulin delivery and a lot of works are to be done to address this problem.

2.9. Gastrointestinal Barriers for Insulin Oral Delivery

Insulin is a protein, and protein absorption through the gastrointestinal track (GIT) is restricted by a natural protective barrier. The intestinal lining includes an absorption barrier, which is composed of mucus and an epithelial wall. The epithelium secretes mucus, which forms a semipermeable barrier for the molecules composing the drug. Furthermore, the epithelial cells has a tight intercellular structure, which prevents the

entry of large molecules, such as insulin. After oral administration, insulin traverses the GIT and experiences severe pH changes, as it encounters both acidic and alkaline mediums as it passes through the GIT (Figure 2 .5). Insulin reaches the stomach in less than a minute, where it encounters a sudden drop in pH from 6.8 in the mouth to 1.2 in the stomach. The acidic and proteolytic degradation fragment the insulin into amino acids, which can be easily absorbed into blood stream via the epithelium. If there is remaining insulin, it could pass into the large intestine (LI). These insulin molecules cannot be absorbed efficiently into the bloodstream due to the tight epithelial junctions, as previously mentioned. In fact, a percentage of less than 2% of the insulin taken orally could reach the bloodstream, which has a negative effect on glucose metabolism [33].



	Function	pH	Time	Hurdle	Strategy
Mouth	Drug entry	6.2 - 7.4	< 1 min	None	None
Esophagus	Passage	6.2 - 7.4	< 1min	None	None
Stomach	Digestion	1.2	02 hrs	Acidic pH and Proteases degrades Insulin	Capsulation of insulin to inhibit degradation
SI	Digestion and Absorption	6.8	02 hrs	Proteases degrades Insulin	Capsulation Insulin to inhibit degradation
LI	Absorption	pH = 7.2	8-12 Hrs	Mucosal tight junction inhibits insulin absorption	Transient opening of tight junction for insulin absorption

Figure 2.5. The segments of gastrointestinal tract (GIT), their functions, pH status, drug passage time, the hurdle presented by them and the strategy needed to overcome the hurdles for effective delivery of insulin orally [33].

Drugs which are administered orally, should be able to endure the chemical conditions in the GIT in order to be absorbed into bloodstream (Figure 2.6). The main gastrointestinal barriers which affects the delivery of insulin orally are as follows:

- PH conditions in GIT range from acidic (pH 1.2 – 3.0) to slightly basic (pH 6.5 – 8.0). Such mediums are damaging for insulin, causing it to lose its activity. This is due to its nature, being a polypeptide exposed to pH-induced oxidation, hydrolysis or deamination (Figure 2.6) [29].
- Different digestive enzymes in the GIT, stomach and small intestine may degrade protein drugs. For instance, the proteolytic enzymes in the GI lumen (Figure 2.6), in addition to those associated with enterocytes (membrane-bound amino peptidases). A percentage of 94-98% of orally administered proteins are metabolized by these digestive enzymes [29, 34].
- The intestinal epithelium is the main barrier facing hydrophilic macromolecules absorption, such as polysaccharides, and nucleic acids. This is due to their large molecular weight and hydrophobicity, which hinder their diffusion through the cell membrane [32].

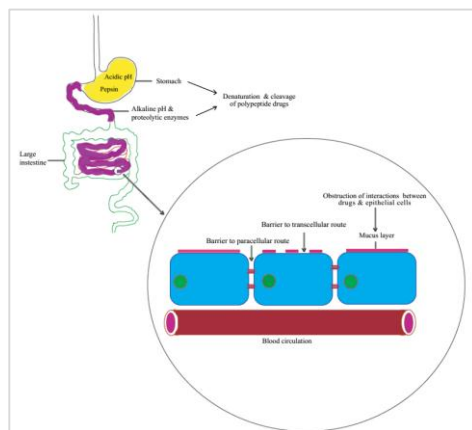


Figure 2.6. Main barriers faces proteins oral delivery [29].

Therefore, designing and developing methods to deal with the hurdles of the GIT has become a necessity. [33] During recent years, attempts have been made to resolve the oral insulin delivery issues. One of the most promising strategies is the encapsulation of insulin into nanoparticles [26].

2.10. Nanoparticle-Based Insulin Oral Delivery

Over the past half century, there has been an explosion in biomedical research, which led to a unique understanding of the pathophysiology of many diseases. Thus, many therapies are being developed using nanoparticle (NP)-based therapeutic approaches, which could have a significant impact on disease treatment and patient outcomes. So far, the design of nanoparticle systems for timely and accurate drug delivery is one of the richest areas of research, which helps increasing therapeutic efficacy of the drug, reducing its side effects and improving patient compliance [35].

Delivering the insulin using nanoparticle system present the solution for the gastrointestinal barriers encountered with the oral insulin delivery. In addition the nanoparticles can control the insulin release and also increase its absorption in the intestine.

Nanoparticles can improve the oral insulin delivery in the GI track in the following aspects:

- Encapsulation the insulin within NP carriers protect the drug against the enzymatic and pH-related degradation in the GI tract [29].
- Encapsulation of the insulin in the nanoparticles increase its permeability to systemic circulation, provide a controlled release and extending the intestinal residence time [18].
- Nanoparticles taken up by cells more efficiently thus present an effective delivery systems [36].

- Nanoparticles have the possibility of integration of both hydrophilic and hydrophobic substances [37].
- Nano particulate systems can also be used for various drug administration routes such as ocular , nasal, parenteral, etc. [38].

2.11. Polymeric Nanoparticles for Insulin Oral Delivery

Among all the nanoparticle systems the polymeric nanoparticles (Figure 2.7) originated from natural or synthetic polymers are one of the most favorable strategies due to their stability, biocompatibility and flexibility. They can be tailored to maintain a controlled drug release into the intended site of action by altering the characteristics and surface properties of polymers [39, 40]. Furthermore, they have a favorable bioavailability, controlled release and less toxic properties [41].

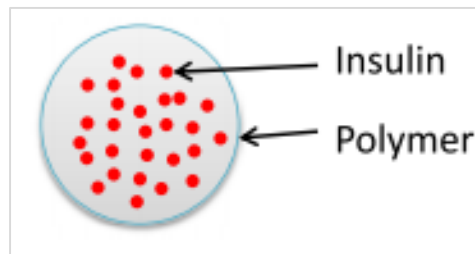


Figure 2.7. Insulin-loaded polymeric nanoparticles [41].

Polymeric nanoparticles offers superior advantages for the oral insulin delivery:

- Polymeric NPs are flexible systems that, enable the modulation of their physical (size, surface morphology) properties as well as its drug release to give the required performance [42].

- Beside protecting the insulin from gastrointestinal environment the polymeric NPs might also enhance the uptake by the intestinal cells (Figure 2.8) [18].
- Polymers have improved pharmacokinetic properties than the drug molecules. They have a better circulation time thus target the tissues more specifically [43].
- The biodegradable Polymeric NPs are approved by the Food and Drug Administration. They characterized by their slow degradation which provide a sustained and controlled release of the drug thus improving release kinetics and prevent carrier accumulation [18].
- Some of the bioactive polymers can be used not to carry the drug but also to provide their own therapeutic benefits [44].
- Polymers used for such drug delivery systems should be nontoxic and non-immunogenic thus provide a safe way for the drugs [43, 44].
- One of the most important properties that make polymers one of the most appropriate materials for the insulin oral delivery is that it can be engineered to mimic biological systems respond to the external conditions such as the change (temperature, light, etc.) and as a conclusion their pharmacokinetic characteristics are change [44].

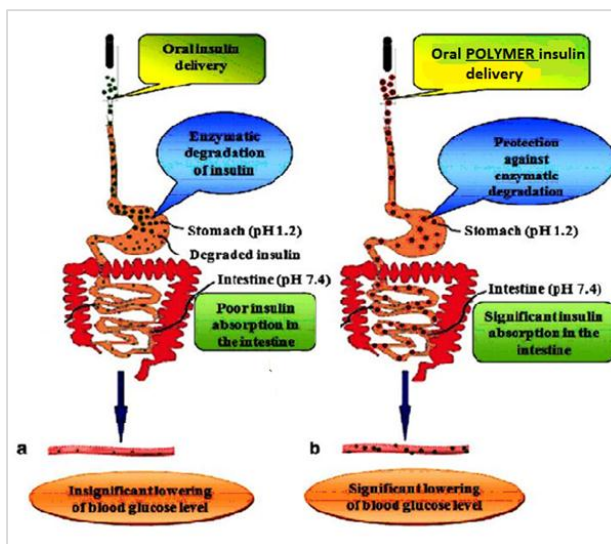


Figure 2.8. The efficiency of the polymeric nanoparticles in protecting insulin from the harsh gastrointestinal environment [18].

Thus, encapsulation of the insulin into polymeric nanoparticles is regarded a convenient method to enhance the bioavailability of orally taken insulin. The polymer choice should be based on the desired release profile, loaded drug, the degradation property, etc [28]. Recently, various polymeric systems have been developed to encapsulate insulin and deliver it via oral route. The synthetic polymers such as poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) and acrylic and natural polymers, such as chitosan, alginate, poly (γ -glutamic acid) and hyaluronic are the commonly used polymers to produce nanoparticles for the delivery of insulin orally [26].

2.11.1. Synthetic Polymers for Insulin Oral Delivery

In drug delivery, the main import feature of the synthetic Polymers is that, their physiochemical features and structures can be controlled to achieve the desired drug release behaviors [45].

2.11.1.1 Poly (lactide-co-glycolide) (PLGA)

PLGA, an aliphatic polyester co-polymer. It is a hydrophobic, biocompatible, biodegradable and provide a sustained release profiles. Thus it commonly used in the field of the insulin oral delivery. PLGA can also combined to other polymers to provide a versatile nanoparticles with various important features that might improve the absorption of load drugs [26].

2.11.1.2 Poly- ϵ -caprolactone (PCL)

PCL have glass transition temperature at about -60°C and low melting point about 60°C . PCL have a high blend-compatibility which made it one of the popular polymers in the biomedical field [46].

PCL is a semi crystalline, hydrophobic, biodegradable, and biocompatible polyester. It has a slower degradation rates than the PLGA-based polymers, so it is an excellent choice when the sustained drug release profiles are needed [26]. PCL has a superior viscoelastic properties and possesses easy formability compared with other synthetic polymers like, PLGA and PLA. Also the degradation productions of the PCL is less acidic than PLGA-based polymers which considered one of its important advantages [18]. All of these along with the low cost of PCL and its preferred mechanical properties makes it an attractive polymer for the insulin drug delivery systems [2].

2.11.2. Natural Polymers

The most widely used natural polymers in drug delivery are the chitosan and alginate. They characterized by their low cost, biocompatibility, abundantly and another superior properties made them an attractive candidates for the oral insulin delivery [47].

2.11.2.1. Chitosan (CS) Nanoparticles

CS is an abundant polysaccharide in nature. It is a cationic copolymer found in fungi, yeast or many types of the shellfish. It is a hydrophilic, biocompatible, easily absorbable, biodegradable and nontoxic polymer. There are a lot of chitosan types with various molecular weights and deacetylation degrees [18, 48].

The most important property that makes chitosan preferred for insulin oral delivery is its mucoadhesive property which prolongs the drug's intestinal residence time [33]. The tight junctions among the epithelial cells are the main barriers for the water soluble drugs absorption. Water soluble drugs like insulin, cannot transport through the epithelial cells. Chitosan can adhere to mucosal surface and opens up the tight junction among the cells (Figure 2.9). Thus chitosan plays as an enhancer for the absorption of these molecules [18].

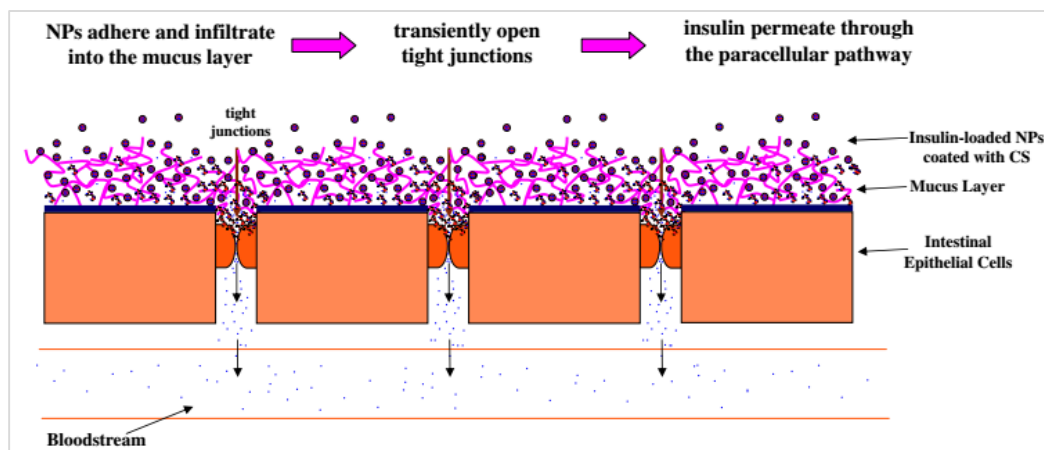


Figure 2.9. Insulin paracellular transport to circulation using a coated chitosan nanoparticles [49] .

Furthermore, CS digested by an intestine enzymes called chitosanase so it has no toxic effect. The cationic nature of chitosan ($pK_a \sim 6.5$) facilitates the encapsulation of negatively charged drugs like, insulin and extends their intestinal residence time [50]. All these unique properties and advantages prove the key role of chitosan in developing a strong system for oral insulin delivery that can overcome the gastrointestinal barriers [33].

2.11.2.2. Alginate (ALG)

Alginate (ALG) also one of the abundant polymers in the nature. It is an anionic natural polysaccharide, extracted from seaweed. It a biocompatible, hydrophilic, biodegradable, low toxic, low immunogenic and good mucoidhesive polymer. In addition to that alginate has some extra properties that made as an excellent candidate for the oral insulin delivery. Alginate have gelling property emerged from its guluronic residues. Crosslinking of the guluronic residues and divalent ions results in ALG gel matrices formation. Furthermore, alginate is a pH-responsive polymer, it shrinks in lower pH and extends in a high pH thus keep the encapsulated drug against the stomach acidic environment [50, 51].

The current trend in the polymer based NPs systems has become towards the controlled drug delivery using stimuli-responsive polymers. Using stimuli-responsive polymers are favorable for regulating glycaemia thus, raise the life quality of Diabetics[52].

2.12. Stimuli-Responsive Delivery of Insulin

The controlled delivery of the drugs is one of the most advantageous drug delivery techniques. It ensures maintaining the effectiveness and reliability of the drug. Recently, using the stimuli – responsive polymers gained a great interest.

Stimuli sensitive or smart polymers are vital type that, exhibit a physiochemical variation in their properties as a response to some types of stimulus such as the pH value, light, ionic factors, magnetic field, temperature, etc. Figure 2.10 demonstrate some of the possible stimulus that can trigger the insulin delivery systems. The applications of the stimuli responsive polymers in the drug delivery systems increased significantly in the latest three decades. They present an interfaces of chemistry and biology [53]. Among these stimuli –responsive strategies the pH-sensitive polymers have been introduced as one of the most vital strategies for insulin oral delivery.

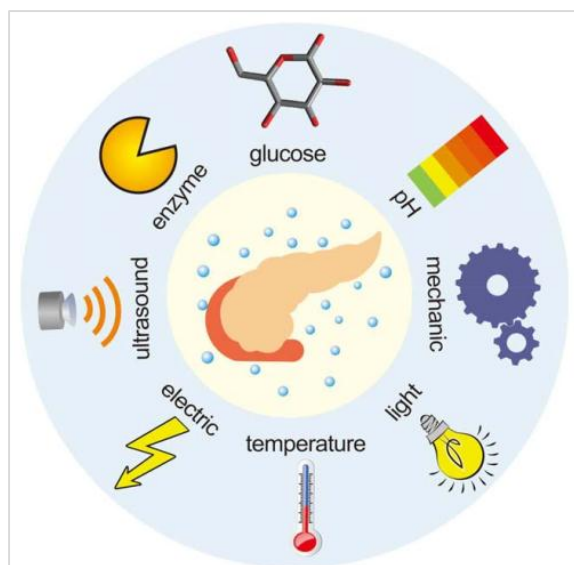


Figure 2.10. Several stimulus to trigger the insulin delivery [52].

2.13. PH-Responsive Systems for Oral Insulin Delivery

In this strategy the insulin release rate from the PH-sensitive polymers is affected by pH values (Figure 2.11). PH-responsive based systems developed to enhance the bioavailability of the drug. Thus the treatment efficiency. These systems protect insulin from the acidic stomach environment and allow the insulin release at the neutral environment of the intestine [54].

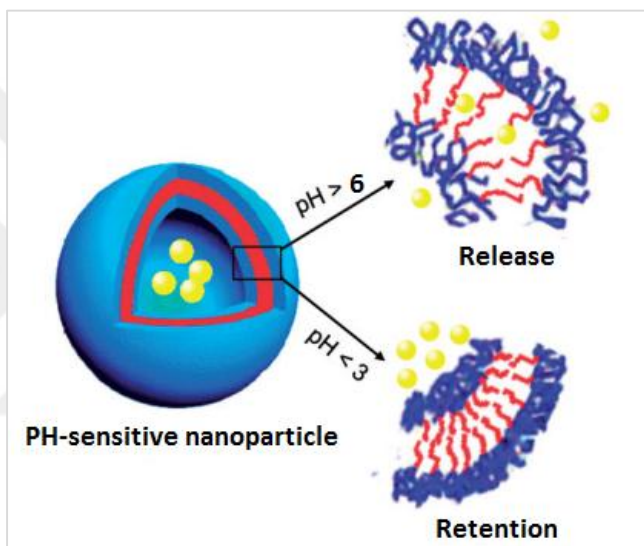


Figure 2.11. PH sensitive nanoparticles at different pH values [55].

Myriad researches and advancements have been made and still are being worked on in the area of oral insulin delivery. We will present some these recent works especially which concerns the pH responsive based polymeric nanoparticle systems for the insulin oral delivery.

2.14. Advancements of the Oral Insulin Delivery Systems

Polymer based nanoparticles for insulin oral delivery have been investigated for the first time in 1988 by Damage et al.[56]. They designed a nanocapsules of Polyalkylcyanoacrylate with a mean size of 220nm for oral insulin delivery. The in-vivo experiments demonstrated that, the nanocapsules orally administration decreased the levels of glucose (for an extended period) in the diabetic rats .Thus, they proved that encapsulating insulin with a biodegradable polymers protect insulin from the proteolytic degradation in the GIT. This was the starting point for using the polymeric nanoparticles as oral drug carrier for insulin.

Recently, different synthetic and natural polymeric nanoparticles developed to deliver insulin orally. The synthetic polymers such as poly (lactic acid) (PLA), poly (lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) and natural polymers, such as chitosan, alginate, poly (γ -glutamic acid) and hyaluronic are the commonly used polymers [26]. Among the natural polymers the polysaccharides chitosan and alginate gained a great interest as an oral delivery vehicles. They characterized by their biocompatibility, low cost, abundantly and another superior properties made them an attractive candidates for the insulin oral delivery [47].

Sarmiento et al. [51] evaluated the pharmacological effect of insulin-loaded alginate/chitosan nanoparticles oral administration on a diabetic rats. The nanoparticles had a mean size of 750 nm. The insulin was released in a pH-sensitive pattern and insulin association efficiency was about 70%. In-vivo experiments in diabetic rats showed that, the oral administration of the nanoparticles dropped the levels of glucose in a more significant manner than that obtained from insulin solution. This study present alginate/chitosan nanoparticles as a significant oral delivery system for insulin.

Another study by Mukhopadhyay et al. [50] investigated insulin-loaded core-shell chitosan-alginate (CS/ALG) nanoparticles as a pH responsive devices for oral insulin administration. In-vitro experiments of release demonstrated that the nanoparticles retained the encapsulated insulin under the gastric simulated conditions and released the insulin in the neutral condition of the intestine. The nanoparticles showed significant hypoglycemic effects in in-vivo studies.

PCL is a popular synthetic polymers in the delivery of drugs. It is a biodegradable and biocompatible polymer characterize by its sustained release behavior. The slow degradation rate of the PCL and its preferred mechanical properties make it favorable for the prolonged drug delivery [26]. Damge et al. [57] investigated the blends of a biodegradable poly- ϵ -caprolactone (PCL) and a polycationic non-biodegradable acrylic polymer (Eudragit® RS) nanoparticles loaded with a commercial insulin (Novorapid®). The in vivo study, showed that the insulin-loaded nanoparticles decreased the glycaemia (for a prolonged period) when administered in fasted diabetic rats and improved the glycemic control. In another study by the same group, the same nanoparticles have been loaded with two different commercial types of insulin Novorapid® and Actrapid®. In vitro study reported that, the nanoparticles exhibited a burst release behavior. In vivo studies showed that the oral administration of the nanoparticles decreased the glycemic effect in the diabetic rats [58].

There are many critical parameters such as molecular weight of polymer, preparation method and the stabilizing agents used, which affect the polymeric nanoparticles properties. Among those parameters, stabilizing agent have an important role in the production of nanoparticles and its properties. Recently, many research studied the influence of adding different stabilizing agents with different concentrations on the morphology and the drug release behaviors for the polymeric nanoparticles[59].

Turk et al. [59] studied the effect of various stabilizing agents, various types of Pluronics (Pluronic F127, Pluronic P85, Pluronic F68, and Pluronic L64) and polyvinyl alcohol (PVA) on the PLGA nanoparticles properties. The influence of these agents concentration on the PLGA nanoparticles properties (size, morphology, charge and shape) were evaluated. Results showed that, the addition of the Pluronic produce nanoparticles with larger, rougher surface than the nanoparticles formed with PVA. Pluronic introduction results with polydisperse nanoparticles distributions on contrary PVA resulted in a monodisperse size distribution nanoparticles. The study showed that the stabilizing agent choice is an effective parameter in determining the nanoparticles properties.

Ma and Song. [60] studied the effect of Pluronic F68 on the morphology and drug release behavior of the PCL microspheres loaded with paclitaxel. The results showed that, the porosity of PCL/F68 microspheres increased and also the drug release increased as the F68 amount increased. Thus, this study suggested F68 as a pore-forming and a drug release enhancer.

The nanoparticles studies not only concerned their preparation methods and characteristics but also extend to study the kinetics mechanisms of the drug release. The kinetics studies of the drug release is very important since it is the key factor of its biological effect.

2.15. Kinetics Characterization of Drug Release Mechanism

“Drug release” defined as the process by which the drug transfer from inside the nanoparticles system into the release medium. The drug release is a complex process that can be affected by many factors such as the release medium, the drug physicochemical characteristics, the structural and properties of the system and the interactions between all these factors [61]. There are many kinetics models that characterize the kinetics and the mechanism of the drug release [62]. These kinetics models present a description for the complex release behavior of the nanoparticles by a simple mathematical forms to simplify the understanding of the drug release mechanisms [61]. In the scope of the thesis the drug release mechanism of the nanoparticles were characterized using some of the most commonly used models to describe the release from the polymeric nanoparticles which include, zero-order kinetics, first order kinetics, Higuchi and Korsmeyer- Peppas kinetics models.

2.15.1. Zero order Model:

Zero-order kinetics model (Equation 2.1) model is express the drug release mechanism when the drug release from the nanoparticles is not depends on its concentration [63]. It describe the release of the drug as a relation of the released drug with the time.

$$F = K_0 t \quad \text{Equation 2.1}$$

F is the drug released at time t, k_0 zero-order constant (concentration/time)[62].

Thus in this model, the graphical presentation of the drug released fraction versus the time of relese will be linear. This model express the systems that have a constant and sustained drug release profiles [64].

2.15.2. First Order Model

In contrary with the zero order model the first order model express the release mechanism in the systems, where the drug release rate is depend on the drug concentration. Drug release mechanism follows the first order first order can be presented by Equation 2.2 [64].

$$\frac{dF}{dT} = -K_1 F \quad \text{Equation 2.2}$$

Where F is drug released at time t and K_1 is the first order rate constant expressed in units of time^{-1} .

By integrating and rearrange Equation 2.2 it be can presented as [12, 64]:

$$\ln(1 - F) = -k t \quad \text{Equation 2.3}$$

As it clear from Equation 2.3, the graphical presentation of released drug natural logarithm against time is linear with a negative slope.

2.15.3. Higuchi model

In 1961 Higuchi defined the first model describing the release of drug from matrix systems [65]. According to Higuchi model, release of drug release from a porous matrix can be described as a diffusion controlled procedure obeying the Fick's law [66]. According to this model, the released amount of drug can be present simply by the following Equation:

$$F = K_H \sqrt{t} \quad \text{Equation 2.4}$$

F is the released drug at t time and K_H is the Higuchi release constant.

Thus, for procedures that diffusion controlled the released amount of drug F exhibit a linear relation with the time square root [63].

2.15.4. Korsmeyer- Peppas Model

Korsmeyer and Peppas (1983) describe the drug release mechanism of the polymeric systems by a simple model. The Korsmeyer and Peppas model present a linear relation between the natural logarithm of the released drug with the natural logarithm of the time. Rendering to Korsmeyer–Peppas model the mechanism of drug release can be found out using the 60 % fraction of the release data [67]. Korsmeyer–Peppas model can be presented simply by Equation 2.5:

$$F = K_{kp} t^n \quad \text{Equation 2.5}$$

By taking the natural logarithm of Equation 2.5, it could be written as Equation 2.6:

$$\ln F = \ln K_{KP} + n \ln t \quad \text{Equation 2.6}$$

F is drug released at t time, K_{KP} is Korsmeyer- Peppas constant of release present geometric and structural properties of the system [63]. n value is exponent of release which is important factor that describe the drug release mechanism (see Table 2.1) [68]

Table 2.1: Korsmeyer -Peppas diffusion exponent (n) (for the spherical nanoparticles) and the associated drug release mechanism.

Diffusion Exponent n values	Drug Release Mechanism
≤ 0.43	Fickian diffusion
$0.43 < n < 0.85$	Non Fickian(Anomalous transport)
Higher than 0.85	Super case II transport

Furthermore, from the Korsmeyer- Peppas model parameters, the mean dissolution time (MDT) of the matrix can be determined. MDT is an important term of that reflects the drug retaining capability of the matrix. Higher MDT indicates a higher drug retaining capability of the nanoparticles matrix [69]. MDT can be calculated by Equation 2.7:

$$MDT = \frac{n}{n+1} K^{\frac{-1}{n}} \quad \text{Equation 2.7}$$

The aim of our study is to develop a novel pH-responsive polymeric system for a controlled insulin oral delivery based on a unique polymeric combination consist of polycaprolactone (PCL) core coated with chitosan (CS) and the alginate (ALG) layers. In order to achieve our objective the first challenge was to deal with the PCL core hydrophobicity which was a barrier for achieving the desired insulin release profile. To address the challenge, the effect of blending the hydrophilic agent Pluronic127 (F127) with the PCL were investigated. Furthermore, the effect of each the CS and ALG layers on the nanoparticles physiochemical properties were discussed. The pH sensitive properties of the system were examined using two different pH environment (1.2 and 7.4) to simulate the physiological conditions in GIT. The release kinetic for all the nanoparticles formulations have been characterized.

3. EXPERIMENTAL METHODES

This chapter will presented the materials used for the particles preparations, discuss the particles preparation methodology and the different measurements devices that used for the particles characterization.

3.1. Materials

The Commercial recombinant human insulins (Actrapid®) used as a model drug, were brought from the pharmacy. Poly- ϵ -caprolactone (PCL; Mw 42,000 Da) was a gentle gift from Prof. Dr. Erhan Pişkin's research laboratory in chemical engineering department at Hacettepe University. Hydrochloric acid, Tween 20 phosphate were purchased from Merck (Darmstadt, Germany). PluronicF127, dichloromethane, ethyl acetate, chitosan (low molecular weight) and sodium alginate were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Sodium hydroxide and methylene blue were purchased from Fluka and acetic acid from Merk. These chemical materials were used without further purification. Ultrapure water purified by Millipore Direct-Q3 UV was used in all experiments.

3.2. Preparation of the Nanoparticles using Double Emulsion Method

There are many ways used to synthesize the NPs for drug delivery. In our study, all nanoparticles have been prepared based on the double emulsion method with just a little modifications in the components of both the water and oil phases.

3.2.1. Synthesis of PCL NPs

PCL nanoparticles were synthesized based on the double emulsion (w/o/w) method, (Figure 3.1). 1 ml of (Actrapid®) aqueous solution of insulin (100 IU/mL) poured into an organic phase consists of 250 mg of PCL dissolved in 10 ml of ethyl acetate (EA). This mixture was sonicated by sonication, using an ultrasound probe (Bandelin sonopuls uw 2070 use small letter) for 15 sec at 50 W output to form the first emulsion (w/o). Then the emulsion poured into 17 mL of (0.1% w/v) Pluronic127 (F127) solution and sonicated in the same conditions for 60 sec to achieve the final emulsion (w/o/w). Then the solvent evaporated under a decrease pressure for 20 min using rotary evaporator (Heidolph Laborota 4000). After that, the NPs were obtained by centrifugation (Centrifuge MPW-251) at 18000 rpm for 30 minutes.

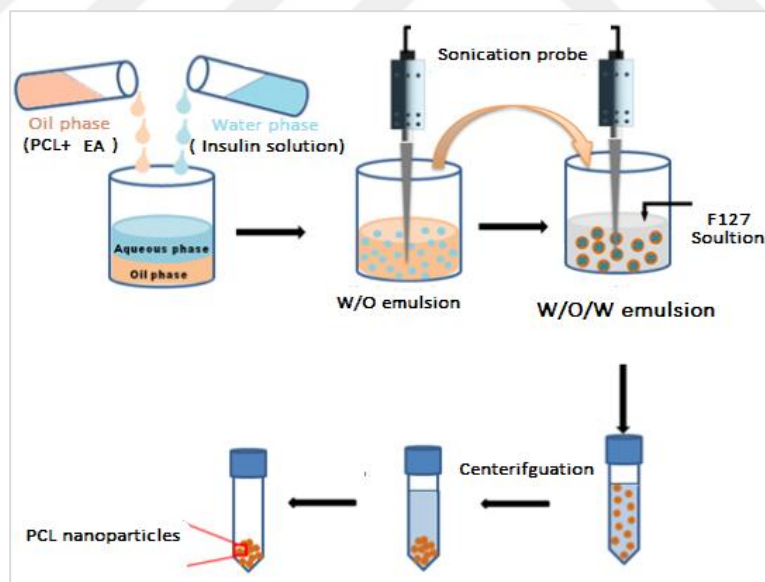


Figure 3.1. Preparation of the PCL nanoparticles using the double emulsion method.

3.2.2. PCL Nanoparticles Bended with F127 Preparation

The PCL/F127 were fabricated based on double emulsion (w/o/w) shown in Figure 3.2. 1ml of insulin aqueous solution (100 IU/mL) poured into the organic phase which was formed by co dissolve PCL and various concentrations of F127 (0.1, 0.5, 1, 5%, w/v) in 10ml of dichloromethane (DCM). The mixture was sonicated, using an ultrasound probe (Bandelin Sonopuls UW 2070) for 15 sec at 50 W output to form the first emulsion. Then this formed emulsion poured into 17 mL of ultrapure water (UP) and sonicated in the same conditions for 60 sec to obtain the final emulsion. The final emulsion vigorously stirred overnight at 800 rpm in the room temperature to evaporate the DCM. The product was collected by centrifugation at 18000 rpm for 30 minutes.

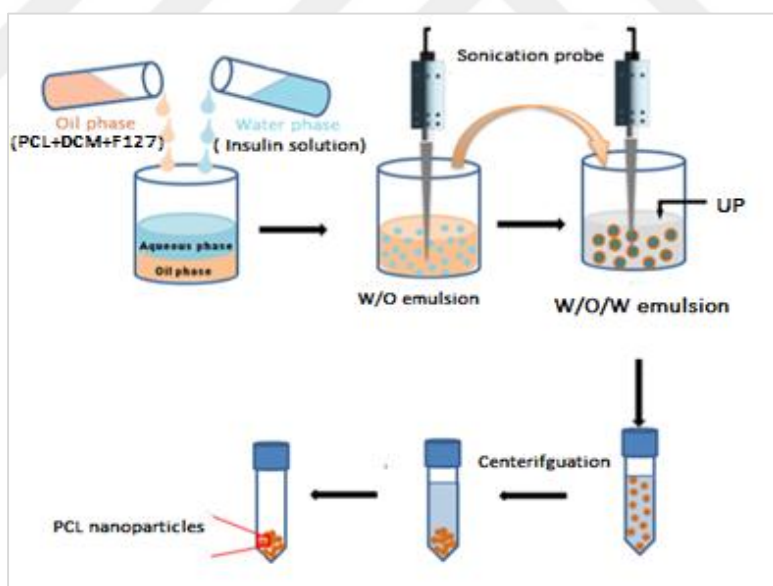


Figure 3.2. Preparation of the PCL/F127 nanoparticles using the double emulsion method.

3.2.3. PCL Nanoparticles with an outer layer of Chitosan (PCL/CS) Preparation

PCL/CS particles were fabricated based on double emulsion (w/o/w) method shown in Figure 3.3. 1 mL of insulin aqueous solution was poured into an organic phase of 250 mg of PCL and (0.1 % w/v) F127 co-dissolved in 10ml of DCM and sonicated for 15 sec to obtain the first emulsion. First emulsion rapidly added to the water phase which, consist of 17 ml of 2% acetic acid solution containing various amounts of chitosan (0.1, 0.2, 0.4 and 0.8 mg/ml). PH of the chitosan solution was adjusted to about 5.5 by the addition sodium hydroxide solution. The mixture is sonicated for 60 sec to obtain the final emulsion. The final product then magnetically stirred overnight at 800 rpm in the room temperature to evaporate DCM. The particles collected by centrifugation at 18000rpm for 30 min.

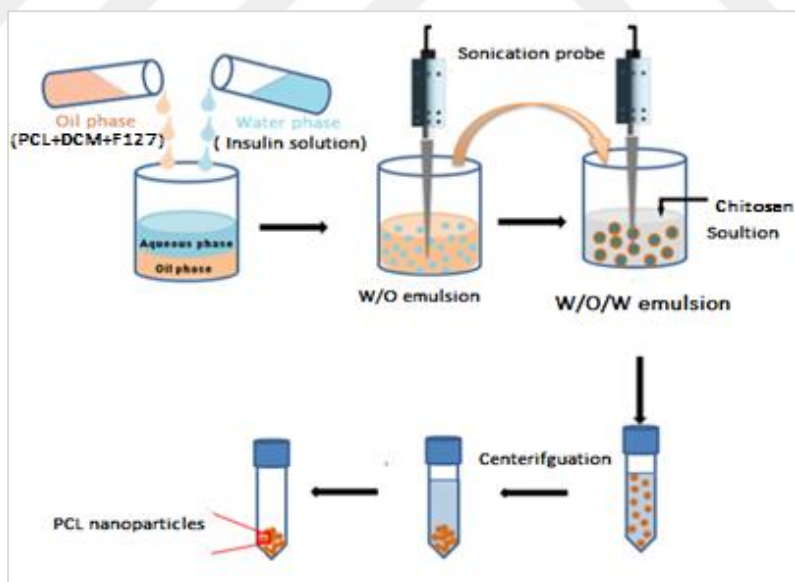


Figure 3.3. Preparation of the PCL/CS particles using the double emulsion method.

3.2.4. Alginate Coated (PCL/CS) Particles Preparation

The PCL/CS/ALG particles prepared by a two-step methodology that have been modified from the method suggested by Rajaonarivony et al. [70].

First Step: Alginate pre-gelation:

The most significant feature of alginate is its ability to form gels when cross linked with divalent ions such as Ca^{+2} [71]. In our study the sodium alginate cross linked with the calcium chloride which resulted in the calcium alginate pre-gel, (Figure 3.4). To prepare the pre gel, an aqueous solutions of (3.35 mg/ml) calcium chloride and ALG aqueous solution of (3 mg/ml). The pH of the alginate solution were adjusted to 5 using a hydrochloric acid solution. 2ml of the CaCl_2 solution was added into 10 ml of sodium ALG solution under magnetic stirring at 1200rpm and ambient temperature for 30 min.

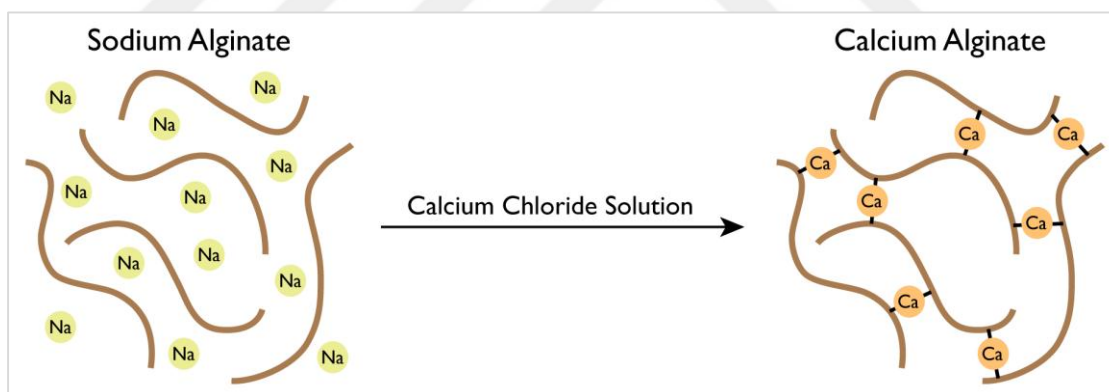


Figure 3.4. Ionotropic pre-gelation of sodium ALG with CaCl_2 [71].

Second Step: ALG and CS electrostatic interaction

4 ml of the pre-prepared PCL/CS particles suspension (after DCM remove) were poured to the resultant calcium alginate pre-gel and stirred at the same conditions for an additional 1 hour.

Thus, the cationic chitosan layer in the outer surface of the PCL/CS particles interact with the anionic alginate. Figure 3.5 show the (CS /ALG) electrostatic interaction.

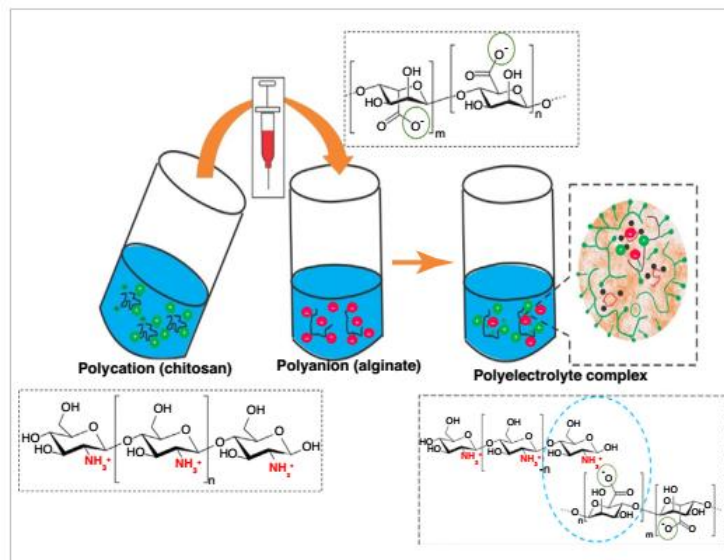


Figure 3.5. The electrostatic interaction between the cationic CS and anionic ALG [72].

3.3. Nanoparticle Characterization

To understand the physiochemical characteristics of the obtained NPs different characterization parameters such as, in vitro drug release behavior and encapsulation efficiency, size and size distribution, morphology and surface topography and the surface charge of NPs have been studied using various techniques. In current section different characterization tools and methods used within the scope of our study will be discussed.

3.3.1. Dynamic Light scattering (DLS) Technique

DLS is one of the most common techniques to measure the nanoparticles sizes. In the dynamic light scattering measurement a laser beam incidence into the nanoparticles suspension. The collision of the incidence beam with the nanoparticles scatter the incidence beam with different angles thus, alter its direction and intensity. (Figure 3.6) shows the block diagram of DLS measuring.

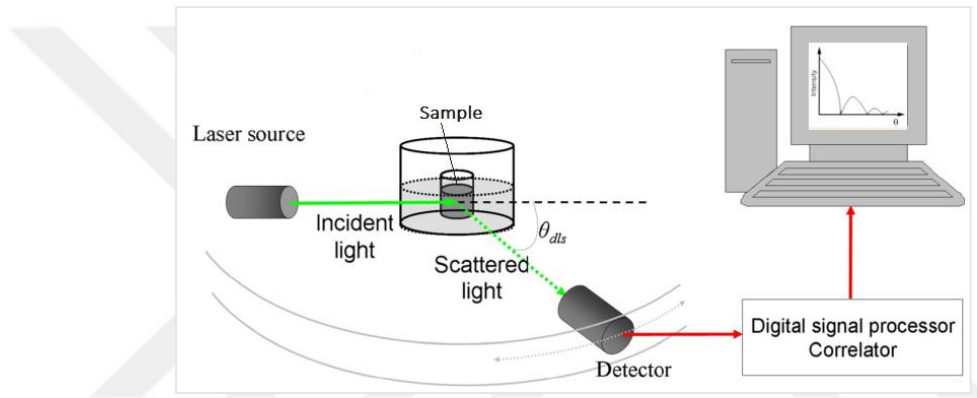


Figure 3.6. Block diagram of the dynamic light scattering measurements [73].

The intensity variation of the incidence beam reflects information about the random motion of the nanoparticles which, can be used for measuring the nanoparticles diffusion coefficients. From the diffusion coefficient of the nanoparticles their hydrodynamic radius (R_H) can be calculated according to Stokes-Einstein equation, (Equation.3.1)

$$D_f = K_B T / 6 \pi \eta R_H \quad \text{Equation 3.1}$$

Where,

K_B : Boltzmann constant

T: temperature of the suspension

η : viscosity of the surrounding media.

DLS measure the hydrodynamic radius of the nanoparticles. DLS have many unique features that make it one of the powerful techniques for the nanoparticles characterization. DLS measurement is a simple process that, an extensive experience is not needed for the measurement performing. Also, it is non-invasive technique, the sample solution can be used again after the measurement. This property is significant especially in the case of the expensive substances, such as enzymes or molecular ligands. In addition to the nanoparticles size, it gives an idea about the size distribution of the nanoparticles. DLS have a high sensitivity to the nanoparticles aggregation thus, it can be used to check the stability of the NPs suspension [73]. In the hand DLS have some limitations in the size ranges it can be used to measure the nanoparticles sizes generally in the range of (2–3000nm). Since the DLS measures the hydrodynamic radius it results in an average nanoparticles sizes slightly bigger due to the nanoparticles contaminations within the sample [74].

In our study, the DLS (ALV-CGS-3 Compact Goniometer) device was used. The samples for the DLS were prepared by a diluting the nanoparticle suspension to about (1:100 v/v) with UP water. The measurements were utilized at angle of 90°.

3.3.2. Zeta Potential Measurement

Zeta potential measure the electrostatic or charge between the nanoparticles. It used to measure the surface charge of nanoparticles. Zeta potential is an indication of the stability of nanoparticles. In our study, the zeta potential were measured using Zeta Meter System 3.0 (Zeta Meter Inc.) with a quartz-teflon GT-2 cell, molybdenum anode and platinum cathode. For the zeta potential measurement the samples were prepared by diluting the NPs suspension to about (1:30) by using UP water. All the measurements were performed in triplicate.

3.3.3. Atomic Force Microscopy (AFM)

AFM is a microscopic technique of imaging, it gives an idea about the surface topography by dragging a probe tip across the surface of sample. The forces played between the tip and the sample surface reflect a topographical map of the surface [75]. The atomic forces between the sample and the tip changing according to the surface height variations which cause a deflection of the cantilever. This deflections cause a signals that convey an information about the surface topography. Thus the sample Surface topographic information is gained from the feedback signal which monitors the deflection of the cantilever as a result of its changing force due to surface height variations. The tip is usually attracted to the surface due to the capillary condensation. Thus the forces plays on the sample surface are caused by the sample/ tip interactions and the shear forces generated from to the lateral scan movements [76].

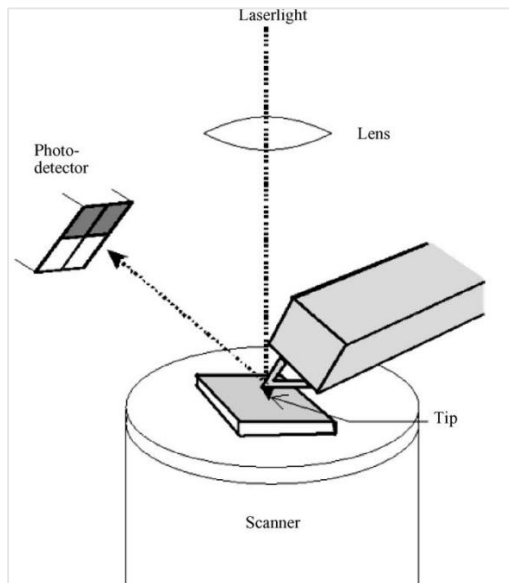


Figure 3.7. Illustration of AFM [77].

As presented in Figure 3.7, the surface of the sample scanned with a tip mounted to a cantilever spring. During the scanning the atomic interaction force between the surface and the tip cause the cantilever to deflect.

The atomic force can be detected by cantilever bending caused by a tip-sample interacting force. Thus plotting the deflection of the cantilever versus its position on the sample the surface topographic image is obtained [77]. AFM can image the surface topography at wide scales ranges of micrometers to sub nanometers. AFM exert a low forces at the surface of the sample and characterized by a high spatial resolution, which is makes AFM a valuable tool [76]. They are two modes for the AFM: contact mode in which, the probe can be contacted to the samples surface, and noncontact mode, in which the probe just hover above (noncontact mode) [75].

In our study, AFM (PSIA Corporation, XE-100E) of a non-contact mode with Cr-Au cantilevers (ACTA 10M) and frequency of 0.37 Hz was used. The AFM samples were prepared by dropping the diluted solution of NPs onto a microscopic slide and keep it to be dried under the normal room conditions.

3.3.4. Scanning Electron Microscopy (SEM)

In SEM microscope a beam of an electron concentrated on the sample surface and the scanned laterally with a parallel lines. The interaction of the electrons lines with the surface of the sample produce a signals, including back-scattered electrons, secondary electrons, X-rays and light. These signals collected to form the image and reflect information about the sample's topography and composition [78].

For our study, the SEM (Zeiss Evo 50 model), in the geology department at the Hacettepe University was used to image the nanoparticles and to take an idea about the surface topography.

3.3.5. Optical Microscope (OM)

The OM use the visible light and a system of lenses to enlarge images of small samples. A light beam focus on the sample surface. The light beam can be absorbs, scatters, or deflects when passed through the samples. The effect of the sample on the light beam passed through it produce the image of the sample. OM gives a general outline of the sample [79].

For our study, the Leica DMI 4000B instrument was used, (Figure3.8). The measurements were carried out with a 100X oil lens using immersion oil to achieve a higher magnification ratio.



Figure 3.8. Leica DMI 4000B model microscope.

3.3.6. Fluorescence Microscopy (FM)

In Fluorescence microscopy fluorescent dyes mixed with the sample solution. The fluorescent in the sample excited by a certain wavelength of light. This fluorescent in turn emits a light of a longer wavelength. The emitted light can be imaged are then transferred to the camera to display the magnified sample image. (Figure 3.9) demonstrate the fluorescent microscopic technique [79].

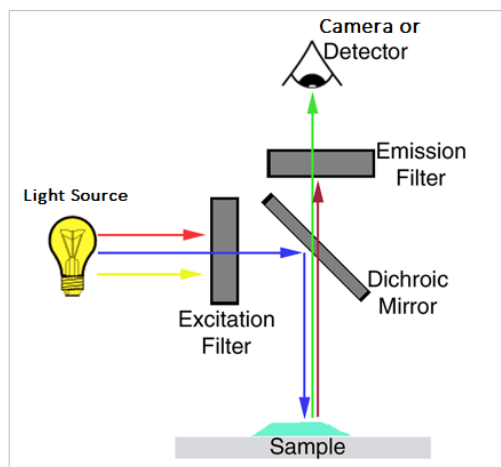


Figure 3.9. Schematic of fluorescent microscopic technique [79].

Leica DMI 4000B microscope support both of optic and fluorescent microscope modes thus the same microscope used again to obtain the fluorescent image. Methylene blue solution was used as fluorescent dye to perform fluorescence microscope measurements.

3.4 Encapsulation Efficiency (EE) of NPs

EE expresses capability of the NPs to entrap the drug successfully. It is the ratio of the amount of the drug that have been encapsulated to the total amount of the drug used for the nanoparticles preparation.

After collecting the prepared nanoparticles by centrifugation, a sample from the nanoparticle supernatant was analyzed using UV-vis spectrophotometer (Thermo Scientific™, GENESYS 10S) with 1 ml quartz cell at a wave length of 271 nm to determine the unencapsulated ampunt of insulin recovered in the supernatant. By knowing the insulin concentration in the supernatant (unencapsulated insulin) and the total concentration of insulin used for the nanoparticles preparation (total insulin) the EE was determined via the following formula (Equation.3.2):

$$(EE. \%) = \left[\frac{\text{total insulin amount} - \text{unencapsulated insulin amount}}{\text{total insulin}} \right] * 100 \%. \quad \text{Equation 3.2}$$

The absorbance of the measured sample then compared with the pre-prepared insulin calibration curve (see Appendix) to determine the free insulin concentration.

3.5. In vitro Insulin Release Behavior

In-vitro insulin release behavior for all nanoparticles formulation were examined via the dialysis membrane method. In this method the insulin loaded nanoparticles were dispersed with 2 ml of a release media of phosphate-buffered saline (PBS; pH = 7.4) in the dialysis membrane. The enclosed dialysis immersed into a flask containing 20 ml of the release media (PBS pH 7.4) and 0.1% (v/v) of Tween®. The flask was incubated at 37°C under continuously magnetic stirring at 150 rpm, to mimic the physiological condition of the body. At defined time intervals of (5, 10, 15, 30, 60, 120, 240, 360 minutes and 24 h), a sample from the solution were withdrawn and replaced with a same volume of fresh PBS to maintain the final volume constant throughout the experiment. The absorbance of the samples was measured by UV-vis spectrophotometer (Thermo Scientific™, GENESYS 10S) with 1 ml quartz cuvette at a wave length of 271 nm and compared with the pre-prepared insulin's calibration curve (Appendix) to determine the released insulin concentration. All release experiments were performed for triple time. Beside PBS release media two other release media that simulate the physicochemical environments in the intestine and stomach were prepared to examine the pH sensitivity behaviors of the PCL/CS and PCL/CS/ALG. The simulated gastric fluid (SGF) with pH of 1.2 were prepared to simulate the gastric environment and simulated intestinal fluid (SIF) with 7.4 pH to simulate conditions in the intestine.

4. RESULTS AND DISCUSSIONS

4.1. PCL Nanoparticles Formation

In our study, the PCL have been chosen to be the core of our polymeric based insulin oral delivery system due to it is superior viscoelastic and physicochemical properties, which made it one of the preferred polymers in this field. In our study, the insulin loaded PCL NPs were synthesized by the double emulsion (w/o/w) method, which is a convenient method for preparation of the polymeric NPs loaded with water soluble drugs such as insulin. The main benefits of this method are: the drugs and therapeutic proteins can be loaded into the nanoparticles in their solution form and no need for drying. This assist a uniform drug distribution inside the carrier matrix; thus improve the release of drug [80]. Furthermore, the (w/o/w) performed under a mild conditions thus, minimize the risk of losing the bioactive agents [81].

In our study, EA have been chosen as organic solvent for PCL dissolving because it have a low deteriorative effect on the drug bioactivity [82]. Pluronic127 (F127) is a water-soluble and oil-soluble surfactant. It is an FDA approved and have been chosen as a stabilizing agent due to its nontoxic effect. The nanoparticles were prepared with two phases: the first phase was the formation of the water-in-oil (w/o) emulsion. In this phase insulin dispersed into the organic phase formed from the PCL dissolution in the ethyl acetate.

The second phase is pouring the first emulsion into an aqueous solution of F127 (0.1 %w/v) and thus resulting final emulsion water-in-oil-in-water (w/o/w) .Then the organic solvent removed under a reduced pressure.

4.1.1. PCL Nanoparticles Characterization

The morphology and the nanoparticles size were characterized using the scanning electron microscope (SEM) and the dynamic light scattering (DLS) technique. The SEM images reflect information about the nanoparticles topography. While DLS displays the hydrodynamic diameter and the size distribution profiles of the nanoparticles.

From the SEM images (Figure 4.1. (A and B)), it can be observed that PCL nanoparticles have a spherical shape with a smooth surface and this was expected as the PCL have a hydrophobic nature. The DLS measurement (Figure 4.2.), showed that the nanoparticles are with a mean diameter of about 335 ± 92 nm with a poly disperse size distribution.

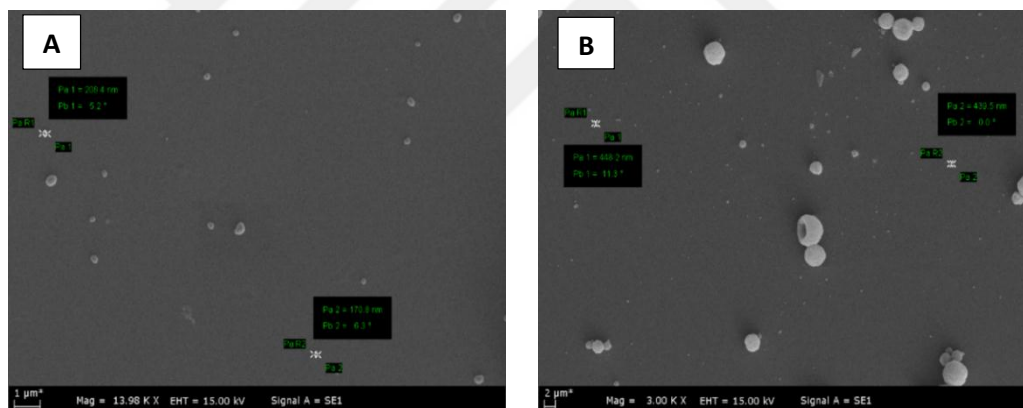


Figure 4.1. SEM images of PCL nanoparticles

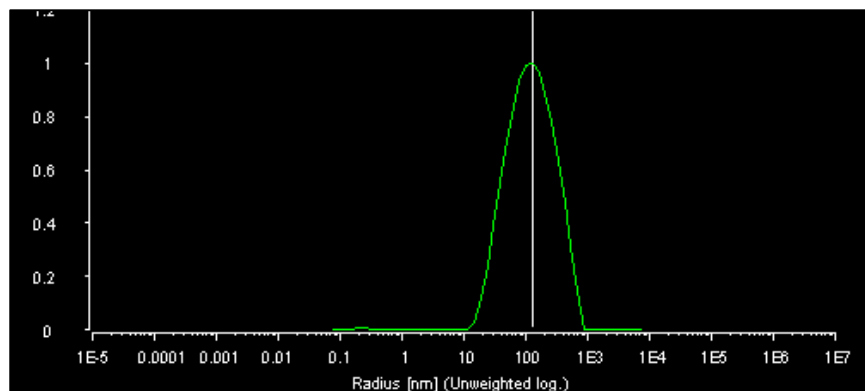


Figure 4.2. Dynamic light scattering image of PCL nanoparticles.

4.1.2. Encapsulation Efficiency and In vitro Release Behavior of PCL nanoparticles

The encapsulation efficiency of the nanoparticles was high, about $82 \pm 1.5\%$ of the total amount of the insulin successfully have been adsorbed into the nanoparticles. The high encapsulation efficiency attributed to the hydrophobic nature of the PCL polymer. The high hydrophobicity of the PCL which present an impediment to the drug passage from inside the nanoparticle matrix to the dissolution medium. Thus, a high amount of the drug can be retained inside the nanoparticle matrix which increase the nanoparticles encapsulation efficiency. The in-vitro behavior of insulin release of the nanoparticles in PBS (pH 7.4) at 37°C for 24 h, is illustrated in Figure 4.3. The insulin release from the PCL nanoparticles starts by an initial burst release then a constant release over the next 6 h and then a plateau of slow and incomplete release up to 24h.

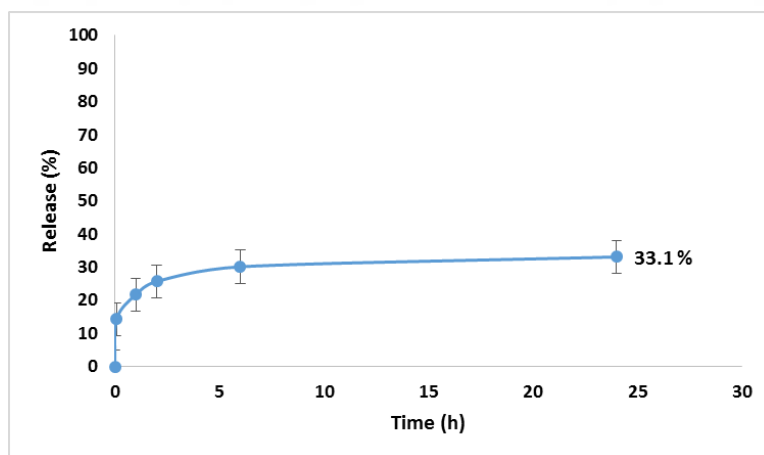


Figure 4.3. In-vitro insulin release profile of PCL nanoparticles for 24h at 37°C ($n = 3$, mean $\pm$ SD).

The burst release at the beginning of the release curve can be attributed to that, the hydrophilic insulin have a strong tendency to immigrate into the dissolution medium thus, accumulate at the nanoparticles surface and result in a significant burst release [83].

As it seen from the release curve, the nanoparticles released just about 33% of the encapsulated insulin within 24h. This low release profile of the PCL can be attributed to the PCL tight structure, which plays as a barrier avoids the sufficient drug diffusion into the dissolution medium.

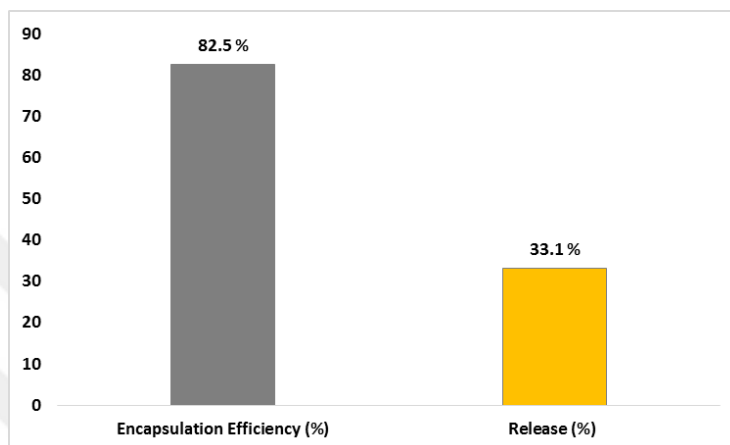


Figure 4.4. Encapsulation efficiency vs the Release percentage for the PCL nanoparticles (n = 3, mean $\pm$ SD).

As it noticed from Figure 4.4, the nanoparticles encapsulation was high whereas the release percentage of the nanoparticles was low. This is reasonable since the high hydrophobicity of the PCL results in a high encapsulation efficiency which means that a higher amounts of the drug retained inside the nanoparticle matrix and just a low amounts of the drug could diffused (released) into the dissolution medium. Hence, it is obvious that the hydrophobic nature of the PCL present a challenge that should be overcome to obtain the desired drug release profiles. The polymer blends is one of the effective techniques for tailoring the polymer properties without affecting its mechanical integrity. For example the water permeability properties and the degree of hydration of some hydrophobic polyesters have been enhanced by blending with a hydrophilic additives [84]. Thus blending a hydrophilic additives into the hydrophobic polymer can significantly modify the drug release behavior of the polymeric matrix [85].

Ma and Song.[60]examined the Poloxamer188 effect on the hydrophobic nanoparticles characteristics. The results showed that, blending Poloxamer188 with the nanoparticles affected the release of the drug.

In our study, the effect of blending the hydrophilic PluronicF127 (F127) with the PCL matrix to enhance the nanoparticles drug release behavior have been investigated.

4.2. F127 Blending into PCL matrix

In order to blend the F127 into PCL matrix, the nanoparticles were reconstructed by introducing the F127 into the organic phase within the PCL matrix, instead of the aqueous phase. As the ethyl acetate cannot dissolve the F127, it replaced with the organic solvent dichloromethane (DCM). DCM have the ability to dissolve a the majority of the biodegradable polymers and characterized by its low boiling point (39.8C^o) which make it easy to be removed by evaporation [82]. The PCL/F127 nanoparticles were fabricated based on double emulsion.

It well known that Pluronic have an amphiphilic structure which means that, it possess both hydrophilic and hydrophobic natures. Due to the hydrophobic Pluronic nature, it strongly get absorbed into the hydrophobic PCL surface. The absorption results in, a molecular dispersion in the PCL core and therefore, changing the smooth PCL surface into a rough one [59]. Then due to the hydrophilic poly ethylene oxide blocks in Pluronic structure, it will be leached out during the aqueous phase formation and solvent-removal leaving a porous structure in the surface of PCL nanoparticles. Thus incorporating Pluronic into the hydrophobic polymers act as pore-forming agent [60, 86]. It expected that the surface roughness and porosity degree will depend on the amount of the F127 have been blended into the polymer matrix.

In our study, a different formulation of PCL/F127 blend nanoparticles with different concentrations (0.1, 1 and 5 %, w/v) of F127 were prepared, to explore the varying F127 concentration impact on the nanoparticles morphology and insulin release behavior.

4.2.1. Effect of F127 concentration on the Nanoparticles Characteristics

The atomic force microscopy (AFM) have been used to study the effect of F127 on the nanoparticles morphology. AFM is a powerful tool that reflects information about the nanoparticles surface topography, through the adhesion between the device tip and the measured surface. AFM measurements were taken to investigate the effects of different concentrations of F127 on the nanoparticles morphology. By looking at the topography and the line profile of the nanoparticles, depicted in the AFM images (Figure 4.5, 4.7 and 4.9) we can take an idea about the topography of the nanoparticles.

The AFM images showed that all the nanoparticles formulation have a spherical shape. From the AFM image depicted in Figure 4.5, it have been noticed that the nanoparticle's surface seems to be smooth. Thus, low concentration 0.1 % F127 did not significantly affect the surface morphology. When the concentration of F127 increased to 0.5 % (Figure 4.7), the porosity degree and the surface roughness as well as the sizes of the nanoparticles increased slightly. In contrast, when the F127 concentration increased highly up to 5%, the degree of the porosity and the surface roughness of the nanoparticles increased clearly. Also, the nanoparticles sizes increased significantly from a nanometers up to about a micro meter (Figure 4.9).

From the AFM images also it can be noticeable that, not only the size but also the polydisparsity of nanoparticles increased as the F127 concentration increased. This have been verified also using the DLS measurements, from the un-weighted distribution of the nanoparticles in the DLS measurements at an angle of 90° (Figures 4.6, 4.8 and 4.10), it was obvious that the nanoparticles size getting bigger and the size distribution of the nanoparticles getting wider when the F127 concentration increased. These results were compatible with the results gained by Türk et al. [59] which studied the effect of a different Pluronic types with different concentrations on the PLGA nanoparticles properties. They stated that, both the sizes and polydispersity index of the nanoparticles increased when the Pluronics introduced into the PLGA nanoparticles. Thus, there is a direct proportional of the Pluronic concentration with the porosity degrees and the sizes of the nanoparticles. The mean size of the different PCL/F127 blend formulations were listed in Table 4.1.

Table 4.1. The mean size of PCL/F127 nanoparticles blend with different F127 concentrations.

F127 concentration (w/v %)	0.1	0.5	5
Mean Sizes(nm)	275.6 ± 13.1	550.2 ± 70.6	1002.9 ± 156.5

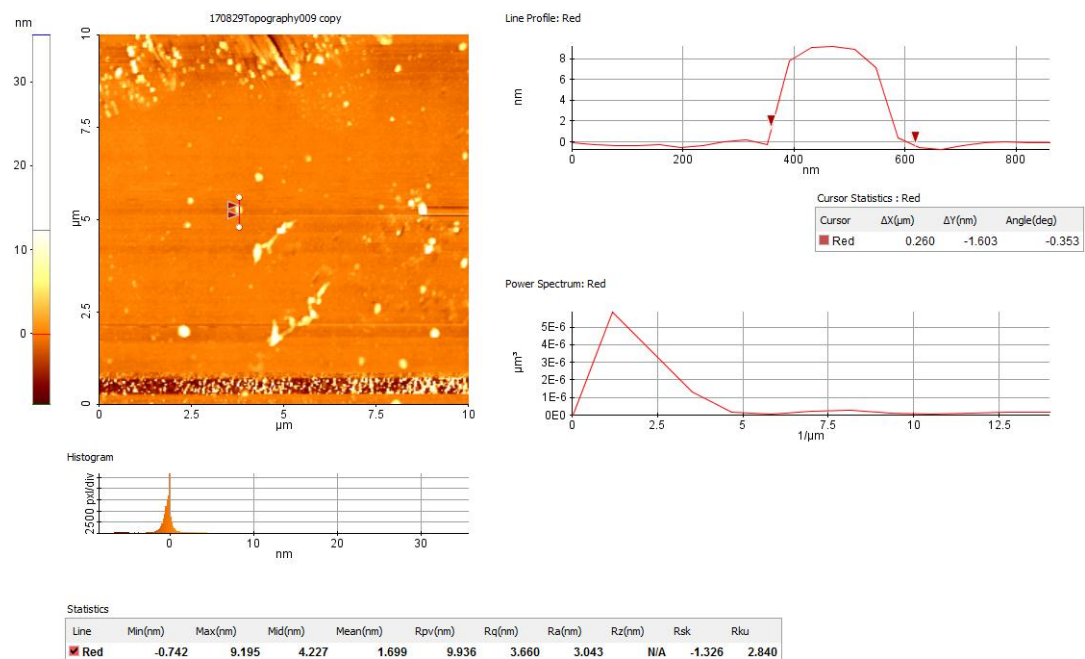


Figure 4.5. The PCL core blended with (0.1 % w/v) of F127.

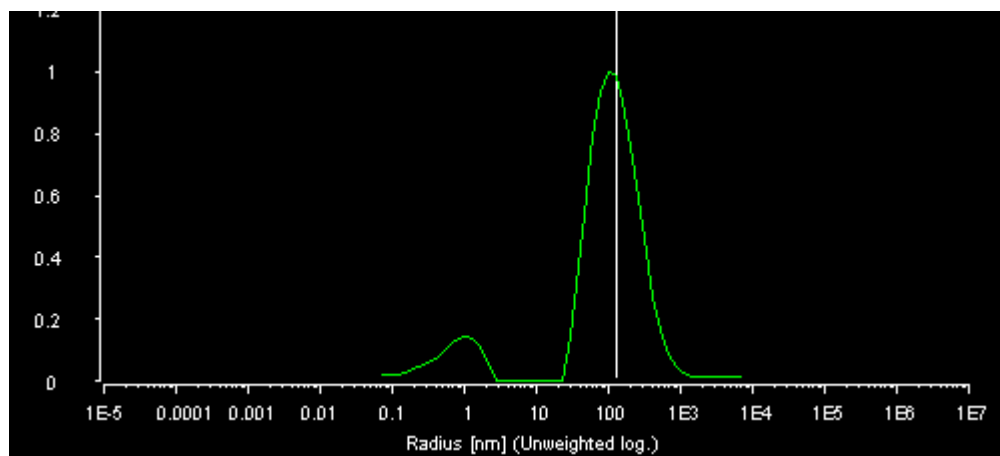


Figure 4.6. Dynamic light of PCL/F127 blend with (0.1 %) of F127.

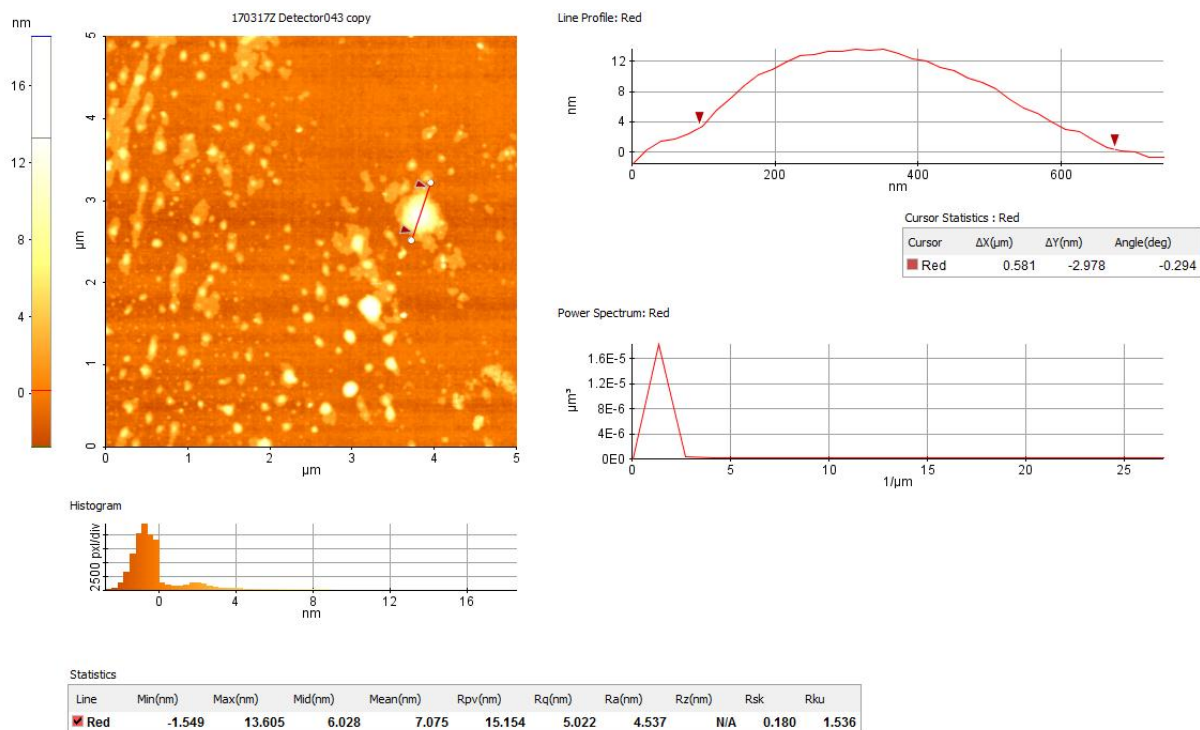


Figure 4.7. The PCL core blended with (0.5 % w/v) of F127.

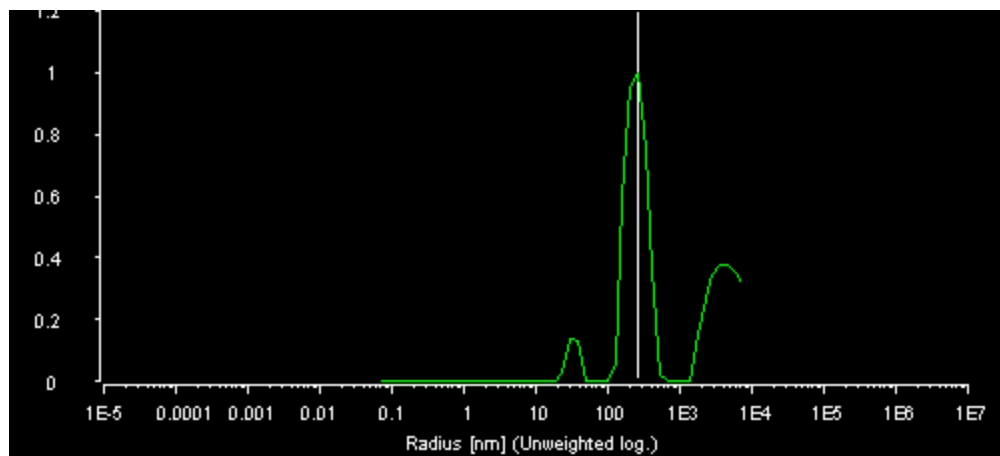


Figure 4.8. Dynamic light of PCL/F127 blend with (0.5 %) of F127

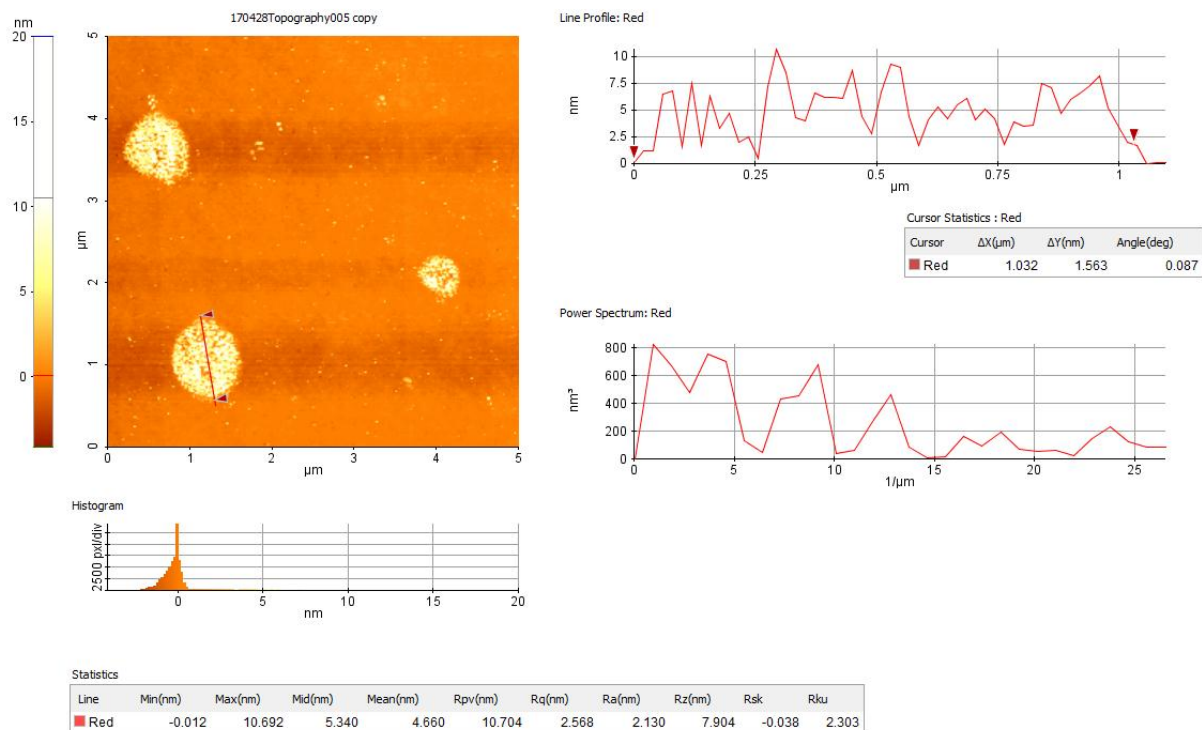


Figure 4.9. The PCL core blended with (5 % w/v) of F127.

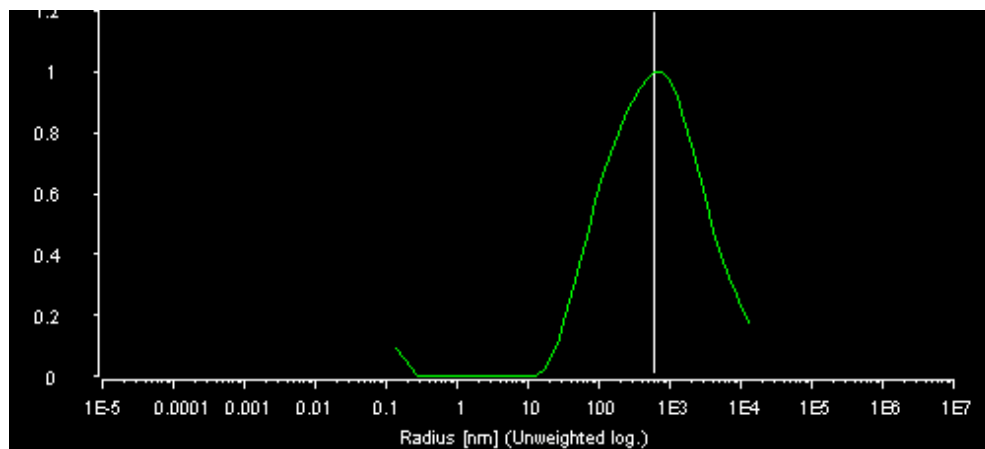


Figure 4.10. Dynamic light of PCL/F127 blend with (5 %) of F127.

4.2.2. Effect of F127 Concentration on Encapsulation Efficiency and the In-vitro Insulin Release Behavior of the Nanoparticles

There was an inverse relationship between the blended F127 concentrations and the encapsulation efficiency of the nanoparticles (see Figure 4.11). This can be explained by that, as the F127 concentration increased the porosity (i.e., space) formed on the nanoparticle's surface increased. This leading to a deficiency in polymer capability to encapsulate the drug, especially for the hydrophilic drugs like, insulin which have a high tendency to migrate to the aqueous phase. Generally, loss of drugs to aqueous phase is more likely to occur in the hydrophilic drugs in comparison with hydrophobic drugs [87].

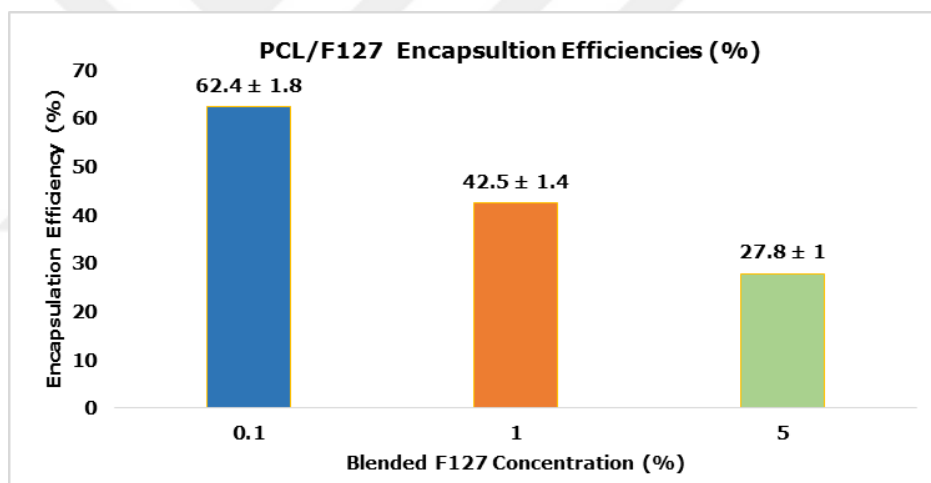


Figure 4.11. Encapsulation Efficiencies (%) of the different PCL/F127 nanoparticles formulations (mean $\pm$ SD, n = 3).

That means the nanoparticles ability to encapsulate a high amounts of drug decreased when the F127 concentration increased and vice versa. This is obvious by looking to the insulin amounts have that been encapsulated in each of the PCL/F127 formulation (see Figure 4.12), taking into account, the same amount of insulin (3.5 mg) were added at the beginning of the preparation of each nanoparticle formulations.

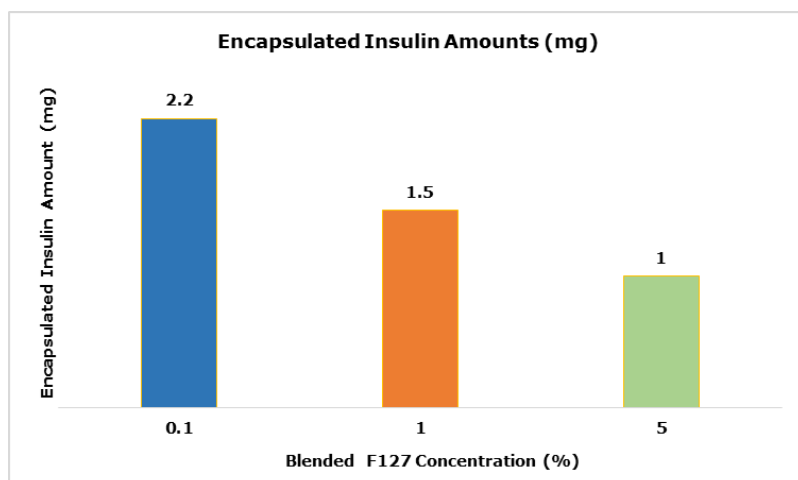


Figure 4.12. The encapsulated amounts of insulin (mg) in each of the PCL/F127 nanoparticles.

The insulin release behavior for the PCL/F127 nanoparticles blended with a different F127 concentrations (0.1, 1 and 5%, w/v) were observed in in-vitro in PBS (pH 7.4) at 37°C for a time period of 120 h. The release profiles of the different PCL/F127 nanoparticles formulations showed that, when the PCL nanoparticles blended with 0.1 % of F127 the nanoparticles released about 45% of the encapsulated insulin. Further increasing the blended F127 concentration to 1% increased the released insulin amount to about 80%. A highly increasing of the F127 concentration up to 5% significantly raised the percentage of insulin release up to 96%, (Figure 4.13). However, for the objective of this research which is to investigate insulin oral delivery all the presented insulin release profiles limited just to the first 24 h, taking into account the intestinal residence time, (Figure 4.14).

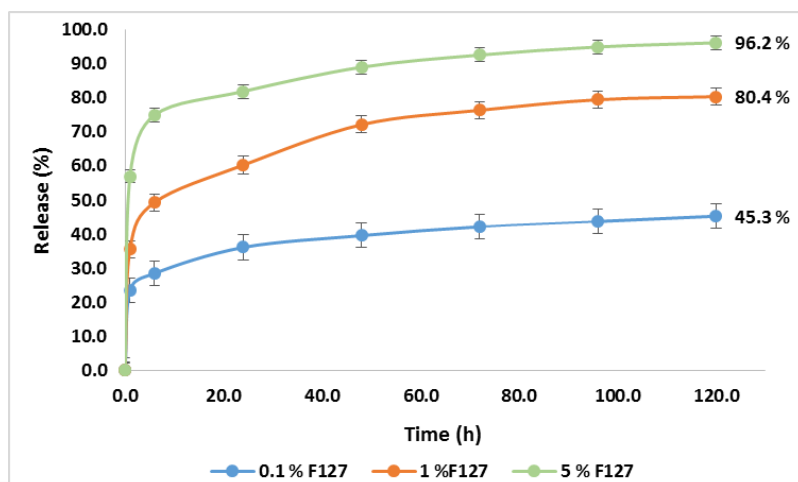


Figure 4.13. Insulin Release from the PCL/F127 nanoparticles with different F127 concentrations for 120 h (n = 3, mean $\pm$ SD).

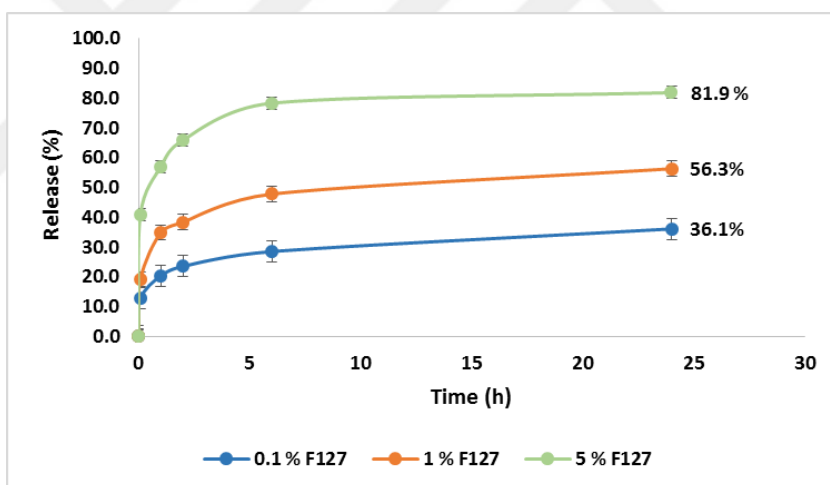


Figure 4.14. Insulin Release from the PCL/F127 nanoparticles with different F127 concentrations within 24 h (n = 3, mean $\pm$ SD).

These results implying, a direct proportionality between the released amounts of the insulin from the PCL/F127 nanoparticles with the blended F127 concentrations. As mentioned earlier the nanoparticles degree of porosity increased as the blended F127 increased. The porous structure allow the diffusion of release medium into the PCL matrix to dissolve the drug and thus facilitate the insulin diffusion from PCL matrix.

When a low concentration of F127 blended into the PCL matrix, just a few and isolated pores formed on the nanoparticles surface this results in a few and isolated interconnected pathways. Therefore, the drug diffusion through the matrix will be difficult. This explains why nanoparticles release just a slight amount of the encapsulated insulin in the case of PCL/0.1 F127. On the contrary, increasing the F127 concentration increased the porous structure which results in forming more interconnecting pathways in the nanoparticles matrices so, it will be more "filled in" with the release medium which facilitate the drug release and this explain the high release percentage of the PCL nanoparticles blend with %5 of F127. Thus the F127 could be used as drug release enhancer agent. These results were consistent with the previous studies by Ma and Song. [60], which suggested that Pluronic blend into the polymer nanoparticle structure create porosity in the nanoparticles surface and enhance the drug-release behavior. It was noticed that, all the nanoparticles formulations release behaviors characterized by an initial burst release followed by a fast sustained release over the next 6 h and then a plateau up to 24h. As previously mentioned the initial burst release can be attributed to the tendency of insulin to concentrate on the nanoparticle surface.

Although the PCL/0.1% F127 nanoparticles encapsulated about 60% of the insulin it had the ability to release just a small percentage of it (about 36%). In the other hand the PCL/5% F127 nanoparticles had the lowest encapsulation efficiency (about 27 %) released about 81% of the encapsulated insulin. Hence, there is a reverse relationship between the encapsulation efficiency of the nanoparticles and their associated release percentages, (Figure 4.15).

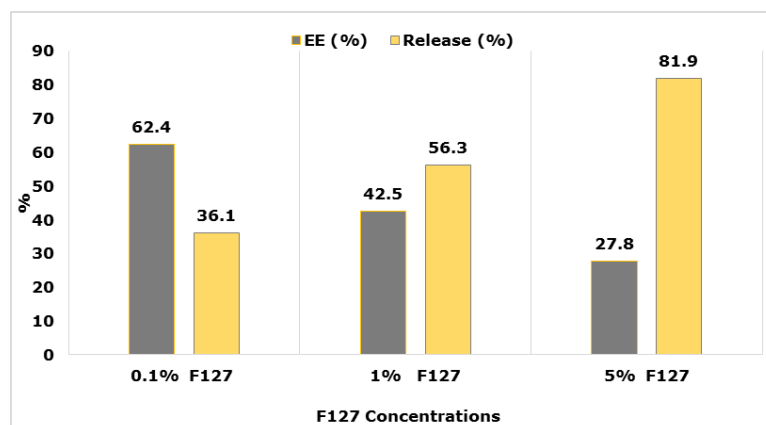


Figure 4.15. Insulin released percentages vs the nanoparticles encapsulation efficiencies.

This can be explained by that, the high porosity structure like in the case of PCL/ 5 %F127 created many interconnecting paths which allowed the release of a high amount of the drug. In contrast with the PCL/0.1 % F127 which have a tight structure that hinder the release of the drug, thus a higher amount of the drug retained inside the nanoparticles (higher encapsulation efficiency).

Figure 4.16 demonstrate, the encapsulated amount of insulin (mg) versus the amounts of insulin (mg) delivered by the different PCL/F127 nanoparticles formulations within the 24h.

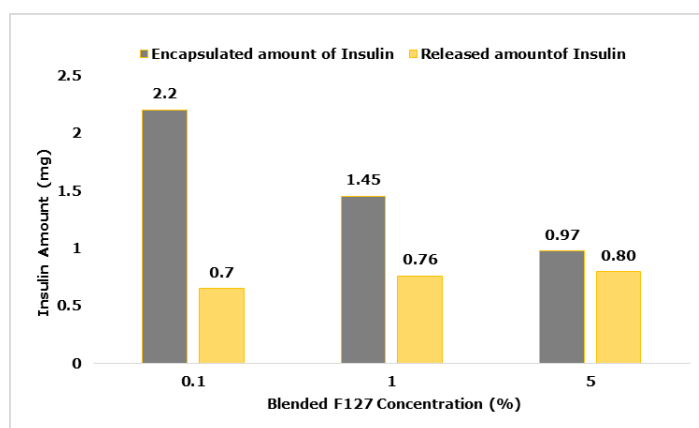


Figure 4.16. Encapsulated amounts versus the released amounts of insulin for 24h, for each of the PCL/F127 nanoparticles formulations.

The PCL nanoparticles blended with 0.1% F127 encapsulate the highest amount of insulin was loaded about 2.2mg while delivered just about 0.70 mg within 24 h, which present just a small division of the encapsulated amount of insulin. While the PCL/ %5 F127 nanoparticles encapsulated just about 1 mg of the insulin and delivered about 0.8 mg within the same period, which present a bigger division of the encapsulated drug amount. Hence, it is noticeable that the released amount of insulin was almost the same.

Since the insulin solubility was higher than the dissolution media concentration, theoretically it was expected that the diffusion will be the main driving force for the release. That true but there is another thing to put in mind which the nanoparticles "porous" structure. Thus it was expected that, PCL/ 0.1% F127 nanoparticles will release the highest amount of insulin under the diffusion influence but that wasn't the case because the diffusional force compensated by the tight and condense structure of these nanoparticles which was a barrier in the way of a sufficient drug release. In contrary, the PCL/ %5 F127 nanoparticles encapsulated just about 1 mg of the insulin and was able to release a bigger division of it about 0.8 mg, this can be attributed to their looser structure which allowed the drug to be released more freely through the porous structure of the nanoparticles and compensated the weak effect of diffusion.

Thus we can say that, the blended F127 concentration negatively affect the drug retaining capability of the nanoparticles. This is reasonable and compatible with the previous discussion about the blended F127 concentration effect on the PCL matrix porosity, which influence the release media penetration into the matrix, which in turn affect the dissolution of the drug into the release media and thus affecting the drug release by diffusion.

Thus, the required insulin dosage can be controlled easily by varying the loaded drug concentrations and more importantly choosing the appropriate F127 concentrations to obtain the desired release profiles.

4.2.3. Characterization of In-vitro Insulin Release Kinetics of PCL/F127 Nanoparticles

In this section the insulin release kinetics for the three PCL/F127 nanoparticles formulations will be discussed based on four different kinetics models (the kinetics models were previously expressed details in chapter two). Zero order model plotted as the amount of insulin released versus time (Figure 4.17 (a), (b) and (c)). First order model, plotted as the released insulin natural logarithm versus time (Figure 4.17 (d), (e) and (f)). Higuchi model, plotted as the released amount of insulin versus the time square root (Figure 4.17 (g), (h) and (i)). To explain the mechanism of drug release according to Korsmeyer–Peppas model, only first 60% of the in-vitro release values were chose and plotted as the natural logarithm of the released amount of insulin versus natural logarithm of time (Figure 4.17 (j), (k) and (l)).

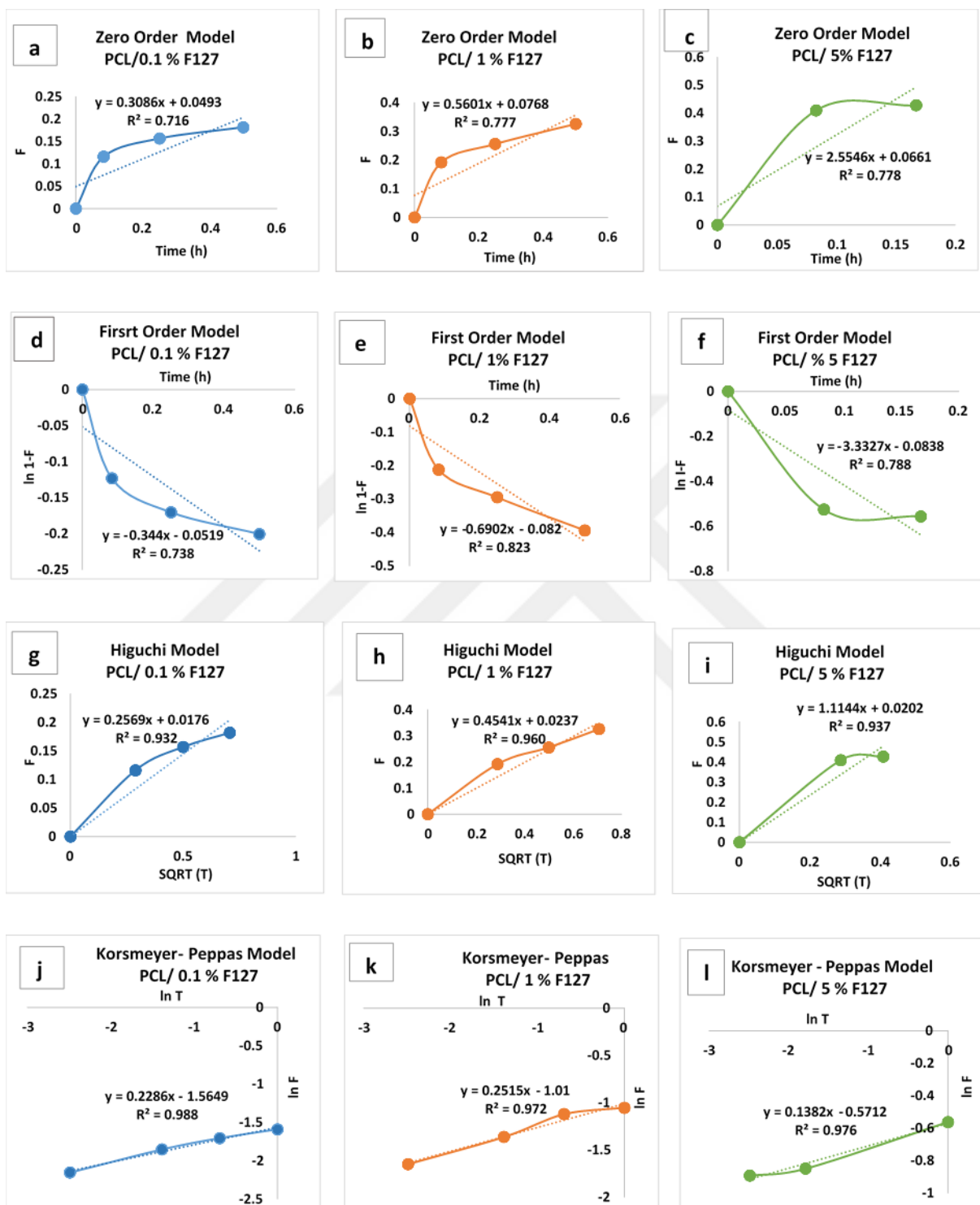


Figure 4.17. Different kinetics models of PCL nanoparticles blended with different F127 concentrations.

The suitable model for describing the drug release mechanism of the nanoparticles is determined according to the regression coefficient value (R^2). All the models parameters for the three PCL/F127 nanoparticles formulations have been presented in the following Table:

Table 4.2: Summary of kinetic models values of the PCL/ F127 nanoparticles with different F127 concentrations.

Formulations	Kinetic Models Parameters						
	Zero Order	First Order	Higuchi	Korsmeyer- Peppas			
	R^2	R^2	R^2	R^2	n	K_{KP}	MDT (h)
PCL/ 0.1 % F127	0.716	0.738	0.932	0.988	0.229	0.21	169.8
PCL/ 1 % F127	0.777	0.823	0.960	0.972	0.251	0.36	11.7
PCL/ 5 % F127	0.778	0.788	0.937	0.976	0.138	0.56	8.1

Due to the regression values (R^2) shown in the Table above, the Korsmeyer-Peppas model own the highest R^2 value followed by the Higuchi model with the second highest value of R^2 . Thus it is concluded that, Korsmeyer-Peppas model is the most fitting model to describe the release behavior of all PCL/F127 nanoparticles formulations. The diffusional exponent of the Korsmeyer- Peppas model 'n' characterize the drug release mechanism. Here as depicted in the Table 4.2, the 'n' values for all the PCL/F127 nanoparticles formulations were less than 0.43 which indicating that, the drug release mechanism following the Fickian law of diffusion. That means the concentration gradient is the driving force for the insulin release.

From the release rate constant values (K_{KP}) summarized in the Table it is clear that, the release rate constant increased proportionally with the F127 concentrations. This supports the results obtained from the in vitro release experiments which indicated that increasing the F127 concentration increases the release rate of the nanoparticles.

The mean dissolution time (MDT) is a term used to describe the average time in which the drug gets dissolved thus, reflects the drug retaining capability of the nanoparticles [69]. Higher MDT corresponds to higher drug retaining capability. As presented in Table 4.2, MDT value was the highest for PCL/0.1% F127 reflecting its higher drug retaining ability, followed by PCL/1 % F127 and the lowest MDT for PCL/5 % F127 which reflected a low drug retaining ability. This is completely consistent with results mentioned in the previous section. Thus all the results obtained by the kinetics analysis are supporting and proving the in vitro results.

From the results obtained at this part, it is clear that the PCL matrix physiochemical properties such as size, encapsulation efficiency and the release behavior can be adjusted by blending with the F127. Thus, F127 not only can be used as stabilizing agent to stabilize the nanoparticles during the preparation process, but also it can be used as both a pore-forming agent and thus an enhancer for the release of drug.

The PCL/F127 nanoparticles blended with (0.1 %, w/v) of F127 were selected to form the core of our system- due to its good characteristics (encapsulation efficiency and mean size) - and all the further steps were built based on it.

4.3. Coating PCL Nanoparticles with Chitosan and Alginate Layers

In order to build an effective system for the oral delivery of the insulin, insulin should be protected from the harsh pH changes through GIT from acidic (pH 1.2) in stomach to neutral (pH 7.4) in the intestine. Thus, the insulin oral delivery systems should possess pH sensitive properties to efficiently deliver encapsulated insulin. To construct our pH sensitive oral insulin delivery system, layers of the natural polymers chitosan (CS) and alginate (ALG) have been added onto the PCL nanoparticles surface.

Due to its preferred properties in the oral drug delivery systems alginate have been chosen to support our system with the desired pH sensitivity properties. However to bind the anionic alginate to the PCL core a cationic layer were needed, so chitosan have been chosen to support the system with the desired positive charge. Chitosan addition not only provide system with the positive surface charge needed but also would added a mucoadhesive properties and enhance the absorption of the insulin by the intestine. Thus our system possess a combination of the unique physicochemical properties of these polymers.

In our study the pH sensitive layer prepared by ionotropic pre-gelation of the alginate with calcium chloride, followed by electrostatic interaction with the cationic chitosan- interactions between the negative carboxylic groups of alginate with the positive amine group of chitosan [88]. One of the most important advantages of this process is that it performed under a mild conditions which protect the encapsulated insulin from the denaturation. Also, the electrostatic interaction between the ALG and CS layers effect avoids the need to use chemical cross-linkers, which reduces the toxicity and other harmful effects that may be caused by the chemical agents so its help to reach a safer physiological system[89].

The PCL/CS/ALG system construction pass through two main stages: first stage was adding chitosan into the outer layer of the PCL nanoparticles. Then adding the formed nanoparticles into the calcium alginate mixture. These two stages and the effect of both chitosan and alginate layers in the nanoparticle's characteristics will be discussed in details.

4.3.1. Chitosan Layer Addition

As mentioned previously adding chitosan not only provide the positive surface to create the electrostatic interaction with the alginate but also facilitate passing of the nanoparticles through tight junction of epithelial cells and extend the drug resent time in the intestine. Moreover, after the oral digestion of the nanoparticles, CS digested by chitosanase enzymes which secreted in the intestine [50].

The CS concentration had been chosen based on the capability to supply the nanoparticle's surface with the positive charge needed to create the electrostatic interaction with the alginate layer. Four CS concentrations of (0.1, 0.2, 0.4 and 0.8, mg/ml) were examined and CS concentration to be used have been chosen according to the created (desired) surface charge over the particle. Zeta potential is a main key to identify surface charge of the nanoparticles.

Zeta potential of the nanoparticles prepared with the different CS concentrations, were measured and the obtained values are demonstrated in Figure 4.18. It obvious that zeta potential of the nanoparticles increased gradually as the CS concentration in the nanoparticles increased.

The CS concentration of (0.8 mg/ml) have been chosen as it is supply the nanoparticles with the positive charge of (+22.8 mV) which could be enough to provide a stable nanoparticles.

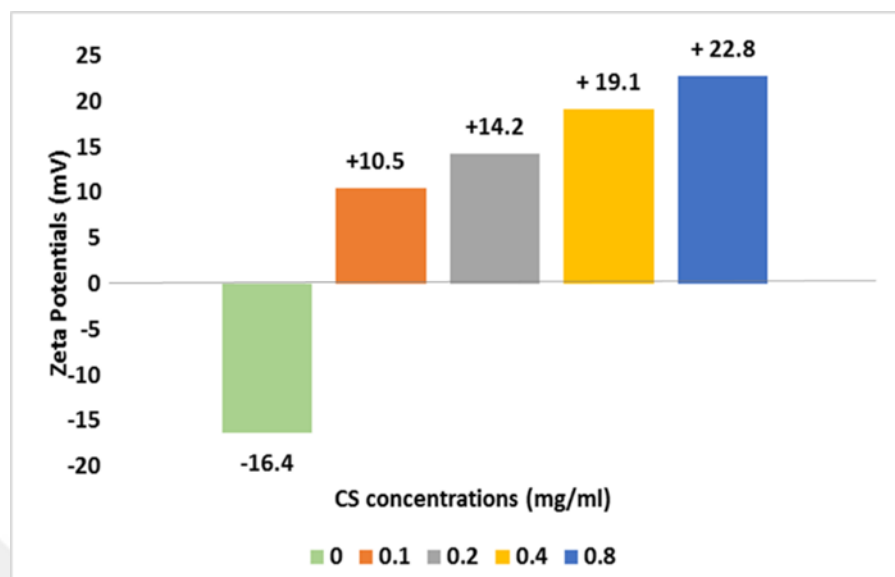


Figure 4.18. The different zeta potentials obtained using different CS concentrations.

4.3.1.1. CS Effect on Nanoparticles Characteristics

From Figure 4.18, it was noticed that before adding the CS layer, the PCL nanoparticles had negative zeta potential of about -16 mV, which may be attributed to the negative charge of the insulin. After CS introduction, the nanoparticles showed a positive zeta potential of about +23 mV. This positive inversion in the zeta potential is attributed to the amino groups in the CS structure which, indicates that PCL nanoparticles were effectively coated by CS layer. The CS layer introduction increased the zeta potential of the nanoparticles thus results in a more stable nanoparticles. PCL/CS particles were characterized by the optic microscope (OM). From OM photographs (Figure 4.19.), it noticed that PCL/CS particles have a spherical shapes with uniform size distribution. The size distribution determination from optical microscopy images using ImageJ software showed that, the mean size of PCL/CS particles were in a range of 1-20 μm with a mean size of 10 μm .

Thus, PCL nanoparticles sizes increased significantly from a nanometers ranges up to the micro meters ranges upon the introduction of the CS. This also can be an indication of the successfully coating of PCL nanoparticles surface with the CS layer.

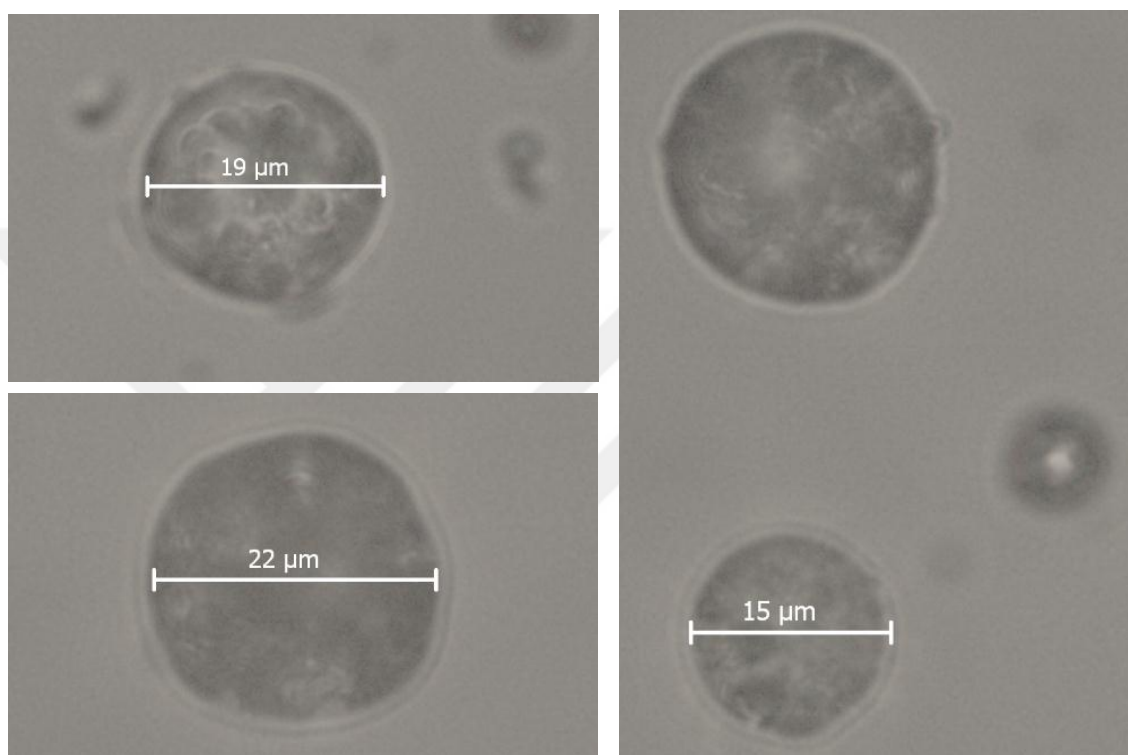


Figure 4.19. OM images of the (PCL/CS) particles.

Thus both the particles sizes and the zeta potential obtained implies that CS have been introduced successfully on the PCL nanoparticles surface.

4.3.1.2. CS Effect on Encapsulation Efficiency and In-vitro Release Behavior of the Particles

Introducing the CS into the PCL nanoparticles structure alters the hydration dynamics of PCL which affected the encapsulation efficiency of the particles. As depicted in Figure 4.20, the encapsulation efficiency of the particles decreased to about 48.2 % upon introducing the CS layer into the nanoparticles structure.

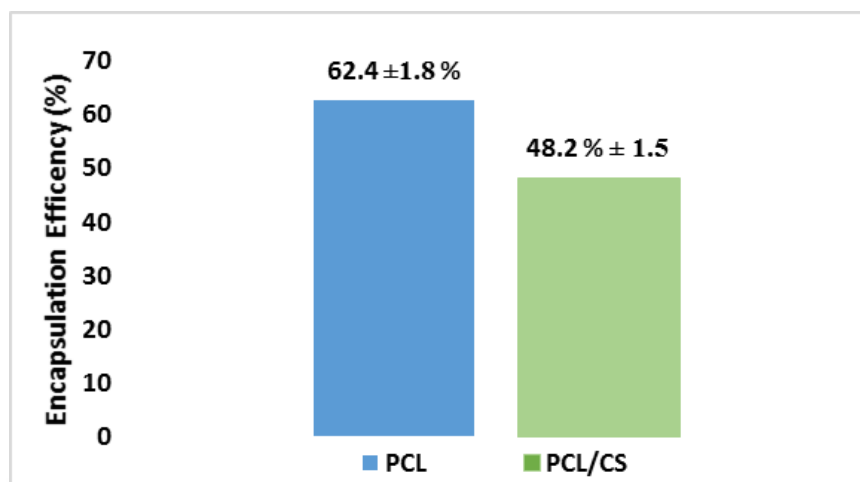


Figure 4.20. Effect of introducing CS on the encapsulation efficiency of the PCL nanoparticles (n = 3, mean ± SD).

The decrease of encapsulation efficiency attributed to the positively charged CS which absorbs some of the negatively charged insulin to the PCL nanoparticles surface during preparation process, therefore leads to leakage of insulin to the external medium. This result was in agreement with the results obtained by Wang et al. [90] which noted that, coating PLGA nanoparticles with chitosan significantly affected the encapsulation efficiency of the nanoparticles.

The in-vitro insulin release profiles of both the PCL nanoparticles and the PCL nanoparticles after the CS layer addition (PCL/CS) were studied in pH 7.4 at 37C° for 24h, to investigate the CS layer addition on the PCL nanoparticles release behavior. The obtained release profiles are depicted in Figure 4.21

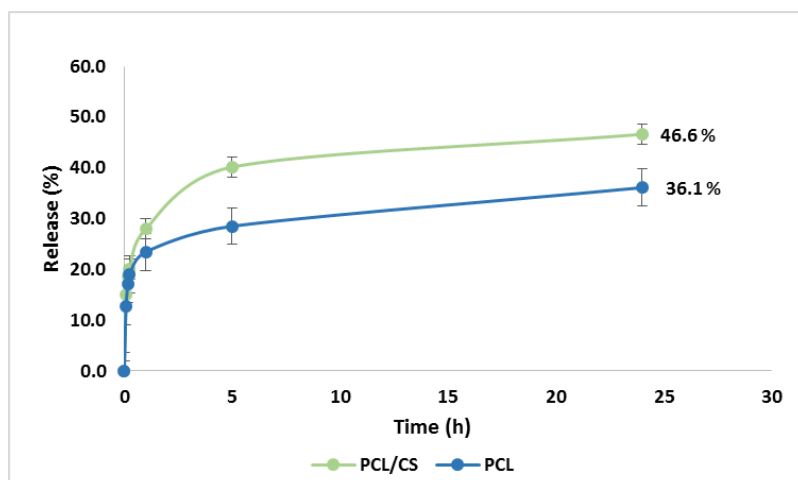


Figure 4.21. Insulin release behavior of PCL and PCL/CS particles (n = 3, mean $\pm$ SD).

As it noticed from the release curves, the chitosan addition increased the release rate of the particles. This attributed to the hydrophilic nature of CS which alters the hydration dynamic of the PCL matrix hence, facilitate the dissolution media penetration into the PCL matrix which in turn, increased the insulin releasing rate. Furthermore, the positive charge of CS on the surface of nanoparticles attract the negatively charged insulin toward the surface thus accelerate the insulin release rate. This result was consistent also with results obtained by Wang et al. [90] and Khanal et al. [91] which investigated the effect of CS on the release rate of PLGA nanoparticles and stated that, modifying the PLGA nanoparticles with CS addition resulted in a higher drug release rates than PLGA nanoparticles alone.

Both PCL and PCL/CS particles were characterized by a first burst release and then a sustained release up to 24. The burst release behavior of the PCL/CS particles was higher than the PCL nanoparticles, this is also attributed to the positive surface charge of CS which strongly attract the insulin into the nanoparticle surface and thus result in a higher burst release [91].

4.3.1.3. Effect of the pH on the Insulin Release behavior of PCL/CS Particles

To study the pH changes effect on the insulin release behavior of PCL/CS particles, the in-vitro release experiment was carried out in two different fluids with a different pH values. A gastric simulated fluid GSF with (pH1.2) and intestinal simulated fluid ISF with (pH 7.4) at 37°C for 24h. The obtained in vitro release profiles are depicted in Figure 4.22.

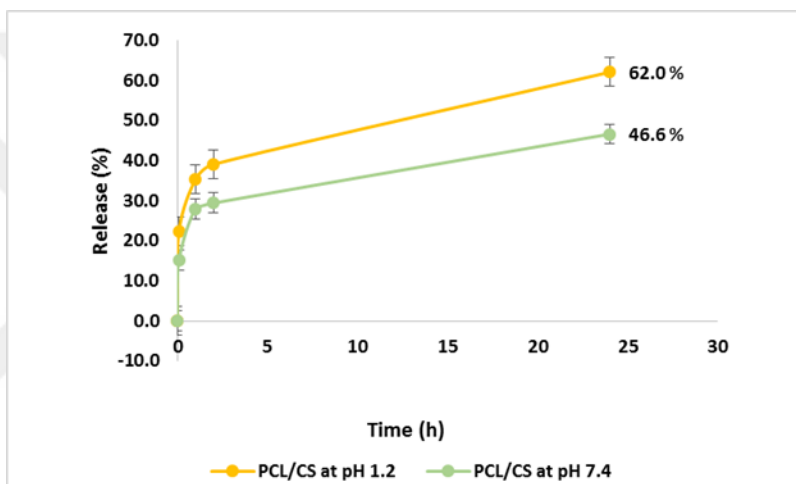


Figure 4.22. Effect of different pH on insulin release from PCL/CS particles (n = 3, mean $\pm$ SD).

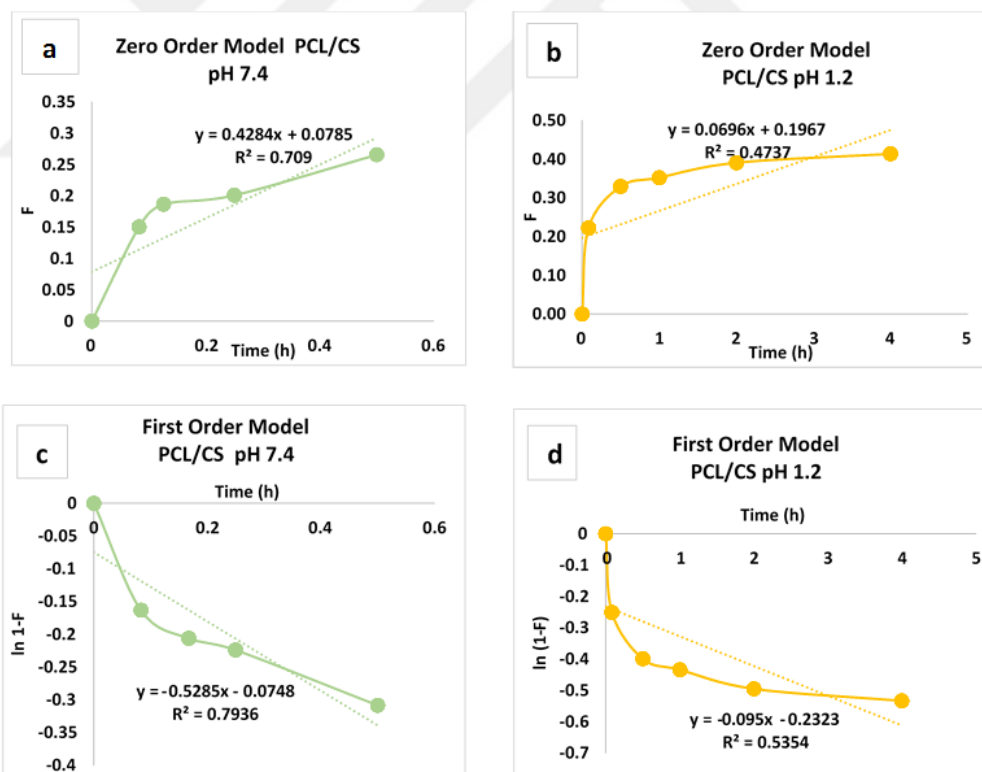
It shows two phases of release, an initial burst release followed by a continued release. At intestinal simulated environment (pH 7.4) only about 46 % of the encapsulated insulin released, while almost more than the half of the insulin amount is retained inside the particles. On the contrary at gastric simulated condition (pH 1.2) the PCL/CS particles released about 62 % of the encapsulated insulin amount. This behavior of the particles can be explained by the following:

At higher pH, the amine group of CS becomes deprotonated and uncharged, thus increase the CS layer viscosity and create an insoluble hydrogel networks [91].

While at lower pH, the $[\text{NH}_3]$ group of CS neutralized by OH^- to form NH_2 , which decrease the repulsion force between the positive charges in the chitosan. Thus, CS layer solubility increased on the acidic conditions [92].

4.3.1.4. Characterization of In-vitro Insulin Release Kinetics of PCL/CS Particles

The release kinetics for the PCL/CS particles at different pH values (7.4 and 1.2) were fitted to the different kinetics models. Zero order model (Figure 4.23 (a) and (b)), first order model (Figure 4.23 (c) and (d)), Higuchi model (Figure 4.23 (e) and (f)) and Korsmeyer–Peppas model (Figure 4.23 (g) and (h)).



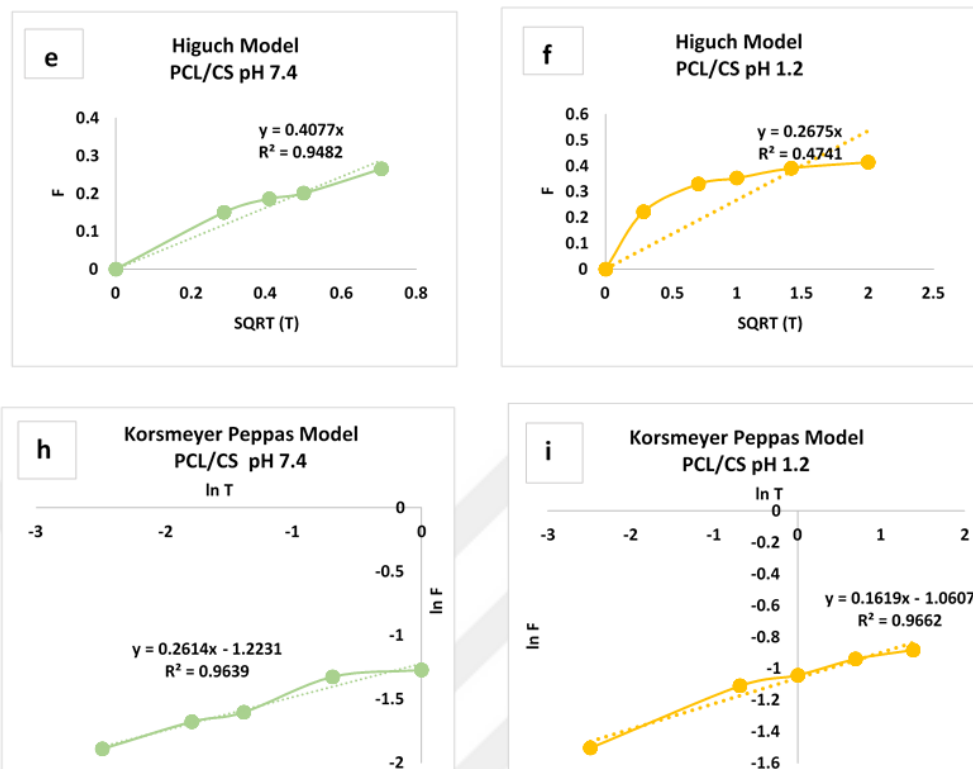


Figure 4.23. The different kinetics models for PCL/CS particles at different pH values (7.4 and 1.2).

The regression coefficients (R^2) and all the models parameters for PCL/CS particles at different pH values are presented in Table 4.3.

Table 4.3. Summarize the regression coefficients (R^2) and model parameters for PCL/CS particles at different pH values

Formulations	Kinetic Models Parameters					
	Zero Order	First Order	Higuchi	Korsmeyer- Peppas		
	R^2	R^2	R^2	R^2	n	K_{KP}
PCL/ CS pH 7.4	0.709	0.794	0.948	0.964	0.261	0.30
PCL/ CS pH 1.2	0.473	0.535	0.474	0.966	0.162	0.35

From the regression values (R^2) summarized in the Table above, the Korsmeyer- Peppas model is the most fitting model to describe the insulin release from the PCL/CS particles in both of the pH environments (7.4 and 1.2). The diffusion exponentials (n) in the both cases were less than 0.43 that means the insulin release mechanism of the PCL/CS particles is obeying the Fickian law of diffusion. It is obvious that, the release rate constants (k_{KP}) for the PCL/CS particles at higher pH (pH7.4) was lower than the release rate constant at pH (pH1.2). These kinetics results are compatible with the in vitro release experiments results which indicated that the release behavior of PCL/CS particles (due to amine group of CS) affected by the pH value. When the PCL/CS particles placed into the lower pH environment, the particles released a higher amount of the encapsulated insulin. Whereas at a higher pH environment the particles were able to release just a small division of the encapsulated insulin.

4.3.2. Alginate Layer Addition

ALG have been chosen due to its preferred pH sensitive properties. It shrinks in the acidic environments, protecting the drug from degradation in the harsh stomach environment. On the contrary, ALG becomes more soluble under the neutral conditions which maintain a sufficient and sustained insulin release in the intestinal environment [50]. To understand the alginate advantages for the oral insulin delivery system, the effect of adding the alginate layer on the particle's characteristics was studied.

According to the previous studies [35, 50] the optimal mass ratio of [chitosan: sodium alginate: calcium chloride] to create a sufficient interaction was about [3.2:30:6.7]. It has been proven that this ratio maintained calcium alginate in the pre-gel phase and ensured the presence of a sufficient amount of the cationic polymer to create a sufficient electrostatic interaction.

4.3.2.1. Alginate Effect on Particles characteristics

The zeta potential presents the degree of the electrostatic repulsive forces between the particles. High (negative or positive) zeta potential reflects the tendency of the particles to repel from each other; thus, the particles seem to have a low tendency to aggregate. On the contrary, low zeta magnitude indicates the tendency of the particles to aggregate. Thus, zeta potential is an indication of the particle's stability [58]. The diagram depicted in Figure 4.24, indicates the zeta potential changes during the preparation of the different particles formulations.

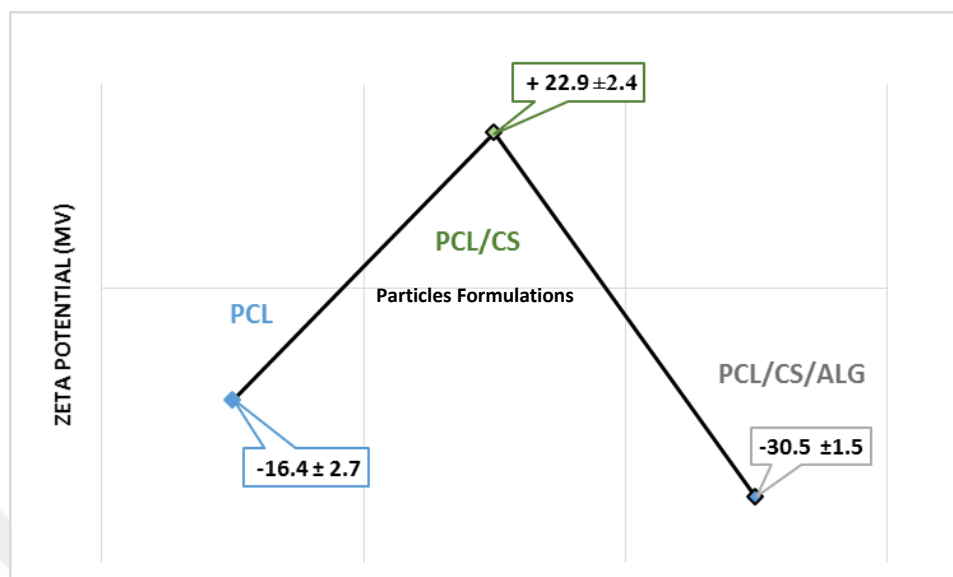


Figure 4.24. Zeta potentials of all the particles formulations ($n = 3$, mean $\pm$ SD).

As noticed from the diagram, the formulations prepared with PCL alone showed a quite negative zeta potential value of about -16 mV which attributed to the negative charge of the insulin. The positively high inversion in the zeta potential value up to about +23 mV, occurred when the CS layer introduced into the PCL nanoparticles. Thus the CS addition supports the system with the positive charge needed to create an electrostatic interaction with the ALG layer. A high negative inversion in the zeta potential magnitude from about +23 mV to about -30 mV were noticed when the PCL/CS particles surface coated with ALG. This attributed to the negative charge of carboxyl groups in the alginate structure. Thus from the zeta potential values it can be understood that, adding the CS and ALG layers into the PCL nanoparticles formulation increased the overall stability of the system.

As depicted from the optic microscope (OM) and fluorescent microscope (FM) photographs (Figure 4.25. and 4.26. respectively), PCL/CS/ALG particles have a spherical shape. The size distribution determination from optical microscopy images using ImageJ software showed that, the particles have a wide size distribution range from (20-150) μm with a mean size of about 50 μm . PCL/CS/ALG sizes were larger than PCL/CS particles, this can be also an indication of the effective coating of the PCL/CS particles by alginate layer.

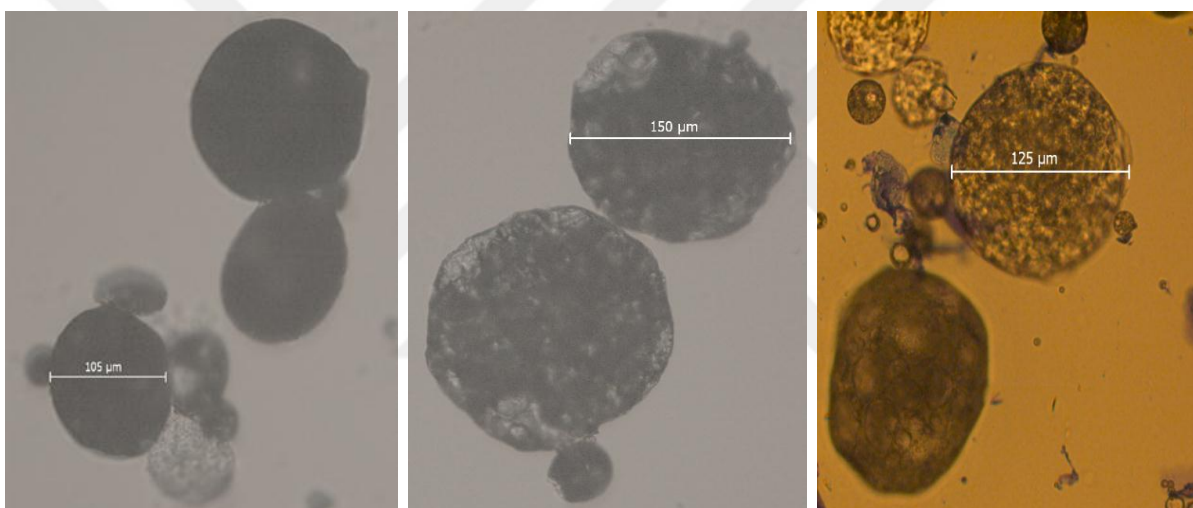


Figure 4.25. OM images of the PCL/CS/ALG particles.

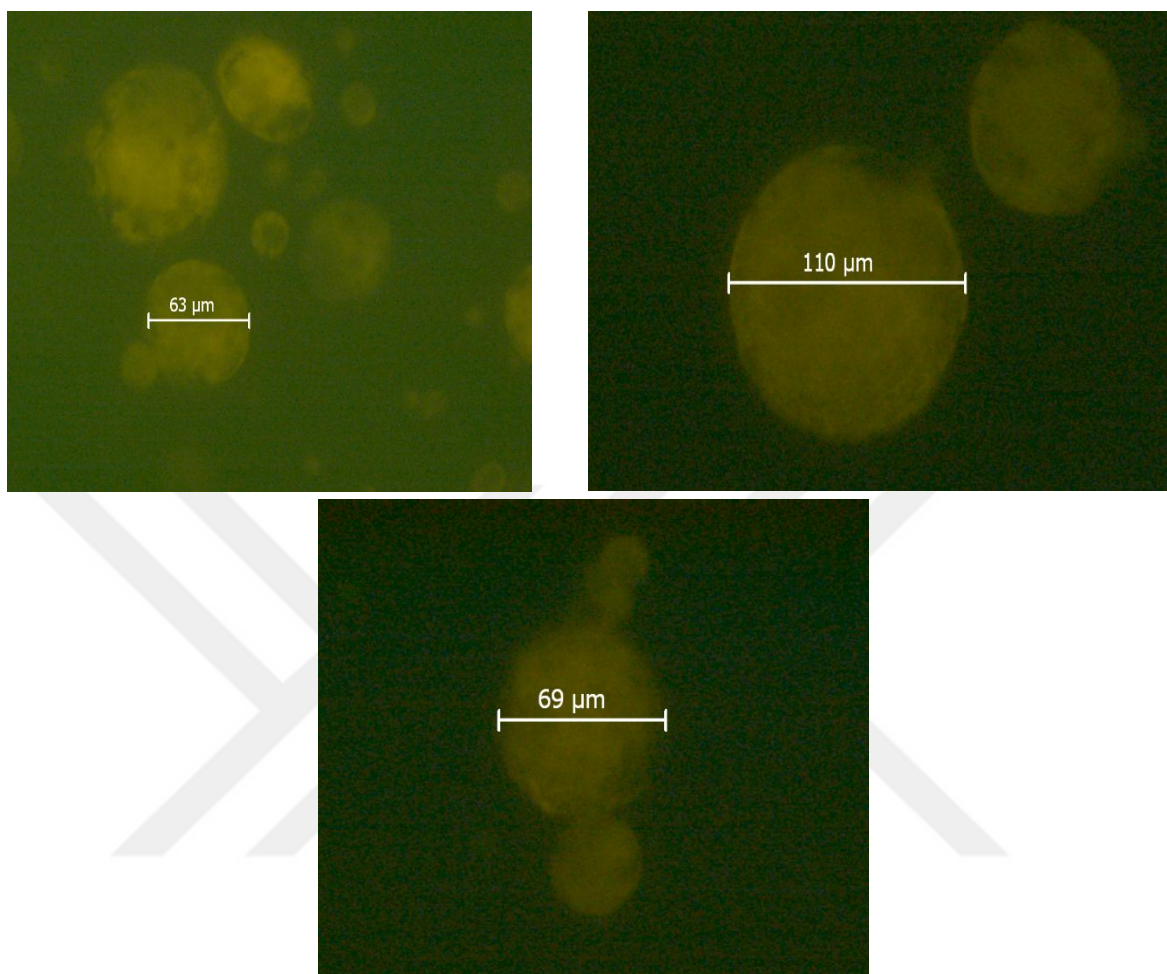


Figure 4.26. FM images of the PCL/CS/ALG particles.

4.3.2.2. Effect of the pH changes on the Insulin Release behavior of PCL/CS/ALG Particles

The effect of the pH changes on the insulin release behaviors from (PCL/CS/ALG) particles were studied in both a GSF (pH 1.2) and ISF (pH 7.4) in 37° C for 24 hours (Figure 4.27).

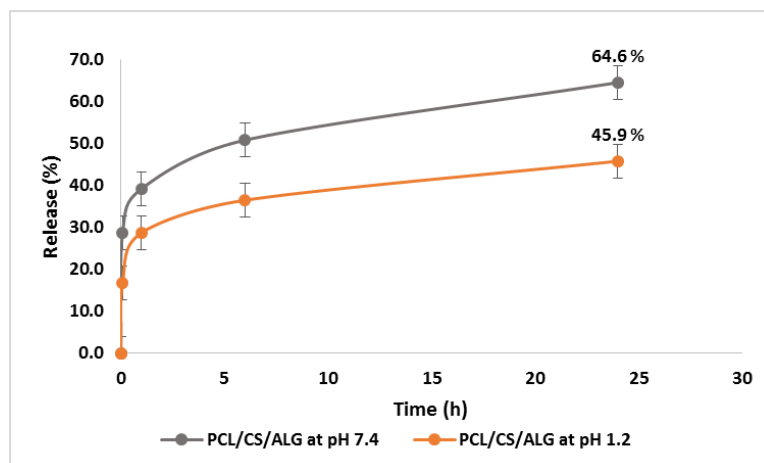


Figure 4.27. In-vitro release profiles of insulin from (PCL/CS/ALG) particles at various pH 1.2 and 7.4 in 37°C (n = 3, mean ± SD).

As noticed from the release profiles above, the total amount of insulin released from the particles in the GSF was about 45 %. While the particles released up to about 65 % of the encapsulated insulin in the SIF. This attribute to the presence of the alginate layer on the particles surface.

At neutral pH, the carboxyl groups ionized to become in the form of (COO⁻ groups). This will induce an electrostatic repulsion force with the negative charges in the alginate hydrogels. This leads the alginate network to be loose and swollen, thus a higher insulin amount could be released. On the contrary, at low pH (below the pKa of alginate which is about 4), the carboxyl groups of the alginate exchanged with the protons in the medium to become COOH form. The interaction between COOH groups (intermolecular hydrogen bond) would tight the alginate network which leads the hydrogel to shrink, thus reduce the drug release [93]. This explains why the insulin release rate from PCL/CS/ALG was much faster in the ISF than release rate in the GSF. It is clear now the ALG layer supplied the system with the desired pH sensitivity properties.

4.3.2.3. In-vitro Studies of the Insulin Release Behaviors in each of the ALG Uncoated (PCL/CS) and ALG Coated (PCL/CS/ALG) Particles at Different pH Values

For further understanding of the pivotal role of ALG layer in the oral insulin delivery, the insulin release behavior of both ALG coated (PCL/CS/ALG) and uncoated (PCL/CS) particles have been compared in different pH environment. The release profiles of insulin from the PCL/Cs and PCL/Cs/ALG particles at ISF (pH 7.4) for 24 h, were studied and the resulted release profiles depicted in (Figure. 4.28).

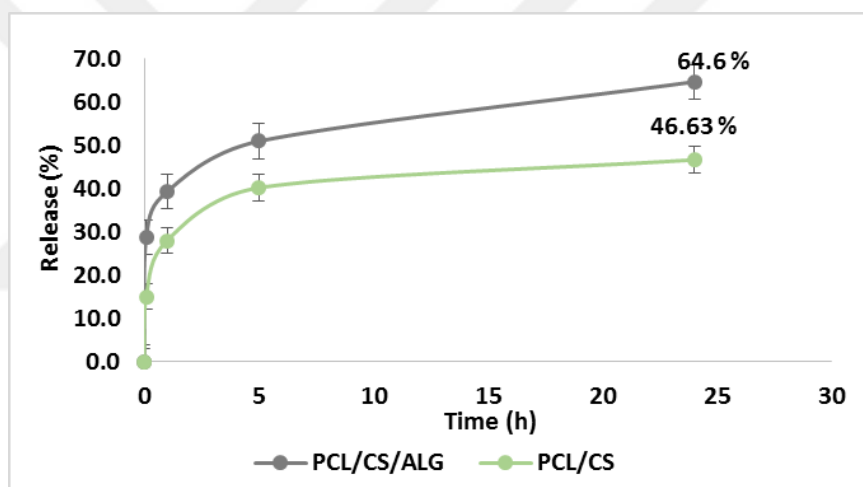


Figure 4.28. In vitro release profiles of insulin from (PCL/CS) and (PCL/CS/ALG) in ISF (pH7.4) (n = 3, mean $\pm$ SD).

At higher pH 7.4, the amine group of the chitosan becomes deprotonated and uncharged, thus increase the CS layer viscosity and create an insoluble hydrogel networks, so just about 46% of the encapsulated insulin released in 24 h [91]. Whereas at the same condition of (7.4), the carboxyl groups of the alginate ionized to create (COO⁻ groups) which, induce an electrostatic repulsion force with the negative charge of the alginate. This leads the alginate network to be loos and hence a higher insulin amounts can be released [93].

This express the higher release rate (about 65 %) of the particles coated with alginate. Which means that alginate layer improved the insulin release behavior at the intestinal conditions.

At gastric simulated environment (pH 1.2) only about 46 % of the encapsulated insulin released from the PCL/CS/ALG while, up to 62 % of the insulin amount released from the PCL/CS, as depicted in Figure 4.29.

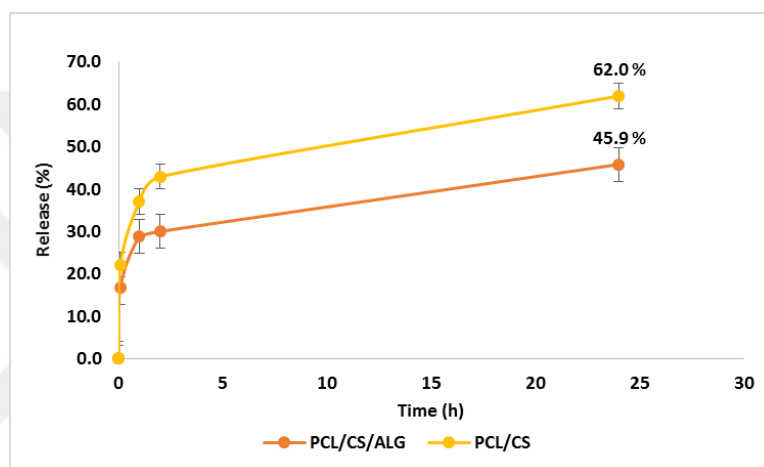


Figure 4.29. In-vitro release of insulin from (PCL/CS) and (PCL/CS/ALG) in GSF (pH 1.2) (n = 3, mean $\pm$ SD).

As mentioned earlier CS have a high solubility on the acidic conditions (pH 1.2) which attributed to the neutralized of it amino group which increase its hydrophilicity. Thus allow more amount of insulin to release. On the other hand at the acidic environment, the carboxyl groups of the alginate become COOH groups and the intermolecular interactions between the COOH groups tight the alginate network [93]. Thus the PCL/CS/ALG particles release just a low amount of the encapsulated insulin at GSF. This indicates that coating the particles with ALG layer improved the insulin release behavior in the low pH environments, thus protect the encapsulated insulin from the harsh environment in the stomach.

These results confirmed PCL/CS/ALG particles pH-sensitivity properties, which protects drug damage in harsh stomach environment as well as control the release of drug in intestine.

4.3.2.4. In-vitro Simulation of the Insulin-loaded PCL/CS/ALG Particles Passage through the GIT

In order to examine the capability of the PCL/CS/ALG system to tolerate the severe pH changes through the GIT, the insulin release behavior of the particles were studied in a pattern that simulates the real tract of the insulin from the stomach into the intestine. Thus, the experiment started with placing the insulin loaded PCL/CS/ALG particles into a GSF (pH 1.2) for 2h (the period of the insulin staying in the stomach). Then the particles transferred into an ISF (pH 6.8). The release behavior of the particles in these conditions depicted in Figure 4.30.

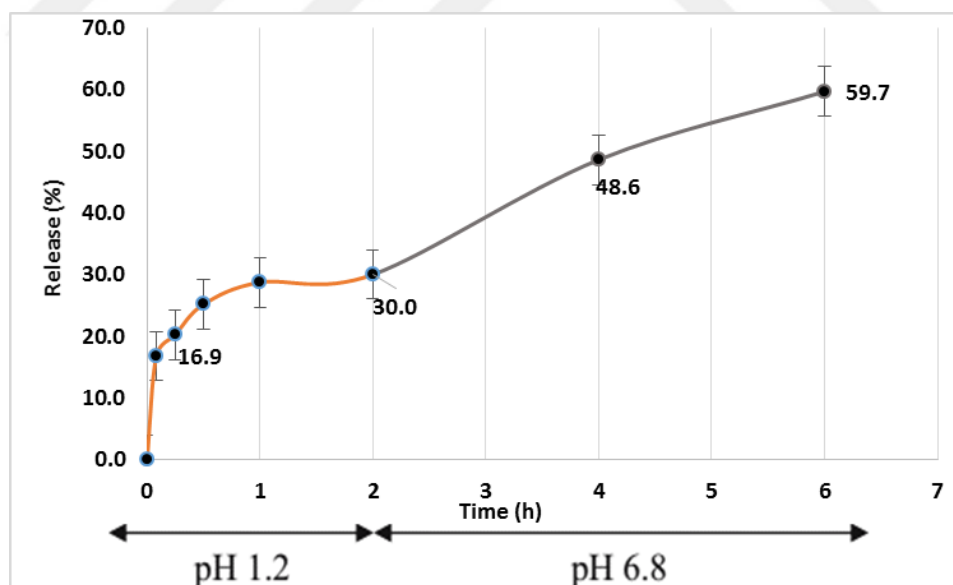


Figure 4.30. Insulin release from PCL/CS/ALG particles in GSF (pH 1.2) for 2h followed by 4 h in ISF (pH 6.8) at 37° C (n = 3, mean ± SD).

As it is clear from the release profiles above, when the particles placed into GSF (pH 1.2), the release profile were characterized by an initial burst release of about 25% followed a sustained release. After 2h in the GSF, just about 30% of the total encapsulated insulin was released from the particles. Which means that, there was still about 70 % of the insulin retained inside the particles. When the particles transferred to ISF (pH 6.8), the release rate increased briskly to about 48% within the first 2 h and then continued until the released amount of insulin reached 60% after 6 h. Therefore, our system proved its effectiveness in protecting the encapsulated insulin from the acidic degradation in the stomach and helped the efficient delivery of insulin in to the intestine. This simulated release experiment results are in compatible with another study by Mukhopadhyay et al. [50] which studied the in-vitro insulin release profile of the CS/ALG nanoparticles at a similar conditions. The obtained results showed that, the nanoparticles minimized the insulin loss in the GI tract.

It have been noticed that all the release profiles of the insulin from all the particles formulations were characterized by an initial burst release followed by a sustained and incomplete release profiles over the 24 h. This attributed to the desorption of insulin from the particles surface thus some insulin amounts will remain on the surface of the particles and will be released once putted into the dissolving media [94].

It is worth mentioning that all the in- vitro release experiments have been conducted in an enzyme –free environments. However, under the normal conditions of the body and with the existence of the chitosan's digestion enzymes (chitosanase) in the intestine it is expected that- after the degradation of the alginate layer- the chitosan layer will be susceptible to the enzymatic degradation, which would increase the release rate of the insulin from the particles.

The required daily dose of insulin for the diabetic is ranged from 0.4 – 1.0 insulin unit per kilogram body weight per day. That means, for a patient weight 70 kg the daily required dose of insulin will equal $(70\text{kg} \times 0.4 \text{ units/kg/d})$ i.e. 28 units per day. This amount administrated as a basal and pre-meal doses. The normal blood glucose levels is between 72 to 108 milligrams per deciliter (mg/dl) when fasting and rise Up to about 140 mg/dl 2 hours after meal. Usually, one unit of insulin is needed to drop the blood glucose by about 50 mg/dl. The required insulin doses determined by the doctor depends on the patient's age, weight, pancreatic activity, daily actives and life style. Also of the type of the used insulin and the route of its administration affect the required dose [95]. For our system the total dose of insulin delivered into the intestine was about 20 insulin unit which close to the required dose.

The main advantage of our system is that the delivered dose can be adjusted very easily by modifying the system's parameters as discussed before. Thus we built a drug delivery system which is able to deliver the drug to the intended target and with a rate determined by the needs of the body.

4.3.2.5. Characterization of In-vitro Insulin Release Kinetics of PCL/CS/ALG Particles

The release kinetics for the PCL/CS/ALG particles at different pH values (7.4 and 1.2) studied using the different kinetics models. Zero order model (Figure 4.31 (a) and (b)), first order model (Figure 4.31 (c) and (d)), Higuchi model (Figure 4.31 (e) and (f)) and Korsmeyer–Peppas model (Figure 4.31 (g) and (h)).

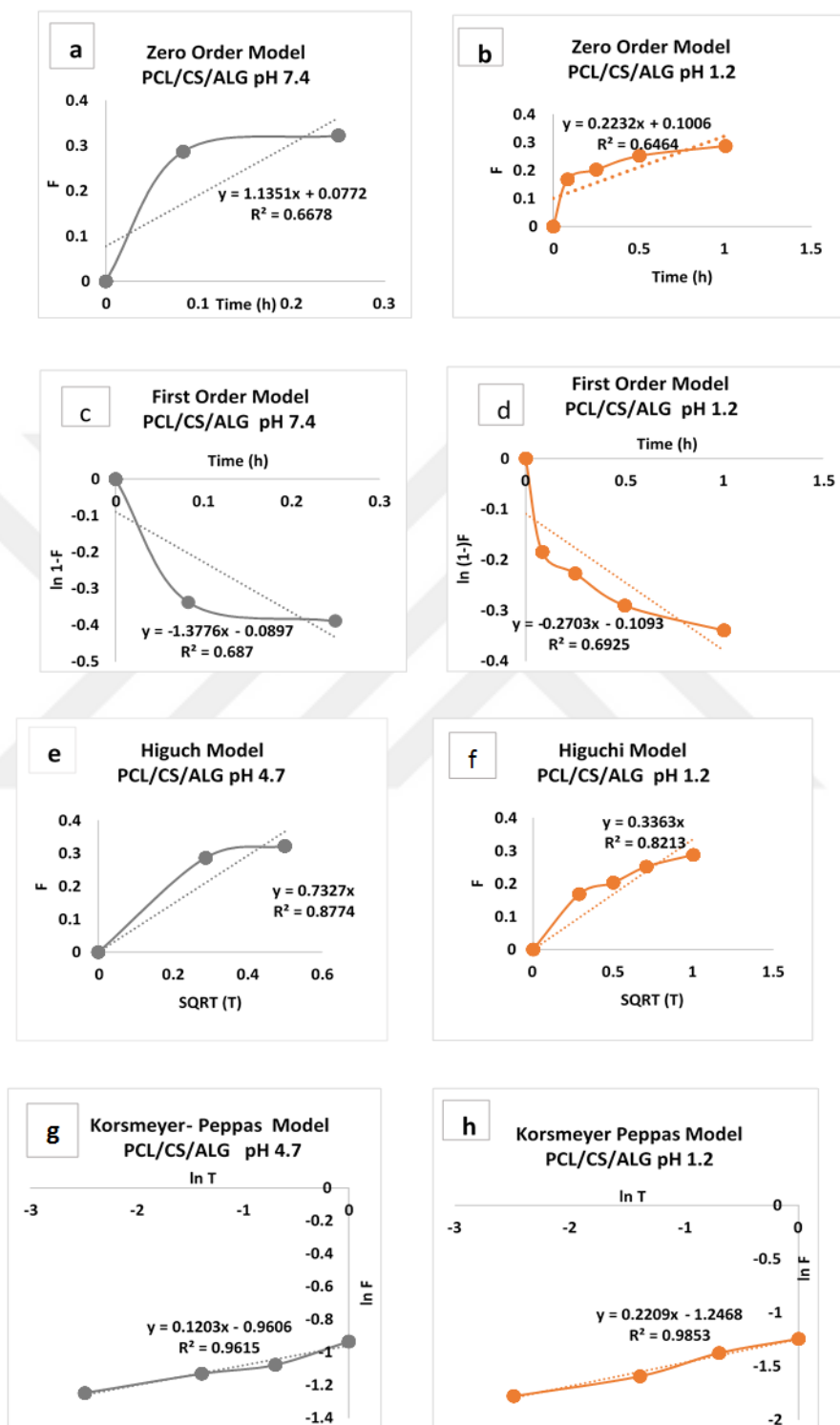


Figure 4.31. The Different kinetics models for PCL/CS/ALG particles at different pH values (7.4 and 1.2).

The regression coefficients (R^2) and all the models parameters for the PCL/CS/ALG particles are summarized in the following Table.

Table 4.4: Summery of kinetic models values of the PCL/CS/ALG particles in different pH environments.

Formulations	Kinetic Models Parameters					
	Zero Order	First Order	Higuchi	Korsmeyer- Peppas		
	R^2	R^2	R^2	R^2	n	K_{KP}
PCL/ CS/ALG pH 7.4	0.668	0.687	0.877	0.962	0.120	0.38
PCL/ CS/ALG pH 1.2	0.646	0.693	0.821	0.985	0.221	0.29

From the regression values (R^2) in the Table above, it is noticeable that the Korsmeyer- Peppas model is the most fitting model to express the release mechanism of the PCL/CS/ALG particles in both the high 7.4 and low 1.2 pH environments. The diffusion exponentials (n) in the both cases were less than 0.43 so that, the insulin release mechanism of the PCL/CS/ALG particles is governed by the Fickian diffusion. By looking for the release constants (k_{KP}) it is clear that the release rate consent for the PCL/CS/ALG particles at the higher pH environment (pH7.4) was higher than the release rate constant at (pH1.2). This kinetics results in consistence with the in vitro release experiments results which indicating that the release rate of the insulin from the PCL/CS/ALG at higher pH environment is higher than its release at lower pH environments.

4.4. Further Discussion of the Release Kinetics of the System

As discussed before, the kinetics of the insulin release for all the particles formulations were studied and analyzed based on four kinetics models, zero order, first order, Higuchi and Korsmeyer- Peppas models to determine the most appropriate model to describe the system's release mechanism. The obtained results indicated that, the insulin release from all the particles formulations (PCL/F127, PCL/CS and PCL/CS/ALG) can be described by the Korsemeyer -Peppas model and obeying the Fickian diffusion mechanism, which attributed the release of the drug mainly to the concentration gradient. This results can be explained as following:

Generally, drug release from the polymers is a combination of the drug diffusion through the polymer matrix and the polymer degradation or bio-erosion. The biodegradable polymer degradation can be described by two erosion forms, surface and bulk erosion. In surface erosion, the polymer degrades from its exterior surface, while in bulk erosion, both the exterior surface and the interior side of the material degrade equally. The biodegradable polymers degradation produce a nontoxic molecules that can be extracted from the body [96]. Most of the natural polymers degrade enzymatically and their degradation produce sugars or amino acids. Whereas, the synthetic biodegradable polymers degrade hydrolytically with a slight enzymatic participation. Polymer hydrolysis depends on the polymer's physical properties like, molecular weight, crosslinking degree, crystallinity, hydrophobicity, etc. [97].

Our system is based on PCL which is biodegrade according to the hydration of its aliphatic ester linkage. This degradation yield a caproic acid which metabolized by the tricarboxylic acid cycle or can be eliminated by direct renal secretion [46].

As it well known that the PCL is a crystalline with a high degree of hydrophobicity thus it degrades in a very slow rates. Chalwa et al. [98] studied the PCL degradation in in-vitro in PBS at 37° C ,they observed that even after 140 days there was no significant decrease in its molecular weight. Which implies that, the degradation effect of the PCL on the Insulin release from the particles can be neglected.

This supports the kinetics analysis results which stated that, the dominant driving force for the insulin release from all the particles formulations was the diffusion effect.

5. CONCLUSIONS

The in-vitro release studies at different pH environments showed that, the insulin release profiles influenced by the associated pH value i.e. the PCL/CS/ALG particles have a pH-sensitive pattern of release, which keep insulin form harsh environment of the stomach. Thus the obtained results confirmed the success of the presented system as a pH-sensitive vehicles for insulin oral delivery. The most important conclusions obtained during the thesis study have been summarized with next items:

- In thesis study scope, all the insulin-loaded particles formulations were prepared by the double emulsion (w/o/w) solvent evaporation method which, performed under a mild conditions thus, minimize the loss of the bioactive agents of the insulin.
- The concentrations of F127 blended with PCL core affect the different physiochemical properties of the PCL nanoparticles.
- F127 not only can be used as stabilizing agent to stabilize the nanoparticles during the preparation process, but also it can be used as a drug release enhancer to obtain a controlled drug delivery system.
- Alginate (ALG) have been chosen to provide the system with the needed pH-sensitivity properties. It shrink in the acidic environments and become more soluble under the neutral conditions which maintain a sufficient and sustained insulin release in intestinal environment.
- CS introduction into the PCL nanoparticles structure not only give the nanoparticles the positive surface charge needed to coat the nanoparticles with ALG, but also provide the system with a mucoadhesive properties that enhance the transportation of the insulin through the gastrointestinal tract.

- The addition of both CS and ALG layers into the PCL nanoparticles structure increased the zeta potential of the nanoparticles, thus increased the stability of the system.
- The electrostatic interaction between the CS and ALG was created due to the ionic interactions between the negative carboxylic groups of alginate with the positive amine group of chitosan.
- The combination of these three polymers (PCL, CS and ALG) rather than using them separately, increased the stability of the system as well as provided the system with a unique characteristics that could not be achieved by using each of them separately.
- All the particles formulations had a spherical shape with a poly disperse size distributions. The average sizes of the PCL nanoparticles were about 255 nm. After the introduction of the CS into the PCL structure the mean diameter of the nanoparticles increased to about 10 μm . A larger sizes of about 50 μm were obtained as the PCL/CS particles coated with the ALG layer.
- The in-vitro insulin release studies in a different pH environments showed that, just a small amount about 45 % of the encapsulated insulin was released in the gastric simulated (low pH 1.2) conditions, while a larger amount about 65 % of the insulin was released in the intestinal simulated (higher pH 7.4) conditions which protect the insulin from the stomach's acidic environment. Thus the, developed system proved its effectiveness as a pH-sensitive vehicle for the oral insulin delivery.
- The in-vitro study of the kinetics release models presented that, the insulin release from all the particles formulations was obeying the Korsmeyer- Peppas kinetic model, which suggested that the Fickian diffusion effect is the dominant force for insulin release from the particles.

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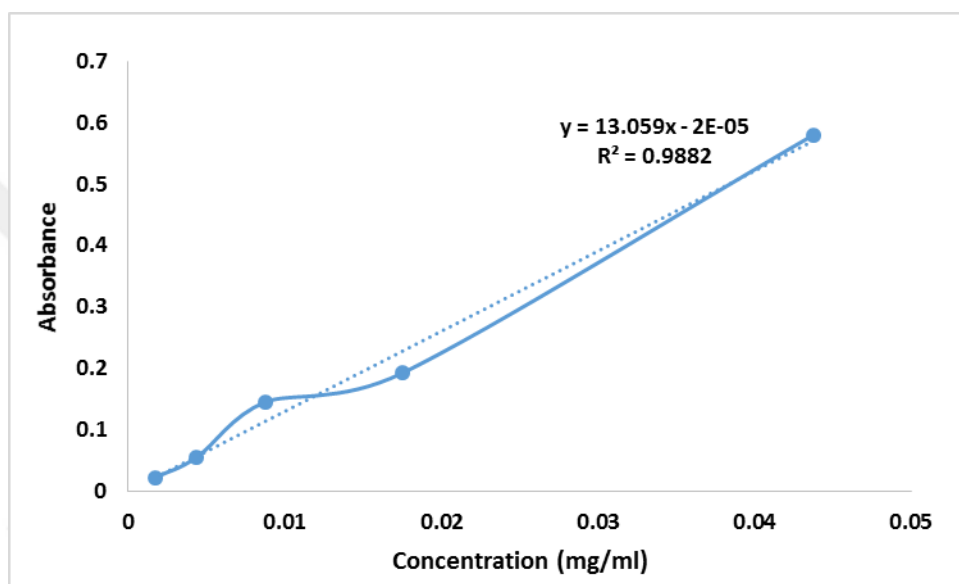
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APPENDIX

Calibration Curve of Insulin



CURRICULUM VITAE

Credentials

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Education

BSc. : Biomedical engineering Department, Sudan University of
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Foreign Languages

English (Good Level)

Turkish (Good Level)

Arabic (Native speaker)

Work Experience

Teaching Assistance 2011-2014, Biomedical engineering Department
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Areas of Experiences

Biometrics, bio signals (phonocardiogram (PCG) and
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Digital signal processing (DSP) and Digital image processing (DIP).

Analogue Electronics and medical instrumentations.

Design and formation of a nanoparticles based drug delivery systems.

Projects and Budgets

Phonocardiogram as a Biometric Signature.

Electrocardiogram as a Biometric Signature.

Preparation and Investigation of pH-sensitive Hydrogels/
nanoparticles for drug delivery.

Publications

Oral and Poster Presentations



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