ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

PREPARATION OF BIOCOMPOSITES USING SPRAY DRYER AND THEIR APPLICATIONS IN DRUG DELIVERY SYSTEMS

Ph.D. THESIS

Erhan ÖZSAĞIROĞLU

Department of Chemical Engineering

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

PÜSKÜRTMELİ KURUTUCU İLE BİYOKOMPOZİTLERİN HAZIRLANMASI VE İLAÇ TAŞINIM SİSTEMLERİNDE KULLANIMI

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Erhan ÖZSAĞIROĞLU, a Ph.D. student of ITU Graduate School of Science, Engineering and Technology student ID 506112002, successfully defended the thesis entitled "PREPARATION OF BIOCOMPOSITES USING SPRAY DRYER AND THEIR APPLICATIONS IN DRUG DELIVERY SYSTEMS", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my dear wife Tuğba and my family,

FOREWORD

The purpose of the research is to produce stable and effective encapsulation material as L-ascorbic acid delivery systems. This thesis is supported and founded by the Istanbul Technical University Scientific Research Projects. I have experienced this period as very interesting and instructive. The whole part of the study was done here in ITU Biotechnology Lab. between 2013- 2015.
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Erhan ÖZSAĞIROĞLU
(Chemical Engineer, MSc.)

TABLE OF CONTENTS

Page

ABBREVIATIONS

AMP	**:** Antimicrobial Peptide
CH	**:** Chitosan
CS	**:** Casein
EGF	**:** Epidermal Growth Factor
FDA	**:** US Food and Drug Administration
IBD	**:** Inflammatory Bowel Disease
NDDS	**:** Novel Drug Delivery Systems
NP	**:** Nanoparticle
PAA	**:** Polyacrylic Acid
PBS	**:** Phosphate Buffer Solution
PCL	**:** Polycaprolactone
PEG	**:** Polyethylene Glycol
PGA	**:** Polyglycolic Acid
PMMA	**:** Poly(methyl methacrylate)
PVA	**:** Polyvinyl Alcohol
SA	**:** Sodium Alginate
SD	**:** Standard Deviation
SEM	**:** Scanning Electron Microscopy
TGA	**:** Thermogravimetric Analysis
UV	**:** Ultraviolet Analysis

LIST OF TABLES

Page

LIST OF FIGURES

Page

PREPARATION OF BIOCOMPOSITES USING SPRAY DRYER AND THEIR APPLICATIONS IN DRUG DELIVERY SYSTEMS

SUMMARY

Biodegradable and biocompatible polymers have been widely used in drug delivery systems with an increasing interest. The main reason to drive biopolymers for top material for drug delivery applications is after drug depletion, the carrier degraded in the body to form products that are easily resorbed or eliminated. On the other hand, the unwanted adverse effects have to be minimized and therapeutic improvement has to be maximized at the same time for an effective and useful drug delivery tool. The only way to obtain improved therapy is that creating very effective drug release in the body and bio-based polymers could provide these vital necessities. Because of these reasons, biopolymers are preferred for drug delivery systems.

The biodegradable polymers are a new class of controlled release polymers developed for the interstitial delivery of drugs to their target site in human body over periods ranging from days to years. These polymers can release molecules of any size in a predictable fashion. Their degradation products are non-cytotoxic and biocompatible. The only way to obtain improved therapy is that creating very effective drug release in the body and bio-based polymers could provide these vital necessities. Because of these reasons, biopolymers are preferred for drug delivery systems.

Controlled drug delivery systems have become increasingly attractive options for inhalation therapies. A large number of carrier systems have been developed and investigated as potential controlled drug delivery formulations to the lung, including drug loaded lipid and polymer based particles. However, there are some extraordinary specifications should have for a drug delivery material. First of all, polymers which are used in a drug delivery system, it has to be biodegradable and biocompatible. For example, polycaprolactone, polyethylene glycol , polylactic acid, chitosan, chitin, and polyglycolic acid and their blends or copolymers are widely used for drug delivery systems because of controllable particle diameter, particle size distribution, and biodegradation rate in human body.

Spray drying is transformation of material from solution state to powder form by spraying feed into a hot air medium. The production of dried particles from a liquid feed in a single processing step makes spray drying a unique and important process. Since then, a tremendous development of the spray drying process with the refinement in the hardware and equipment configuration and improved understanding of fluid dynamics has made it versatile technique operational in diverse industrial fields. Spray drying is widely used for the drying of heat-sensitive foods, pharmaceuticals, and other substances mainly due to rapid solvent evaporation and homogeneous particle size distribution. At last decade, spray dryers have been preferred for pharmaceutical industry by combining drug delivery systems with micro-particle uniform distributed polymeric materials. Furthermore, amorphous solid dispersions, soluble complexes, encapsulated systems, solid self-emulsifying

systems and nano-dispersions of poorly soluble drugs prepared by spray drying are primary solubilization strategies.

The present work concerns the preparation and characterizations of biodegradable, biocompatible biopolymer based composites and obtain uniform particle size distribution at the same time. For achieving of the goal our study, we used polycaprolactone, polyethylene glycol, chitosan, casein, and sodium alginate to prepare drug delivery system and spray dryer will be our tool to obtain microspheres. Triple mixture of the polymers are used such as polycaprolactone-polyethylene glycol-chitosan, polycaprolactone-polyethylene glycol-casein, and polycaprolactone-polyethylene glycol-sodium alginate. First of all, we are evaluated effects of spray drying conditions and composition of the microencapsulating formulation. Secondly, the most uniform distributed particle size microsphere are selected and drug active ingredient is loaded to it. L-ascorbic acid is an active ingredient for the study. After that, drug encapsulation and drug release studies are performed. Drug release experiments are maintained at different pH solutions (pH 2.5, 7.4, and 9.6). Finally, drug release kinetics are determined by widely used equations to describe the degradation kinetics; Zero-order, First-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas. Furthermore, L-ascorbic acid release mechanism from microspheres is also determined. Release profiles of three microspheres produced are obeyed to previously developed kinetic models to perform possible release mechanisms; The Korsmeyer–Peppas model is the best described each release scenario.

Encapsulation and drug release ratio are easily adjusted by changing structure of microspheres. Particularly, release behavior of microspheres are changed by replacing biopolymers in the drug structure. By this way, released L-ascorbic acid ratio in different release mediums are adjusted.

The main reason of using of ternary polymer mixture is that to obtain best performance from a drug material because these polymer has some superior features but they are not enough separately. The importance of the study is producing of a stable and effective drug encapsulation system using ternary polymer mixture by spray dryer Our study is proposed as an alternative or adjuvants for controlling release of L-ascorbic acid. By this way, we can achieved higher drug loading and drug release efficiency in our study.

PÜSKÜRTMELİ KURUTUCU İLE BİYOKOMPOZİTLERİN HAZIRLANMASI VE İLAÇ TAŞINIM SİSTEMLERİNDE KULLANIMI

ÖZET

Biyoteknolojik uygulamaların önemli bir bölümünü oluşturan biyomalzeme üretimi günümüz mühendislik teknolojisinin güncel konularından biridir. Üretilen biyomalzemelerin biyouyumlu olması, toksik ve kanserojen olmaması, kimyasal açıdan kararlı olması, yeterli mekanik dayanıma sahip olması istenen özelliklerdendir. Seramik, metalik, polimerik ve kompozit malzemeler genellikle biyomalzeme olarak kullanılmaktadır.

Biyopolimerler, toksik etki yaratmadan bozulabilmeleri ve biyouyumlu olabilmeleri bakımından ilaçtan yapay organlara birçok alanda kullanılmaktadır. İlaç taşınım sistemlerinde biyopolimerlerin tercih edilme sebeplerine yukarıda bahsi geçen özelliklerin yanı sıra mekanik ve fiziksel açıdan kolay işlenebilir ve dayanımlarının ayarlanabilir olmaları da eklenebilmektedir. Kullanıldıkları biyolojik alanlara örnek olarak ilaç salım sistemleri, doku ve organ nakilleri ve ortopedik uygulamalar verilebilmektedir. Biyopolimerlerin kullanım alanlarına doku mühendisliği uygulamaları, tarımsal uygulamalar, ambalaj sektörü, medikal uygulamalar ve kontrollü ilaç salınım sistemleri örnek olarak verilebilir. Biyopolimerlerin ilaç kapsülasyon, vücutta taşınım ve kontrollü salınması ile ilgili sistemlerde kullanılmalarının sebepleri, ilacı taşıyıp bıraktıktan sonra kolaylıkla vücutta absorbe edilebilmeleri ve böylece vücuttan hızlı bir şekilde atılabilmeleridir. Ayrıca, vücutta bozunmalarıyla ortaya çıkan bileşenlerin toksik etkisi yoktur. Bunun yanı sıra fazla dozda alımdan kaynaklanan ilaç yan etkileri azaltılırken, ilacı etkinlik bölgesine taşıyarak terapötik etkisinin arttırılması oldukça önemli bir konudur. Ayrıca, özellikle hassas ilaç etken maddelerin bozunmadan etkinlik bölgesine taşınabilmesi amacıyla enkapsülasyonu ilaç taşıma sistemlerinin avantajları arasında sayılabilir.

İlaç taşıma sistemi, bir etken maddenin ilaç salınım süresinin ve hızının ayarlanabilmesini sağlayan, böylece ilaç yan etkisini minimize edebilmemize yardımcı olan araç veya formülasyon olarak tanımlanabilir. İlaç taşıma sistemleri ve artan ilgilerinin sebebi düşük doz alımında bile vücutta yeterli etkiyi sağlayabilmeleridir. Ayrıca ilaç plazma konsantrasyonunun istenen süre ve kararlılıkta olmasına da yardımcı olurlar. Genel olarak ilaç taşıma sistemlerinde etkin salınım veriminin sağlanabilmesi ve bahsi geçen üstün özelliklerin elde edilebilmesi için tek bir polimer değil de polimer karışımları kullanılır.

İlaç taşıma sistemi olarak kullanılabilmesi için herhangi bir polimerin belirli üstün yapısal özelliklerinin bulunması gerekmektedir. İlk olarak, kullanılacak olan polimer türlerinin biyobozunur ve biyouyumlu olmaları gerekmektedir. Bu tür polimerlere örnek olarak polikaprolakton, polietilen glikol, polilaktik asit, kitosan, kitin ve poliglikolik asit gibi polimerler ve bunların karışımlarından yada kopolimerlerinden oluşan malzemeler yaygın olarak kullanılmaktadır. İkinci diğer en önemli özellik ise

ilaç kapsül maddelerinin partikül boyutlarının kontrol edilebilir ve yoğunluklarının ayarlanabilir olmasıdır ki vücutta her organ ve doku için istenen özellikler değişebilmektedir.

Püskürtmeli kurutucular malzemeleri akışkan formdan kuru tanecikli forma dönüştüren ve bunu püskürttüğü akışkanı sıcak bir gaz ortamında kurutan yapılardır. Püskürtmeli kurutucular kurutma işlemini çok hızlı yaptıklarından ve homojen partikül boyut dağılımına sahip malzemeler üretilmesine olanak tanıdığından dolayı birçok farklı alanda kullanılabilmektedir. Kurutulacak maddelerin sıcak havaya nispeten daha kısa süre maruz kalması ve akışkanlaştırılan havanın ısı transferi kapasitesinin artmasıyla daha düşük sıcaklıklarda kurutma sağlanabilmesi dolayısıyla, ısıya hassas gıdaların ve ilaçların üretilmesinde püskürtmeli kurutucular sıklıkla kullanılmaktadır. Özellikle de son yıllarda mikropartikül temelli ilaç taşıma sistemlerinin geliştirilmesinde tercih edilen bir yöntem olmuştur. Ayrıca püskürtmeli kurutucular ile suda çözünürlüğü düşük olan ilaç etken maddelerinin dahi kapsülasyonu yapılabilmektedir.

Sağlıklı bir gelişim, diş, kemik ve deri için vazgeçilmez bir bileşen olan C vitamini olarak da adlandırılan L-askorbik asit (3-keto-L-gulofuranolaktan); demirin absorblanması, kolajen sentezi, kan damarlarının yapısal gücünün sürdürülmesi, bazı aminoasitlerin metabolizmasında ve adrenal bezlerin hormon sentezi ve salgılaması gibi metabolik fonksiyonlarda görev alan karbonhidrat benzeri bir kimyasal maddedir. Ancak L-askorbik asit kolay okside olabilen ve bozulabilen bir malzemedir. Oksijen varlığı, sıcaklık yükselmesi, güneş ışığı ve ultraviyole ışıklara maruz kalma, bakır ve demir gibi ağır metallerin varlığı, güçlü asit ve alkali koşullar gibi durumlarda bu oksidasyonun derecesi artmaktadır. Çalışmamızda L-askorbik asidin bozulmadan ve stabil kalarak enkapsüle edilmesi amaçlanmıştır.

Bu çalışmanın amacı, polikaprolakton, polietilen glikol, kitosan, kazein ve sodyum aljinat polimerlerinin mikrokürecik yapısında hazırlanması ve ilaç taşıma sistemi olarak kullanımının incelenmesidir. Bu sayede biyobozunma, biyouyumluluk ve kapsülün stabilitesi gibi problemler de tekli değil de üçlü polimer kompoziti ile aşılabilmesi beklenmektedir. Bu amaçla ilk aşamada üçlü polimer karışımından oluşan çözeltiler püskürtmeli kurutucuda kurutularak mikrokürecikler üretilmiştir. Püskürtmeli kurutma işleminde giriş sıcaklığı ve besleme debisi parametrelerinin partikül boyutu, partikül verimi ve yüzey alanı üzerine etkisi incelenmiştir. Sonraki aşamada, üretilen mikrokürecik lere ilaç yüklemesi için uygun koşullar belirlenmiştir. İlaç etken maddesi olan L-askorbik asit kullanılmıştır. Son olarak ilaç yüklü mikrokürecik lerin asidik, nötral ve alkali ortamlarda ilaç salınım özellikleri incelenmiş ve ilaç salınım kinetiği parametreleri belirlenmiştir.

İlk aşamada, polimerik mikrokürecikler elde etmek için üçlü polimer karışımı püskürtmeli kurutucu ile kurutulmuştur. Püskürtmeli kurutucuda çözeltinin besleme debisinin ve kurutma havası sıcaklığının değiştirilerek mikroaprtiküller morfolojisi üzerindeki etkisi incelenmiştir. En etkili kurutma parametreleri (sıcaklık ve debisi) kullanılan polimer karışımının türüne göre değişmiştir. Çözelti besleme debisi ve kurutma havası sıcaklığı değiştirilerek elde edilen mikropartiküllerin SEM ve partikül boyut analizleri yapılmıştır. Üretilen mikrokürecik termogravimetrik analiz ile yapısal olarak incelenmiştir.

Çalışmanın ikinci aşaması ilaç yüklü partiküllerin üretilmesidir. Dolaylı ilaç yükleme metodu ile seçilen mikrokürecik lere L-askorbik asit yüklemesi yapılmıştır. Yüklenen

ilaç miktarları UV analiz ile belirlenmiştir. İlaç yükleme koşullarında mikrokürecik miktarı ve L-askorbik asit çözeltisi derişimleri değiştirilmiştir.

Çalışmanın son aşamasında ise L-askorbik asit yüklü mikroküreciklerin farklı pH ortamlarında kontrollü ilaç salınımı incelenmiştir. L-askorbik asit yüklü partiküllerin üç farklı pH ortamında da sekiz saat süresince ilaç salınımını deneyleri gerçekleştirilmiştir. En yüksek ilaç salınım oranı 2 saat içerisinde % 93 ile pH 2.8 ortamında elde edilmiştir. İlaç salınım miktarları mikrokürecik yapısına göre değişmektedir. Ayrıca yapılan deneyler göstermiştir ki mikrokürecik yapısı değiştirilerek ilacın en etkin olacağı salınım ortamı da manipüle edilebilmektedir. Böylece L-askorbik asit yüklü ilaçların insan vücudunda istenilen salınım süresi ve ilaç salınım noktaları elde edilmesine olanak sağlanabilmektedir.

Çalışmalarımız süresince elde edilen veriler ışığında söylenebilir ki ilaç enkapsülasyon malzemeleri seçiminde tek polimere bağlı kalınmasındansa birden fazla polimer karışımından oluşan bir yapı kullanmak avantaj sağlamaktadır. Bu sayede ilaç yükleme ve ilaç salınım oranları ayarlanabilmekte/değiştirilebilmektedir. İlacın istenilen salınım ortamında daha etken olması da sağlanabilmektedir. Ayrıca püskürtmeli kurutucu kullanılarak mikrokürecik eldesi de daha homojen ve düşük partikül boyut dağılımına sahip yapılar elde etmemize olanak vermiştir. Böylece etkili enkapsüle sistemleri üretmemiz kolaylaşmıştır.

L-askorbik asit gibi kolayca çevresel etkenlerden dolayı yapısal bozulmaya uğrayan ancak hayati öneme sahip ilaç etken maddelerinin kaplanması ve taşınmasında çalışmalarımız esnasında geliştirdiğimiz yöntemlerin kullanılabilir olduğu görülmektedir. Çalışmamız verimli ve stabil ilaç sistemlerinin elde edilmesine yardımcı olabilecek bir yol önermektedir.

1. INTRODUCTION

Nowadays, pharmaceutical industry has been focused on some specific biodegradable and biocompatible polymers which are polycaprolactone (PCL), polyethylene glycol (PEG), chitosan (CH), polyglycolic acid (PGA), polylactic acid (PLA), sodium alginate (SA), and chitin [1]. Biodegradable and biocompatible polymers have many advantages for drug delivery systems such as porous surface, encapsulation efficiency, high drug release ratio, high stability, low side effects, and good drug-encapsulation material interaction. Drug delivery research aims to conveniently administer complex drugs to the target tissue in the biological system in a more stable and reproducible controlled way so that it would achieve higher activity at a minimal dose for prolonged period at the site devoid of side effects. Entrapment of a drug into a polymeric system may protect the drug from inactivation and help to retain its activity for prolonged durations, decrease its toxicity, dosing frequency and offers flexibility in administration [2].

Particulate drug carriers can act as delivery vehicles for drugs through various routes of administration [3]. On the other hand, the undesirable adverse effects have to be minimized and therapeutic improvement has to be maximized at the same time for an effective and useful drug delivery tool [4-7]. The only way to obtain improved therapy is that creating very effective drug release in the body and bio-based polymers could provide these vital necessities. Because of these reasons, biodegradable polymers are preferred for drug delivery systems.

Drug encapsulation in colloidal delivery systems is an efficient approach to improve the pharmacokinetics of hydrophilic drugs [8]. These carriers encompass a broad range of dispersion systems ranging from submicron emulsions to colloidal particles, such as biopolymers (PCL, PEG, CH, CS, SA, and starch) aiming to protect the drug against degradation, sustain drug release, increase patient comfort by avoiding repetitive bolus injections or the use of perfusion pumps and reduce side effects [9-11].

It is always a fact that performance of a drug delivery system is about encapsulation and release efficiency. One of the basic requirements for the controlled and balanced release of the medicament in the body is its ideal spherical shape of polymeric particles and narrow distribution of their sizes. The size and shape of the particles play key role in their adhesion and interaction with the cell [12]. Another important issue for drug delivery systems is that stability at outside of the human body and sufficient degradation of drugs inside of human body. To overcome this problem, mixture of biopolymer for drug delivery systems could be an enormous alternative because biodegradation rate is easily adjustable by blends of polymers. At these view of points, preparing of a drug easily biodegradable, biocompatible inside the body and stable outside the body is the main problem and most important goal for pharmaceutical industry.

L-ascorbic acid, commonly called vitamin C, is a highly abundant and essential metabolite for plants and animals. It is an important dietary supplement for some animals, such as primates and humans, which lack the capacity to synthesize L-ascorbic acid. It has attracted considerable attention from the scientific community due to their numerous beneficial effects for human health, which include antioxidant and immune booster effects [13]. Unfortunately, while L-ascorbic acid provides beneficial therapeutic effects, its stability also easily effected temperature, sun-light and O_2. Thus, encapsulation of L-ascorbic acid would provide a stable and highly effective drug [12].

Spray drying is known to be a convenient one-step process for the continuous conversion of liquid formulations into dry particulates, which is well-established in many relevant branches such as the chemical, food or pharmaceutical industry, due to the high flexibility to manipulate particle properties (e.g. size and morphology) [14]. It is the process of contacting an atomized stream to be dried with a gas stream that is at a higher temperature than the liquid stream. The higher temperature of the gas stream causes evaporation of the liquid from the droplets, forming particles. It results in microspheres with good quality, low fluid activity (water etc.), easier handling, and storage [15-19]. Equipment is readily available and production costs are lower than most other methods. Another advantage of spray drying is that it is possible to control particle size and morphology by varying process parameters and formulation. This is of great

importance for the preparation of powders for drug encapsulation. Recently, the use of this process to manufacture hollow, micron and sub-micron particles has also been demonstrated [15, 17]. Therefore, spray drying can be considered as an interesting alternative to any other methods for the preparation of amorphous solid dispersions, soluble complexes, encapsulated systems, solid self-emulsifying systems and nano-dispersions of poorly soluble drugs [20-22].

On the basis of these remarks, aim of our work is encapsulation of L-ascorbic acid in microspheres made by biocompatible and biodegradable polymers that are PCL, PEG, CH, CS, and SA. (PCL-PEG-CS) in order to obtain (i) production of effective and stable encapsulation material (ii) enhancing of encapsulation and (iii) increasing of drug release ratio simulated with different pH mediums. We used ternary polymer mixture to obtain best performance from an encapsulation material because a combination of these materials could achieve superior characteristics than each component individually and spray dryer was our tool to obtain microspheres. Another importance of the study was encapsulation of L-ascorbic acid, essential vitamin for humans. For achieving of the goal our study, we used PCL-PEG-CH, PCL-PEG-CS, and PCL-PEG-SA to prepare drug delivery systems. First, we evaluated effects of spray drying conditions and composition of the microencapsulating formulation. Secondly, the uniform distributed particle sized microsphere was selected and drug was loaded to it. L-ascorbic acid is the active ingredient for the study. After that, drug encapsulation and drug release efficiencies were calculated and best drying conditions were determined. Drug release studies were accomplished at different pH mediums (2.8, 7.4, and 9.6).

2. SUMMARY OF LITERATURE

2.1 Biopolymers for Drug Delivery Systems

Polymers and polymer based materials have been widely used for biomedical applications, particularly drug delivery systems, artificial tissues, and prosthesis. The main reasons of highly preference of polymer on biomedical area are that higher elasticity and strength, biocompatibility and biodegradability, adjustable hydrophobicity and hydrophilicity, and porosity. Biocompatibility and biodegradability are the most important factors that affect a materials using area on biomedical applications. One of the main areas of usage of biodegradable polymers is related to their clinical applications, and knowledge of the toxicity of the products released during their biodegradation is certainly of great concern. In vitro degradation tests in simulated physiological solutions are a means of forecasting the interactions between body fluids and the biopolymer, and to study its stability and degradation rate. Biocompatibility includes interaction between human cell and drug, toxicity of a drug; biodegradability means throwing easily of drug from the body [23].

The basic principles for development of drug delivery systems are easily integration of active ingredient into drug, achieving of higher drug release ratios, and non-toxic effects of drug capsule to cells after its biodegradation. The last ten years, production of more effective, biocompatible, and biodegradable drug systems have been increasingly interest for pharmaceutical industry [24-29]. Nowadays, polyester based biopolymers are used for drug delivery and encapsulation systems and some examples of these polymers are polycaprolactone, polyethylene glycol, polylactic acid, polyglycolic acid, polyacrylic acid (PAA), poly(methyl methacrylate) (PMMA), chitosan, chitin, casein, alginate, and starch [29].

2.1.1 Polycaprolactone as an encapsulation agent

PCL, a semi-crystalline linear aliphatic polyester approved by the US Food and Drug Administration (FDA), has attracted much attention to be used in the controlled drug delivery due to non-toxicity and low cost [30]. Besides, the degradation of polycaprolactone does not result in an acid environment and its degradation products can be solubilized by body fluids and removed by phagocytosis. PCL is prepared by the ring-opening polymerization of the cyclic monomer ε-caprolactone and structure of polymer chain is shown in Figure 2.1.

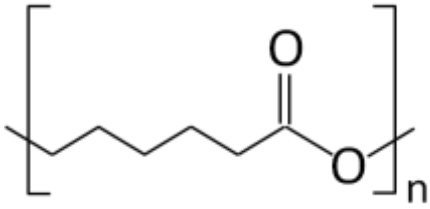


Figure 2.1 : Polycaprolactone chain structure [27].

Some applications of PCL for biomedical area are surgical suture, bone and tissue engineering systems, and drug delivery systems with other bio-based polymers. In the past four decades, several studies have been published relating to the biocompatibility of sutures made from aliphatic polyesters [27]. A block copolymer of PCL with glycolide, offering reduced stiffness compared with pure polyglycolide, is being sold as a monofilament suture by Ethicon, Inc. (Somerville, NJ), under the trade name Monacryl [31].

Because of controllable biodegradability, easily shaped, and producible in different porous size, PCL has more advantages than other biopolymers for biomedical applications [32]. Due to a higher permeability of PCL, it is blended with other polymers to improve stress, crack resistance, dyeability and control over release rate of drugs.

Within the last decades, PCL polymers have been major area of interest to develop controlled delivery systems especially for peptides and proteins [27]. The matrix material of bioresorbable microparticles can be decomposed into non-toxic and low molecular weight species concomitant with release of the drug which are then metabolized or absorbed by the organism. It is no surprise that considerable research interest is now focused on the application of biodegradable microparticles for controlled drugs release. Among them, polycaprolactone is one of the more widely

utilized. The advantages of PCL include its high permeability to small drug molecules, and its negligible tendency to generate an acidic environment during degradation as compared to PLA and PGAs [33]. PCL microspheres can be prepared by several different methods and spray drying is common technique, which is reviewed by Freiberg and Zhu [34]. Uniform distributed microspheres and production of drugs with effective encapsulation ratio are easily achieved by this way.

2.1.2 Polyethylene glycol as an encapsulation agent

PEG is a bio-based polyether class polymer, although its monomer is toxic for human tissues. PEGs (in Figure 2.2) are known as compounds with widespread industrial and medical applications.

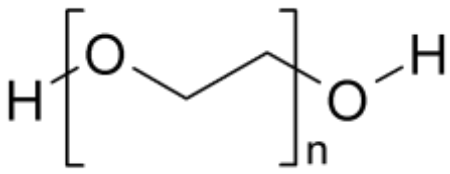


Figure 2.2 : Polyethylene glycol chain structure [35].

Today, it is an indispensable material for drug industry and it is one of the first polymers which are used for drug delivery systems. The advantages of PEG than other polymers are it can be easily interacted active ingredients with weak hydrogen bonds and contributes to development of stable drug delivery systems [35, 36]. Furthermore, PEG based copolymers play a crucial role as a biomedical material for biomedical applications, because of its biocompatibility, biodegradability, thermo-sensitivity and easy controlled characters [37].

PEGylation (also often styled pegylation) is the process of both covalent and non-covalent attachment or amalgamation of PEG polymer chains to molecules and macrostructures, such as a drug, therapeutic protein or vesicle, which is then described as PEGylated (pegylated) [38]. PEGylation is routinely achieved by incubation of a reactive derivative of PEG with the target molecule. The covalent attachment of PEG to a drug or therapeutic protein can "mask" the agent from the host's immune system (reduced immunogenicity and antigenicity), and increase the hydrodynamic size (size in solution) of the agent which prolongs its circulatory time

by reducing renal clearance. PEGylation can also provide water solubility to hydrophobic drugs and proteins.

A common method of preventing the biological adhesion of proteins and cells is to coat the artificial surfaces with PEG polymers. This ability to generate protein-resistant surfaces is often attributed to the fact that PEG chains are highly mobile and flexible as well as hydrated. Due to the high mobility of the surface-attached and swollen PEG chains, it is impossible for the protein molecules to penetrate onto the surface layer, reach the surface, and then prevent a cell adhesion response to the PEG-variant biomaterials with an adsorbed protein layer [39]. Surface-attached PEG chains can assume a wide range of conformations after swelling in water, and they required a certain volume. As the protein approaches the surface, it compresses the PEG chain, which leads to an entropy loss of the polymer chains (reduction of the degree of freedom) and accordingly to a decrease in the Gibbs free energy of the system [40]. This makes the diffusion of the protein into the layer energetically unfavorable. In particular, cell-repellent surfaces are only valid for very long PEG chains in the so-called brush regime, based on a high grafting density.

2.1.3 Chitosan as an encapsulation agent

Chitosan is a poly-cationic biopolymer as shown in Figure 2.3 and it is generally obtained by alkaline deacetylation of chitin, which is the main component of the exoskeleton of crustaceans, such as shrimps [41]. It may also be chemically modified to expand its properties and it has several advantages such as low cost and high commercial availability [42-44]. Since chitosan consists of molecules with multiple positive charges, this polysaccharide can interact with molecules that have negative charges, such as glycosaminoglycans present in the extracellular matrix [44]. Moreover, it has another important feature, which is about higher antimicrobial activity of chitosan. These features make chitin and chitosan ascendant in drug delivery applications.

CH has structural characteristics similar to glycosaminoglycans. It has interesting biopharmaceutical characteristics such as pH sensitivity, biocompatibility, biodegradability, muco-adhesivity, low toxicity and low immunogenicity. Due to these favorable properties, the interest in chitosan and its derivatives as tumor-targeted drug-delivery carriers has been increased in recent years [42]. CH allows

specific chemical modifications to form a wide range of derivatives since it has primary amine groups in the C-2 position and the hydroxyl groups in the C-6 position of its monomeric units. Chemically modified chitosan derivatives have great utility in the formulation of tumor-targeted drug-delivery carriers.

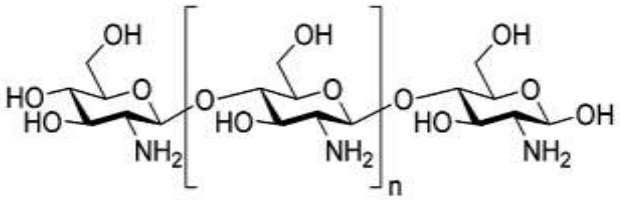


Figure 2.3 : Chitosan chain structure [42].

CH is considered to be one of the most widely used biopolymers for nanoparticle (NP) preparation because of its unique structural features [45]. It promotes cross-linkage with various cross-linking agents, such as glutaraldehyde, sodium tripolyphosphate (TPP), geneipin, etc., to provide an efficient network to entrap the drug molecules into the NPs. Since these NPs exhibit relatively long blood circulation times and low uptake by the reticuloendothelial system due to their smaller size, they can target the tumor sites through the disorganized and defective vascular architecture in tumor tissue, known as the enhanced permeability and retention effect. This is often referred to as passive targeting. One major limitation with passive tumor targeting alone is its inability to achieve a sufficiently high level of drug concentration at the tumor site, resulting in lower therapeutic efficacy and eliciting adverse systemic effects. To further improve delivery efficiency and cancer specificity, a strong emphasis has been placed on developing chitosan-based NPs with active tumor-targeting ability. Active targeting can be achieved by functionalizing chitosan and its derivatives with tumor-targeting ligands such as folic acid, antibodies, peptides, hyaluronic acid, biotin and avidin, which can recognize and bind to specific receptors that are unique to cancer cells.

CH is extensively studied in brain scaffold preparations and spinal cord implantable systems [46]. Moreover, it has displayed multiple bioactivities in various investigations carried out in the last decade. It is also widely employed in nanoparticle preparation for transportation of loaded drugs to targeted organs in case of cancer, diabetes, lung diseases and CNS disorders. CH and its degradation products, at the molecular level, exhibits anti-Alzheimer mechanisms majorly by

prevention of phosphorylation of c-Jun N-terminal kinase caused by αβ-amyloid, inhibition of pro-inflammatory cytokines and blockage of nitric oxide synthase [47]. Moreover, biodegradability, biocompatibility, flexibility in surface modification and ease of multiple preparation methods are added advantages of chitosan, which makes it an attractive nano-shells forming material to achieve successful delivery of drugs and nucleic acids to brain interstices.

Various studies showed that the enzymatic grafting of some phenols onto CH backbone such as chlorogenic acid or hexyloxyphenols using tyrosinase enzyme as catalyst was effective to obtain water-soluble CH derivatives at physiological pH [48]. These CH derivatives can be promoted to use in the pharmaceutical field for the production of drugs. The same enzyme was also used to catalyze the enzymatic covalent grafting of a de novo designed coil peptide onto chitosan to specifically capture proteins tagged with the partner coil peptide such as the epidermal growth factor (EGF). This enzymatic approach was considered as a method to obtain easily a pure and safe product for biomedical applications such as gene delivery and regenerative medicine. Moreover, the *M. thermophila* laccase was used to produce CH derivatives by the grafting of oxidation products of ferulic acid and ethyl ferulate onto CH particles in phosphate buffer at pH 7.5. This enzymatic process improved the HUVEC cell adhesion on chitosan derivative films compared to CH film, promising to use in tissue engineering [49] Microbial transglutaminase (MTGase) was also used as enzymatic catalyst to bind collagen peptide with chitosan or with carboxymethylated CH. The enzymatic grafting of collagen onto CH contributed to enhance antioxidant properties and promote L929 fibroblasts growth. Consequently, these CH derivatives could be potential wound dressings for clinical applications and showed the potentiality to repair skin in cosmetic, biomedical and pharmaceutical fields.

CH is also a treatment agent for humans with phenolic compounds on Fe toxic effects. In fact, under certain physiological conditions, increased iron absorption from the diet or due to genetic disorders like hemochromatosis, iron overload or the presence of “free” iron occurs [50]. Free iron causes toxic oxygen agents in the body via fenton chemistry. Another study discussed the use of *Trametes hirsute* laccase to catalyze the grafting of phenolic compounds onto CH in sodium citrate buffer at pH 4.5. The microspheres prepared by those functionalized CH derivatives presented

high iron-chelating abilities. These microspheres could be extensively investigated for drug delivery vehicles for controlled release and targeting studies of almost all class of bioactive molecules in medicine [50].

Peng *et al*. have prepared chitosan/αβ-glycerophosphate thermo-sensitive hydrogels for the sustained delivery of venlafaxine hydrochloride and studied the optimization of the formulation [51, 52]. Release mechanism is investigated by applying various mathematical models to the in vitro release profiles. Overall, drug release from the hydrogel shows best fit in first-order model and drug release mechanism is diffusion-controlled release. The results present that higher strength and glycerophosphate concentration result in higher initial release and rate constant, which support the hypothesis that the kinetic gelation mechanism of this system is nucleation and growth [53]. Furthermore, biodegradable poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) nanoparticles containing insulin phospholipid complex are loaded in chitosan/β-glycerophosphate hydrogels for long-term sustained and controlled delivery of insulin. In the in vitro release studies, only 19.11% of total insulin is released from the nanoparticle-loaded hydrogel within 31 days. Most importantly, the hypoglycemic effect of nanoparticle-loaded hydrogel following subcutaneous injection in diabetic rats lasts for >5 days, much longer than the effect caused by free insulin-loaded chitosan/β-glycerophosphate or other long-acting insulin formulations.

At present, there are several reports available regarding the production and use of chitosan nanoparticles as a delivery matrix for the release of pesticides in agriculture [54]. As an example, Paula *et al.* prepared and characterized microspheres composed of chitosan and cashew tree gum, which were used as carriers of the essential oil of *Lippia sidoides*, which possesses insecticidal properties [55]. The findings indicated the suitability of chitosan for use as matrices to carry bio insecticides designed to control the proliferation of insect larvae. Similarly, microcapsules of alginate and chitosan were prepared, characterized, and evaluated as a carrier system for imidacloprid. The particles obtained were stable and imidacloprid was encapsulated with an efficiency of around 82%. In release assays, it was shown that the release time of the encapsulated insecticide was up to eight times longer, compared to the free insecticide, and that alterations in the concentrations of alginate and chitosan affected the release profile.

2.1.4 Casein as an encapsulation agent

Caseins in Figure 2.4 are members of a paralogous group of unfolded phosphoproteins, some of which share the ability to sequester amorphous calcium phosphate through phosphate centers [56].

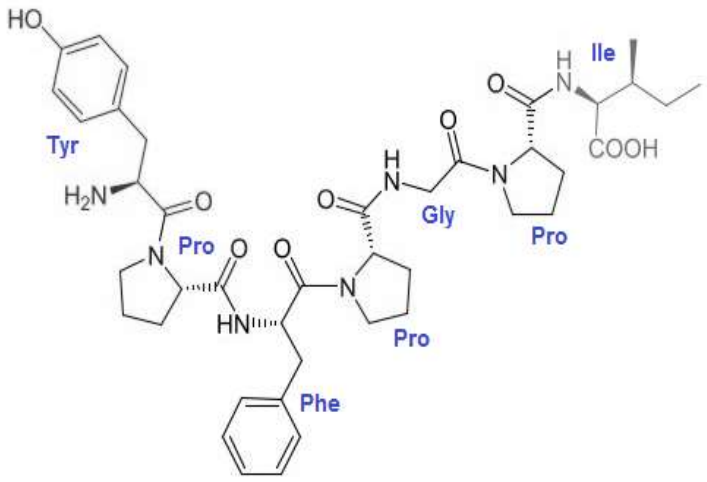


Figure 2.4 : Casein from bovine milk chain structure [57].

From a product technological and dairy industry point of view the caseins are by far the most important and valuable component of milk. The main dairy products as liquid milk, cheese and yoghurt derive their textural, sensory and nutritional properties from the caseins. Replacing caseins by plant proteins leads to products with different textural and sensorial quality [58]. The protein fraction of the CS micelles, which represents ~ 93% of its dry mass, is composed of four individual gene product components, denoted α_{s1}-, α_{s2}-, β- and κ-casein, which differ in primary structure and type and degree of post-translational modification. The remainder of the micellar solids consists of inorganic material, collectively referred to as colloidal calcium phosphate or micellar calcium phosphate.

Horne indicated that treating the CSs as block copolymers is an approach that grew out of studies of their behavior on adsorption to hydrophobic interfaces [59]. As regards the self-association of these proteins, it is speculated that the hydrophobic regions interact intermolecularly in solution, rather than compact themselves into a folded form. This further implies that they have a certain rigidity that inhibits such folding. Such intermolecular hydrophobic interaction leads naturally to the detergent-like micellar structure observed for β-casein in many studies, with a central

hydrophobic core and the hydrophilic peptides forming a hedgehog-like external coating.

For structure and chemical properties CS micelles, Horne also described that from the biofunctional viewpoint, the CS micelle is the ultimate level of self-association that involves all of the CSs [58]. CS micelles differ from the polymers of the individual caseins in one crucial aspect; they contain inorganic calcium phosphate as small microcrystalline inclusions, termed nanoclusters. Because the casein molecule has to be readily available to exert such control, this is offered by the proponents of the rheomorphic hypothesis as a reason for its open, flexible structure. The interaction would, therefore, conform to the inducible binding pathway where folding follows binding to the appropriate ligand.

The widespread or universal distribution of micelles in milk, suggests that they have some physiological or nutritional significance over the nutritional value of the proteins per se. Two benefits are apparent [60]:

1. Calcium and phosphate are required for the development of teeth and bone and growth rate is positively correlated with the concentrations of Ca and Pi. However, calcium phosphate has low solubility at the pH of milk with which it is supersaturated; therefore, calcium phosphate would be expected to precipitate in the mammary gland, with the formation of etopic stones that would block the ducts of the gland, resulting in the death of the organ and perhaps of the animal. By forming micelles, casein maintains the excess calcium phosphate in a colloidally stable state. Thus, the casein micelles may be regarded as a device by which to enable the secretion of milk with a high concentration of calcium phosphate in a "soluble" form.
2. Presumably, the micelles are designed to be coagulated in the stomach of the neonate by chymosin, a proteinase designed for this function. Coagulation delays the entry of milk constituents into the small intestine, thereby improving digestibility. Furthermore, the coagulum acts as a buffer to facilitate nursing at intervals that may be very long (24 h) for some species, e.g., the hare.

Strube *et al.* showed that CSs are also usable for biofilm applications because of a great potential of CS in many applications including biocompatibility, multi-

functional surfaces, anti-fouling, and biodegradability [61]. In all previous coating applications though, casein is simply used as a binder agent. The presented approach brings a completely new aspect to the use of casein in this area, as it utilizes the complex structure of the micelles and their ability to change its solubility. More precisely, it mimics the natural process of the enzymatic casein cleavage, in order to form biological coatings with high level of control. The crucial point is the cleavage of the casein micelles in direct proximity to the support surface. This prevents uncontrolled agglomeration and precipitation. Instead of that, the cleaved micelles deposit on the support surface due to hydrophobic interactions.

Bovine CS is also used to obtain antimicrobial peptide (AMP) which has received increasing interest due to the emergence of bacterial resistance and the potential toxicity of chemical food preservatives [62]. There is increasing evidence that AMPs act through different mechanisms, among which the membrane action mode. Although the specific membrane action target of AMPs is not fully understood, it is generally believed that the membrane binding process is the initial step when AMPs exert bactericidal activity. Zhang *et al.* demonstrated in their study that the affinity interaction between AMPs and the bacterial cell membrane plays a vital role in the antibacterial action.

2.1.3 Sodium alginate as an encapsulation agent

Alginates are anionic biopolymers extracted from 100% organic sources like brown seaweeds. Sodium salt of alginate is also extremely versatile and adaptable biomaterial, with great potential for use in biomedical applications such as drug encapsulation materials. Their extracellular matrix-like features as seen in Figure 2.5 have been key factors for their choice as biopolimers for cell delivery at tissue regeneration and for drug delivery at encapsulation systems. A variety of strategies to decorate them with other biofunctional components to modulate their biophysical properties has been developed recently, which further allow their tailoring to the desired application [63].

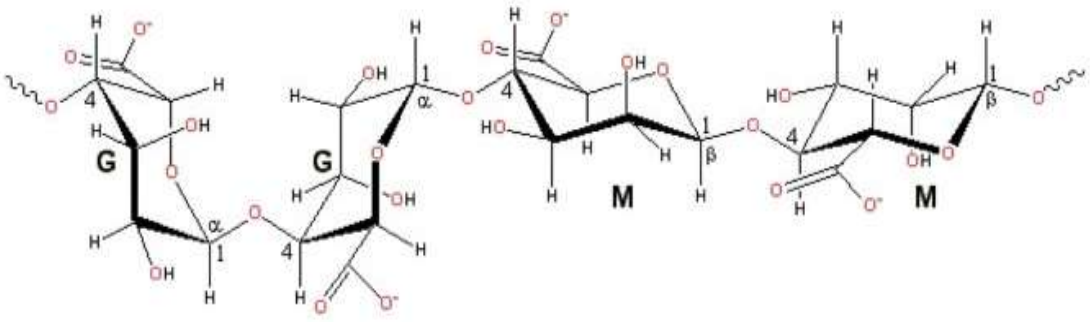


Figure 2.5 : Alginate chain structure [63].

SA is of the most popular natural polymers which has been investigated for wound dressing application incorporating with polyvinyl alcohol (PVA) polymer as either main or additional component to the dressing structure due to its high water swelling ability which impacts the local wound environment beyond moisture management [64]. Kim *et al.* have used PVA/alginate hydrogel containing nitrofurazone for wound dressing purposes, where they have used the freeze–thawing method to crosslink PVA/SA blended polymer [65]. They have reported that the increase of SA concentrations in PVA hydrogel films, increased the swelling ability, elasticity, and thermal stability of PVA/SA hydrogel films while, significant decreases in gel fraction%, and mechanical properties of PVA/SA hydrogel film were found with increased SA contents. They have also conducted the bio-evaluation of PVA/SA hydrogel films, and they revealed that increased SA contents resulted in the protein adsorption *in vitro* increases, indicating the reduced blood compatibility. Furthermore, *in vivo* experiments showed wound size reduction in rats, indicating a better wound healing ability proportionate to the amount of SA incorporated into PVA hydrogel films.

Levic *et al.* have made an efficient encapsulation matrix for d-limonene encapsulation using crosslinking of PVA/SA by the freeze–thawing method, followed by a calcium ionic interaction between alginate and $CaCl_2$ solution but these hydrogels do not apply for wound dressing but for food processing application [66]. This study showed that increasing SA concentration decreased the gelation (%), maximum strength and break elongation, but it resulted in an increment in the swelling ability, elasticity and thermal stability of the hydrogel film. However, SA content had an insignificant effect on the release profile of clindamycin from the PVA/SA film, whereas PVA/SA-clindamycin improved the healing rate of artificial wound in rats.

Tarun and Gobi presented a new concept for the synthesis of the nanocomposite web of calcium alginate and PVA with varying proportions using an electrospinning technique for wound healing application [67]. They have demonstrated that the blended nanofiber composite web which has the maximum calcium alginate content, showed the maximum water vapor transmission rate indicating the PVA/SA nanofiber web helps in maintaining the local moist environment for accelerating wound healing. Furthermore, *in vivo* experiments on rats exhibited apparently new epithelium formation without any harmful reactions, when the wound is covered with the PVA/SA nanofiber. This study has classified wound dressing material formation which is based on PVA/SA into three categories as follows: (a) dressings containing a significant proportion of SA to improve the gelling properties of the dressing in use, (b) dressings obtained by the freeze-dried PVA/SA film or γ-ray irradiation techniques, and (c) dressings formed depending on the bond between an exuding wound and an ion-exchange reaction, occurring due to the calcium ions in the dressing and sodium ions in the serum or wound fluid [64].

Stulzer et al. showed encapsulation efficiency of SA in microparticle system to provide controlled drug release [68]. In this study, SA and CH is used microparticle agent for encapsulation of a high molecular weight molecule rifampicin. The microparticles are prepared by gelatinizing method during a 30-min period in contact with the precipitating solution and were then filtered, washed with distilled water and dried at room temperature. The results obtained showed that the SA/CH microparticles represent an efficient system for the controlled-release of rifampicin. At acidic pH, the release of 20% of the drug occurred in 2 h, and at pH 6.8 a rapid increase in the release rate was observed up to 100%.

Kim et al. are described with detailed models using of SA hydrogels for bone tissue engineering applications in their paper [69]. Alginate hydrogels or scaffolds can be prepared by diverse range of cross-linking agents that are majorly calcium-based substances. When sodium alginate is placed into the calcium-based ion, the calcium ion replaces the sodium ion in the polymer; each calcium ion is cross-linked with two moieties of polymer strands. Alginate scaffolds are being actively explored for their ability to facilitate the regeneration of other tissues and organs, including skeletal bone, skin, nerve, liver and pancreas. Different kinds of methods including lyophilization, electrospinning and cross linking, have been extensively studied to

prepare the alginate scaffolds. Traditional method to produce the alginate biopolymer scaffold is freeze dry-lyophilization method.

Bioactive glasses comprise of glass-ceramic biomaterials and are extensively studied materials for implantation in the human body to repair/replace the defective or diseased bone. Mishra *et al.* prepared the scaffold *viz.* bioactive glass-polyvinyl alcohol-alginate biocomposite materials by surfactant foaming, having low-density 0.92 g/cm^3 along with pore size 200–500 μm [70]. Synthesized biocomposite shows excellent biocompatibility with fibroblast cells with compressive strength of 1.64 MPa and elastic modulus of 18 MPa. In another study, Luo *et al.* reported mesoporous bioactive glass and concentrated alginate paste scaffolds by 3D plotting techniques [71]. Addition of mesoporous silica material increased the mechanical strength, apatite mineralization and cytocompatibility properties of the composite scaffold. In addition, dexamethasone was incorporated for the delivery purpose for better bone regeneration. Compressive strength of 50% mesoporous bioactive glass/alginate scaffolds was reported to be significantly higher than pure alginate scaffolds. Enhanced mineralization and protein adsorption was observed on addition of bioactive glass in alginate. Furthermore, human periodontal ligament fibroblast and osteosarcoma cells were viable, adhered and proliferated well on the alginate-nanobioactive glass composite scaffolds in comparison to the control alginate scaffolds.

Different kinds of biocomposite materials have been established to improve the bioactive properties of the scaffold *via* chemical modification, including the immobilization of functional cell-adhesive ligands and bioactive molecules such as enzymes, drugs and cytokines. SA is widely utilized in medical area for tissue engineering and regeneration due to its biocompatibility, non-thrombogenic nature, easily handled from nature, mild and physical gelation process, and the resemblance of its hydrogel matrix texture and stiffness to that of the extracellular matrix. The use of bioactive molecules (growth factors, cytokines, and stem cell mobilizing factors) has always been of interest in the field of therapeutic myocardial regeneration. The effects exerted by these molecules are relevant to almost every target in regeneration strategies, such as cell survival and proliferation, vascularization apoptosis inhibition, progenitor/stem cell migration, and directed stem/progenitor cell differentiation. In addition, systemic administration requires high doses of the protein

in order to achieve optimal concentration at the specific target site, due extremely low protein stability in the circulation which leads to fast elimination of the protein. Moreover, most of these molecules have pleiotropic functions, emphasizing the need for careful, local and time-adjusted interventions. Thus, biomaterials could be engineered to produce a sustained delivery system for bioactive molecule combinations. Due to the Cohen and Ruvinov, in such a system, the biomaterial will provide structural temporary matrix support and direct the formation of functional tissue *in situ* [72]. Simultaneously, it will serve as a temporary depot for sustained delivery and presentation of bioactive molecules with spatial and controlled distribution of the desired agent to induce multiple reparative/regenerative processes. In general, due to low protein adsorption and its highly porous and hydrophilic nature, the pristine unmodified alginate matrix yields fast and unpredictable protein release kinetics. Thus, several modification designs and engineering schemes were applied for the use of alginate in protein delivery.

2.2 L-Ascorbic acid as an active ingredient

L-ascorbic acid is a naturally occurring organic compound with antioxidant properties. It was originally called L-hexuronic acid, but when it was found to have vitamin C activity in animals. It is a lactone which contains six carbons and an essential vitamin for humans because of antioxidant properties and cofactor agent in enzymes [73]. Chemical structure of L-ascorbic acid was shown in Figure 2.6.

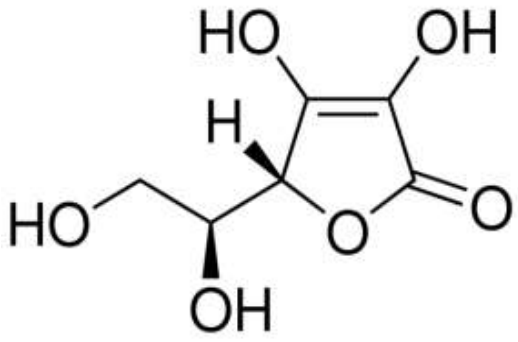


Figure 2.6 : Structure of L-ascorbic acid [73].

L-ascorbic acid appears in so many biologic applications in human body, for example in bone, teeth, cartilage, and skin as collagen agent; in enzymes as cofactor

agent [74]. However, it is a very sensitive material and pH, temperature, oxygen, metal ions, UV, and X-ray radiation can easily damage its structure.

L-ascorbic acid is a required nutrient for a variety of biological functions. Humans and other primates have lost the ability to synthesize ascorbic acid due to a defect in L-gulono-1,4-lactone oxidase, an enzyme that catalyzes the conversion of L-gulonolactone into ascorbic acid [75]. Humans, primates, and a few other animals (e.g., guinea pigs) depend on the diet as a source of L-ascorbic acid to prevent the L-ascorbic acid deficiency disease, scurvy, and to maintain general health. Another healthy effect of L-ascorbic acid can be attributed to its biological functions as a cofactor for a number of enzymes, most notably hydroxylases involved in collagen synthesis, and as a water-soluble antioxidant.

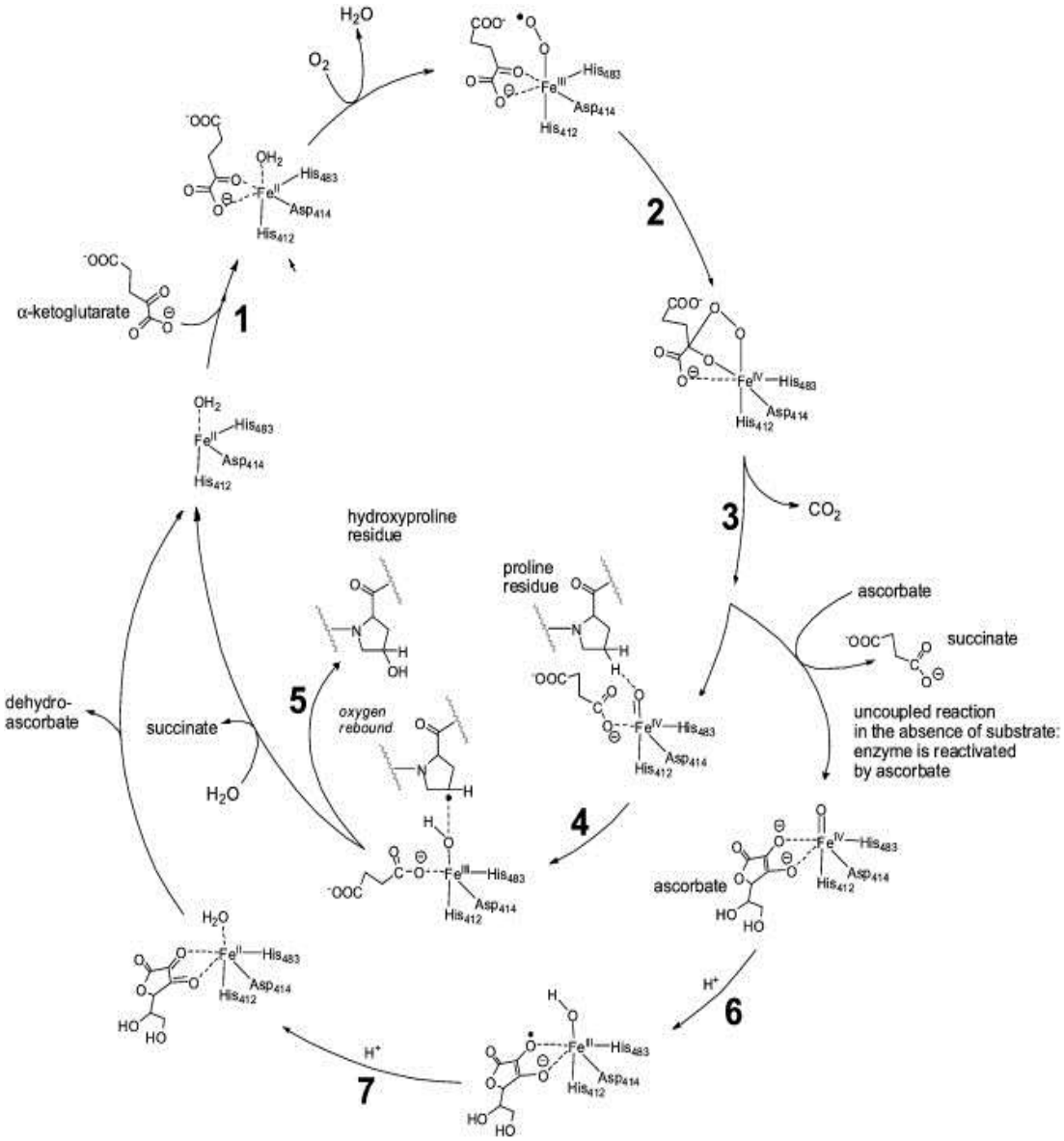


Figure 2.7 : L-ascorbic acid acts as a cofactor for prolyl 4-hydroxylase [76].

It can also function as a source of the signaling molecule, hydrogen peroxide, and as a Michael donor to form covalent adducts with endogenous electrophiles in plants. One of them the functions and the underlying mechanisms is illustrated in Figure 2.7 [75].

Since the discovery of L-ascorbic acid, the number of its known biological functions is continually expanding [77]. Both the names ascorbic acid and vitamin C reflect its antiscorbutic properties due to its role in the synthesis of collagen in connective tissues. L-ascorbic acid acts as an electron-donor keeping iron in the ferrous state thereby maintaining the full activity of collagen hydroxylases; parallel reactions with a variety of dioxygenases affect the expression of a wide array of genes as well as *via* the epigenetic landscape of cells and tissues. In fact, all known physiological and biochemical functions of L-ascorbic acid are due to its action as an electron donor.

The repeatedly ageing effects of oxidants has led to negative impression on prolong lifespan, so antioxidant dietary is suggested recently. L-ascorbic acid is an essential antioxidant in human diets and is widely used for supplementation. It is one of the most important water soluble and easily handled antioxidants; ever since the free radical theory of ageing was discovered forward 50 years ago [78]. Therefore, humans and animals depend on their diet as a source of L-ascorbic acid to prevent scurvy, produce essential enzymes and maintain general health. It is included primarily through the consumption of herbals such as fruit, vegetables; some artificial drinks and supplements. It has been shown to be important for the synthesis of collagen, carnitine, and catecholamines; the impaired production of which leads to the symptoms associated with scurvy. The health promoting effects of L-ascorbic acid can be attributed to its biological functions as a co-factor for a number of enzymes, such as hydroxylases involved in synthesis of collagen in the body, and as a water-soluble antioxidant for anti-ageing. It donates two electrons from a double bond between the second and third carbons of the 6-carbon molecule as seen in Figure 2.7. It is hypothesized that by donating its electrons, L-ascorbic acid prevents proteins and other cellular compounds from being oxidized, so it helps anti-ageing [79].

There has been considerable interest regarding the association of L-ascorbic acid with the common cold. Banerjee *et al.* studied effect of L-ascorbic acid on prevention of influenza and they indicates in their article that mega doses of L-ascorbic acid

(3 g/d) have been shown to prevent cold and flu symptoms in students 18–30 y of age; on the other hand, lower doses of L-ascorbic acid have not been shown to prevent cold symptoms in a number of trials [80]. They indicates that doses of L-ascorbic acid in excess of 1 g/d taken shortly after onset of a cold symptom did not decrease the duration or severity of cold symptoms in healthy adult volunteers when compared with a L-ascorbic acid dose lower than the minimum recommended daily intake. In a controlled trial of 226 patients with influenza A, 114 patients received L-ascorbic acid 300 mg/d, and 112 patients served as controls; outcomes measured were development of pneumonia and duration of hospital stay. Pneumonia was reported in two subjects in the treatment group and 10 in the control group, and hospital stays for influenza or related complications averaged 9 d in the L-ascorbic acid group and 12 d in the control group.

Another interest of usage area for L-ascorbic acid is anti-tumor treatments because of its antioxidant effects [81]. By disarming biologically damaging molecular fragments known as free radicals, L-ascorbic acid can fight devastation effect of aging and many chronic diseases. Free radicals also offer benefits, however, such as ridding the body of germs and damaged cells. By curbing these activities, a new animal study finds, antioxidants can aid cancer growth. Because of these reasons, it has become the ost wanted antioxidant of the nutritional world [82]. Free radicals of reactive oxygen and nitrogen species that have potential to damage nucleic acids and promote carcinogenesis and they are generated in response to high concentration of L-ascorbic acid can catalyze lipid peroxidation and are cytotoxic to cancer cells. Within the body, antioxidant levels act as a signal, controlling cell division. In healthy cells and benign tumors, oxidants tend to increase cell proliferation, whereas antioxidants inhibit it. By contrast, the malignant tumor environment can be so strongly oxidizing that it is damaging and triggers cell death by apoptosis. In this case, antioxidants may help tumor cells proliferate and survive, by protecting the cells against oxidation and stimulating the malignancy to grow. L-ascorbic acid presents a double-faced character in that it exhibits a pro-oxidant activity arising from its routine antioxidant property. Apart from reducing oxidizing sources like H_2O_2, L-ascorbic acid also reduces metal ions like Fe^{3+} and Cu^{3+}, the process during which free radicals are generated. This reaction in which L-ascorbic acid generates highly reactive free radicals in presence of transition metal ions is called Fenton reaction [81].

$2Fe^{3+}$ + L-ascorbic acid → $2Fe^{2+}$ + DHA (2.1)

$2Fe^{2+} + 2H_2O_2 \rightarrow 2Fe^{3+} + 2OH^- + 2OH^*$ (2.2)

These hydroxyl radicals are reported to interact with DNA inducing its damage by causing breaks in phosphodiester backbone and modification of DNA bases. This property of pro-oxidant activity inducing cytotoxicity has been employed in many studies in the prevention and treatment of cancers and is proposed to be dose-dependent.

2.3 Drug Encapsulation Techniques

New drug delivery technologies are revolutionizing the drug discovery; development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this regard, novel drug delivery systems (NDDS) have many benefits, which includes improved therapy by increasing the efficiency and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site specific delivery to reduce the unwanted adverse effects.

The basic principles for development of drug delivery systems are easily integration of active ingredient into drug, achieving of higher drug release ratios, and non-toxic effects of drug capsule to cells after its biodegradation. The last ten years, production of more effective, biocompatible, and biodegradable drug systems have been increasingly interest for pharmaceutical industry [18,23]. Nowadays, polyester based biopolymers are used for drug delivery and encapsulation systems and some examples of these polymers are PCL, PEG, PLA, PGA, polyacrylic acid (PAA), poly(methyl methacrylate) (PMMA), chitosan, chitin, and starch [83].

It is another important issue for drug delivery systems is that stability at outside and sufficient degradation of drugs inside of human body. To overcome this problem, polymeric based drug delivery systems could be an enormous alternative because biodegradation rate is easily adjustable by blends of polymers [84]. In this way, stability, effective biodegradation rate, and higher drug release contents for a drug could be achieved simultaneously.

Stability of a drug is depends on protection capacity of active ingredient, thus if a polymer is used for an encapsulation system, it interact temporarily stable between active ingredient to deliver system on targeted cells before releasing.

At these view of points, preparing of a drug, which is easily biodegradable, biocompatible inside the body and stable outside the body, is the main problem and most important goal for pharmaceutical industry.

Encapsulation may be defined as a process to entrap one substance within another substance, thereby producing particles with diameters of a few nm to a few mm. The substance that is encapsulated may be called the core material, the active agent, fill, internal phase, or payload phase. The substance that is encapsulating might also be called the coating, membrane, shell, carrier material, wall material, external phase, or matrix. Two main types of encapsulates might be distinguished, i.e., the reservoir type and the matrix type [12].

The most widely used encapsulation techniques and some related applications are summarized in Table 2.1.

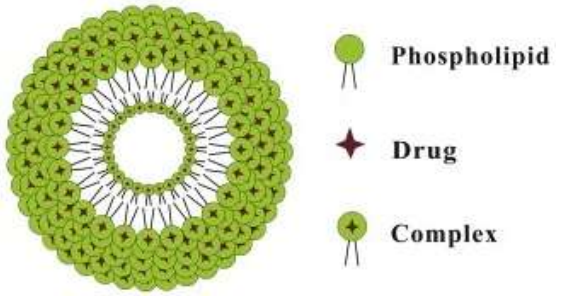
Table 2.1 : Main conventional techniques used for the encapsulation of bioactives and food ingredients [85].

Technique	Concept	Examples of applications
Spray drying	Drying of the encapsulated material dispersed in the shell material. Encapsulation by starch, polysaccharides, maltodextrins and/or proteins. This technique produces particles between 10 and 100 μm.	Encapsulation of flavor compounds, polyphenols and vitamins
Spray cooling/chilling	Incorporation of the core material in the warm and liquefied shell material (often-vegetable oils). Relatively low-cost encapsulation technique. Through product atomization, and consequently cooling, micro-capsules are formed.	Encapsulation of flavor compounds, minerals, vitamins and probiotics
Freeze drying	Co-lyophilization of the core and the shell materials after a homogenization process. Normally this technique produces non-uniform particles.	Encapsulation of flavor compounds, fatty acids and probiotics
Extrusion	Formation of core material droplets that became microparticles after immersion in a hardening bath with the shell material. Normally the shell material is a glassy carbohydrate matrix. The core material may be released in a high temperature medium. The encapsulation efficiency is small, moreover the produced particles show high stability and an extended shelf-life.	Encapsulation of flavor compounds, vitamins and food ingredients (lactic acid)
Spinning disk	Passage through a spinning disk of a suspension of the core material in the shell material. During processing, the shell material forms a thin film around the core material particles. Production of particles from 20 μm to few millimeters of diameter.	Encapsulation of cells (yeast)

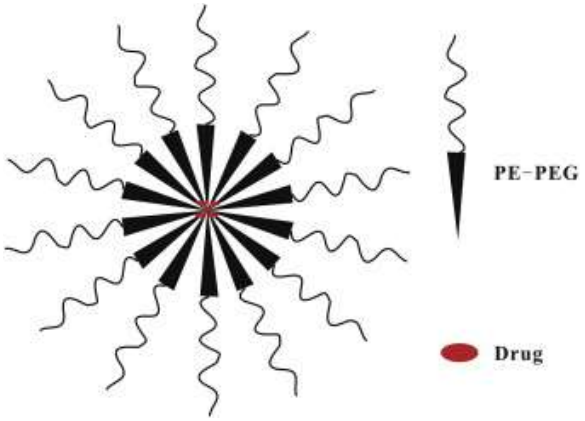
Table 2.1 (continued) : Main conventional techniques used for the encapsulation of bioactives and food ingredients [85].

Technique	Concept	Examples of applications
Supercritical fluid extraction	This technique is similar to spray drying, except that the shell material and the core material are solubilized/dispersed in a supercritical fluid.	Encapsulation of heat-sensible cores as vitamins and polyphenols
Fluidized bed	Solid particle encapsulation (100 μm to few mm). The shell material is atomized onto core material fluidized by an upward stream of air.	Encapsulation of acidulates, vitamins and cells
Co-crystallization	Spontaneous crystallization of a supersaturated solution of sucrose simultaneously with the addition of the core material, forming a crystalline irregular network, allowing the encapsulation into the pores of the network.	Encapsulation of acids, flavor compounds, antioxidants and minerals
Co-acervation	Phase separation of one or many polyelectrolytes from a solution and deposition of the colloidal particles around the active ingredient suspended or emulsified in the same reaction media. When hydrocolloids are used, they can be cross-linked using appropriate chemical or enzymatic agent.	Encapsulation of fatty acids and flavonoids
Liposomes	Spherical particles consisting of a membranous system formed by one or more concentric bi-layers of lipids (often phospholipids). They can be used in the entrapment, delivery and released of water-soluble, lipid-soluble and amphiphilic materials.	Encapsulation of vitamins, enzymes and peptides
Inclusion	Supra-molecular association through non-covalent interactions of a ligand ("encapsulated" compound) into a cavity formed by a "shell" material (e.g., cyclodextrins).	Encapsulation of vitamins, flavor compounds and essential oils

Liposomes, micelles and microparticles (as seen in Figure 2. 8 (a), (b), (c), respectively) are the most attractive ways to produce effective drug delivery systems.



(a)



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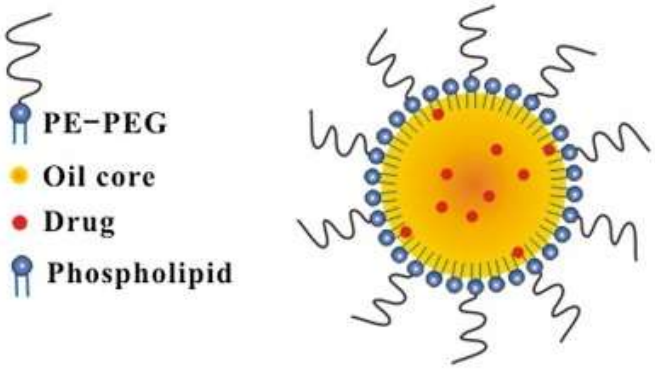


(c)

Figure 2.8 : Schematic representation of the structures of **(a)** liposomes, **(b)** micelles, **(c)** microparticles [86].

It is always a fact that performance of a drug delivery system is about encapsulation and release efficiency. One of the basic requirements for the controlled and balanced release of the medicament in the body is its ideal spherical shape of polymeric particles and narrow distribution of their sizes. The size and shape of the particles play key role in their adhesion and interaction with the cell. The most important problem for polymer based drug delivery systems is lower drug

loading and drug release efficiency [87]. On the other hand, drug loaded microspheres have to be lower particle size (about 10 μm) and well distributed.

Emulsion solvent evaporation technique is widely used in the field of particulate carriers' development. It consists of the formation of a simple or a double emulsion and the subsequent evaporation of the organic solvent which leads to the precipitation of the polymer and the obtaining of the particles. More precisely, first, the polymer is dissolved in a volatile and non-miscible organic solvent such as chloroform, ethyl acetate, toluene, tetrahydrofuran or dichloromethane. This organic phase is then dispersed by high-speed homogenization or by sonication in an aqueous phase that contains a surfactant. Once an oil-in-water (o/w) emulsion is obtained, the evaporation of the organic solvent allows its diffusion to the outer phase leading to the formation of the particles. This method of simple emulsion solvent evaporation is generally used for the encapsulation of hydrophobic drugs. Besides, drug delivery systems which are obtained by oil/water emulsion based mixture, has man disadvantages. The main reason of this problem is using of toxic organic solvents in these systems, so it could have a potential harmful effect on human body. Furthermore, organic solvents-active ingredients interactions may have adverse side effect for drugs [88]. Thus, using water as a solvent for drug preparation has become more important since last decade. Contribution of water on zero toxicity and higher biocompatibility of drugs for human cells makes it more important for drug delivery synthesizing systems.

Novel technologies are required for delivery of new drug molecules in order to reduce their side effects, optimize their efficacy, and enhance patient compliance. Due to the Farokhi et al., recently, the use of nanotechnology has led to the development of many novel carriers capable of controlled release and targeted delivery of a wide range of small molecules, proteins, peptides, and genes [89]. These devices can have many different structures, including quantum dots, dendrimers, fullerenes, ferritin, and nanoparticles. Among them, nanoparticles based on biodegradable and biocompatible polymers have potential applications in cancer therapy and as sustained drug delivery vehicles. These carriers can also be designed as low toxicity systems with suitable physical and chemical structures and specific targeting properties.

Another important study about nanoparticles on drug delivery systems by Hua *et al.* are described that improved oral drug delivery design has drastically improved the colonic bioavailability of drugs, that is, these formulations are effective at reaching and releasing drug specifically in the colon [90]. However, in order for a drug to have therapeutic efficacy it must be localized to the site of action within the colon. Conventional oral formulations can be adversely affected during active inflammatory bowel disease (IBD) or following intestinal resection, and have limited efficacy and specificity for diseased colon tissue versus healthy colon tissue. In addition, despite coverage of the colonic surface (including diseased tissue), there is no guarantee that the drug is effectively taken up into the tissue and cells at the site of inflammation. Pharmaceutical strategies utilizing nano-delivery systems as carriers for active compounds have shown promising results in addressing the physiological changes in IBD, and exploiting these differences to enhance specific delivery of drugs to diseased tissue. Therefore the use of nanotechnology in formulation design may further improve the efficacy of therapeutics by allowing inflammation-specific targeting and uptake within the colon. Furthermore, reducing the size of drug delivery carriers to the nanometer scale has been shown to improve colonic residence time in inflamed intestinal regions and provide additional benefits for IBD therapy

Nano-delivery systems have been designed to passively or actively target the site of inflammation. These systems have been shown to be more beneficial than conventional formulations, because their size leads to more effective targeting, better bioavailability at diseased tissues and reduced systemic adverse effects. Hence, nano-delivery systems have been found to have similar or improved therapeutic efficacy at lower drug concentrations in comparison to conventional formulations [91]. Although size is an important factor in targeting the colon, additional strategies to enhance drug delivery to inflamed intestinal mucosa and achieve maximal retention time in tissues are being explored.

Liposomes are most commonly studied and used encapsulation systems because of fabrication of liposomes form food-grade components using relatively simple laboratory-scale methods [92]. On the other hand, liposomes production is really hardo to scale indusctrial level. Furthermore, their poor physical stability under the environmental conditions is another problem. In additionally, encapsulation efficiency of liposomes is generally low for hydrophilic active ingredients, Because

of these reasons mentioned above, liposome productions are not effective and economic at large scale.

Microencapsulation has been defined as “the technology of packaging solid, liquid and gaseous materials in capsules at micro-scale that release their contents at controlled rates over prolonged periods of time” [93]. Microencapsulation technique is used in various applications like pharmaceutics, cosmetics and food industries. In food applications, several purposes are targeted; firstly, the stabilization of the encapsulated product against damage caused by external conditions such as light or heat during processing during storage; secondly, flavor release control; thirdly, colur and taste protection; finally, reactive species separation [94]. Microencapsulation within spherical microparticles possesses many benefits over other encapsulation geometries, including a high surface area to volume ratio, a high resistance to mechanical stress, a relatively short diffusion path length, and access to a number of implantation sites by injection. Moreover, while microencapsulation process, chemical structure of the active ingredient and/or food compounds should not be changed. With this aim in mind, the challenges that must be met by encapsulation are multiple: i) increasing stability of encapsulation, ii) not effecting on sensitivity of drug compounds, iii) improving the release ratio of drug compounds.

Hag *et al*. and Kowalczuk *et al.* indicate that the mechanism of microencapsulation can occur within unimolecular micelles in three different ways [95, 96]. The guest can be encapsulated in the core, in the shell, or at the interface of the core and the shell of unimolecular micelles as shown in Figure 2.9 (a), (b), (c). In addition to this, it was found that unimolecular micelles, although they represent single-molecule micelles per se, do not necessarily transport their cargo in a unimolecular fashion. Instead, the microencapsulation can occur in a fourth way, whereby the guest is encapsulated within aggregates of several unimolecular micelles (Figure 2.9 (d)). The fourth microencapsulation mechanism is therefore not unimolecular but supramolecular.

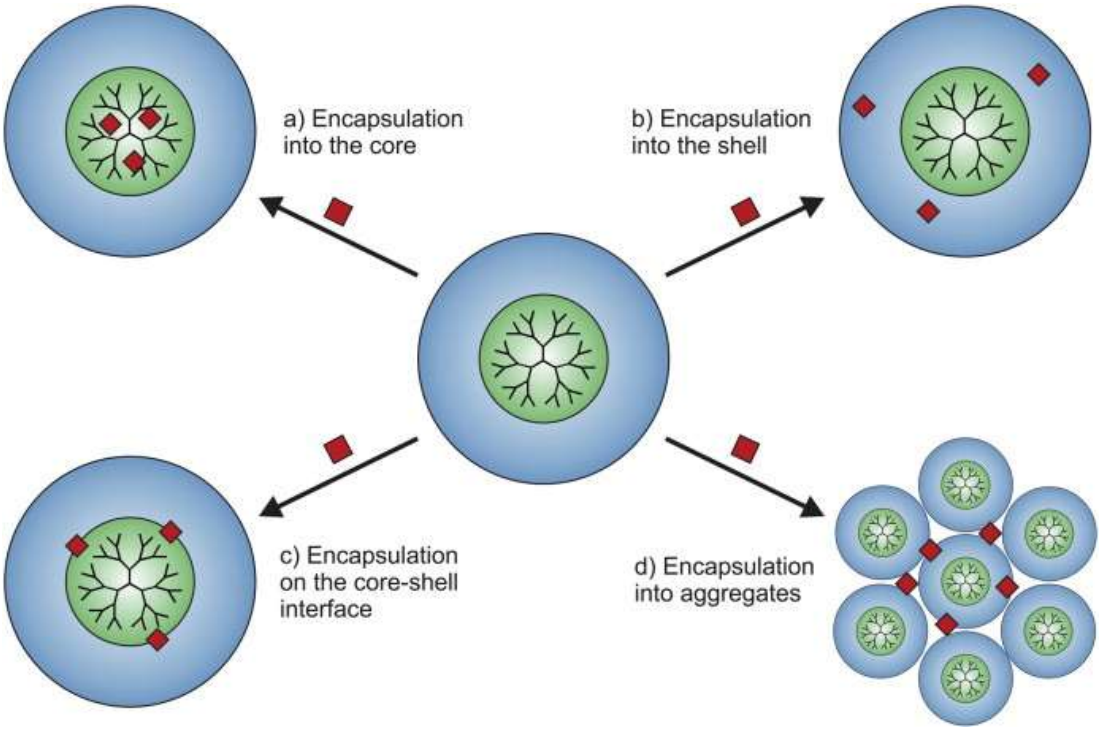


Figure 2.9 : Four different microencapsulation mechanisms in core–shell architectures [95].

Depending upon the mechanism of transport, the size of the micro-carrier could also vary, which is an important factor that needs to be considered for the design of a successful drug delivery system. For instance, transport through supramolecular self-assembly results in larger aggregates. In general, the size of the micro-carriers that transport through unimolecular mechanisms varies from a few nanometers to tens of nanometers depending on the molecular weight of the system.

The reservoir type has a shell around the active agent. This type is also called capsule, single-core, mono-core or core-shell type. Application of pressure can lead to breakage of the reservoir type of encapsulates and thus to the release of its contents. Poly- or multiple-core type of encapsulates with several reservoir chambers in one particle also exist. The active agent in the matrix type is much more dispersed over the carrier material; it can be in the form of relatively small droplets or more homogenously distributed over encapsulation. Active agents in the matrix type of encapsulates are in general also present at the surface, in contrast to those in the reservoir type [96-100].

Inhalation of powder compounds is an attractive concept minimizing the side effects by its pulmonary selectivity. Controlled drug delivery systems have become increasingly attractive options for inhalation therapies. A large number of carrier

systems have been developed and investigated as potential controlled drug delivery formulations to the lung, including drug loaded lipid and polymer based particles. However, there are some extraordinary specifications should have for a drug delivery material [101].

Heng et al., Peltonen et al., and Vehring were also showed encapsulation composite or material should be in amorphous phase for physical stability of drugs [102-104]. Because of good biodegradation behavior in human body and contribution on homogeneous distribution of active ingredients in matrix of amorphous polymers, they are preferred in pharmaceutical industry. In addition, these studies showed that amorphous composites could be easily obtained by spray dryer. Another positive impact of using of spray dryer is that achieving lower particle diameter, well distributed microspheres and as possible as higher surface area in only one process.

In the study of Bee et al. was demonstrated that ester and ether bonds contained polymer are more effective for using of encapsulation material such as cellulosic and lingo-cellulosic polymer chitosan, chitin etc. [105]. Moreover, they were emphasized that when developing solid-dispersion formulations of poorly soluble active ingredients, one must do the following: comparison of the functional groups of both polymer and active ingredients; comparison of solubility parameters with polymer and active ingredients; analyzing the permeability of active ingredients in the polymer matrix. Consideration of only one parameter is not a good approximation to produce a stable drug because all factors such as solubility, permeability, toxicity, productibility, efficiency, and interaction between polymer and active ingredients must be handled at the same time. On the other hand, Taylor et al. also showed that polymer hygroscopicity is another important parameter for physical stability of drugs [106]. Absorption of moisture from air of a drug means that initiation of phase separation in the drug, so activity and stability of drug decreases dramatically. Thus, using of highly hydrophobic polymer should be a must in encapsulation materials. For these systems, water was found to disrupt drug-polymer interactions, which, coupled with the ability of water to increase molecular mobility, led to phase separation.

2.4 Drug Release Mechanism and Kinetics

Drug release has been an important topic in the field of drug delivery for decades. With advancement in material design and engineering, novel materials with increasing complexity and more functions have been introduced into the development of drug delivery devices and systems [107]. Both naturally derived and synthetic macromolecules are extensively used in controlled drug release to maximize bio-efficacy. Control release is a useful tool to maintain constant drug levels in the human body.

Drug release from polymeric carriers involves several mechanisms [107]. Depending on the polymeric structure, the drug might be released by diffusion through the polymeric matrix or through pores present in the carrier. Erosion of the carrier surface or bulk erosion can also lead to drug release. The high surface area to volume ratio of polymeric carriers contributes to a rapid drug release from the matrix which is called a "burst". This phenomenon can be reduced by drug complexation with various agents before encapsulation as well as by adding lipid aid excipients to the formulation [108]. Complexing agents or lipid aid excipients interact with the encapsulated compound and these interactions increase encapsulation efficiency as they decrease the burst.

Types of controlled drug release systems are listed below:

1. Dissolution controlled systems
    a) Encapsulation dissolution controlled system.
    b) Matrix dissolution controlled systems.
2. Diffusion controlled systems
    a) Reservoir controlled systems.
    b) Matrix controlled systems.
3. Dissolution and diffusion controlled release systems
4. Water penetration controlled systems
    a) Swelling controlled systems
    b) Osmotically controlled systems.
5. Chemically controlled release systems
    a) Erodible systems
    b) Drug covalently linked with polymer

6. Hydro gels
7. Ion - exchange resin controlled release systems

2.4.1 Dissolution controlled release systems

The dissolution of a drug administered in the solid state is a pre-requisite for efficient subsequent transport within the human body. This is because only dissolved drug molecules/ions/atoms are able to diffuse, e.g. through living tissue [109]. In this system, dissolution is the rate controlling step which is shown in Figure 2.10

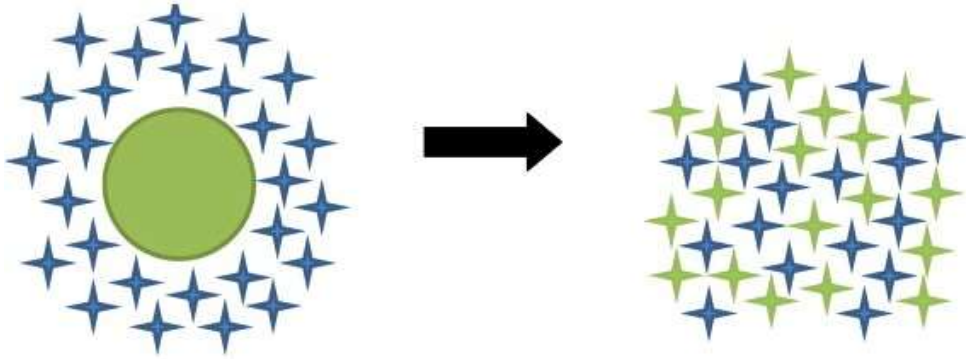


Figure 2.10 : Dissolution of a solid drug particle in an aqueous liquid [109].

The drug release occurs by dissolution and/or erosion of encapsulation material matrix. It is of two types and they are;

a) Encapsulation dissolution controlled system
b) Matrix dissolution controlled system

On the first method, the drug particles are encapsulated by microencapsulation technique by coating agents like cellulose, CH, PEG, PLA, polymethacrylates, waxes etc. the dissolution rate of capsulation depends upon the solubility and thickness of the coating.

Matrix systems depend on dispersion of active ingredients homogeneously into the release medium. Waxes such as beeswax, carnauba wax, hydrogenated castor oil are employed as matrix systems release agents and they allow controlled release of active ingredients by altering the porosity of rate.

2.4.2 Diffusion controlled release systems

Diffusion is a process of moving molecules from a solution of high concentration to low concentration. There are two basic devices that are driven by diffusion in controlled release systems. In these devices, the drug is released either by passing through the pores or between polymer chains, and these are the processes that control the release rate. Diffusion controlled release devices;

a) Reservoir diffusion system
b) Matrix diffusion system

Active ingredients are dispersed homogeneously into the matrix diffusion systems (monolithic devices), and it is released by diffusion from the polymer into the release mediums as shown in Figure 2.11. The release rates of monolithic devices decrease as a function of time and distance.

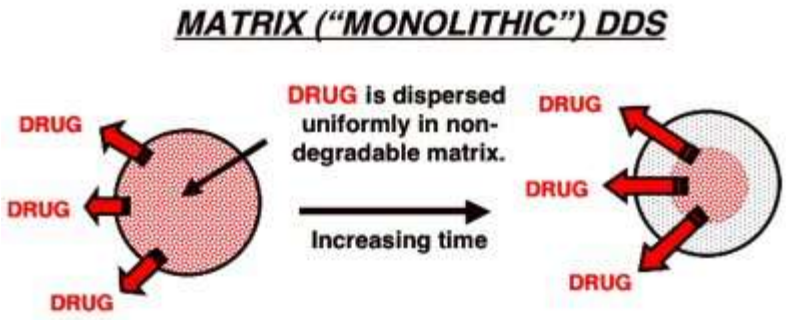


Figure 2.11 : Matrix diffusion systems [110].

Reservoir diffusion systems are also called as membrane-controlled systemssurrounds the active ingredients and released drugs by diffusion through the release mediums (Figure 2.12).

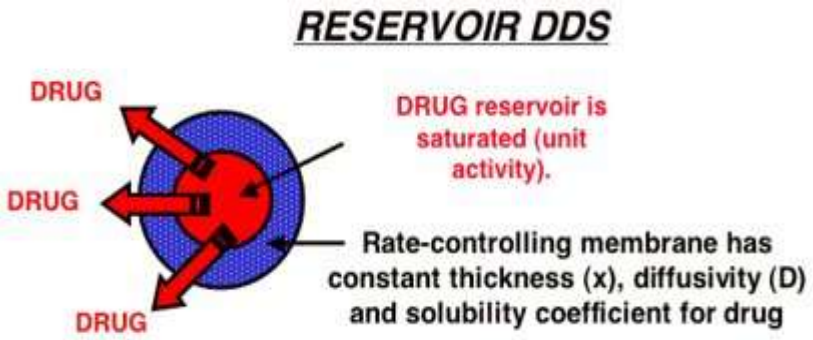


Figure 2.12 : Reservoir diffusion system [110].

2.4.3 Dissolution and diffusion controlled release systems

In dissolution and diffusion controlled release systems, the drug core is coated/capsulated in a partially soluble membrane. Pores in the encapsulation system are generated due to dissolution of parts of the membrane which;

- Drug dissolution performed by entry of aqueous medium into the encapsulation system
- Allowing diffusion of drug out of the encapsulation system.

An example of obtaining such an encapsulation system is using a mixture of biopolymers like PCL, PEG, CH, CS as in our study.

2.4.4 Water penetration controlled systems

Water diffusion (penetration) into the system is obtained by controlling on the penetration of water into the system. On the other hand, swelling controlled release systems are determines as drug is initially dry and when it is placed in the body absorb water or other body fluids and swell. Swelling-controlled devices usually incorporate drugs in a hydrophilic polymer and/or gels that are stiff or glassy when dry, but swells when placed in an aqueous environment (Figure 2.13). A typical oral capsule or pill (obtained commercial) is usually a swelling-controlled device. These drugs are easy to handle and produce, but the release rates are often not steady and effective.

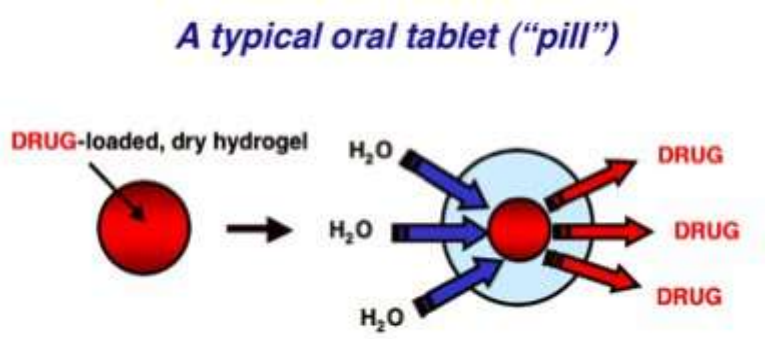


Figure 2.13 : A typical swelling controlled release system [111].

2.4.5 Osmotically controlled release systems

Osmotically controlled release systems (Figure 2.14) are used to encapsulate osmotically active drug or a combination of an osmotically inactive drug with an osmotically active salt e.g. NaCl within a semi permeable membrane made from biocompatible polymer, e.g. cellulose acetate. Osmotically controlled release systems

generates osmotic pressure, so active ingredient in the core is pumped out continuously with a prolonged period of time. This type of drug system dispenses drug solutes continuously at a zero order rate.

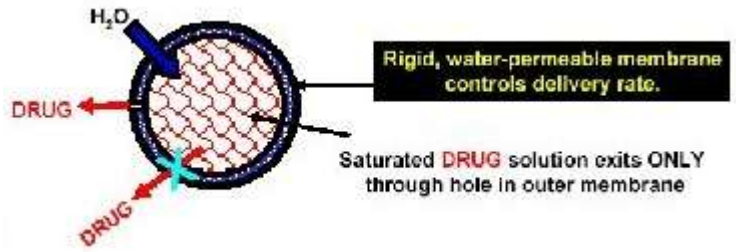


Figure 2.14 : Elementary osmotic released system [112].

2.4.6 Chemically controlled release systems

Chemically controlled release systems are different drug release systems mentioned above. They change their chemical structure, when exposed to biological fluid. Mostly, biodegradable and biocompatible polymers are used for chemically controlled release systems by degradation of polymer structure as a result of hydrolysis and/or erosion of the polymer chains into biologically safe and progressively smaller moieties. It is of two types and they are;

- Erodible systems
- Pendent chain system

In erodible systems, drug release mechanism occurs by erosion of polymer. Erosion may be two types as seen in Figure 2.15 and they are;

a) Bulk erosion process
b) Surface erosion process

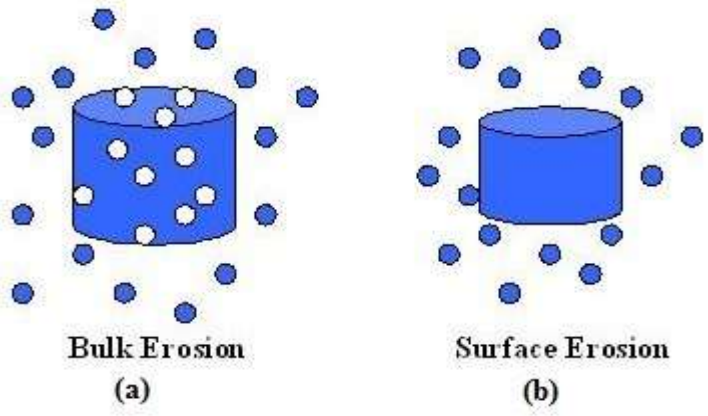


Figure 2.15 : Drug release **(a)** bulk erosion, **(b)** surface erosion [113].

Bulk erosion is polymer degradation may occur through bulk hydrolysis of the polymer which means entire polymer matrix are degraded by this way. On the other hand, surface erosion is polymers like polyorthoesters and polyanhydrides etc. occurs degradation only at the surface of the polymer, resulting in a limited drug release ratio that is proportional to the surface area of the delivery system.

Pendent chain systems consist of copolymers, polymeric blends and composites with the drug attached to the backbone chains. The drug is released from the entire core by hydrolysis or enzymatic degradation of the linkages. Zero order can be obtained and the cleavage of the drug is the rate controlling mechanism. Example for polymers used in pendent chain systems like chitosan, PEG etc.

2.4.7 Hydrogels controlled release systems

Hydrogels are water swollen three-dimensional structures handled by primarily hydrophilic polymers. Because of chemical or physical cross-links, they are insoluble in water. The physical cross-links include crystallites, entanglements or weak associations like hydrogen bonds or Vander Waals forces. These cross-links supply strength, physical integrity and network structure. Chemically instable and weak active ingredients, proteins and peptides are encapsulated and easily handled by hydrogels . Because of their nature and different specifications, hydrogels can be used in many different types of controlled release systems. This unique diffusion-controlled release mechanism as shown in Figure 2.16.

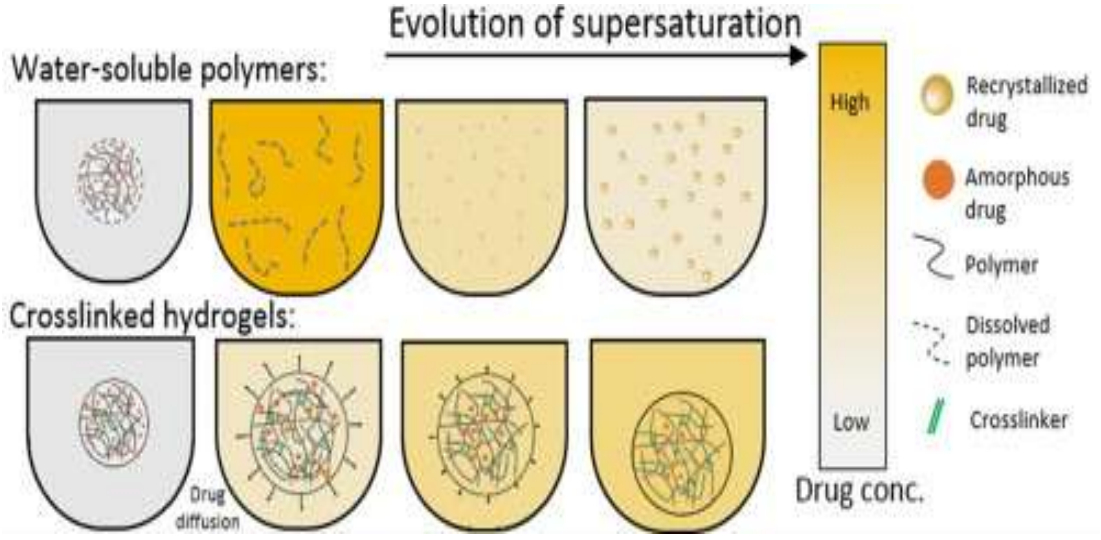


Figure 2.16 : Hydrogels in controlled drug release systems [114].

Hydrogels regulate the dissolution of encapsulated active ingredients (mostly amorphous) from the hydrogel matrix to maintain an elevated level of release for a prolonged period of time [114].

2.4.8 Ion exchange resins controlled release systems

Ion-exchange resins controlled release systems are produced to provide the controlled release of an ionic (or ionizable) active ingredients. The system is prepared by absorption of an ionized drug into the ion-exchange resin granules such as Amberlite, and consequently, particles are filtrated from the alcoholic medium; at final stage, the drug loaded granules are coated with a water permeable polymer, e.g. a modified copolymer of polyacrylic and methacrylic ester or homopolymer like PEG, and then spray drying the coated powders to handle the polymer capsulated drug resin preparation.

Prolong drug release in long time and release efficiency of the ion-exchange resin controlled release systems depend on combination of the principle that positively or negatively charged pharmaceuticals with appropriate resins to yield insoluble polysalt resinates. Release mechanism for anionic and cationic ion-exchange resinates are demonstrated below [115].

$$R - SO_3^- H^+ + H_2N - A \leftrightarrow R - SO_3^- - H_3N - A \quad (2.3)$$

$$R - N^+H_3OH^- + HOOC - B \leftrightarrow R - N^+H_3 - OOC - B + H_2O \quad (2.4)$$

$$H_2N - A \rightarrow basic\ drug, R - SO_3^- H^+ \rightarrow cationic\ exchanges, HOOC - B \rightarrow acidic\ drug \quad (2.5)$$

$$R - NH_3^+ OH^- \rightarrow anion\ exchange\ resins \quad (2.6)$$

A cationic active ingredients are combined with an anionic ion-exchange resin e.g. a resin with a SO_3^- group. Hydronium ion (H^+) in the gastrointestinal fluid penetrates to the durg material and activites the system for release of cationic drug from the core complex**.**

An active ingredients are combined with a cationic ion exchange resin, e.g. a resin with a [$N(CH_3)_3^+$] group. Chloride ion (Cl^-) in the gastrointestinal fluid penetrates the to the durg material and activites the system for release of anionic drug from the core complex.

2.4.9 Release kinetics

The mathematical models are used to evaluate the kinetics and mechanism of drug release from the microspheres that produced by us. The model that best fits the release data is selected based on the correlation coefficient (R^2) value in various models and the highest 'R^2' value is considered as the best fit of the release data.

Mathematical models are [116]:

1) Zero order release model
2) First order release model
3) Hixson-Crowell release model
4) Higuchi release model
5) Korsmeyer – Peppas release model

Zero order, as cumulative amount of drug released versus time, describes concentration-independent drug release rate from the formulation (Equation 1):

$$C = k_0 t \tag{2.7}$$

where k_0 is the zero-order rate constant expressed in units of concentration/time and t is the time in hours.

First order, as log cumulative percent drug remaining versus time, describes concentration-dependent drug release from the system (Equation 2):

$$Log\ C = Log\ C_0 - kt/2.303 \tag{2.8}$$

where C_0 is the initial concentration of drug and k is the first-order constant.

Higuchi's model, as cumulative percentage of drug released versus square root of time, describes the release of drugs based on Fickian diffusion as a square root of time-dependent process from swellable insoluble matrix (Equation 3):

$$Q = kt^{1/2} \tag{2.9}$$

where k is the constant reflecting the design variables of the system.

Hixson-Crowell cube root law, as the cube root of percentage drug remaining versus time, correlated the release from systems with polymer erosion/dissolution resulting in a change in surface area and diameter of particles or tablets (Equation 4):

$$Q_0^{1/3} - Q_t^{1/3} = k_{HC} t \tag{2.10}$$

where Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in the tablet, and k_{HC} is the rate constant for the Hixson-Crowell rate equation.

Korsmeyer-Peppas (*Power Law*) is derived a simple relationship which described drug release from a polymeric system (Equation 5).

$$M_t/M_w = k_{Kp}t^n \quad (2.11)$$

where M_t/M_∞ is fraction of drug released at time t, k_{KP} is the rate constant and n is the release exponent. To find out the mechanism of drug release, first 60% drug release data are fitted in Korsmeyer-Peppas model. The n value is used to characterize different release mechanisms as given in Table 2.2 for different shaped matrices.

Table 2.2 : Exponent *n* and release mechanism of different geometries [116].

Exponent, *n*			Drug release mechanism
Thin Film	**Cylinder**	**Sphere**	
0.5	0.45	0.43	Fickian diffusion
$0.5 < n < 1.0$	$0.45 < n < 0.89$	$0.43 < n < 0.85$	Anomalous transport
1.0	0.89	0.85	Case-II transport
> 1.0	> 0.89	> 0.85	Super Case-II transport

Fickian diffusion refers to diffusion controlled release mechanism; on the other hand, anomalous diffusion or non-Fickian diffusion is obtained combination of both diffusion and erosion controlled rate release. Case-II transport or super case-II transport refers to the erosion of the polymeric chain.

2.5 Spray Drying in Drug Delivery Systems

Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with a hot gas. This is the preferred method of drying of many thermally sensitive materials such as foods and pharmaceuticals. A consistent particle size distribution is a reason for spray drying some industrial products such as catalysts. Air is the heated drying media; however, if the liquid is a flammable solvent such as ethanol or the product is oxygen-sensitive then nitrogen is used.

Spray drying is one of the major industrial drying technologies. It is applied by many industries because of its ability to convert a liquid product into a dried powder in a

lenient single step; in addition, it allows to control to temperature and the particle formation process very accurately [117].

2.5.1 Background of the spray drying process

Briefly, a polymer solution is dropped (dispersed) into a hot air stream and the solvent (water) evaporates on the way to the bottom leaving a dried particle. The main advantage is the combination of particle formation and drying in one step. The disadvantages from the viewpoint of biological control are the variations in particle shape and size distribution, high temperatures and fast drying rates that normally do not allow for encapsulation of drying-sensitive living cells such as non-spore forming microbials [118]. Protectants and other additives may be added before or during the drying process. Especially in the food industry, spray-drying is used for the preservation and concentration of microorganisms. In biological control, too, spray drying is used to produce formulations with extended shelf-life. Figure 2.17 shows schematic presentation of a spray dryer.

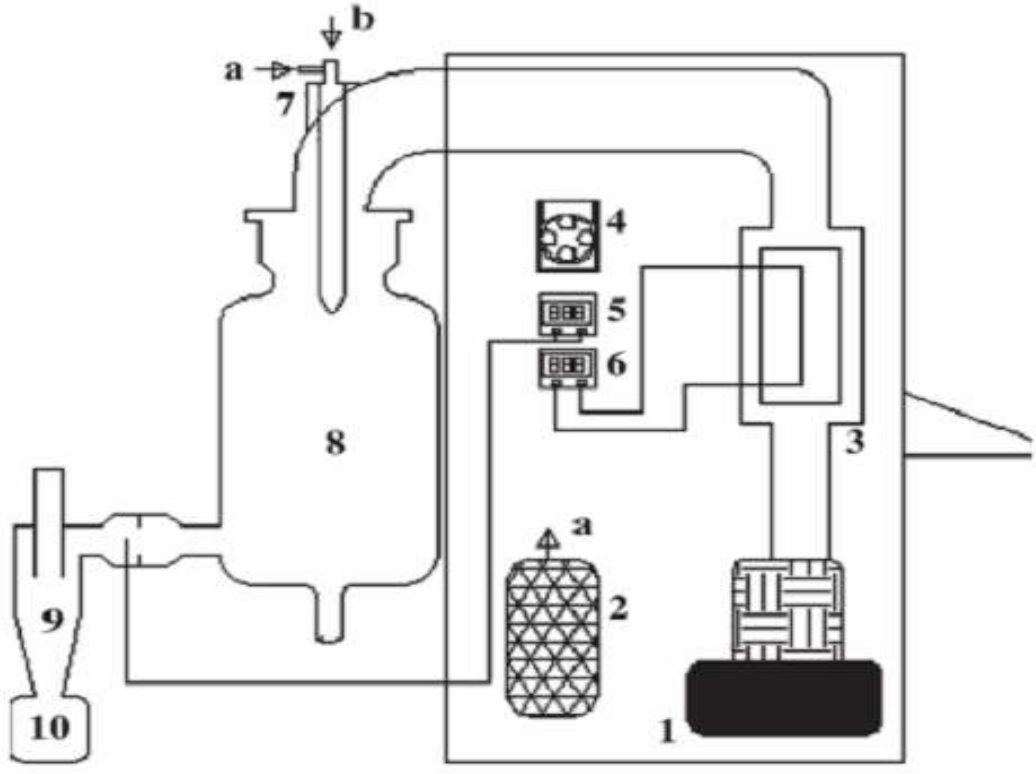


Figure 2.17 : Schematic diagram of the spray dryer used: **(1)** blower+air filter; **(2)** air compressor; **(3)** heater; **(4)** peristaltic pump; **(5)** temperature control; **(6)** inlet thermocouple; **(7)** atomizer: **(a)** compressed air; **(b)** feed microencapsulating composition; **(8)** drying chamber; **(9)** cyclone; **(10)** dry product collector [119].

The solid is usually collected in a drum or cyclone. The liquid input stream is sprayed through an atomizer into a hot vapor stream and vaporized. Solids form as moisture quickly leaves the droplets. The atomization part is used to obtaine the desired characteristics by performing the optimum conditions to a dried powder for evaporation. Nozzles and rotary atomizers are used to form sprays. It is usually used to make the droplets as small as possible, maximizing heat transfer and the rate of water vaporization. Droplet sizes can range from 20 to 180 μm depending on the nozzle. There are two main types of nozzles: high-pressure single fluid nozzle (50 to 300 bars) and two-fluid nozzles: one fluid is the liquid to dry and the second is compressed gas (generally air at 1 to 7 bars) [120].

The central element of a spray dryer is the drying chamber. In drying chamber, the sprayed polymer solution is compared with hot gas (air or an inert gas), resulting in the evaporation of over 95% of the water contained in the droplets in a very limited times like seconds [120]. Droplet contact time with hot air and/or inert gas, contact angle are directly affected the properties of dried poweder such as particle shape and diameter. The type of contact (current, co-current and cross) between the spray and the air/inert gas is determined by the position of the atomizer relative to the hot air/inert gas. As a general orientation of the spray dryers, nozzle headers are located at the top of the dryer and spray down.

Moisture evaporation (liquid chasing) takes place in two stages. During the first stage, saturation of wet droplet is performed by hot air/inert gas and the temperature of hot air/inert gas equals to bulb temperature. Evaporation takes a constant rate during drying stage and moisture in the drop to replace the liquid evaporated sufficiently [121]. After moisture of the droplet is reduced to nearly zero by hot air/inert gas, the second stage begins by production a dried shell to form at the surface. At the following stage, evaporation depends on the diffusion of moisture through the shell that is increasing in thickness. Evaporation phase is dominant at the first stage and it falls rapidly during the second stage. Different polymer and/or composites have differing evaporation and particle-forming characteristics. On the other hand, solvent type of the polymers and/or composites results as same consequences mentioned before. The resulting particles may be uniform distributed spheres, or porous and irregularly shaped.

Following completion of drying, the particles of product must be separated from the hot drying air/inert gas [121]. The particles simply fall to the bottom of the chamber, so the separation is accomplished by the way. A small fraction of the particles may be remain entrained with the air and/or may be sticked together to the drying chamber of the spray dryer. Cyclones, bag filters, and electrostatic precipitators are the final separation stage equipments. Some spray dryers contain wet scrubbers that are often used to purify and cool the air so that it can be released to atmosphere.

Reciprocating compressors of the spray dryers can be either stationary or portable, can be single or multi-staged, and can be driven by electric motors or internal combustion engines [121]. Small reciprocating compressors from 5 to 30 horsepower (hp) are commonly seen in automotive applications and are typically for intermittent duty. Larger reciprocating compressors well over 1,000 hp (750 kW) are commonly found in large industrial and petroleum applications. Discharge pressures can range from low pressure to very high pressure (>18000 psi or 180 MPa).

Heaters in the spray dryers exist for all states of matter, including solids, liquids and gases and there are 3 types of heat transfer: Convection, Conduction and Radiation [121].

2.5.2 Historical development of spray dryers

From the 1870s through the early 1900s over a period of several decades, the development of spray drying equipment itself and drying techniques are revised and evolved [122]. The first spray dryer equipments are used nozzle atomizers, with rotary atomizers. Until the 1920s, because of many difficulties of continuous operation at spray dryers, commercial operations did not grow up and become profitable. On the other hand, commercial operations have just become profitable and practically feasible by the second decade of the twentieth century. First commercial applications are made at food industry such as milk powder production. After that, manufacturers are developed their designs and made spray dryers sophisticated to accommodate heat-sensitive products, emulsions and mixtures. Reduction of transportation expense by spray dryers at food and pharmaceutical industries, they became more popular during World War II. These improvements in spray drying led

to developments in the technology that greatly expanded the range of products like drugs and foods ingredients could be successfully spray dried [122].

Since early years, spray drying technology has developed tremendously and some of the major achievements have been to divide the drying process into several stages [123]. Integration of fluidized bed to the spray dryer provides to overcome problem of fluidization of moist solids at the entrance.

2.5.3 Spray dryer types

2.5.3.1 Co-Current flow spray dryers

In a co-current dryer as seen in Figure 2.18, the hot air and fluid feed is entering the dryer chamber in the same direction [124]. The co-current flow enables the particles to have a lower residence time within the system, so heat-sensitive fluids are used easily in this system and the particle separator (typically a cyclone device) operates more efficiently.

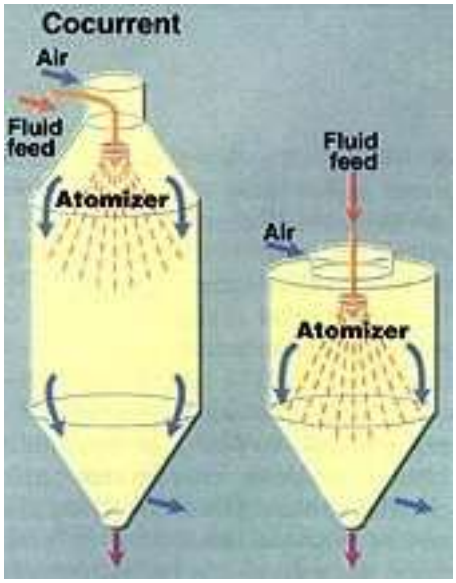


Figure 2.18 : Co-current flow spray dryer [121].

In the co-current flow system, spray evaporation is rapid, and the temperature of the drying air is reduced rapidly by the vaporization of solvent such as water, acetic acid, chloroform etc.

2.5.3.2 Counter current flow spray dryers

Counter-current spray dryers as seen in Figure 2.19, are used for the manufacture of thermally stable powders, such as detergents, and the fluid feed and hot air flowing

in opposite directions to each other into the drying chamber. Spray evaporation is rapid, heat transfer is more efficient [125].

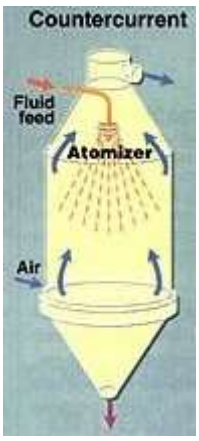


Figure 2.19 : Co-current flow spray dryer [121]

A counter-current dryer offers more rapid evaporation, efficient heat transfer and more energy saving than a co-current design because the driest particles are in contact with hottest air, this design is not suitable for heat-sensitive products.

2.5.3.3 Mixed flow spray dryers

The gas flows down and the feed sprays up, and then comes down with the gas in mixed flow spray dryers (Figure 2.20). It allows for extra drying time, and decreasing the overall spray dryer height required; on the contrary, large particle size powders are produced by this way [121].

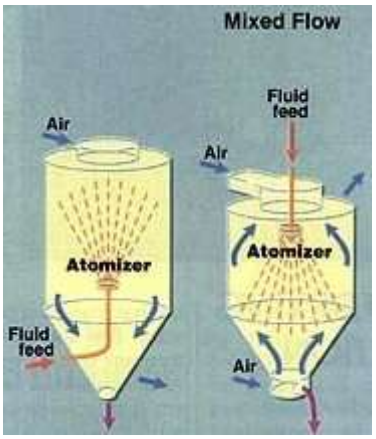


Figure 2.20 : Mixed flow spray dryer [121].

In the mixed flow systems, drying performance is not affected by product type or amount as so-current and/or counter current flow systems. However, thermally stable and resistant materials should be used due to the thermal degradation risk because the driest particles are exposed to the highest gas temperatures.

2.5.3.4 Open cycle spray dryers

In an open cycle spray dryer as seen in Figure 2.21, is the most commonly used design. The drying air is drawn from the atmosphere, heated, sent to the drying chamber and then exhausted to the atmosphere. In brief, it is an open system.

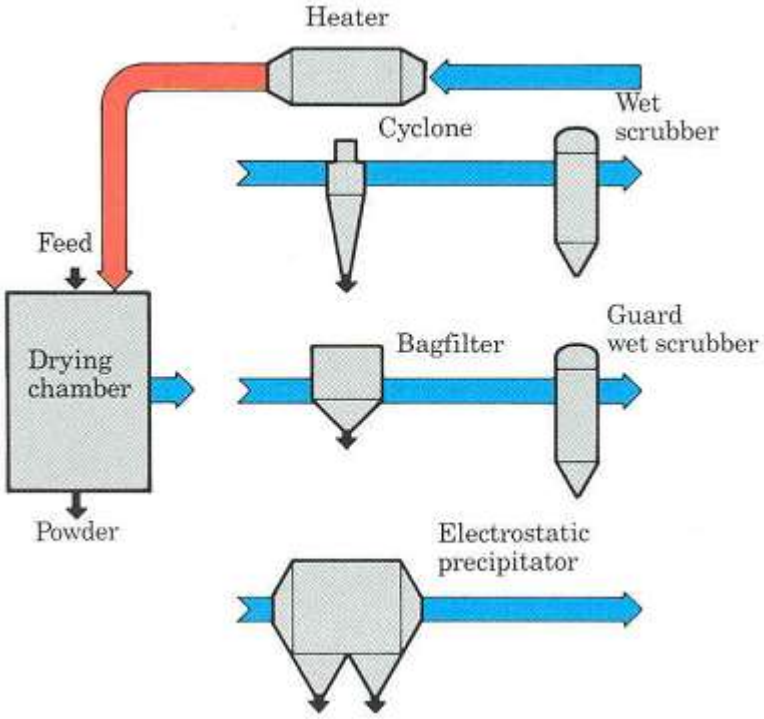


Figure 2.21 : Open-cycle spray dryer [126].

2.5.3.5 Closed cycle spray dryers

Nitrogen gas as an inert atmosphere is usually used as hot gas in a closed-cycle spray dryers and nitrogen gas is circulated within the unit. Therefore, flammable organic solvents can be used in the drying chamber and solvent recovery is provided by this way.

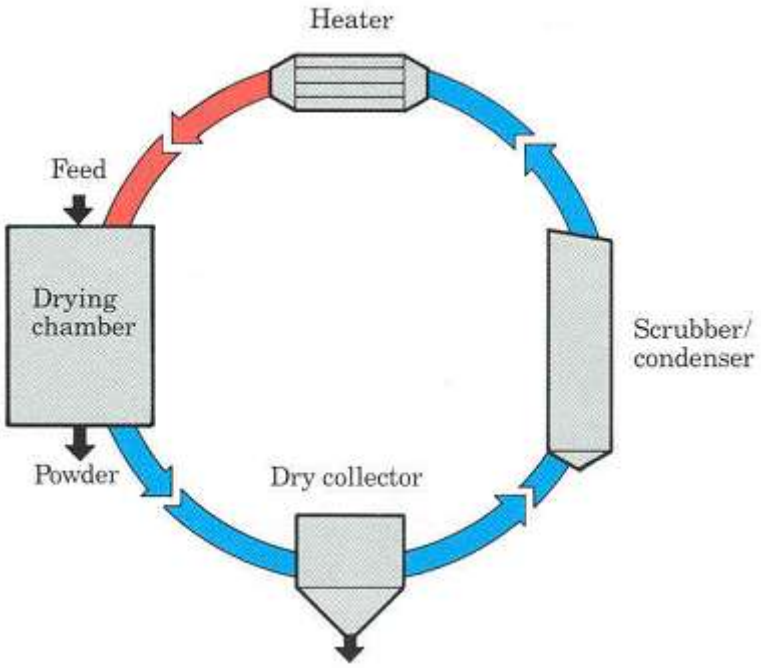


Figure 2.22 : Closed-cycle spray dryer [126].

This system facilitates easy production of a solid dispersion, which can enhance the solubility and bioavailability of poorly water soluble solids. Figure 2.22 shows schematic view of a closed cycle spray dryer.

2.5.3.6 Semi-closed cycle spray dryers

Semi-closed cycle dryer design is a hybrid configuration of both open and closed cycle dryers and it is not air/inert gas tight. If odor is generated during drying, the small volumes of air vented from the system can be effectively and economically incinerated. Solvent recovery is also obtained, but less than closed system dryers. Figure 2.23 shows schematic view of a semi-closed cycle spray dryer.

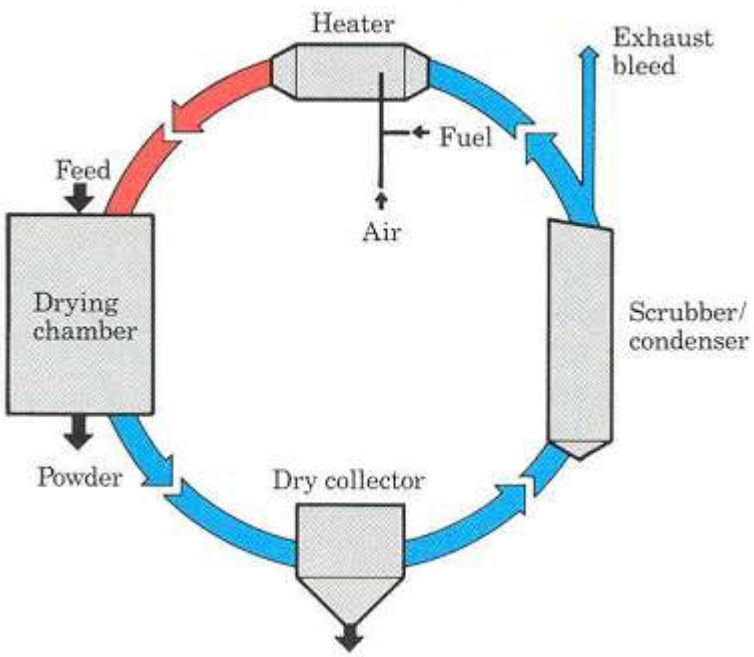


Figure 2.23 : Semi-Closed-cycle spray dryer [126].

2.5.3.7 Single stage spray dryers

One-stage drying is defined as the spray drying process where the product is dried to the final moisture content in the spray drying chamber, as shown in Figure 2.24. The basic and the most used system is a single stage co-current spray dryer with rotary or nozzle atomization.

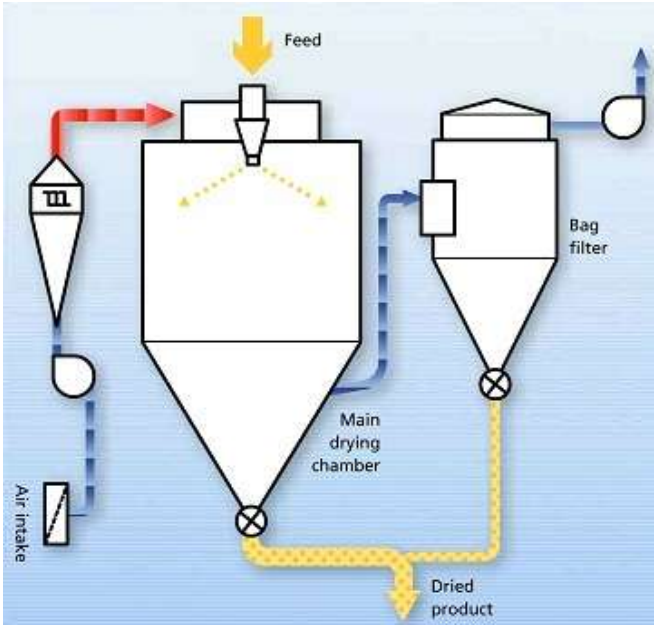


Figure 2.24 : Single stage spray dryer [127].

2.5.3.8 Multi stage spray dryers

The multi stage drier consists of a spray drier with an external vibrating fluid bed placed below the drying chamber. The product can be removed from the drying chamber with a higher moisture content, and the final drying takes place in the external fluid bed where the residence time of the product is longer and the temperature of the drying air lower than in the spray dryer. Consequently the two stage drying process was introduced which proved to be superior to the traditional single stage drying in terms of product quality and cost of production. Figure 2.25 shows schematic view of a multi stage spray dryer.

Figure 2.25 : Multi stage spray dryer [127].

2.5.3.9 Vertical spray dryers

The air enters the spray dryer vertically through the air disperser at high velocity, ensuring optimal mixing of the atomized droplets with the drying air. The evaporation takes place instantaneously during the passage vertically down through the drying chamber. In this first stage drying, the particles are not completely dried. Vertical flow spray dryer is shown in Figure 2.26.

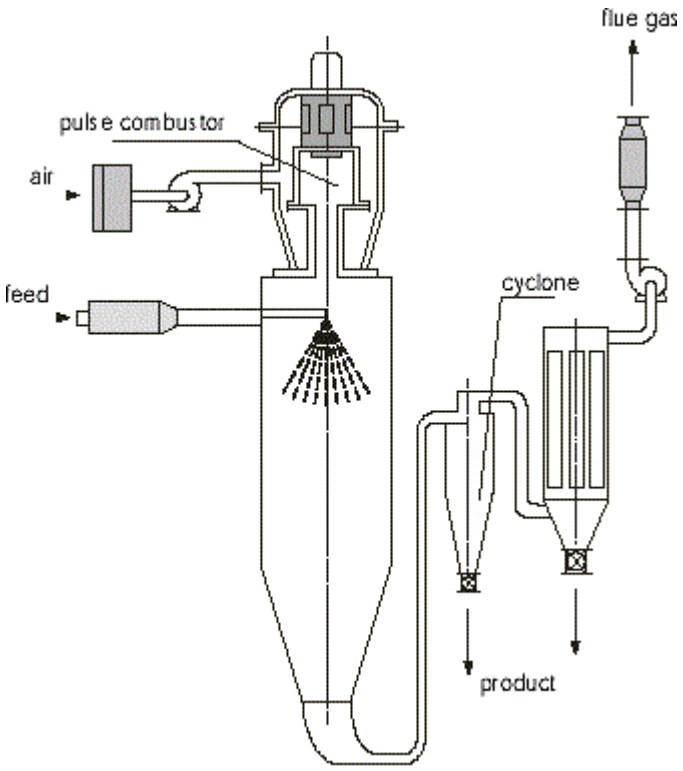


Figure 2.26 : Vertical spray dryer [128].

2.5.3.10 Horizontal spray dryers

The horizontal type spray dryers are generally smaller than other dryers with the same capacity. The high-pressure pump in liquid feed system brings the liquid up to atomization pressure before the liquid is sprayed horizontal by a number of nozzles into the drying chamber. Horizontal flow spray dryer is shown in Figure 2.27.

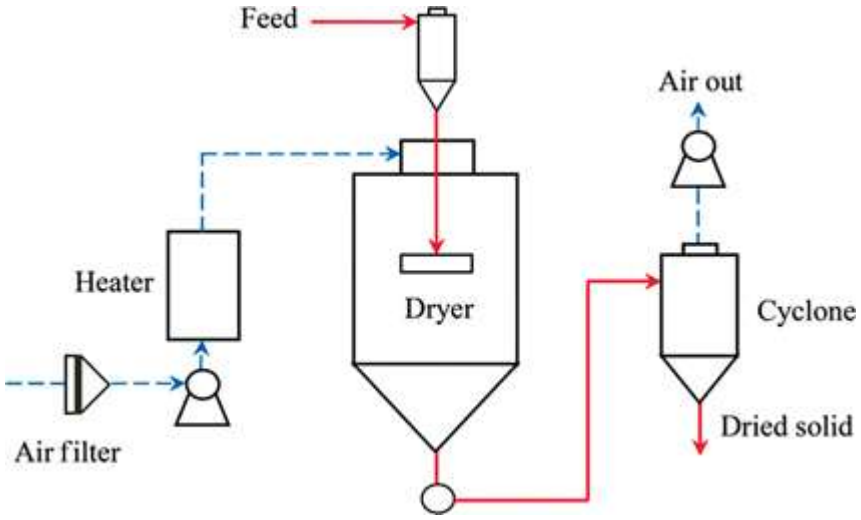


Figure 2.27 : Horizontal spray dryer [129].

3. EXPERIMENTAL STUDIES

3.1 Materials

Polycaprolactone (Sigma-Aldrich): It is used to produce microspheres in polymer solutions. It is obtained from Sigma-Aldrich and number average molecular weight (M_n) 10,000 g/mol and weight average molecular weight (M_w) 14,000 g/mol.

Polyethylene glycol (Sigma-Aldrich): PEG-6000 type polyethylene glycol is used in polymer our studies and it is used to produce microspheres in solutions.

Chitosan (Sigma-Aldrich): Medium molecular weight type chitosan is used in polymer solutions.

Casein (Sigma-Aldrich): Casein from bovine milk is used in our studies and it is used to produce microspheres in polymer solutions.

Sodium Alginate (Sigma-Aldrich): Alginic acid sodium salt from brown algae is used to produce microspheres in polymer solutions.

L-Ascorbic acid (Sigma-Aldrich, ACS reagent, ≥99%): L-Ascorbic acid is active ingredient in the drug delivery system.

Acetic Acid (Merck, %100): Its mixture with formic acid is used to solve polymers to prepare encapsulation mixtures.

Formic Acid (Merck, %98): Its mixture with acetic acid is used to solve polymers to prepare encapsulation mixtures.

2,6-Dichloroindophenol sodium salt hydrate (Sigma-Aldrich): It ($C_{12}H_6Cl_2NNaO_2$ xH_2O) is a reagent for the titration of L-ascorbic acid.

Oxalic acid (Merck, %99): It is an organic compound with the formula $H_2C_2O_4$ and it is a colorless crystalline solid that forms a colorless solution in water. It is used to prepare stabilization solution to determine L-ascorbic acid in UV.

Phosphate buffer solution (PBS) is a buffer solution commonly used in biological research. It is a salty solution containing sodium phosphate, sodium chloride and potassium phosphate. The buffer helps to maintain a constant pH.

0.1 M Acetic Acid PBS, pH 2.8: It is used for drug release fluid in our studies. To prepare 0.1 M PBS at pH 2.8; 2.86 ml glacial acetic acid is dissolved in 350 ml water. Volume is completed to 0.5 L with 1 M HCl and 1 N NaOH is used to adjust pH at 2.8.

0.1 M PBS, pH 7.4: It is used for drug release fluid in our studies. To prepare 0.1 M PBS at pH 7.4; 0.26 g KH_2PO_4, 2.17 g $Na_2HPO_4.7H_2O$ and 8.71 g NaCl are dissolved in 800 ml water. Volume is completed to 1 L with 1 M HCl and 1 N NaOH is used to adjust pH at 7.4.

0.1 M $NaHCO_3$ PBS, pH 9.6: It is used for drug release fluid in our studies. To prepare 0.1 M PBS at pH 9.6; 4.2 g $NaHCO_3$ is dissolved in 350 ml water. Volume is completed to 0.5 L with 1 M HCl and 1 N NaOH is used to adjust pH at 9.6.

3.2 Equipments

3.2.1 Spray dryer

Yamato ADL 310 lab scale spray dryer (seen in Figure 3.1) to obtain microspheres.

Figure 3.1 : Yamato ADL 310 lab scale spray dryer [130].

Table 3.1 also shows specifications of Yamato ADL 310 type lab scale spray dryer [130].

Table 3.1 : Specifications of spray dryer [130].

Evaporation Capacity	Maximum 1300 ml/h
Temperature Control Range	40 ^{o}C – 200 ^{o}C
Dry Air Flow Rate	Maximum 0.7 m^3/min
Feed Flow Rate	Maximum 28 ml/min
Spray Nozzle	Only for Liquid and Air

3.2.2 Particle size analyzer

Malvern Mastersizer 2000 is used to obtain particle size diameter and its distribution. Two procedures are involved in measuring a sample on the Mastersizer; first, as shown in Figure 3.2, there is the capturing of the scattering pattern from the sample by optical unit and this is known as the "measurement" [131].

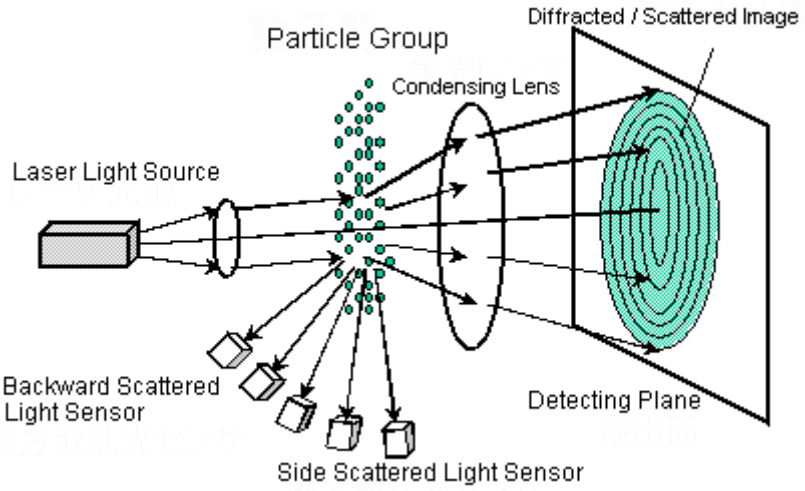


Figure 3.2 : Basic Optical System of a Laser Diffraction Particle Size Analyzer [131].

The detector within the optical unit is made up of many individual detectors. Each detector is collect the light scattering from a particular range of angles. Second, once the Malvern software is used to analyze the measurement which is completed in the first stage and the raw data contained.

3.2.3 Scanning electron microscopy

Scanning Electron Microscopy (SEM, Model: Jeol, JSM-6390 LV) is used to obtain morphological structures of microspheres. SEM (in Figure 3.3) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid

specimens [132]. The signals that derive from electron-sample interactions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample. Particle diameters and the morphology are determined by this way.

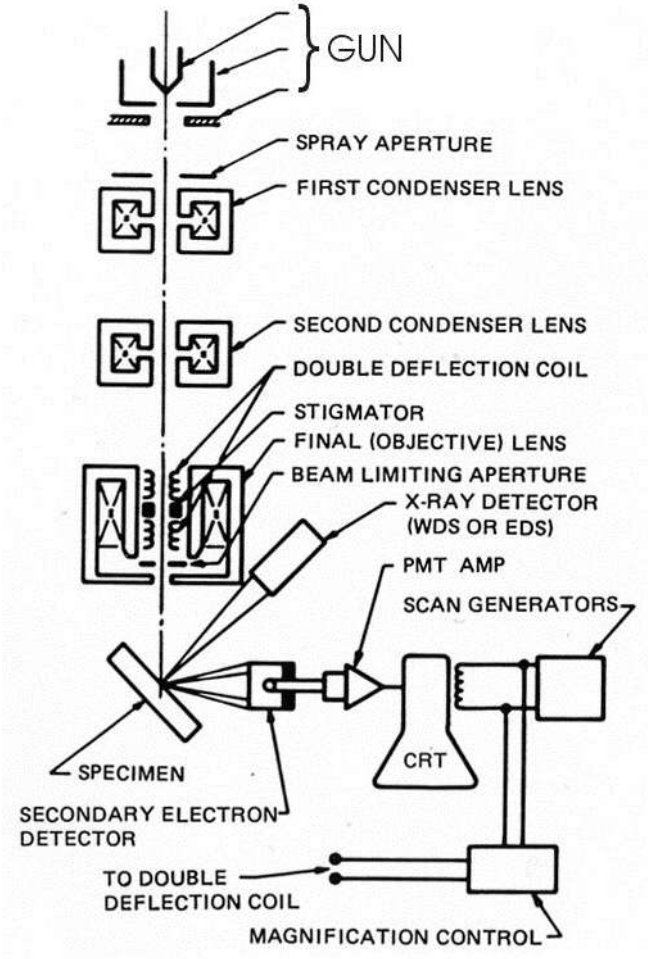


Figure 3.3 : Schematic drawing of SEM [132].

3.2.4 Thermogravimetric analysis

Thermogravimetric analysis or thermal gravimetric analysis (TGA, Perkin-Elmer Diamond TG/DTA) is used to analyze microsphere structure. Thermogravimetric Analysis is a technique in which the mass of a substance is monitored as a function of temperature or time as the sample specimen is subjected to a controlled temperature program in a controlled atmosphere [133]. Figure 3.4 shows a typical TGA instrument schematic view.

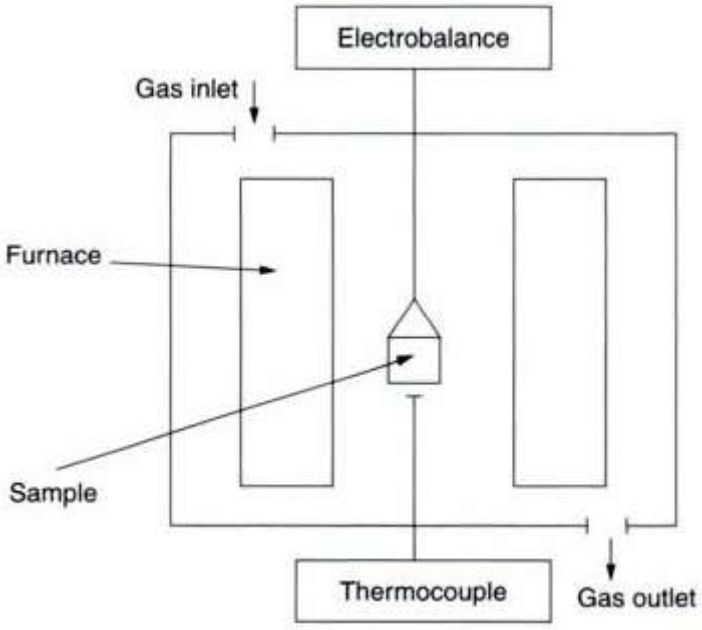


Figure 3.4 : Schematic of a typical TGA [133].

In our study, the microsphere sample is placed in furnace and it is burned in the N_2 atmosphere without O_2 with the ramp 30 ^oC/min up to 800 ^oC. TGA scan is obtained from the measurement to determine decomposition temperature and char yield.

3.2.5 Ultraviolet analysis

Ultraviolet Analysis (UV, Model: UV Mini 1240 SHIMADZU, Single Beam) is used to determine drug encapsulation efficiency and drug release ratios.

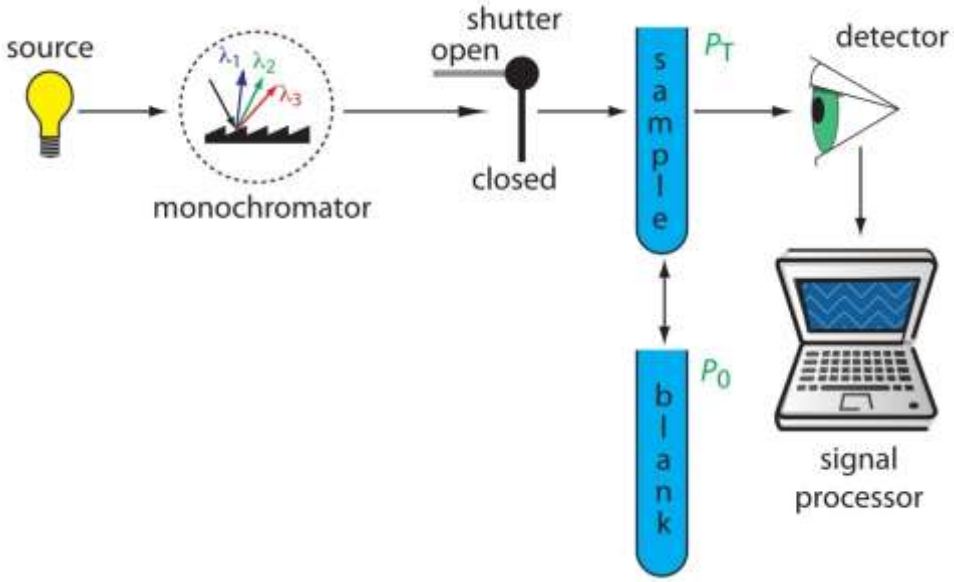


Figure 3.5 : Schematic diagram of a fixed-wavelength single-beam spectrophotometer [134].

The simplest spectrophotometer is a single-beam instrument equipped with a fixed-wavelength monochromator as seen in Figure 3.5 [134]. The instrument is calibrated to 0% T while using a shutter to block the source radiation from the detector. After opening the shutter, the instrument is calibrated to 100% T using an appropriate blank. The blank is then replaced with the sample and its transmittance measured.

3.2.6 Other equipments

Other equipments that are used in our studies are listed below:

- Magnetic stirrer, Ikamag RH
- Oven, Binder
- Heater, Ikamag RCT Classic
- Analytical balance, And, Gr-200
- Shaking water bath, Juloba SW 22
- pH meter, Inolab WTW

3.3 Methods

3.3.1 Preparation of polymer mixture

PCL+PEG+CH, PCL+PEG+CS, and PCL+PEG+SA polymer mixture solutions are prepared by acetic acid-formic acid solutions with 3:7 (v/v) ratios for ~24 h, followed by a filtration step (1.5 μm; 934 AH® Binderless Glass Microfiber Filter Media). The value of 3:7 (v/v) acetic acid:formic acid is determined by previous studies [26, 135, 136]. Polymer amounts are 10 wt% and mixtures are prepared by 1:1:1 (wt/wt/wt) ratio.

3.3.2 L-Ascorbic acid determination procedure

L-ascorbic acid determination procedure is based on the 2,6-dichloroindophenol sodium salt titration method [137]. Firstly, 100 mg L-ascorbic acid is dissolved in 100 ml, 0.4 wt% oxalic acid stabilizer solution to determine calibration curve. The stock L-ascorbic acid solution is treated by 2,6-dichloroindophenol sodium salt. The method of quantitative determination is based on selective oxidation of L-ascorbic acid to dehydroascorbic acid with 2,6-diclorphenolindophenol as seen in Figure 3.6.

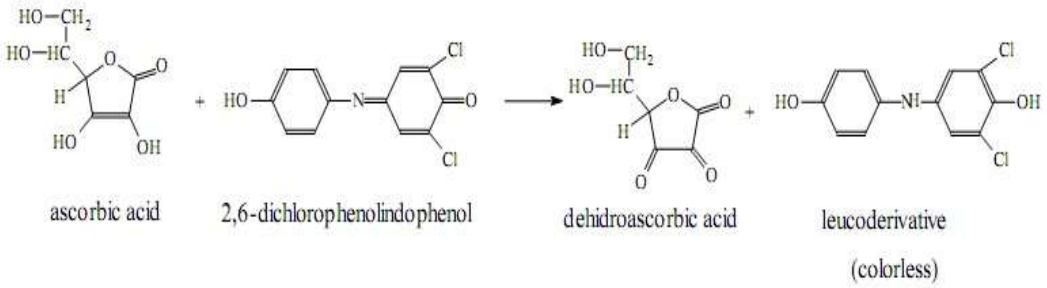


Figure 3.6 : L-ascorbic acid reduction by the oxidized dye the 2,6-dichloroindophenol [138].

Oxidized different L-ascorbic acid stock solutions with the 2,6-dichloroindophenol are analyzed by UV at 518 nm. On the other hand, standard blind solutions are prepared and absorbance values of 2,6-diclorphenolindophenol solutions are also determined at 518 nm. Absorbance values between L-ascorbic acid stock solutions and 2,6-diclorphenolindophenol solutions are subtracted and calibration curve are prepared. Figure 3.7 shows calibration curves of L-ascorbic acid solutions at 518 nm.

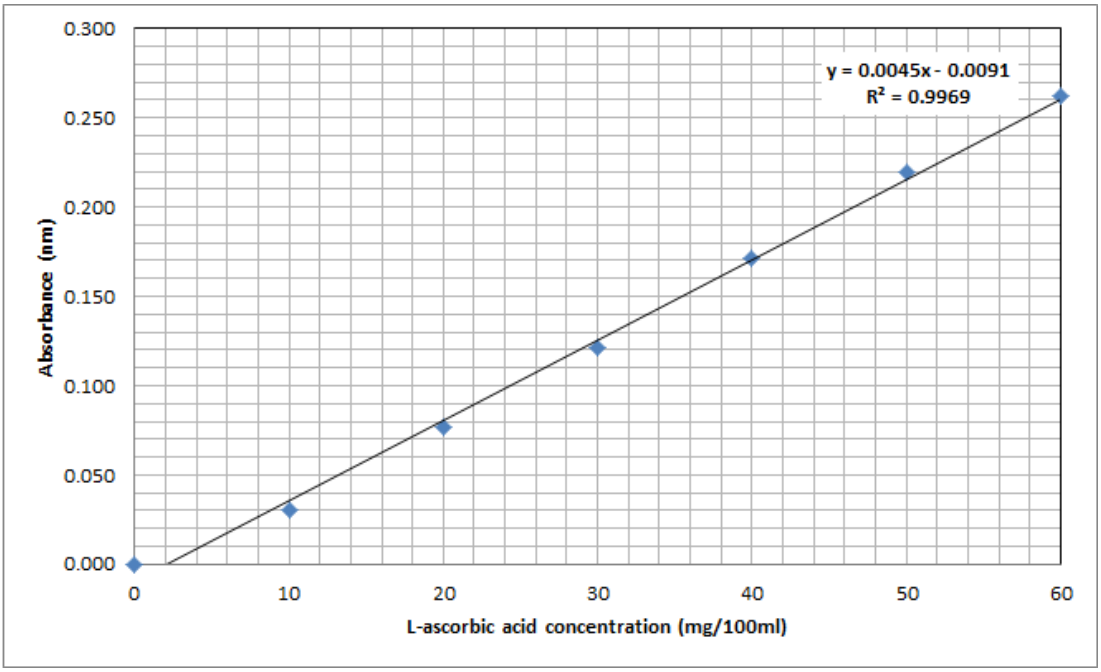


Figure 3.7 : Calibration curve of L-ascorbic acid solutions.

3.3.3 Drying studies

Although in industrial spray drying processes, dispersions with high solid concentration are recommended (>30%) to reduce the costs and increasing drying efficiency, in this study dispersions with low concentration of total solids are selected in order to avoid obstruction of the pneumatic nozzle when using blends with the highest concentration of gum [139].

Polymer solutions are fed to spray dryer (Yamato ADL 310 lab scale spray dryer) by a peristaltic pump and first of all, best drying conditions are determined by changing drying temperature (120 ^{o}C, 135 ^{o}C, and 150 ^{o}C) and feed flow rate (3 mL/min, 6 mL/min, and 9 mL/min). Atomizer pressure is constant at 1 barg. Drying efficiency is calculated by Equation 3:1:

$$Microsphere\ Efficiency\ wt\% = \frac{Dried\ particle\ amount}{Total\ polymer\ amount\ in\ mixture} x100 \quad (3.1)$$

3.3.4 Drug encapsulation studies

Particle diameter and particle size distribution are determined and L-ascorbic acid in different weight ratios are loaded to the microspheres that have the lowest particle diameter. L-ascorbic acid loading studies are done by indirect loading (pH 7.0) of drug to microspheres at 25 ^{o}C and 200 rpm. L-ascorbic acid solutions are 5 wt%, 10 wt%, and 15 wt%, respectively. Encapsulation efficiency is calculated by Equation 3:2 [22]:

$$Encapsulation\ efficiency\ \% = \frac{Final\ L{-}ascorbic\ acid\ amounts}{Initial\ L{-}ascorbic\ acid\ amounts} x100 \quad (3.2)$$

Liquid phase is analyzed by UV at 518 nm to calculate Equation 3:2 [140].

3.3.5 Drug release studies

Calculations in drug release step of the study are also performed by UV. 0.5 mg blend/1 mL release medium with different pH PBS solutions (pH 2.8, 7.4, and 9.6) ratio is used. Polymeric drug is dispersed in the release medium and incubation occurred at 25 ℃ with shaking. Drug release is calculated by Equation 3:3 [22].

$$Drug\ release\ \% = \frac{C\ (t)}{C\ (0)} x100 \quad (3.3)$$

where;

C(t) refers to drug amount in any time and C(0) to drug amount at t=0.

4. RESULTS AND DISCUSSIONS

4.1 PCL+PEG+CH Encapsulation Systems

Yields and particle size distributions with standard deviation are shown for all studies in Figure 4.1 and Figure 4.2, respectively. Due to the Figure 4.2, Metasizer analyses are done 3 times and particle diameter distribution is determined by standard deviation of the results. Final values demonstrate particle size (µm) with standard mean of PCL-PEG-CH microspheres. Figure 4.1 determines that drying efficiency increase by decreasing of flow rates at 120 ^{o}C and 135 ^{o}C; on the contrary drying efficiency increase by increasing of flow rate at 150 ^{o}C. The reason of these results is that increasing drying temperature resulted in agglomeration of polymer mixture, so contact time with polymer mixture and drying temperature had adversely effect on diameter distribution [22]. Due to these results, 120 ^{o}C and 3 ml/min are the best drying conditions because of the lowest particle diameter with 19,143±0,023 µm and highest surface area with 0.897 m^2/g surface area in this study.

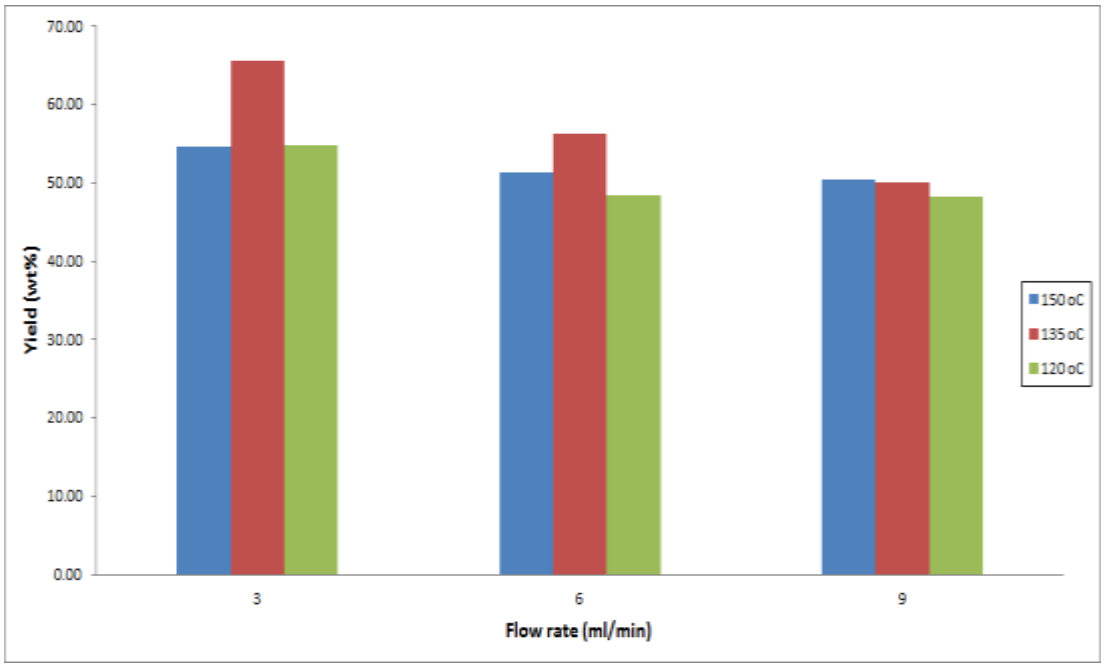


Figure 4.1 : Yield (wt%) at different drying temperature (^{o}C) and flow rates (ml/min).

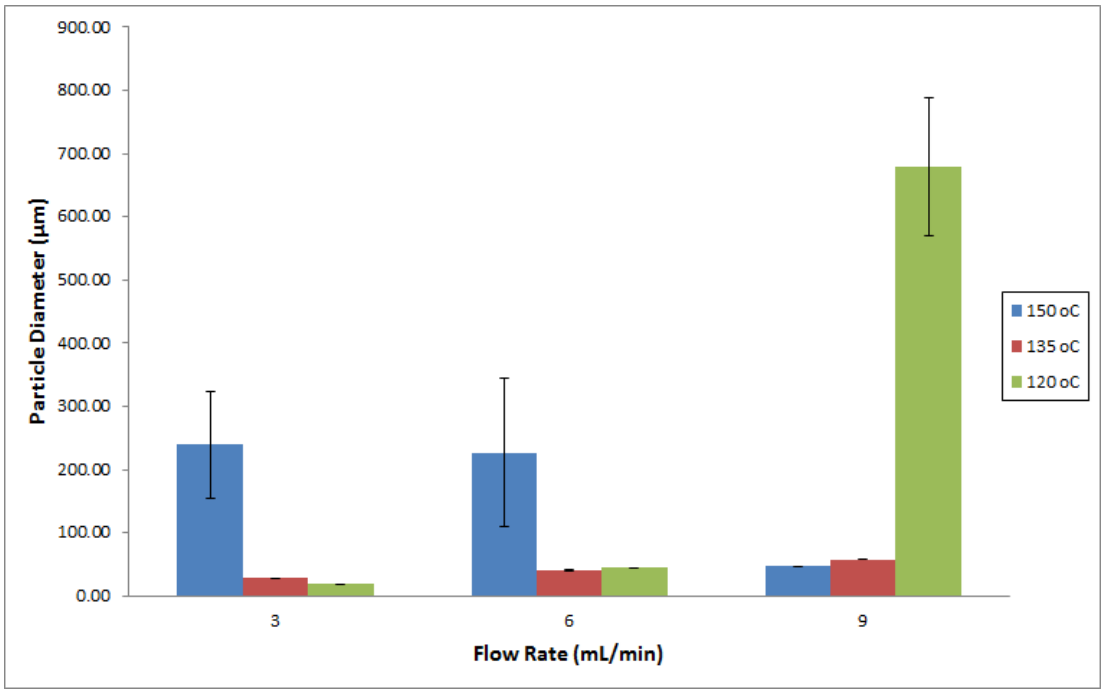


Figure 4.2 : Particle diameter (µm) with standard deviations at different drying temperature (^{o}C) and flow rates (ml/min).

SEM micrographs are shown in Figure 4.3 for 120 ^{o}C-3 ml/min, 135 ^{o}C-3 ml/min, and 150 ^{o}C-9 ml/min. It is easily seen that agglomeration increases by increasing of drying temperature. Porous structure of microsphere is also easily shown in all SEM micrographs. This porous structure is an advantage because drug loading capacity of porous spheres is higher than other spherical structures [16].

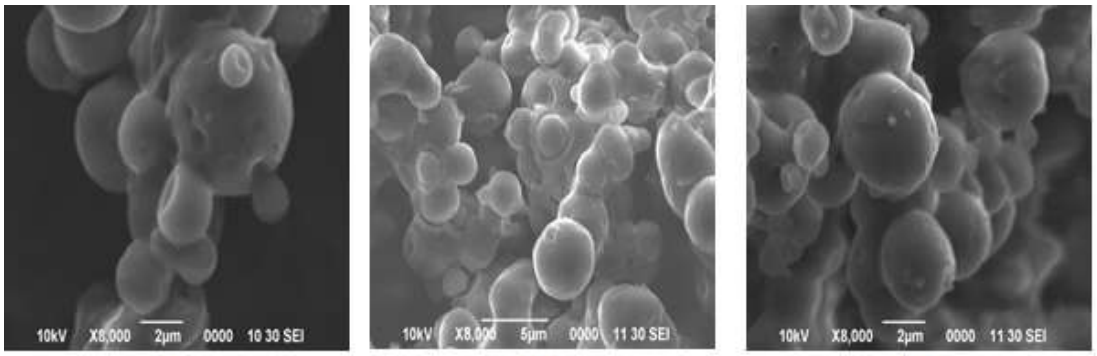


Figure 4.3 : SEM micrographs **(a)** 120 ^{o}C, 3 ml/min; **(b)** 135 ^{o}C-3 ml/min; **(c)** 150 ^{o}C, 9 ml/min.

The sample is placed in furnace and it is burned in the N_2 atmosphere without O_2 with the ramp 30 ^{o}C/min up to 800 ^{o}C. TGA scan is obtained from the measurement to determine decomposition temperature and char yield. TGA curves for PCL-PEG-CH bio-blend and L-ascorbic acid loaded bio-blend are shown in Figure 4.4 and Figure 4.5, respectively.

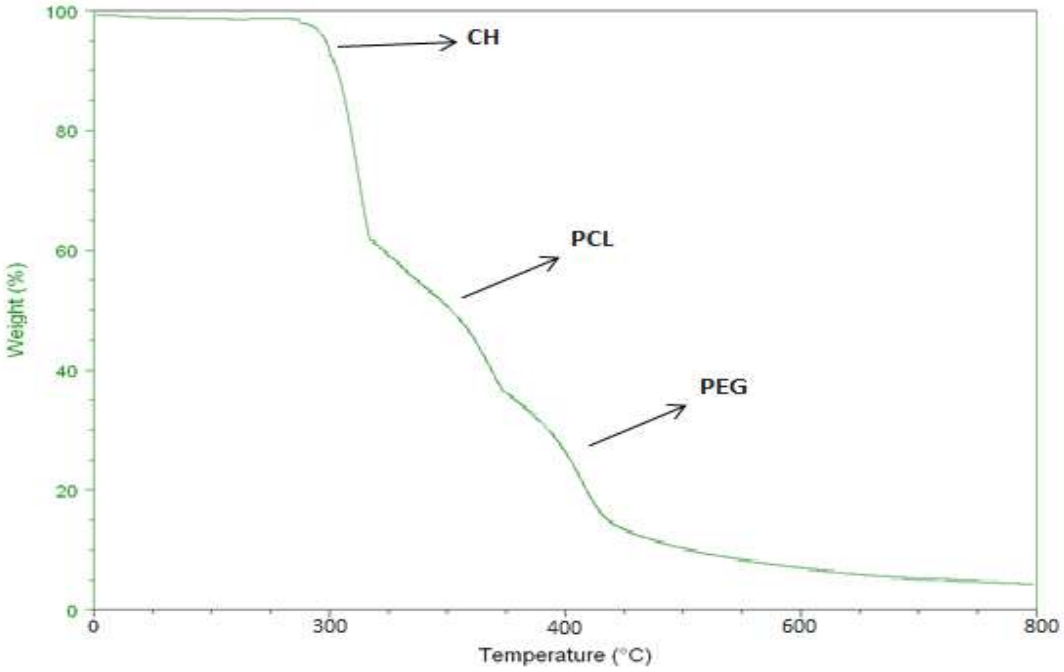


Figure 4.4 : TGA of PCL-PEG-CH microsphere at 120 ^{o}C, 3 ml/min.

It is easily seen that TGA curves in Figure 4.4 and Figure 4.5 are blends because thermal degradation curves goes down step by step. Figure 4 illustrates PCL-PEG-CH blend thermal degradation and it begins about 300 ^{o}C with CH degradation, following at 350 ^{o}C with PCL degradation and finalized at 400 ^{o}C with PEG-6000 degradation. L-ascorbic acid (Figure 4.5) shows that the decomposition starts at around 190 °C. Four thermal degradation steps are identified and allocated to the L-ascorbic acid and polymer fractions, respectively, based on the TGA curves.

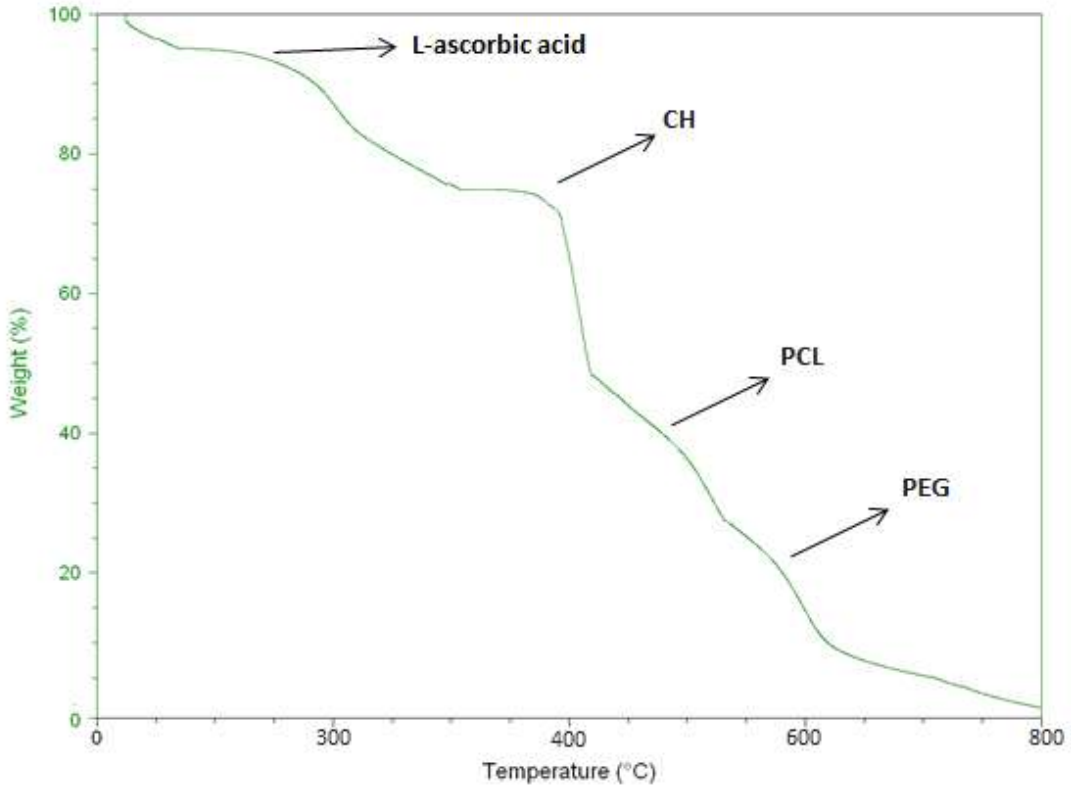


Figure 4.5 : TGA curve of L-ascorbic acid loaded microsphere.

Drug release studies are performed under three different L-ascorbic acid concentrations (5 wt%, 10 wt%, and 15 wt%) with different loading time and PCL-PEG-CH particle amounts. Microsphere obtained at 120 ^{o}C and 3 ml/min is used in drug loading studies because this microsphere had the lowest particle diameter and also best distributed particles due to the Metasizer analyses (see also in Figure 4.1 and 4.2). Results are shown in Figure 4.6 and Figure 4.7 for effects of loading time and effects of particle amount, respectively. All measurements are carried in triplicate and values are presented as the mean ± standard deviation (SD).

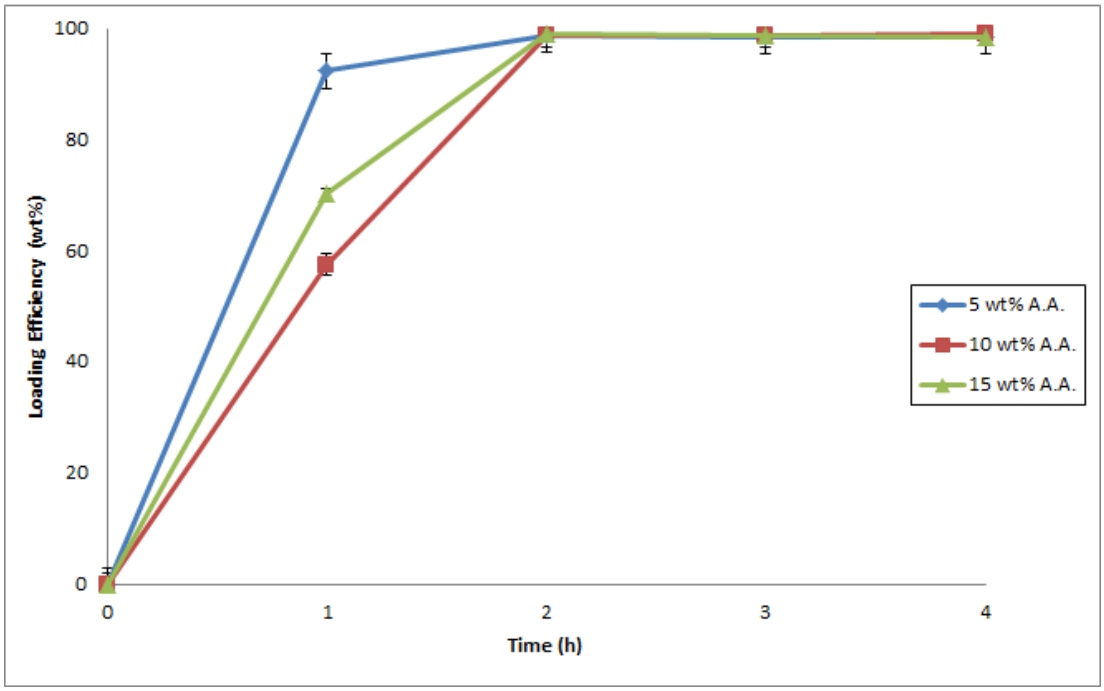


Figure 4.6 : Effects of drug loading time for L-ascorbic acid on PCL-PEG-CH.

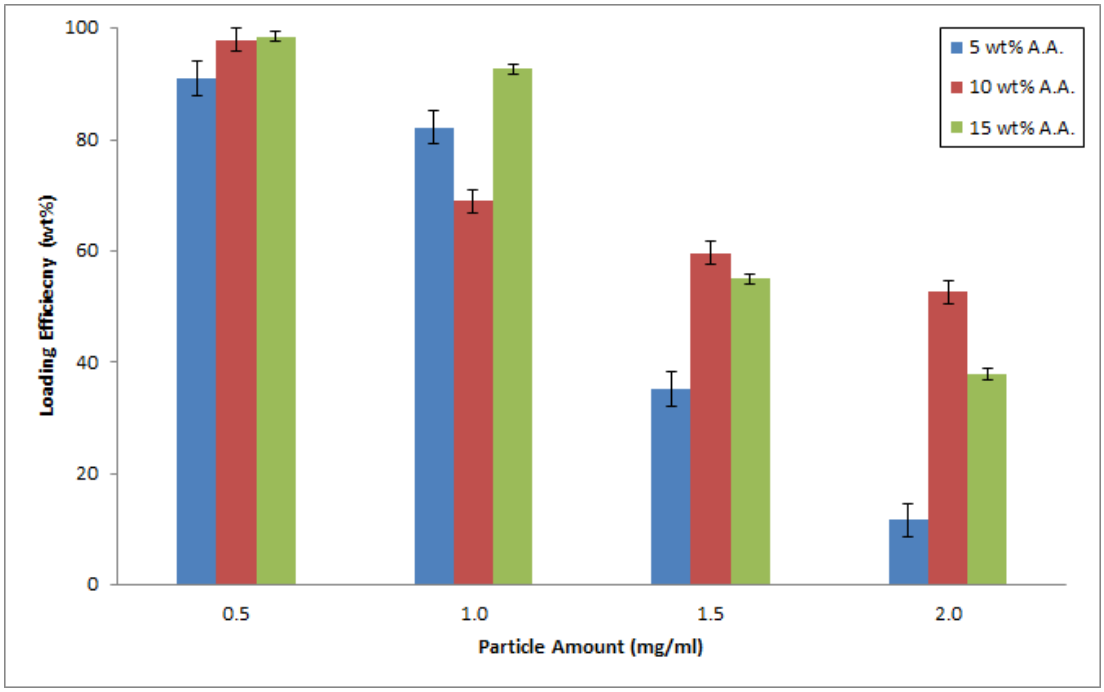


Figure 4.7 : Effects of particle amount for L-ascorbic acid loading on PCL-PEG-CH.

Drug loading experiments are carried out at 25 ^{o}C and 200 rpm and Figure 4.6 demonstrates that L-ascorbic acid concentrations are rapidly increased in 2 h and stabilized from 2 h to 4 h. We achieved significant results in this part and L-ascorbic acid loading is 99.13 wt% due to UV results. SD values are calculated ±3.0, ±2.0 and ±1.0 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L-ascorbic acid solutions, respectively.

We also determined the effects of particle amount on drug loading efficiency by changing particle amounts from 0.5 mg to 2 mg by increasing of 0.5. Results are showed in Figure 4.7. 0.5 mg PCL-PEG-CH in 15 wt% L-ascorbic acid experiment is the most effective study. We also revealed that increasing particle amount decreased drug loading efficiency. The explanation of the result is that increasing particle amount concluded agglomeration of microspheres, so particle surface area is decreased and it affected drug loading efficiency in bad direction. SD values are calculated ±3.0, ±2.0 and ±1.0 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L-ascorbic acid solutions, respectively.

Drug release studies are performed for the most loaded microsphere obtained in 15 wt% L-ascorbic acid solution by 0.5 mg particle amount. Drug release studies are obtained at three different release medium by changing pH from 2.8 to 9.6 and result are shown in Figure 4.8.

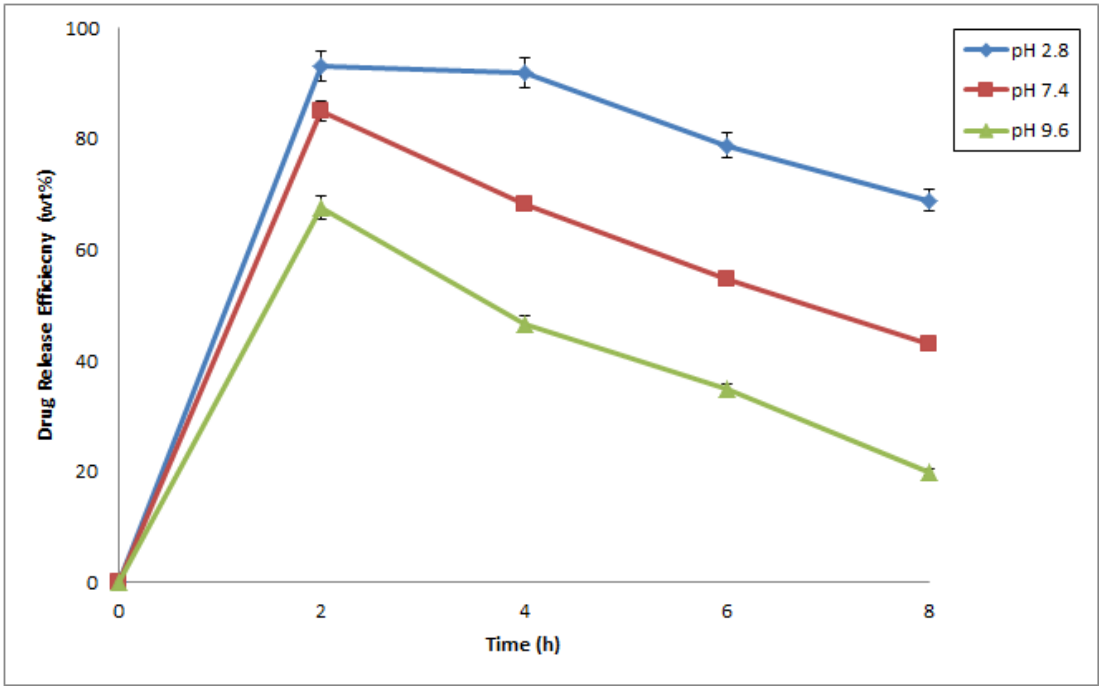


Figure 4.8 : Drug release studies at different release mediums in 8 hours.

We achieved peak release points in just 2 hours in release mediums and Figure 4.8 also illustrated that decreasing pH increased drug release efficiency. Drug loaded microspheres contains PCL-PEG-CH bio-based polymers and particularly interaction of PCL and CH in acidic medium is better than higher pH values [141]. By this way, L-ascorbic acid release remarkably increased in pH 2.8 (max. 93.18%) as shown in

the figure above. SD values are calculated ±3.0, ±2.0 and ±3.0 for drug loading environment at pH 2.8, pH 7.4 and pH 9.6 release mediums, respectively.

To describe drug release mechanism more precisely, there is a more comprehensive but still very simple semi-empirical formulations, called zero order kinetics, first order kinetics, Higuchi, Hixson-Cromwell, and the Korsmeyer-Peppas power law [142, 143]. Thus, the drug release data are fitted to these kinetic models to analyze the release kinetics and the mechanism from the polymeric drugs. Based on the best correlation coefficient values, the most appropriate model is selected to explain the release behavior of the drug. Drug release kinetics are illustrated with different kinetic models at pH 2.8, pH 7.4, pH 9.6 as seen in Figure 4.9, Figure 4.10, and Figure 4.11, respectively. The values of the release exponent (n), kinetic rate constant (k) and the correlation coefficient (R^2) are tabulated in the Table 4.1. L-ascorbic acid release from microparticles exhibits very high correlation with the Korsmeyer–Peppas semi empirical model, with $R^2 > 0.98$. The values of "n" determined by the Korsmeyer–Peppas semi empirical model, ranged from 1.0008 to 1.2475 as tabulated in the Table 4.1. The results indicate that the formulations exhibit super case-II transport mechanism (n>0.85), so the drug release is governed by non-Fickian diffusion, the dominant mechanism for drug transport is due to polymer relaxation as the gels swells [144].

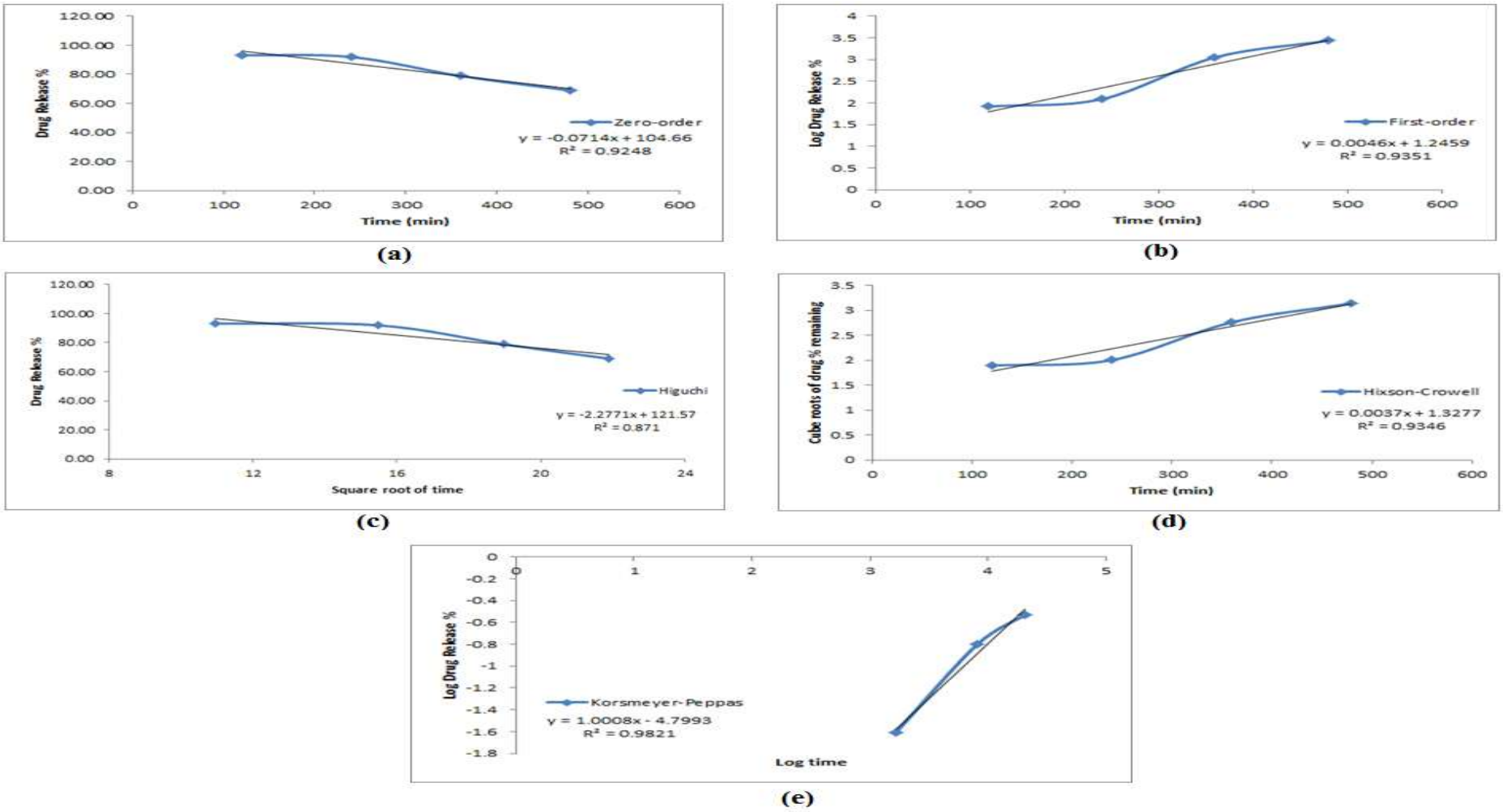


Figure 4.9 : L-ascorbic acid release kinetics at pH 2.8 with different models from the PCL-PEG-CH microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

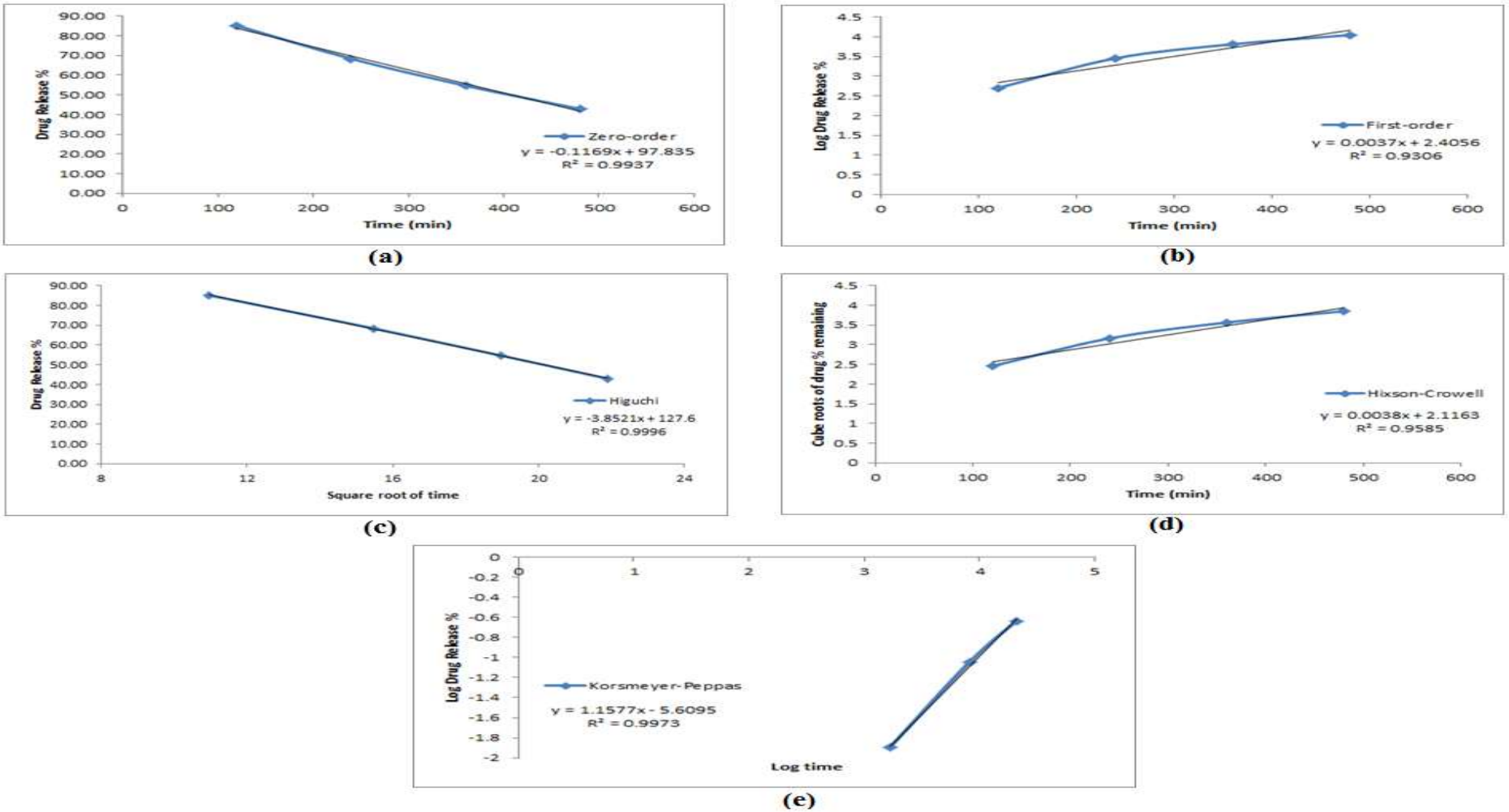


Figure 4.10 : L-ascorbic acid release kinetics at pH 7.4 with different models from the PCL-PEG-CH microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

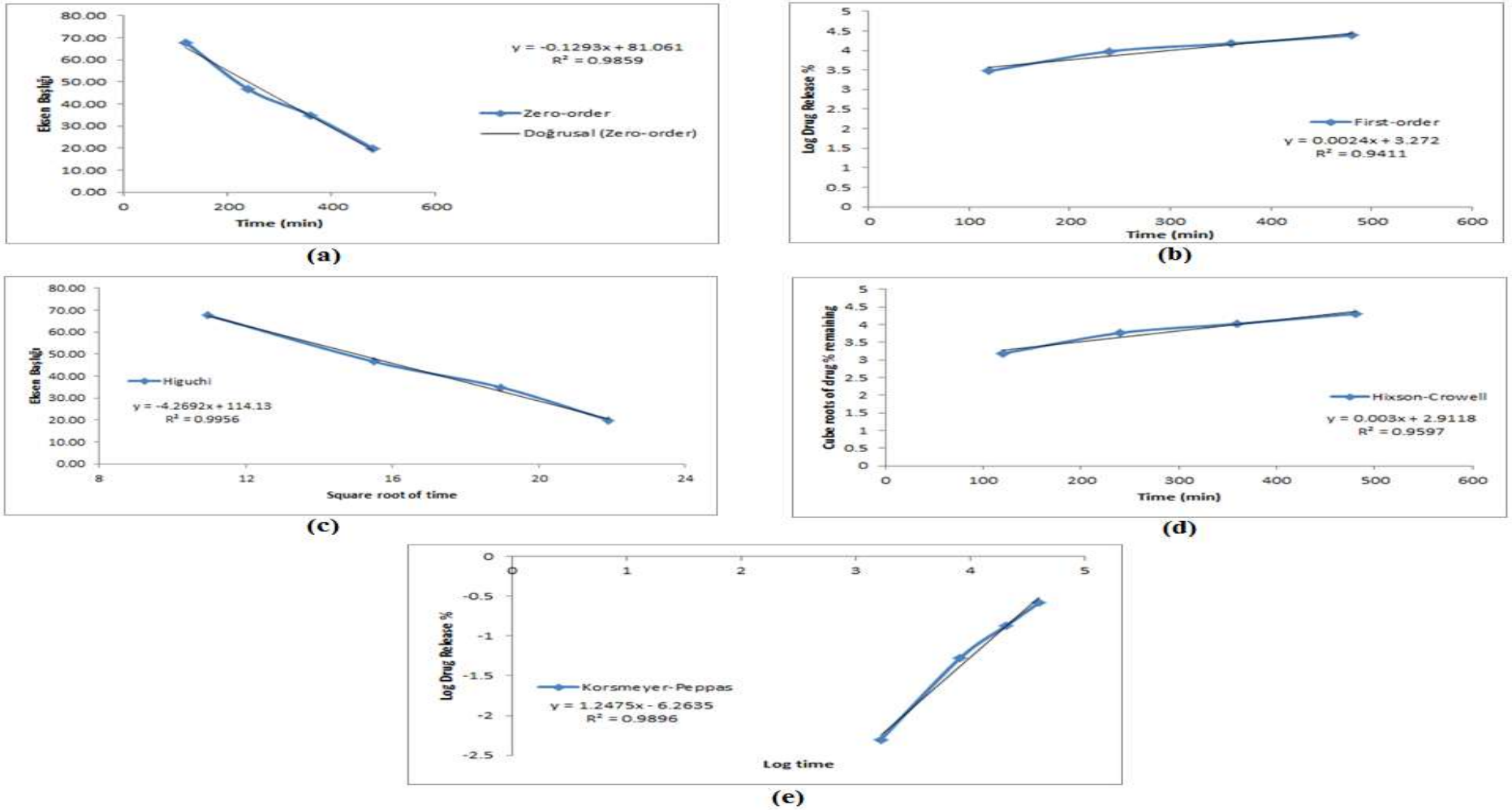


Figure 4.11 : L-ascorbic acid release kinetics at pH 9.6 with different models from the PCL-PEG-CH microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

Table 4.1 : Kinetic parameters of L-ascorbic acid release from the PCL-PEG-CH.

pH	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
	k_0 (min^{-1})	R^2	k_1 (min^{-1})	R^2	k_H ($min^{-1/2}$)	R^2	k_{HC} (min^{-1})	R^2	n	R^2	k_{HP} (min^{-n})
2.8	0.0714	0.9248	0.0046	0.9351	2.2771	0.8710	0.0037	0.9346	1.0008	0.9821	0.0082
7.4	0.1169	0.9937	0.0037	0.9306	3.8521	0.9996	0.0038	0.9585	1.1577	0.9973	0.0037
9.6	0.1293	0.9859	0.0024	0.9411	4.2692	0.9956	0.003	0.9597	1.2475	0.9896	0.0019

4.2. PCL+PEG+CS Encapsulation Systems

Yield and particle size distribution with standard deviation are shown for all studies in Figure 4.12 and Figure 4.13, respectively. Due to the Figure 4.13, Mastersizer analyses are done 3 times and particle diameter distribution is determined by standard deviation of the results. Final values demonstrate particle size (µm) with standard deviation of PCL-PEG-CS microspheres. Figure 4.12 determines that drying efficiency increase by decreasing of flow rates at 120 ^{o}C, 135 ^{o}C and 150 ^{o}C. The cause of these results are that increasing drying temperature resulted in agglomeration of polymer mixture, so contact time with polymer mixture and drying temperature had adversely effect on diameter distribution [145]. Due to these results, 135 ^{o}C and 3 ml/min are the best drying conditions because the lowest particle diameter and highest surface area are also obtained with 27.540±0.656 µm particle size with 0.512 m^2/g surface area in this study. The yields from spray drying have also been significantly decreased at higher flow rates due to the less contact time with hot drying air [146].

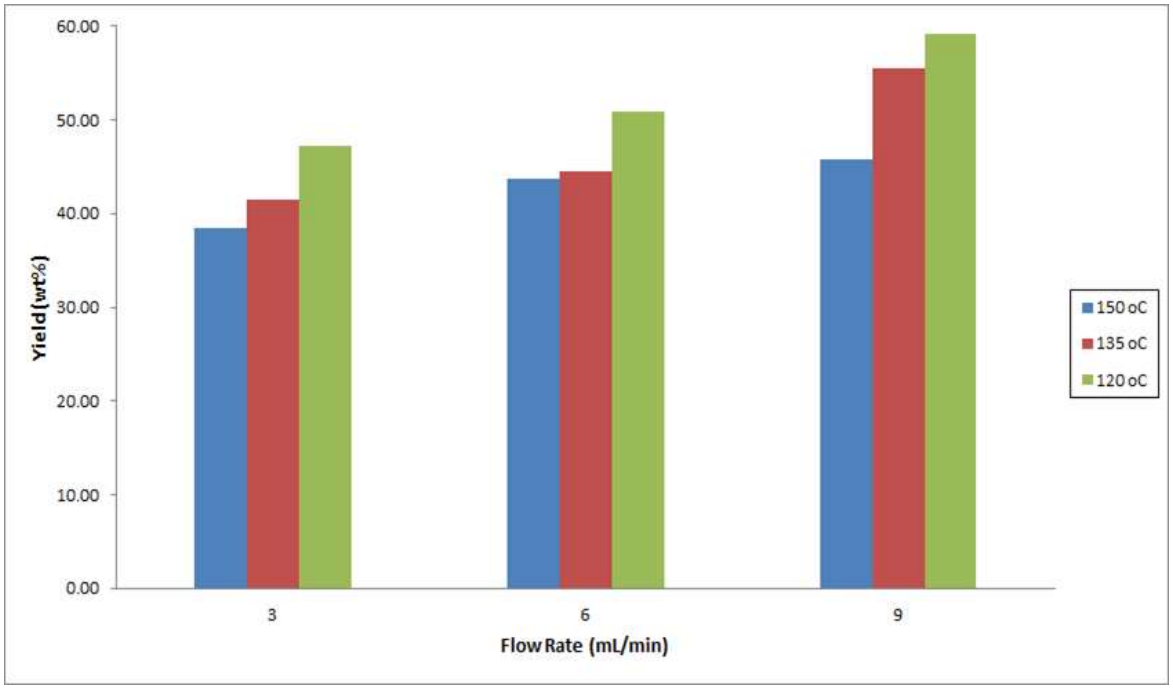


Figure 4.12 : Yield (wt%) at different drying temperature (^{o}C) and flow rates (ml/min).

Figure 4.12 shows the tendency of the yield to be higher when the spray rate was low. Additionally, particle size diameters are increased at higher spray rates as seen

in Figure 4.13. It is revealed that we have to avoid higher spray rates because of its reverse effect on encapsulation material.

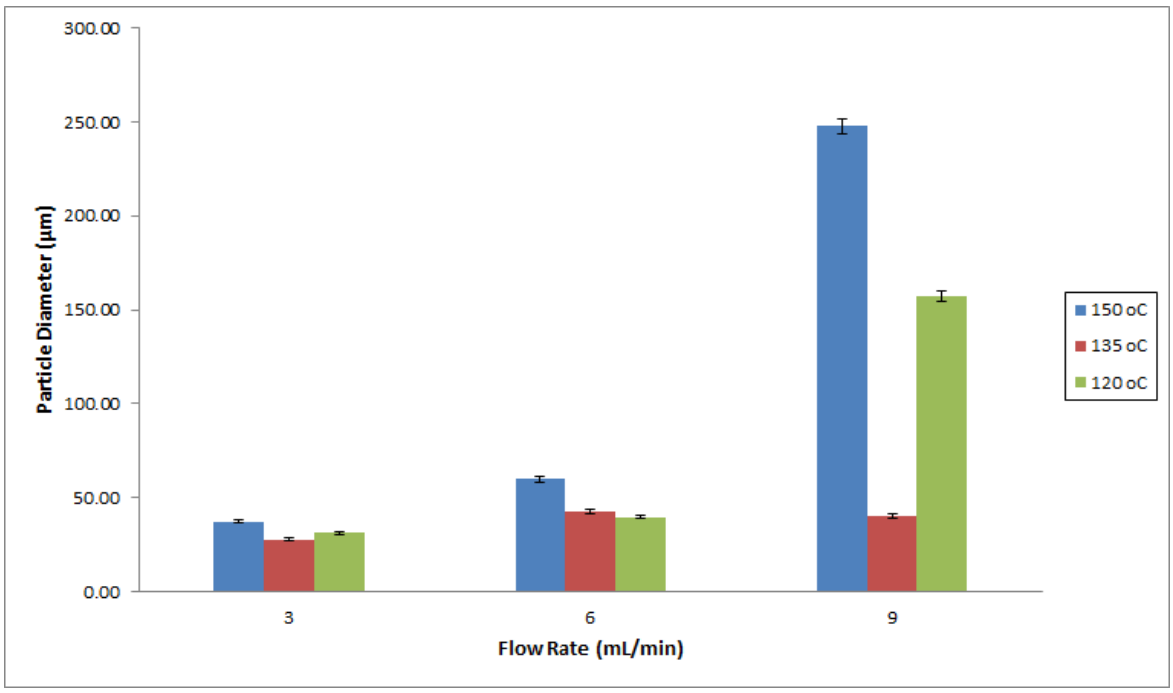
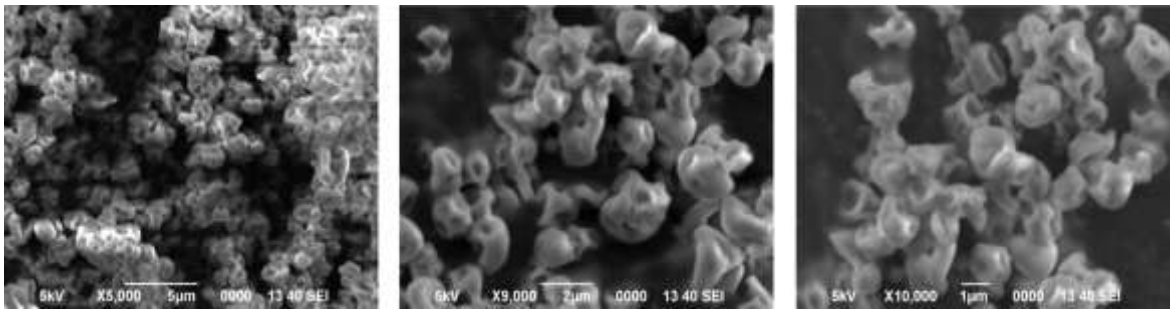


Figure 4.13 : Particle diameter (µm) with standard deviations at different drying temperature (^{o}C) and flow rates (ml/min).

SEM micrographs are shown in Figure 4.14 for PCL-PEH-CS microsphere obtained at 135 ^{o}C and 3 ml/min.



(a) **(b)** **(c)**

Figure 4.14 : SEM micrographs of PCL-PEH-CS microsphere **(a)** 5,000X; **(b)** 9,000X; **(c)** 10,000X.

Observing the external morphology, particles show a porous and spherical-like shape and various sizes with no apparent cracks or fissures, which is an advantage, since it implies that capsules have higher surface area and permeable for active ingredient (L-ascorbic acid). The variety in size is a typical characteristic of particles produced by spray drying. However, we can say that particles are distributed homogeneously as seen in Figure 4.14. The mixtures of different wall materials are influenced on

microparticles morphology [147, 148]. As well, the picture shows that there are no agglomeration and adherence between microspheres during drying process.

TGA curve for PCL-PEG-CS bio-blend is shown in Figure 4.15. The sample is placed in furnace and it is burned in the N_2 atmosphere without O_2 with the ramp 30 ^oC/min up to 800 ^oC. TGA scan is obtained from the measurement to determine decomposition temperature and char yield.

When heating a ternary blend (PCL-PEG-CS), three well-separated peaks are obtained on the TGA thermogram as shown in Figure 4.15. Customarily, the peak temperatures are referred to the degradation temperatures of the blending components [149]. Figure 4.15 shows encapsulation material thermal degradation and it begins about 200 ^oC with CS degradation, following at 350 ^oC with PCL degradation and finalized at 400 ^oC with PEG-6000 degradation.

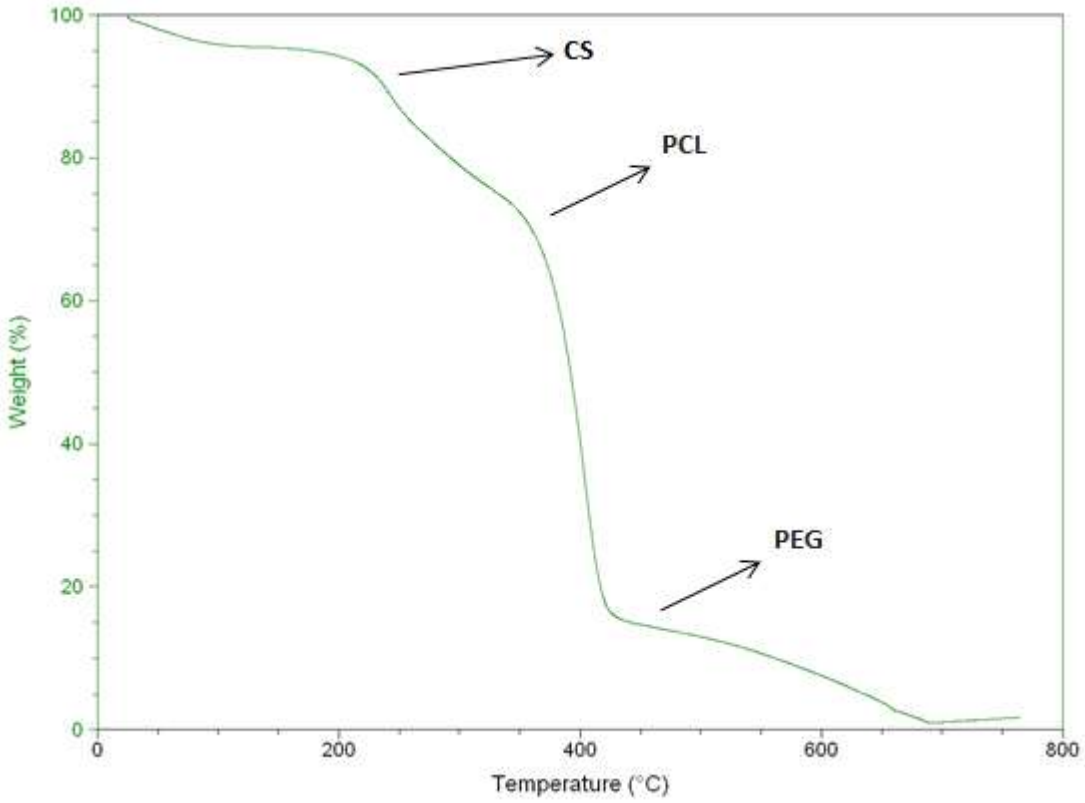


Figure 4.15 : TGA of PCL-PEG-CS microsphere obtained at 135 ^oC, 3 ml/min.

Drug encapsulation studies are performed under three different L-ascorbic acid concentrations (5 wt%, 10 wt%, and 15 wt%) at pH 7.0 with different loading time and PCL-PEG-CS particle amounts. It is expected that encapsulation efficiency of a drug changes depending on structures of the microspheres [150]. Particle diameter also affects the encapsulation efficiency. Drug-encapsulated amount depends on

surface area of the microspheres. Microsphere obtained at 135 ^{o}C and 3 ml/min is used in drug loading studies because this microsphere had the lowest particle diameter and also best distributed particles due to the Mastersizer analyses (see also in Figure 4.12 and 4.13). Figure 4.16 and Figure 4.17 indicate that how much L-ascorbic acid is encapsulated due to the time and particle amount, respectively. All measurements are carried in triplicate and values are presented as the mean ± standard deviation (SD).

Figure 5 shows drug absorbance is achieved peak value (84.05% loading efficiency) in one hour; thereafter it is smoothly decreased in all L-ascorbic acid solutions. SD values are calculated ±4.0, ±2.5 and ±1.5 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L-ascorbic acid solutions, respectively.

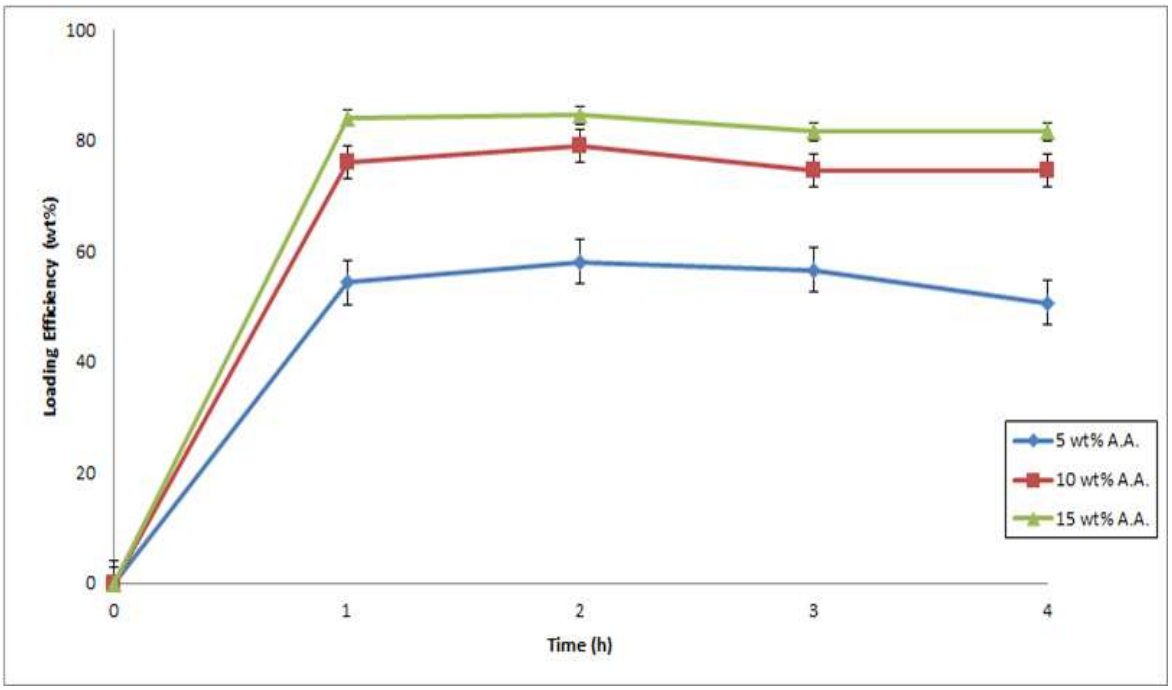


Figure 4.16 : Effects of drug loading time on encapsulation of L-ascorbic acid.

Although there is no significant difference, Figure 6 indicates that the encapsulation efficiency tended to increase from 0.5 to 2.0 mg particle/mL L-ascorbic acid solution. On the contrary, increasing weight percent of L-ascorbic acid in solution significantly increased loading rates. It changes from 49.93% to 84.05% by PCL-PEG-CS microsphere amounts from 0.5 to 2.0 mg particle/mL solution. It is also facts that low diameter and well distributed structure of microspheres are reasons to gain high L-ascorbic acid loading values [151]. Thus, when highly water-soluble drugs, such as L-ascorbic acid, are encapsulated using ternary polymer blends, %

encapsulations achieved are expectably high. SD values are calculated ±5.0, ±2.9 and ±1.5 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L-ascorbic acid solutions, respectively.

In-vitro release profiles of L-ascorbic acid from the microspheres produced by PCL-PEG-CS are shown in Figure 4.18. Experimental uncertainties in the % drug released, based on three replicates, are approximately changed from 3.4% to 5.7%.

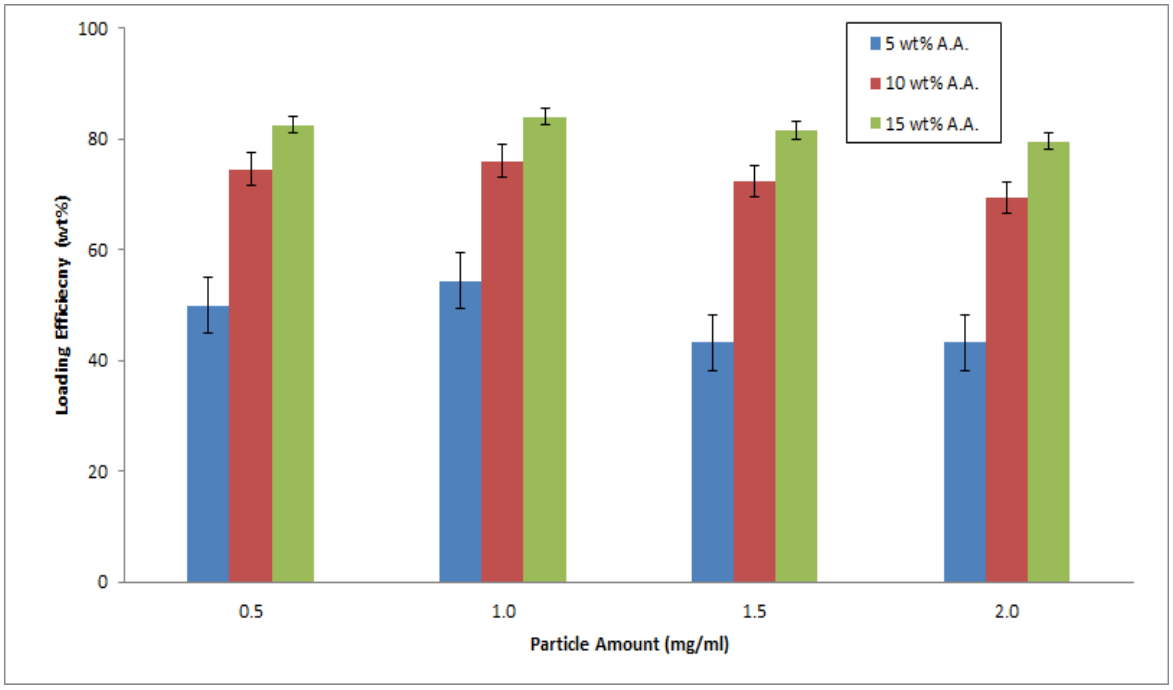


Figure 4.17 : Effects of particle amount on encapsulation of L-ascorbic acid.

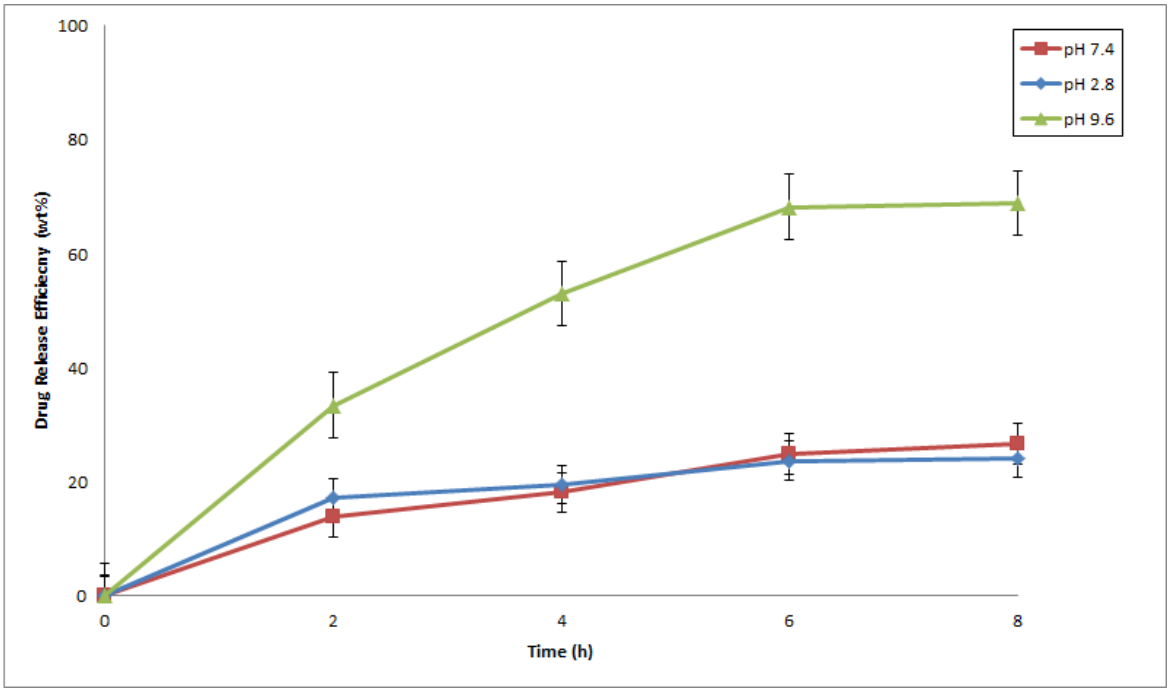


Figure 4.18 : L-ascorbic acid release with different pH mediums in 8 hours.

The % drug release initially in 2 h is 17.21 ± 3.36 at pH 2.8, 13.84 ± 3.50 at pH 7.4 and 33.39 ± 5.7 at pH 9.6. Maximum drug release ratio achieved in 6 h is 68.21 ± 5.7 at pH 9.6. The results indicate that L-ascorbic acid in the bioblend based microspheres are easily degraded higher pH values particularly at basic scale. Previous studies are showed that casein is soluble at basic pH values because of this reason L-ascorbic acid release are more effective at pH 9.6 [152]. In additionally, since CS degraded L-ascorbic acid release is increased, it also indicates CS and L-ascorbic acid interaction is stronger than PCL and PEG. Due to the previous studies, our experiments showed that results are very encouraging particularly with high drug release ratio for a new L-ascorbic acid delivery drugs by PCL-PEG-CS microsphere [153-155].

To describe drug release mechanism more precisely, there is a more comprehensive but still very simple semi-empirical formulations, called zero order kinetics, first order kinetics, Higuchi, Hixson-Cromwell, and the Korsmeyer-Peppas power law [142, 143]. Thus, the drug release data are fitted to these kinetic models to analyze the release kinetics and the mechanism from the polymeric drugs. Based on the best correlation coefficient values, the most appropriate model is selected to explain the release behavior of the drug. Drug release kinetics are illustrated with different kinetic models at pH 2.8, pH 7.4, pH 9.6 as seen in Figure 4.19, Figure 4.20, and Figure 4.21, respectively. The values of the release exponent (n), kinetic rate constant (k) and the correlation coefficient (R^2) are tabulated in the Table 4.2. L-ascorbic acid release from microparticles exhibits high correlation with the Korsmeyer–Peppas semi empirical model, with $R^2 > 0.94$. The values of "n" determined by the Korsmeyer–Peppas semi empirical model, ranged from 0.259 to 0.553 as tabulated in the Table 4.2. The results indicate that the formulations at pH 2.8 and pH 7.4 exhibit Fick diffusion mechanism ($n<0.5$), so the drug release is governed by diffusion. The formulation at pH 9.6 exhibits anomalous transport (i.e non-Fickian diffusion mechanism), so the drug release is governed by both diffusion of the drug and dissolution of the polymeric network [143].

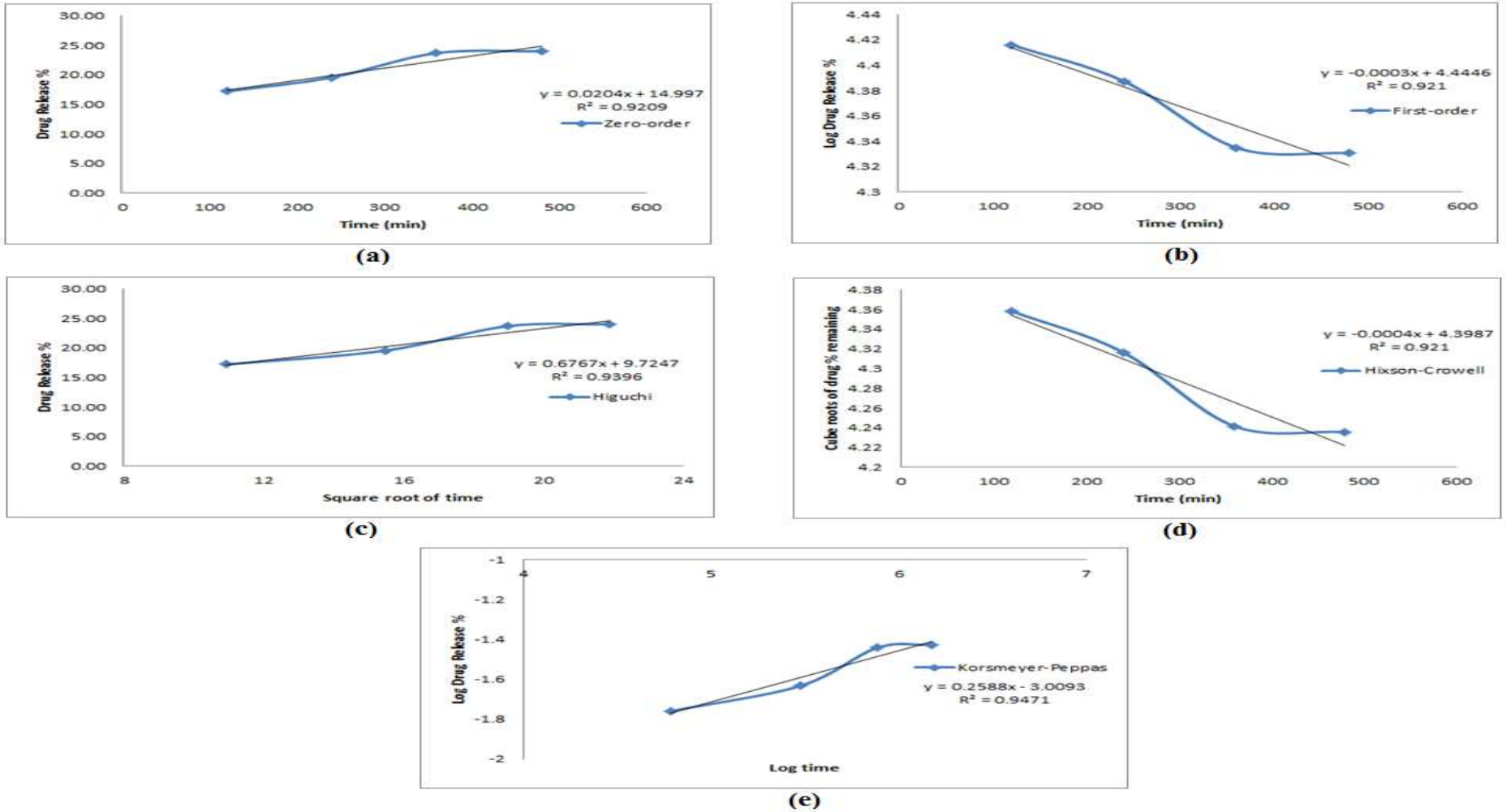


Figure 4.19 : L-ascorbic acid release kinetics at pH 2.8 with different models from the PCL-PEG-CS microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

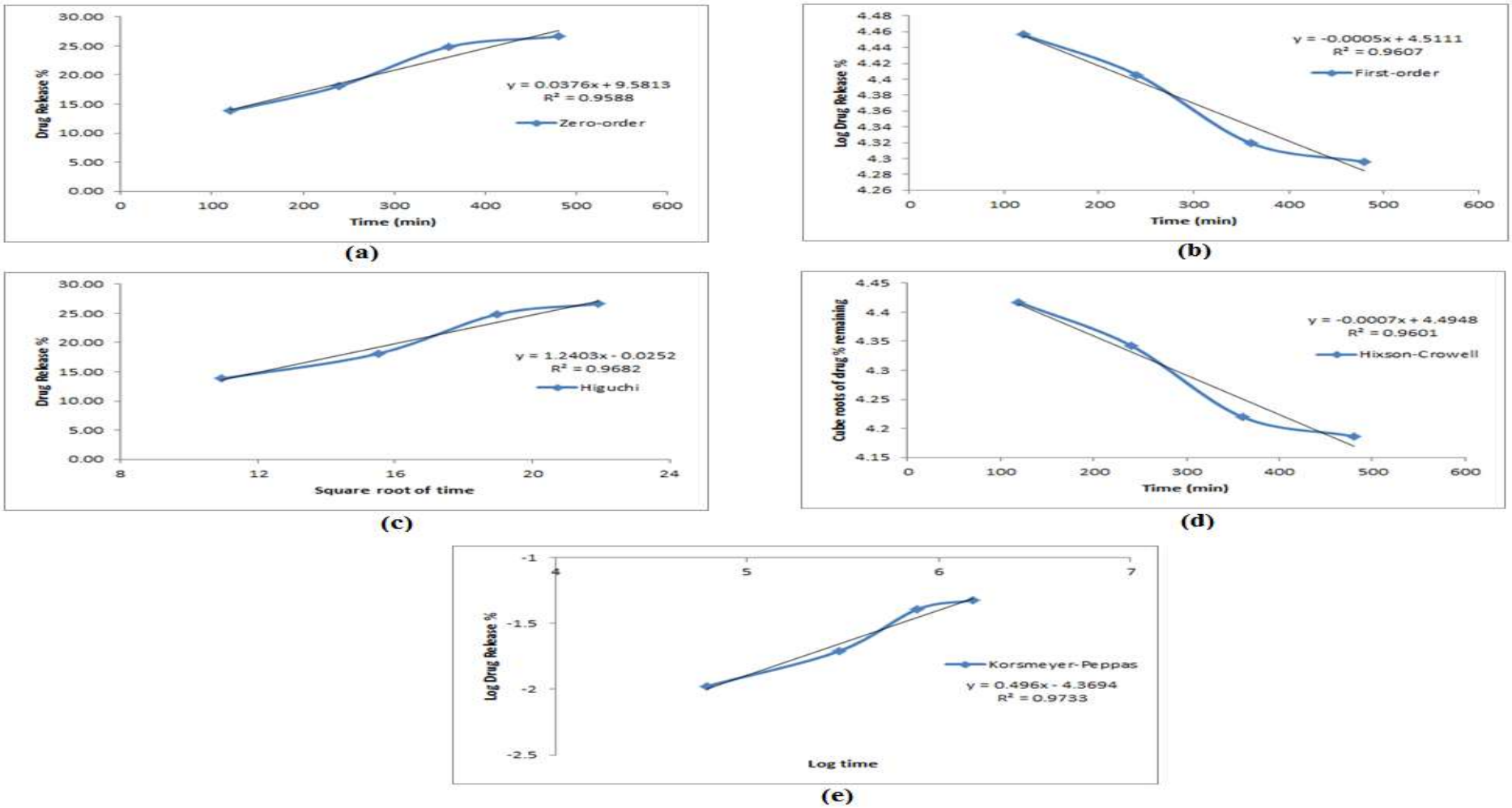


Figure 4.20 : L-ascorbic acid release kinetics at pH 7.4 with different models from the PCL-PEG-CS microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

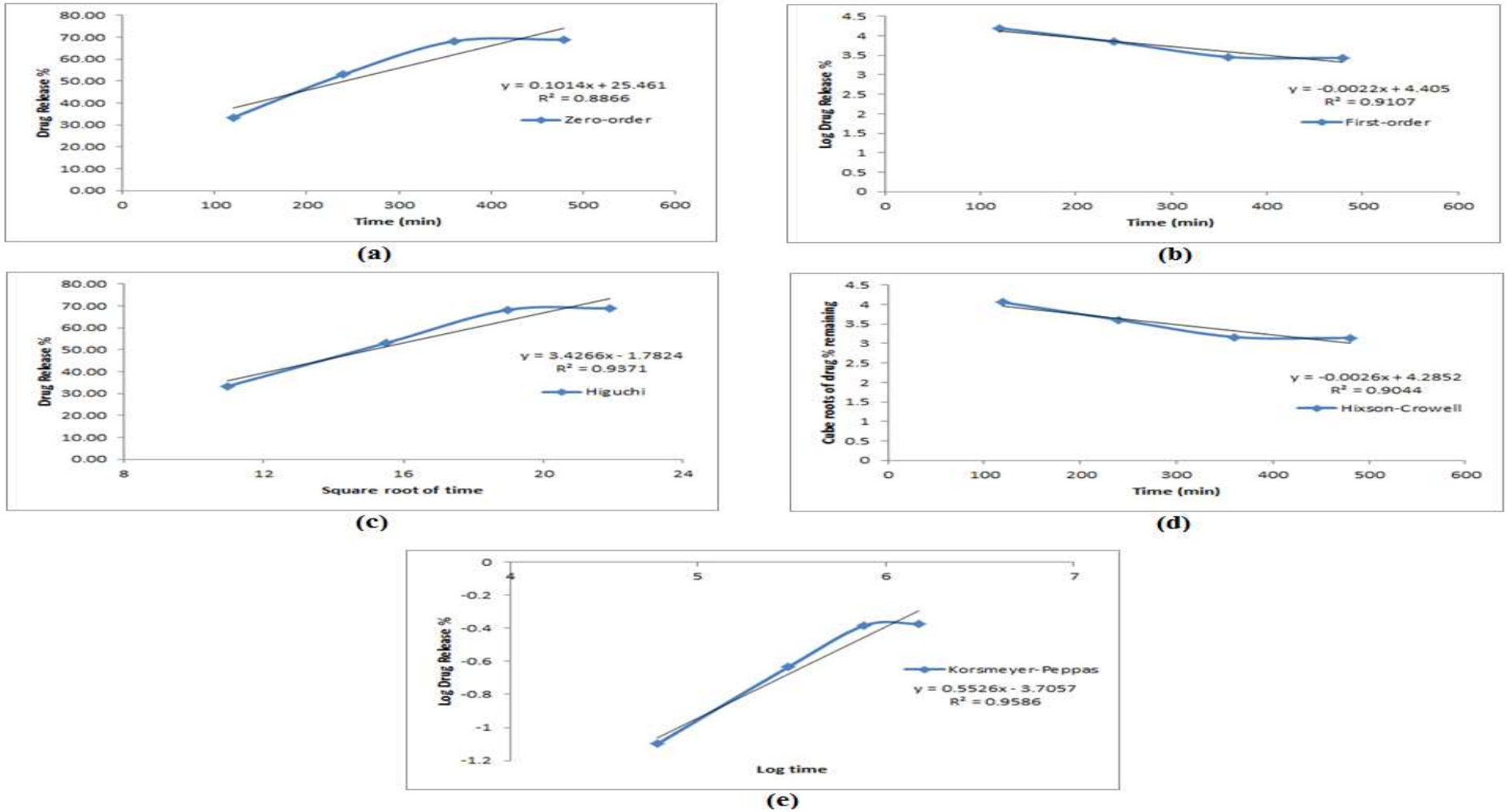


Figure 4.21 : L-ascorbic acid release kinetics at pH 9.6 with different models from the PCL-PEG-CS microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

Table 4.2 : Kinetic parameters of L-ascorbic acid release from the PCL-PEG-CS.

pH	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
	k_0 (min^{-1})	R^2	k_1 (min^{-1})	R^2	k_H ($min^{-1/2}$)	R^2	k_{HC} (min^{-1})	R^2	n	R^2	k_{HP} (min^{-n})
2.8	0.0204	0.9209	0.0003	0.9210	0.6767	0.9396	0.0004	0.9210	0.2588	0.9471	0.0493
7.4	0.0376	0.9588	0.0005	0.9607	1.2403	0.9682	0.0007	0.9601	0.4960	0.9733	0.0127
9.6	0.1014	0.8866	0.0022	0.9107	3.4266	0.9371	0.0026	0.9044	0.5526	0.9586	0.0246

4.3 PCL+PEG+SA Encapsulation Systems

As the last part of the study, yield of PCL-PEG-SA experiments is shown in Figure 4.22. Peak points are shown at 3 ml/min, 9 ml/min, and 6 ml/min flow rates at 120 ^{o}C, 135 ^{o}C, and 150 ^{o}C, respectively. It seems low flow rate is more effective at low drying temperature; on the other hand, flow rate is increased efficiency is decreased.

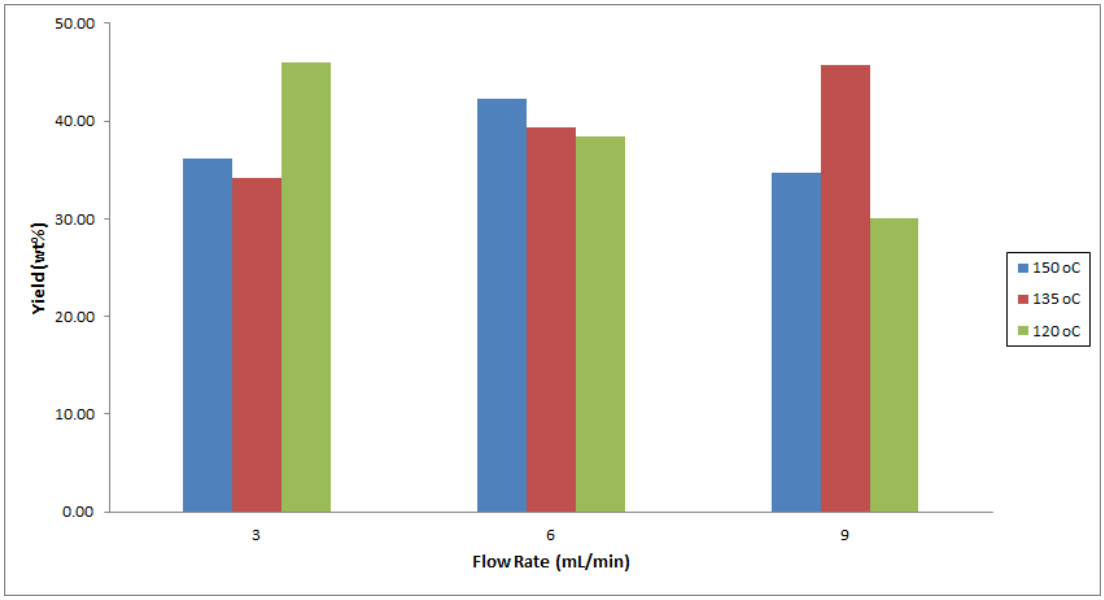


Figure 4.22 : Yield (wt%) at different drying temperature (^{o}C) and flow rates (ml/min).

Figure 4.23 shows particle distribution with standard deviations for PCL-PEG-SA studies and the lowest particle diameter and the highest surface area are obtained at 120 ^{o}C and 9 ml/min drying conditions with 37.924±0.416 µm particle size with 0.461 m^2/g surface area. Although microparticle yield is low at higher flow rates, particle diameter is getting decreased significantly due to the less contact time with hot drying air.

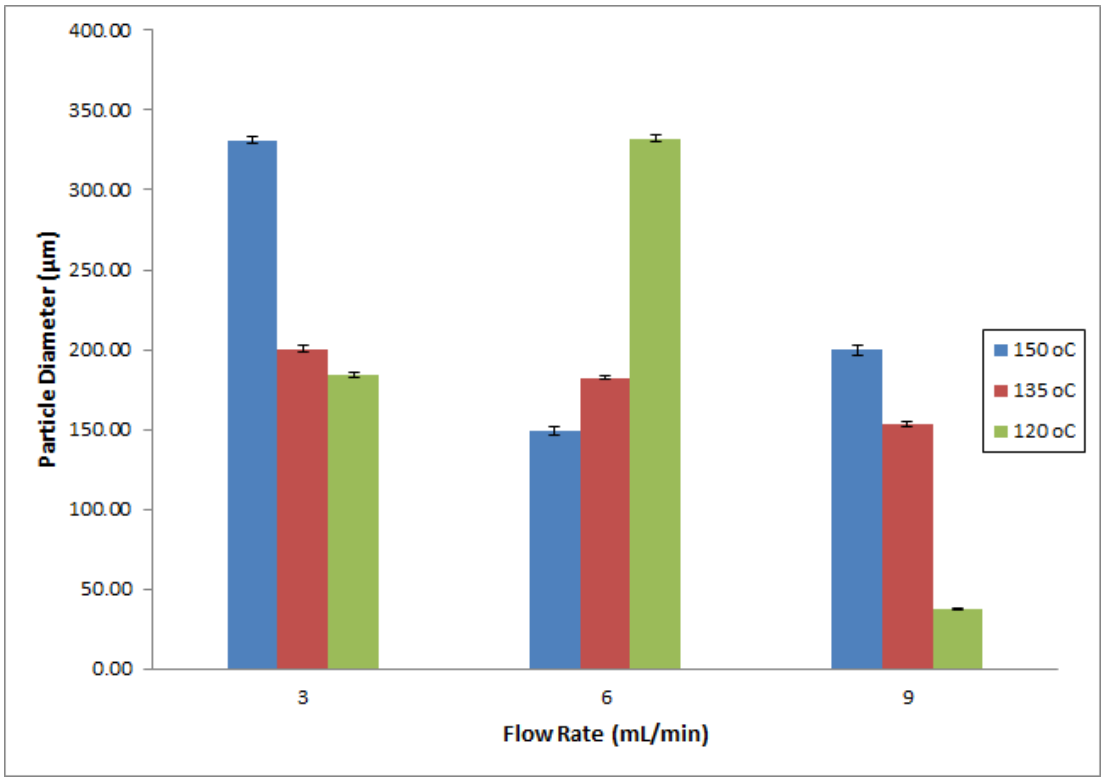


Figure 4.23 : Particle diameter (µm) with standard deviations at different drying temperature (^{o}C) and flow rates (ml/min).

SEM micrographs are shown in Figure 4.24 for PCL-PEH-SA microsphere obtained at 120 ^{o}C and 9 ml/min.

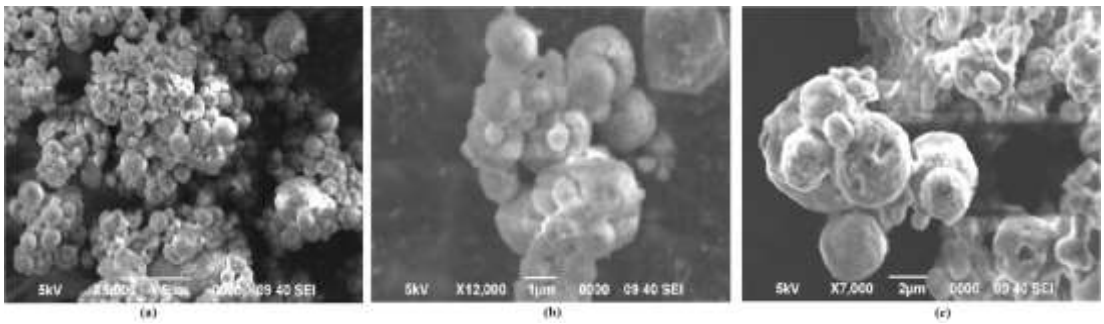


Figure 4.24 : SEM micrographs of PCL-PEH-SA microsphere **(a)** 5,000X; **(b)** 12,000X; **(c)** 7,000X.

Observing the external morphology, particles show a porous and spherical shape and various sizes with no apparent cracks or fissures, which is an advantage, since it implies that capsules have higher surface area and permeable for active ingredient (L-ascorbic acid). The mixtures of different wall materials are influenced on microparticles morphology. As well, the picture shows that there are no agglomeration and adherence between microspheres during drying process.

TGA curve for PCL-PEG-SA microspheres is shown in Figure 4.25. The sample is placed in furnace and it is burned in the N_2 atmosphere without O_2 with the ramp 30 ^oC/min up to 800 ^oC. TGA scan is obtained from the measurement to determine decomposition temperature and char yield.

When heating a ternary blend (PCL-PEG-SA), three well-separated peaks are obtained on the TGA thermogram as shown in Figure 4.25. Customarily, the peak temperatures are referred to the degradation temperatures of the blending components. Figure 4.25 shows encapsulation material thermal degradation and it begins around 180-200 ^oC with SA degradation, following at 350 ^oC with PCL degradation and finalized at 400 ^oC with PEG-6000 degradation.

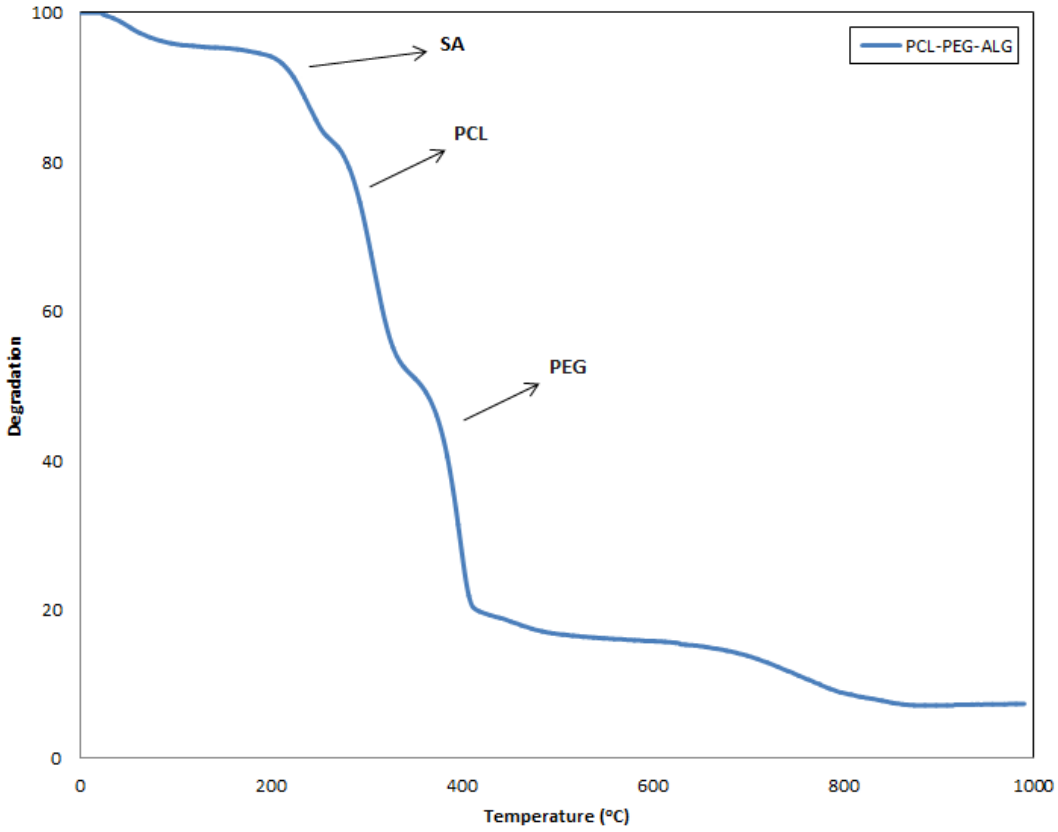


Figure 4.25 : TGA of PCL-PEG-SA microsphere obtained at 120 ^oC, 9 ml/min.

Drug encapsulation studies are performed under three different L-ascorbic acid concentrations (5 wt%, 10 wt%, and 15 wt%) at pH 7.0 with different loading time and PCL-PEG-SA particle amounts. It is expected that encapsulation efficiency of a drug changes depending on structures of the microspheres. Particle diameter also affects the encapsulation efficiency. Drug-encapsulated amount depends on surface area of the microspheres. Microsphere obtained at 120 ^oC and 9 ml/min is used in

drug loading studies because this microsphere had the lowest particle diameter and also best distributed particles due to the Mastersizer analyses (see also in Figure 4.22 and Figure 4.23). Figure 4.26 and Figure 4.27 indicate that how much L-ascorbic acid is encapsulated due to the time and particle amount, respectively. All measurements are carried in triplicate and values are presented as the mean ± standard deviation (SD).

Figure 4.26 shows drug absorbance is achieved peak value (82.57% loading efficiency) in one hour; thereafter it is smoothly decreased in all L-ascorbic acid solutions. SD values are calculated ±5.4, ±4.7 and ±2.7 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L-ascorbic acid solutions, respectively.

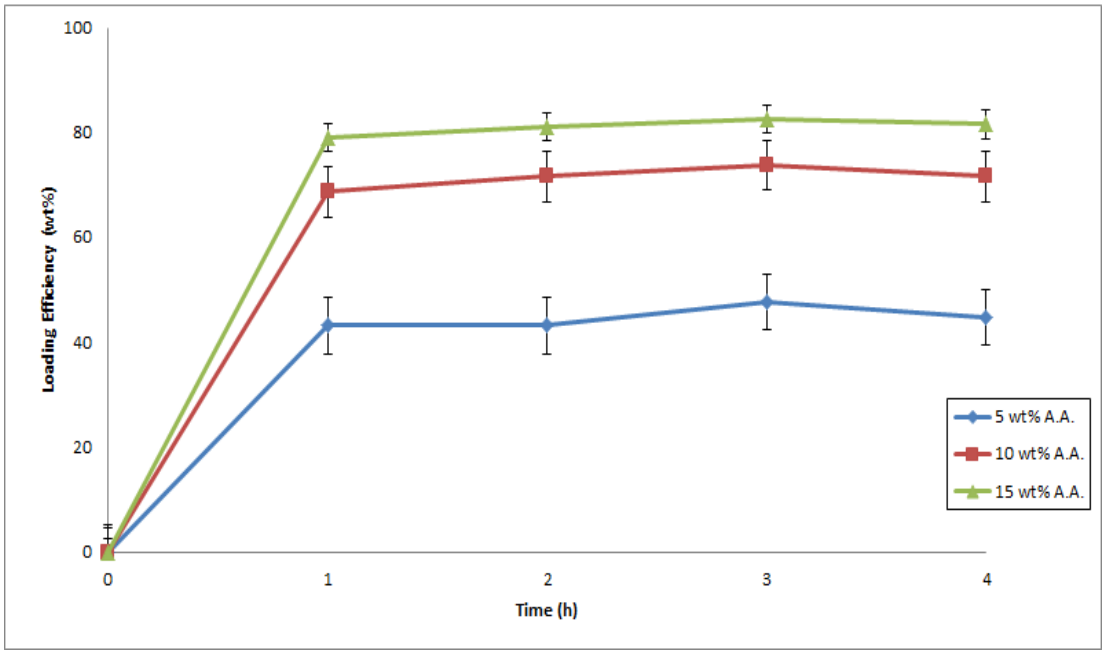


Figure 4.26 : Effects of drug loading time on encapsulation of L-ascorbic acid.

Although there is no significant difference, Figure 4.27 indicates that the encapsulation efficiency tended to increase from 0.5 to 2.0 mg particle/mL L-ascorbic acid solution. On the contrary, increasing weight percent of L-ascorbic acid in solution significantly increased loading rates. It changes from 43.26% to 82.57% by PCL-PEG-SA microsphere amounts from 0.5 to 2.0 mg particle/mL solution. It is also facts that low diameter and well distributed structure of microspheres are reasons to gain high L-ascorbic acid loading values. Thus, when highly water-soluble drugs, such as L-ascorbic acid, are encapsulated using ternary polymer mixtures, % encapsulations achieved are expectably high. SD values are calculated

±5.4, ±7.8 and ±5.5 for drug loading environment at 5 wt%, 10 wt% and 15 wt% L-ascorbic acid solutions, respectively.

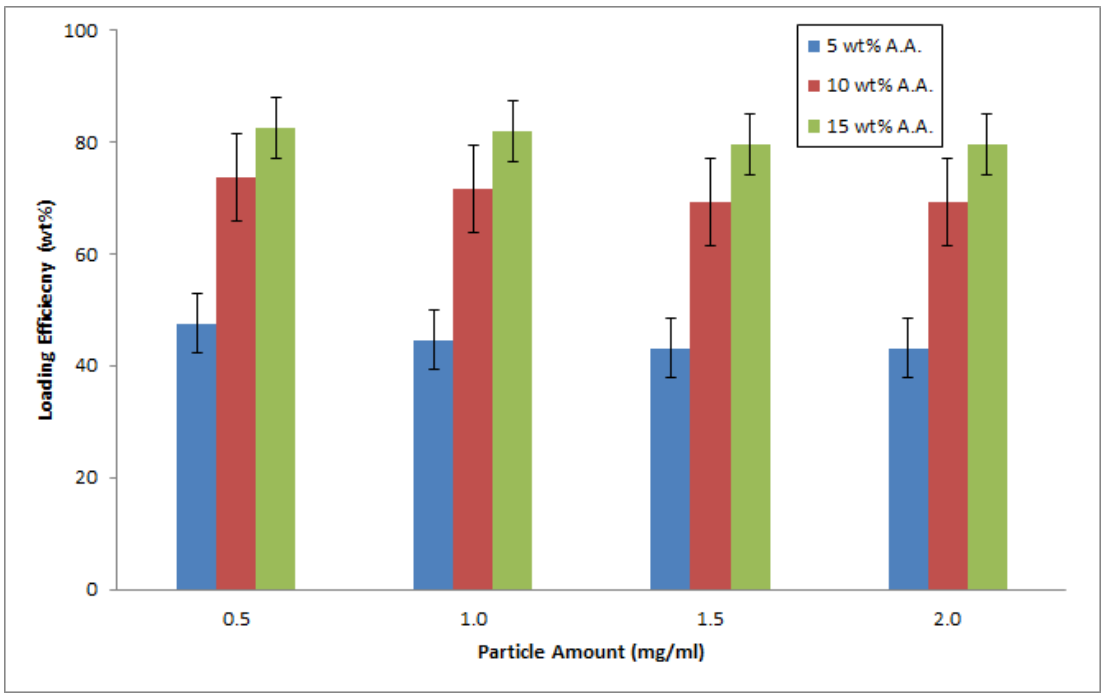


Figure 4.27 : Effects of particle amount on encapsulation of L-ascorbic acid.

In-vitro release profiles of L-ascorbic acid from the microspheres produced by PCL-PEG-SA are shown in Figure 4.28. Experimental uncertainties in the % drug released, based on three replicates, are approximately changed from 1.4% to 4.4%.

The % drug release initially in 2 h is 5.00 ± 4.35 at pH 2.8, 11.00 ± 3.23 at pH 7.4 and 6.76 ± 1.41 at pH 9.6. Maximum drug release ratio achieved in 6 h is 34.27 ± 1.41 at pH 7.4. The results indicate that L-ascorbic acid in the PCL-PEG-SA microspheres are easily degraded nearly neutral pH values. Previous studies are showed that SA is soluble at neutral pH values because of this reason L-ascorbic acid release are more effective at pH 7.4. In additionally, since SA degraded L-ascorbic acid release is increased, it also indicates SA and L-ascorbic acid interaction is stronger than PCL and PEG. Due to the previous studies, our experiments showed that results are very encouraging particularly with high drug release ratio for a new L-ascorbic acid delivery drugs by PCL-PEG-CS based blends.

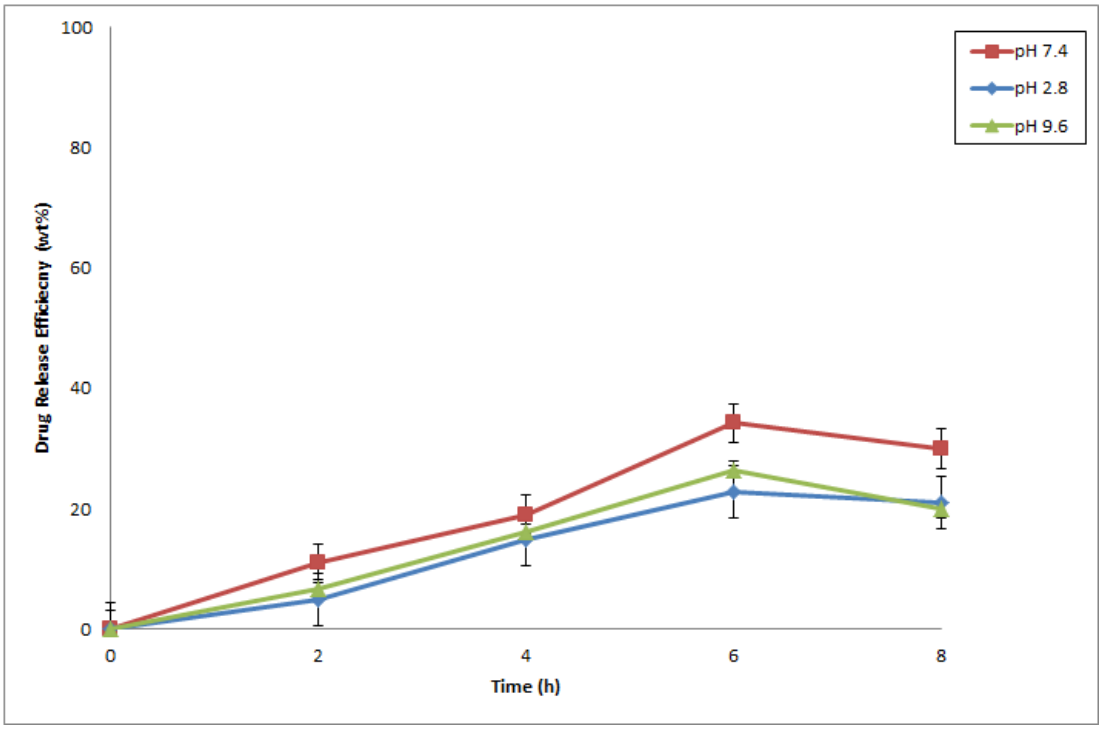


Figure 4.28 : L-ascorbic acid release with different pH mediums in 8 hours.

To describe drug release mechanism more precisely, there is a more comprehensive but still very simple semi-empirical formulations, called zero order kinetics, first order kinetics, Higuchi, Hixson-Cromwell, and the Korsmeyer-Peppas power law [142, 143]. Thus, the drug release data are fitted to these kinetic models to analyze the release kinetics and the mechanism from the polymeric drugs. Based on the best correlation coefficient values, the most appropriate model is selected to explain the release behavior of the drug. Drug release kinetics are illustrated with different kinetic models at pH 2.8, pH 7.4, pH 9.6 as seen in Figure 4.29, Figure 4.30, and Figure 4.31, respectively. The values of the release exponent (n), kinetic rate constant (k) and the correlation coefficient (R^2) are tabulated in the Table 3.3. L-ascorbic acid release from microparticles exhibits high correlation with the Korsmeyer–Peppas semi empirical model, with $R^2 > 0.82$. The values of "n" determined by the Korsmeyer–Peppas semi empirical model, ranged from 0.8152 to 1.1075 as tabulated in the Table 4.3. The results indicate that the formulations at pH 2.8 and pH 9.6 exhibit super case-II transport mechanism (n>0.85), so the drug release is governed by non-Fickian diffusion, the dominant mechanism for drug transport is due to polymer relaxation as the gels swells [144]. On the other hand, the formulation at pH 7.4 exhibits anomalous transport, so the drug release is governed by both diffusion of the drug and dissolution of the polymeric network [143].

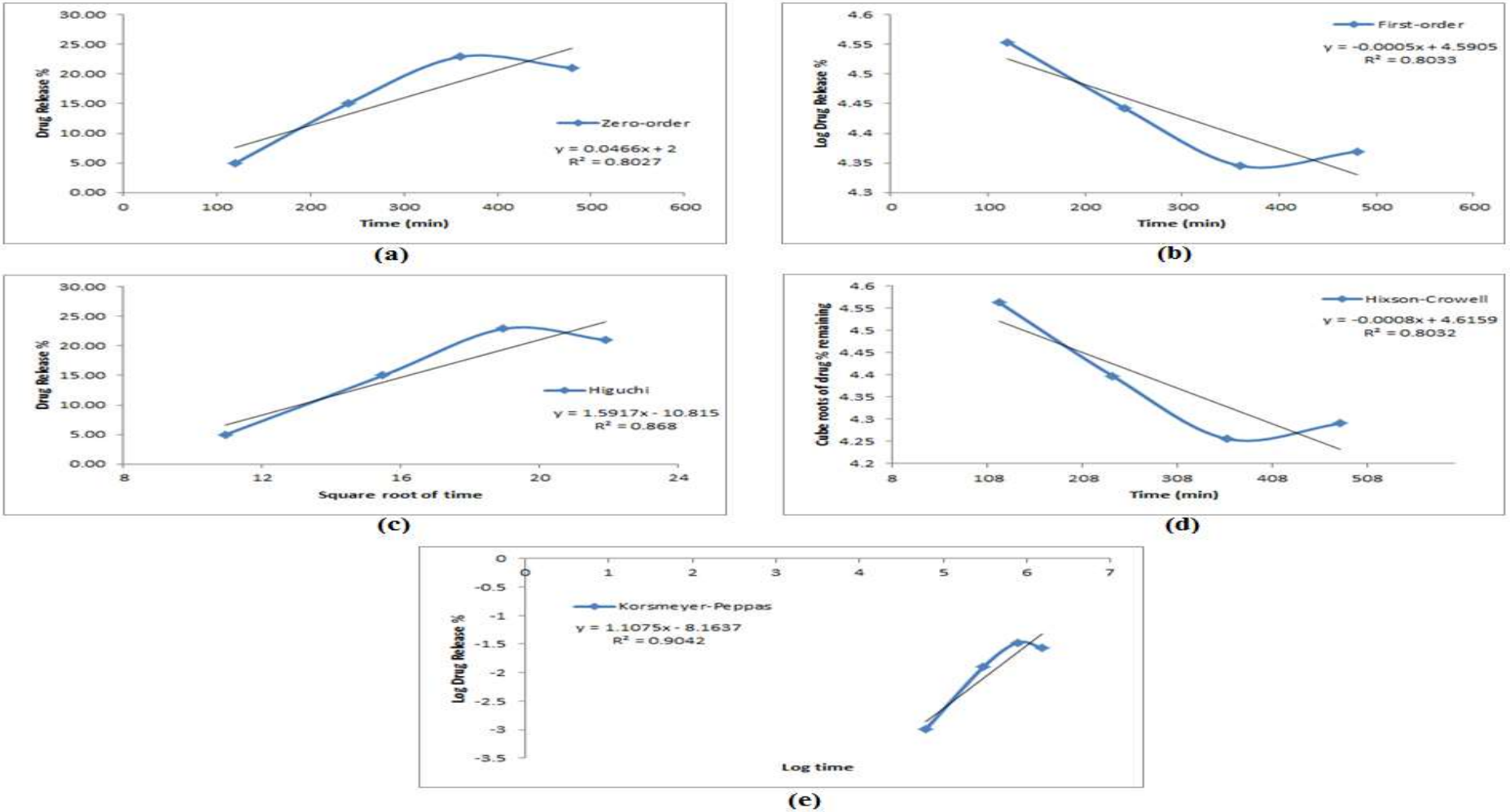


Figure 4.29 : L-ascorbic acid release kinetics at pH 2.8 with different models from the PCL-PEG-SA microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

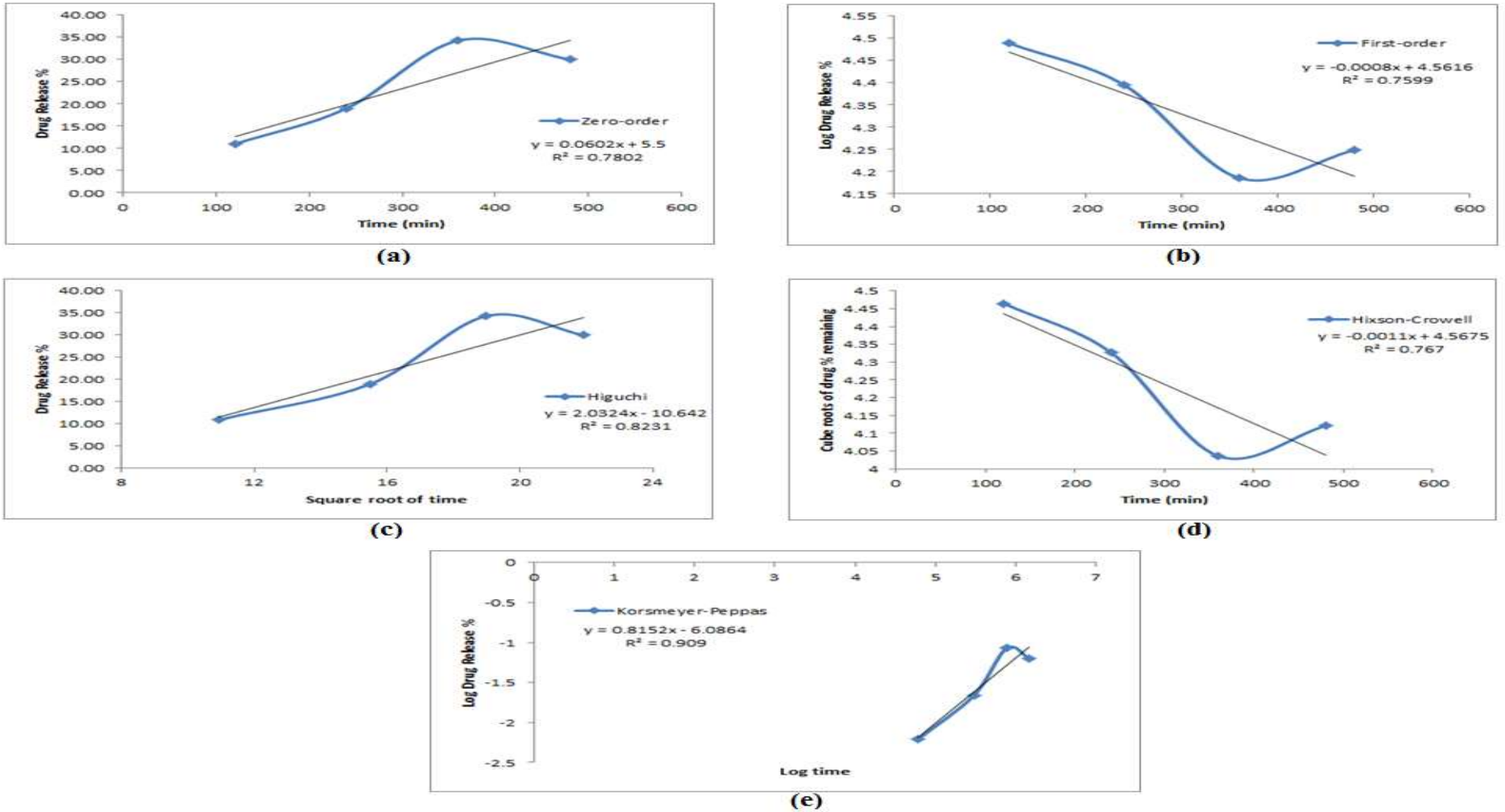


Figure 4.30 : L-ascorbic acid release kinetics at pH 7.4 with different models from the PCL-PEG-SA microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

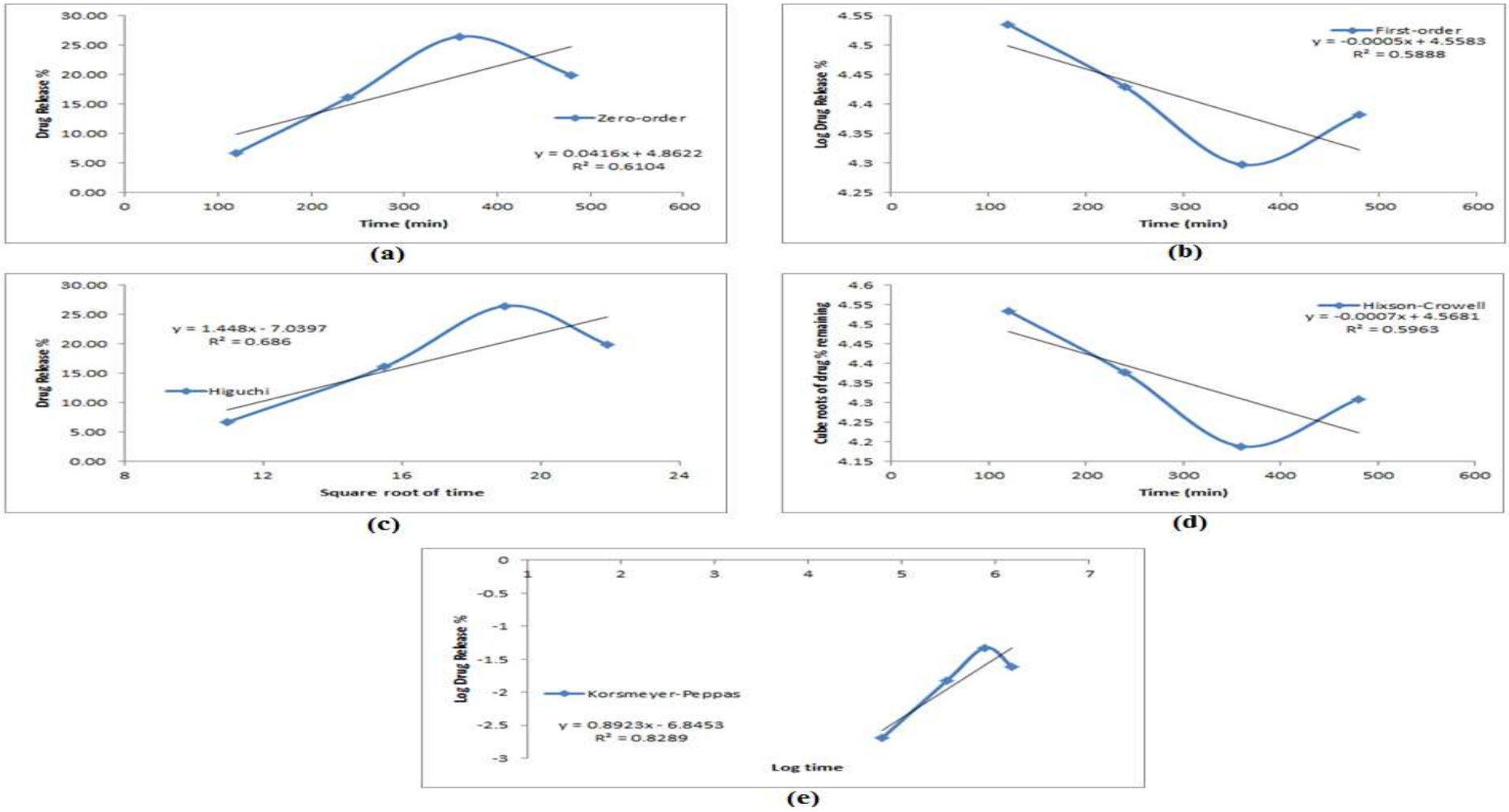


Figure 4.31 : L-ascorbic acid release kinetics at pH 9.6 with different models from the PCL-PEG-SA microspheres **(a)** Zero-order **(b)** First-order **(c)** Higuchi **(d)** Hixson-Cromwell **(e)** Korsmeyer-Peppas.

Table 4.3 : Kinetic parameters of L-ascorbic acid release from the PCL-PEG-SA.

pH	Zero-order		First-order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
	k_0 (min^{-1})	R^2	k_1 (min^{-1})	R^2	k_H ($min^{-1/2}$)	R^2	k_{HC} (min^{-1})	R^2	n	R^2	k_{HP} (min^{-n})
2.8	0.0466	0.8027	0.0005	0.8033	1.5917	0.8680	0.0008	0.8032	1.1075	0.9042	0.0003
7.4	0.0602	0.7802	0.0008	0.7599	2.0324	0.8231	0.0011	0.7670	0.8152	0.9090	0.0023
9.6	0.0416	0.6104	0.0005	0.5888	1.448	0.6860	0.0007	0.5963	0.8923	0.8289	0.0011

5. CONCLUSIONS AND RECOMMENDATIONS

It is always a fact that the encapsulation efficiency, loading capacity, and drug release behavior are the most important issues for drug delivery systems. We suggest an alternative solution to solve the problems and these are using spray dryer to reduce particle diameter, to obtain narrow particle size distribution and to produce blends made by ternary polymer mixture to get best performance from the encapsulation material. In the core of the study, encapsulation materials are changed and effects on loading, release and release behavior are investigated.

To make a clear comparison of all experimental results, different parts of the study is listed below. Table 5.1 shows drying results and differences between PCL-PEG-CH, PCL-PEG-CS, and PCL-PEG-SA microspheres.

Table 5.1 : Comparison of drying studies.

	Temperature (^oC)	Flow rate (ml/min)	Particle Size (μm)	Surface Area (m^2)	Efficiency (wt%)
PCL-PEG-CH	120	3	19.143±0.023	0.897	54.73
PCL-PEG-CS	135	3	27.540±0.656	0.512	41.42
PCL-PEG-SA	120	9	37.924±0.416	0.416	30.10

Due to the Table 5.1, using of PCL-PEG-CH polymer mixture in a spray dryer is more effective to produce microspheres. Low particle size and high surface area is one of the most important features for an encapsulation material and PCL-PEG-CH microspheres are more advantageous than PCL-PEG-CS and PCL-PEG-SA microspheres. On the other hand, low agglomeration and loss in drying chamber of PCL-PEG-CH microspheres result in higher efficiency than PCL-PEG-CS and PCL-PEG-SA microspheres.

Table 5.2 indicates differences on drug loading studies for three different encapsulation materials.

Table 5.2 : Comparison of drug loading studies.

	Operating Conditions	Efficiency (wt%)	Loading Rate (mg/ml)
PCL-PEG-CH	25 ^{o}C, 200 rpm, 2h	99.13±1.00	0.5/1.0
PCL-PEG-CS	25 ^{o}C, 200 rpm, 1h	84.05±1.50	1.0/1.0
PCL-PEG-SA	25 ^{o}C, 200 rpm, 1h	82.57±2.68	0.5/1.0

L-ascorbic acid loading has reached saturation point about 1 hour for PCL-PEG-CS and PCL-PEG-SA microspheres; besides, it is a little bit longer (2 hours) for PCL-PEG-CH. After peak points, loading rates of L-ascorbic acid is stabilized for all microspheres because loading capacity of microspheres are reached. On the other hand, because of the lowest particle size and the highest surface area, L-ascorbic acid loading ratio is higher in PCL-PEG-CH microsphere than the others. Furthermore, highly porous structure of chitosan polymer provides high loading rates for PCL-PEG-CH microspheres. By this feature of PCL-PEG-CH microspheres, it also reduces loading rates for per microsphere to drug solution.

Table 5.3 : Comparison of drug release studies.

	Efficiency (wt%)	Time (h)
PCL-PEG-CH	pH 2.8: 93.18±3.0 pH 7.4: 85.11±2.0 pH 9.6: 67.71±3.0	2 2 2
PCL-PEG-CS	pH 2.8: 23.68±3.36 pH 7.4: 26.62±3.50 pH 9.6: 68.92±5.70	6 8 8
PCL-PEG-SA	pH 2.8: 22.91±4.35 pH 7.4: 34.27±3.23 pH 9.6: 26.49±1.41	6 6 6

Drug release rates differences between microspheres and release mediums are listed in Table 5.3 and also in Figure 5.1. Results showed that drug release rates can be easily adjusted by changing structure of encapsulation material. The residence time of L-ascorbic acid drug in body depends on dissolution of the polymeric network and we achieved different drug residence time by changing encapsulation materials. Increasing of dissolution of encapsulation materials increased drug release rates into the release mediums. By this way, L-ascorbic acid release rates and release medium are manipulated.

Table 5.4 simulates n values and drug release behavior of microspheres.

Table 5.4 : Comparison of release kinetics and release behaviors.

	"n" value	Release Behavior
PCL-PEG-CH	pH 2.8: 1.0008 pH 7.4: 1.1577 pH 9.6: 1.2475	Super Case-II Transport Super Case-II Transport Super Case-II Transport
PCL-PEG-CS	pH 2.8: 0.2588 pH 7.4: 0.4960 pH 9.6: 0.5526	Fickian Diffusion Anomalous Transport Anomalous Transport
PCL-PEG-SA	pH 2.8: 1.1075 pH 7.4: 0.8152 pH 9.6: 0.8923	Super Case-II Transport Anomalous Transport Super Case-II Transport

The results showed that L-ascorbic acid release is generally governed by non-Fickian diffusion, the dominant mechanism for drug transport is due to polymer relaxation and it means structure of microspheres resulted dissolution in the release medium.

Drug release refers to the process in which drug solutes migrate from the initial position in the polymeric system to the polymer’s outer surface and then to the release medium [156]. This seemingly simple process is affected by multiple complex factors such as the physicochemical properties of the solutes, the structural characteristics of the material system, release environment, and the possible interactions between these factors. We chosen to change material matrix, so release kinetics and rates are dramatically changed at different release mediums.

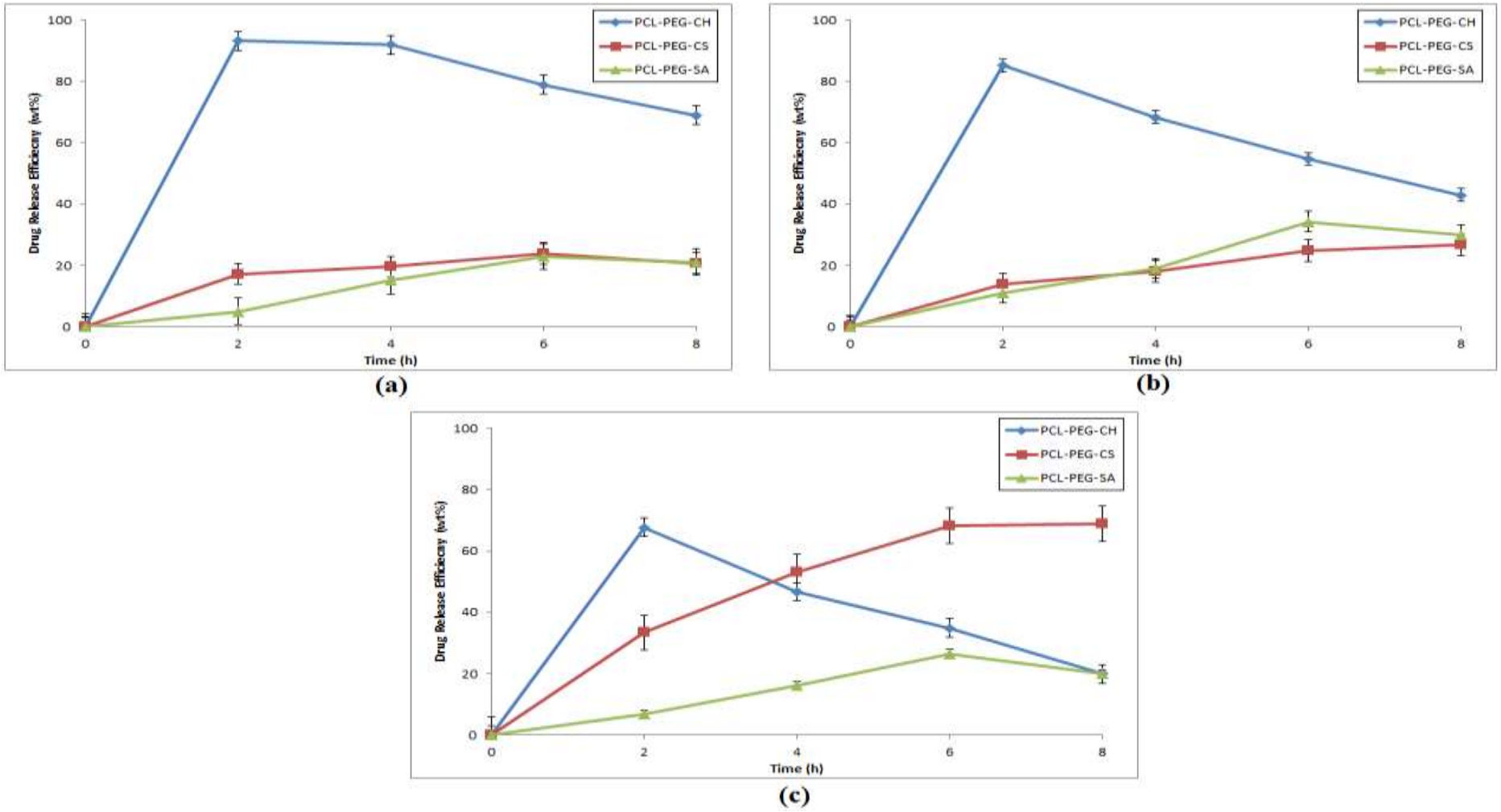


Figure 5.1 : Comparison of drug release studies **(a)** Release in pH 2.8 **(b)** Release in pH 7.4 **(c)** Release in pH 9.6.

Several pharmaceutical products based on biodegradable delivery systems have been approved by the FDA, including hormones, anti-tumor drugs and antibiotics [157]. In general, biodegradable polymers contain labile bonds such as ester, amide, and anhydride bonds that are prone to hydrolysis or enzymatic degradation. PCL, PEG, CH, CS and SA are biodegradable and biocompatible polymers that we used to produce microspheres. By this way, toxic effect of encapsulation materials in body after and/or during drug release will be eliminated.

L-ascorbic acid (vitamin C) an antioxidant which protects the body against oxidative stress. Oxidation reactions produce free radicals which can start chain reactions that damage cells, so it slows down the conversion of irritants into cancer-causing substances. On the other hand, L-ascorbic acid has many benefits for body such as it involved in the production of collagen; it plays a significant role in the healing of wounds, cuts and grazes; individuals with adequate levels of vitamin C are thought to be better able to fight off infections compared to people with vitamin C deficiency [158]. We used L-ascorbic acid (vitamin C) as an active ingredient in our study because in the rapidly expanding market of dietary supplements, it is possible to find L-ascorbic acid in many different forms with many claims regarding its efficacy or bioavailability. However, L-ascorbic acid drugs or supplements are exist as mineral ascorbates are more likely to be found in combination with other mineral ascorbates, such as sodium ascorbates, calcium ascorbates, potassium ascorbates etc. When mineral salts of ascorbic acid are taken, both the ascorbic acid and the mineral appear to be well absorbed, so it is important to consider the dose of the mineral accompanying the ascorbic acid when taking large doses of mineral ascorbates. Unfortunately, taking large doses of mineral ascorbates causes accumulation of lots of minerals into the kidneys and finally, it results kidney stone. This problem is solved by producing of polymeric encapsulation materials. PCL, PEG, CH, CS and SA are biodegradable polymers and they do not provoke accumulation in kidneys or any other organ in a body.

The current study investigates the fabrication of L-ascorbic acid drugs by microspheres for advanced spray-drying applications. In comparison the spray dryer method to others, we obtained much more uniform microspheres and lower particle diameters. Significantly, the experimental results indicate that to obtain particle diameters (19.143±0.023 μm with 0.897 m^2/g surface area for PCL-PEG-CH;

27.540±0.656 μm with 0.512 m^2/g surface area for PCL-PEG-CS; 37.924±0.416 μm with 0.416 m^2/g surface area for PCL-PEG-SA) does not need to any additive effects during drying process. Furthermore, the microspheres can be straightforwardly transformed into the L-ascorbic acid loaded drugs. The dissolution rates of the drugs from microspheres are clearly improved at low, high or nearly neutral pH values by changing encapsulation materials. The shift of the mechanism from diffusion controlled to an anomalous transport changing the pH of the medium from acidic to basic conditions as seen drug release kinetic studies. It is also indicated that drug release ratio can be adjustable by this way, thus preventing the drug release until the target has been achieved. Towards the aim of the study, improving the vitamin C drugs, our method provides easily procurable and effective solution.

Because of unstable and very sensitive L-ascorbic acid, producing vitamin C drugs are difficult. However, another significant result of our study is achieving 93.18% (the highest level) L-ascorbic acid release in 2 hours.

We achieved the aim of the study by production of a stable and very effective L-ascorbic acid loaded drug using biodegradable polymer solutions by spray dryer. By production of porous and high surface area microspheres, we succeeded higher drug loading and drug release efficiency in our study.

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