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Synthesis of polymeric microparticles loaded with the anti-inflammatory drug delivery

Presented by:

TERMOUL Chahrazed

GUERACHA Manel

Proposed jury members:

Mr. L. MAHMOUDI	MCB/UHB-Chlef	President
Mr. K. MOKHNACHE	MCA/UHB-Chlef	Examiner
Mrs. Kh. ZANOUNE	MCA/UHB-Chlef	Supervisor
Mr. M. DELLALI	MCA/UHB-Chlef	Co-supervisor

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Dedications

I dedicate this humble work to my dear mother, Fatima, who has always been by my side—listening, supporting, watching over me, and above all, encouraging me. Words cannot express my gratitude; you are my source of strength.

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Termoul chahrazed



Dedications

We only embarked on beginnings by His facilitation, and we only reached the endings by His guidance, and we only achieved our goals by His grace.

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To the one who made paradise beneath her feet and eased my hardships with her prayers.

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Abstract

This work is part of an innovative approach aimed at developing drug delivery systems based on natural polymers, with a view to sustainable biomedical solutions. The drug delivery system is designed in the form of polymeric microparticles composed of chitosan (Cs) and pectin (PEC). These natural biopolymers were selected for their complementary properties and their ability to form stable microparticles through ionotropic gelation. Two anti-inflammatory active ingredients were targeted for encapsulation: diclofenac sodium (DS), a widely used synthetic molecule, and ginger essential oil (GEO), a natural compound known for its antioxidant and anti-inflammatory properties.

The resulting microparticles exhibited diameters ranging from 200 to 600 μm , high swelling capacity, and encapsulation efficiency exceeding 80%. In vitro release profiles revealed a prolonged release, reaching 50 to 60% after 24 hours at pH 7.4. These results confirm the potential of the Cs/PEC system as a promising platform for natural, targeted, and controlled release therapeutic applications.

Keywords:

Biopolymers, Drug delivery systems, Chitosan, Pectin, Ionotropic gelation, Microparticles, Encapsulation.

Résumé

Ce travail s'inscrit dans une démarche innovante visant à développer des systèmes d'administration de principes actifs à base de polymères naturels, dans une optique de solutions biomédicales durables. Le système d'administration de médicaments est conçu sous forme de microparticules polymères composées de chitosane (Cs) et de pectine (PEC). Ces biopolymères naturels ont été sélectionnés pour leurs propriétés complémentaires et leur capacité à former des microparticules stables par gélification ionotropique. Deux principes actifs à activité anti-inflammatoire ont été ciblés pour l'encapsulation : le diclofénac sodique (DS), molécule de synthèse largement utilisée et l'huile essentielle de gingembre (GEO), un composé naturel reconnu pour ses propriétés antioxydantes et anti-inflammatoires.

Les microparticules obtenues présentent un diamètre de 200 à 600 μm , une forte capacité de gonflement, et une efficacité d'encapsulation supérieure à 80 %. Les profils de libération in vitro ont révélé une libération prolongée, atteignant 50 à 60 % après 24 h à pH 7,4. Ces résultats confirment le potentiel du système Cs/PEC comme plateforme prometteuse pour des applications thérapeutiques naturelles, ciblées et à libération contrôlée.

Mots-clés :

Biopolymères, Systèmes d'administration de médicaments, Chitosane, Pectine, Gélification ionotropique, Microparticules, Encapsulation, ,.

الملخص

يندرج هذا العمل في إطار مقاربة مبتكرة تهدف إلى تطوير أنظمة لإيصال المواد الفعالة تعتمد على البوليمرات الطبيعية، وذلك في سياق البحث عن حلول طبية حيوية مستدامة. تم تصميم نظام إيصال الدواء على شكل جسيمات دقيقة بوليمرية مكونة من الكيتوزان والبكتين. وقد تم اختيار هذين البوليمرين الطبيعيين نظراً لخصائصهما المتكاملة وقدرتهما على تكوين جسيمات دقيقة مستقرة عبر تقنية التغليف الهلامي الأيونوتروبي

تم استهداف مادتين فعاليتين لهما نشاط مضاد للالتهاب من أجل التغليف والتنشيط : ديكلوفيناك الصوديوم، وهو مركب اصطناعي واسع الاستخدام، والزيت العطري للزنجبيل، وهو مركب طبيعي معروف بخصائصه المضادة للأكسدة والمضادة للالتهاب ، تتراوح أقطار الجسيمات الدقيقة المحضرة بين 200 و600 ميكرومتر، وتتميز بقدرة عالية على الانتفاخ، ونسبة التغليف تتجاوز 80%. وقد أظهرت اختبارات التحرر واطلاق المادة الفعالة، حيث تم تحرير ما بين 50 إلى 60% بعد 24 ساعة عند درجة حموضة 7.4. تؤكد هذه النتائج الإمكانيات الواعدة لنظام الكيتوزان والبكتين كمنصة فعالة للتطبيقات العلاجية الطبيعية المستهدفة وبنظام تحرر متحكم فيه

الكلمات المفتاحية : الكيتوزان البكتين، الجسيمات الدقيقة الأديوية، توصيل أنظمة

التغليف، البوليمرات الحيوية

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PCL: Polycaprolactone

PLA: Polylactic acid

PLGA: Polylactide-co-glycolide

PGA: Polyglycolic acid

PHBV: Poly (3-hydroxybutyrate)-co-3-hydroxyvalerate

PHB: Poly hydroxyl butyrate

PPDO: Polyp-dioxanone

PAA: Polyacrylic acid

PA: Polyamides

PEG: Polyethylene glycol

PES: Polyortho esters

PVA: Polyvinyl alcohol

DDSs: Drug delivery system

GlcN: D-glucosamine

GlcNAc: N-acetyl-D-glucosamine

GalA: D-galacturonic acid

NCAP: Nematic curvilinear aligned phase

MPs : Microparticles

P-MPs : Polymeric-microparticles

API: The active pharmaceutical ingredient

NSAIDs: Non-steroidal anti-inflammatory drugs

Eos: Essential oils

CS: Chitosan

PEC: Pectin

TPP: Tripolyphosphate

DS: Diclofenac sodium

FTIR: Fourier-Transform Infrared Spectroscopy

BCS: Biopharmaceutics Classification System

GEO: Ginger essential oil

KBr: Potassium bromide

UV: UV-Visible spectrophotometry

Vis: visible

PBS: Phosphate-buffered saline

EE: Encapsulation efficiency

Ref: Release efficiency

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General Introduction

General Introduction

Pain, whether acute or chronic, represents a major global public health concern, affecting a significant portion of the population. Due to its high prevalence, non-steroidal anti-inflammatory drugs (NSAIDs) remain among the most widely used treatments, owing to their well-established analgesic, antipyretic, and anti-inflammatory properties [1]. Among these, diclofenac is commonly prescribed for the management of mild to moderate inflammatory pain, particularly in cases of osteoarthritis, rheumatoid arthritis, or postoperative pain. It is widely employed in clinical settings via oral, topical, or parenteral administration due to its rapid and effective therapeutic action.

However, despite their recognized efficacy, NSAIDs present several clinical limitations. Prolonged or repeated administration is frequently associated with adverse effects, including gastrointestinal irritation, renal impairment, hepatic toxicity, and an increased risk of cardiovascular events [2]. Diclofenac, in particular, has a relatively short biological half-life (1–2 hours), a high affinity for plasma proteins, and undergoes rapid metabolism and elimination. These pharmacokinetic properties require frequent dosing, which increases the likelihood of dose dependent side effects.

To address these limitations, the development of innovative drug delivery systems (DDS) has emerged as a promising strategy to enhance the therapeutic profile of conventional drugs while minimizing their toxicity. In this regard, the use of biopolymers as drug carriers is particularly attractive due to their biocompatibility, biodegradability, and ability to ensure controlled and sustained drug release.

Drug delivery is a key field in nanomedicine, aiming to transport active pharmaceutical ingredients to specific target sites in the body, thereby improving therapeutic outcomes and reducing systemic side effects [3]. The efficiency of such systems depends on various parameters, including the route of administration, the nature of the drug, and the structural and physicochemical properties of the carriers. Micro/nanoparticles based on biopolymers have been extensively used as a support for drug carriers due to their significant advantages for efficient delivery [4]. There are two structural types of polymeric Microparticles: microcapsules and microspheres [5]. During this study, the experimental part was focused on microspheres as microparticles with a spherical shape and smooth texture, which are adequate for delivering anti-inflammatory agents.

In the present study, the drug delivery system is designed in the form of polymeric microparticles composed of chitosan and pectin. These natural biopolymers were selected for their complementary properties and their ability to form stable microparticles through ionotropic gelation. Microparticulate systems offer numerous advantages, such as high encapsulation efficiency, protection of the active ingredient from degradation, and sustained drug release. These features are particularly beneficial in the case of diclofenac, which requires frequent dosing and presents notable systemic toxicity.

Chitosan (CS) is one of the biopolymers that can form micro/nanoparticles with unique properties [6]. CS received great interest for applications in the medical and pharmaceutical field due to its attractive properties like biocompatibility, biodegradability, and non-toxicity. It is also known for other properties, such as analgesic, mucoadhesiveness, hemostatic, hydrophilicity, antimicrobial activity, and antioxidant [7–9]. CS based microparticles (MPs), due to their simple preparation process, are of great interest and are widely exploited due to their suitability as promising vectors in the pharmaceutical industry for controlled drug delivery [10].

Essential oils (EOs), including ginger essential oil (GEO) extracted from ginger [6], have been utilized for various applications over the years. Their natural aromatic properties make them suitable for flavoring agents. Ginger EO is recognized for its antimicrobial and antioxidant properties, yet its instability against environmental factors such as oxygen, light, moisture, and pH during production and storage limits its effectiveness. Consequently, novel methods are essential to safeguard these natural compounds for biomedical applications [7].

The main objective of this study is to develop a polymeric drug delivery system in the form of microparticles, prepared via the ionotropic gelation method, for the encapsulation of two anti-inflammatory agents:

- Diclofenac (DS), a synthetic non-steroidal anti-inflammatory drug (NSAID);
- Ginger Essential Oil (GEO), a natural bioactive compound with well documented anti-inflammatory properties.

This approach aims to harness the functional advantages of both biopolymers (chitosan and pectin), while proposing a multifunctional delivery system with potential applications in anti-inflammatory therapy.

This thesis is organized into two parts:

The first part is a bibliographic research being composed of two chapters:

- Chapter I, presenting a general overview of biopolymers, their classification, properties, and biomedical relevance.
- Chapter II, which focuses on polymeric microparticles as drug delivery systems, with emphasis on the specific roles and advantages of chitosan and pectin.

The second part composed also to two chapters:

- The third chapter (Materials and methods) details the raw materials, equipment, processing methods, and characterization techniques employed.
- Chapter IV: presents the obtained results and their discussions.

Finally, a general conclusion and suggestions for future are presented.

Theoretical Part

Chapter I

Polymers are natural or synthetic macromolecules consisting of repeating units. Biopolymers are a specific class of polymeric materials derived from living organisms. The use of these biopolymers has grown significantly in recent years due to their renewability, abundance, biodegradability, low immunogenicity, and other unique properties. This chapter focuses on several types of biopolymers, including polysaccharides (such as cellulose, chitosan, and pectin) and proteins. The objective of this chapter is to present the structure and significance of biopolymers. It will also discuss their physical and chemical properties, and finally, their applications.

I.1. Definitions

I.1.1. Polymer

The term polymer is derived from the ancient Greek word “Polus” which means many and “meros” which means parts. Polymers are macromolecules composed of repeating units. Monomers have the ability to react with another molecule of the same type or different type at suitable conditions to form a polymer chain [11]. Their large molecular weights lead to unique physical and mechanical properties including rigidity and viscosity. Polymers can be considered as important components of highly functional materials in the fields of chemical, electronic, optical, pharmaceutical and medical industries.

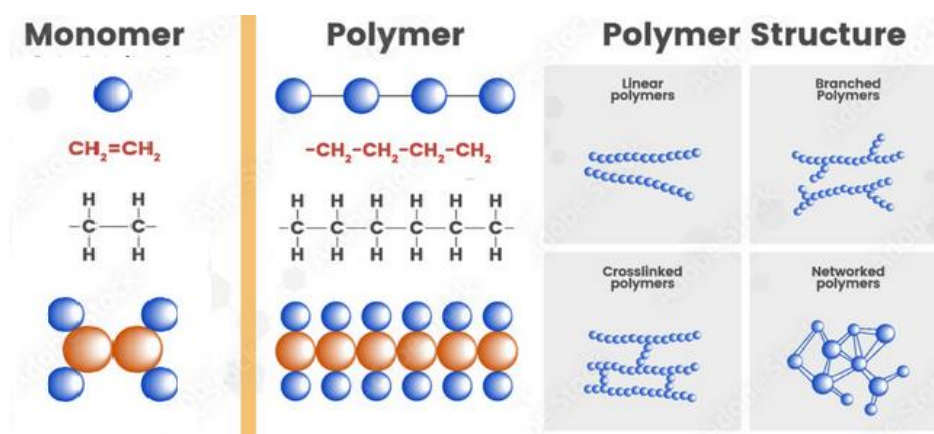


Figure I.1: Polymer structure

I.1.2. Biopolymer

The prefix "bio" in the term ‘biopolymers’ signifies that these polymers are inherently produced from living matter. Comprising of chain-like structured molecules either linearly or branched/cross-linked, biopolymers have found their way into revolutionizing the world of

materials, the term derives from the Greek words *bios* (life) and *polymeres* (many parts) [12]. Biopolymers are macromolecules consisting of numerous repeating subunits, often referred to as polymeric biomolecules [13]. By definition, biopolymers are materials of biological origin, obtained from natural sources such as plants, animals, microorganisms, or other living organisms. These materials are generally flexible, elastic, or fibrous. Biopolymers may easily degrade biologically and are either chemically obtained from natural sources or fully biosynthesized by living organisms [14]. They contain a variety of functional groups, including hydroxyl, amide, amino, phosphate, and phenol groups. Due to their inherent biodegradability and biocompatibility, biopolymers have garnered significant attention in materials science and biotechnology

I.2. Classification of biopolymers

Biopolymers can be classified according to various scales. These classification are based on their origin, a number of monomeric units, on the bases of degradability [15].

I.2.1. On the basis of origin

1. **Natural Biopolymers:** These are biopolymers naturally biosynthesized by living organisms. They are derived directly from biological systems without significant modification.

2. **Synthetic Biopolymers:** These are polymers synthesized from renewable biological resources through controlled chemical processes.

3. **Microbial Biopolymers:** These are biopolymers specifically produced by microorganisms through metabolic processes.

I.2.2. Based on Monomeric Units:

1. **Polysaccharides:** These are polymers consisting of carbohydrate monomers arranged in linear or branched chains. *Anionic:* Alginate, Heparin, and Pectin. *Neutral:* Dextran, Starch, Cellulose. *Cationic:* Chitosan, Pullulan

2. **Proteins:** Proteins are polymers composed of amino acid monomers linked via peptide bonds. Notable examples include Gelatin, Collagen, Wheat Gluten

3. **Polynucleotides:** These biopolymers are long chains of nucleotides, typically composed of 13 or more monomeric units. DNA and RNA are primary examples.

I.3. Natural biopolymers

Natural polymers are macromolecules that are inherently present in nature and can be extracted from biological sources such as plants and other natural materials [16]. These polymers are broadly categorized into polysaccharides and proteins [17]. Compared to synthetic polymers, natural polymers exhibit superior biocompatibility and biodegradability, making them more suitable for biomedical applications [18]. Their key advantages include widespread availability, mucoadhesiveness, non-toxicity, and cost-effectiveness [19].

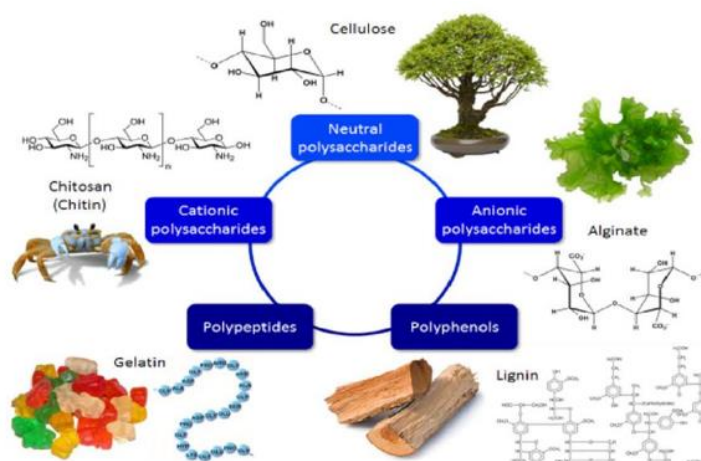


Figure I.2. Sources of Natural Biopolymers [20]

I.3.1. Polysaccharides

Polysaccharides are complex carbohydrate macromolecules found across various biological systems [21]. Structurally, they consist of long chains of monosaccharide units connected via O-glycosidic bonds [22]. As one of the most abundant classes of biopolymers in nature, they play critical roles in structural support, energy storage, cellular signaling, and biological recognition. Polysaccharides are naturally derived from plants, animals, microorganisms, and algae, making them important in various biological and biomedical applications.

Polysaccharides can be classified according to different criteria, primarily based on their monomeric composition, origin, and biological function.

1.3.1.1. Classification Based on Monomer Composition

- **Homopolysaccharides** : These polysaccharides are composed of only one type of monosaccharide unit repeated along the polymer chain. Examples: Cellulose, starch (amylose and amylopectin), glycogen, and chitin.
- **Heteropolysaccharides**: These are composed of two or more types of monosaccharides. Examples: Hyaluronic acid, heparin, and chondroitin sulfate.

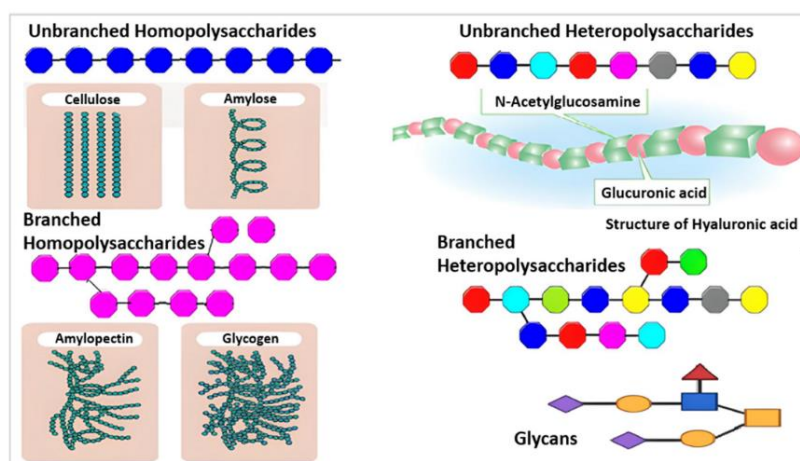


Figure I.3. Different monosaccharides are represented by various colors, including branched and unbranched homopolysaccharides and heteropolysaccharides [23].

1.3.1.2. Classification Based on Origin

Polysaccharides are also classified according to their natural sources:

- **Plant-derived polysaccharides**: cellulose, starch, pectin.
- **Animal-derived polysaccharides**: glycogen, chitin.
- **Microbial polysaccharides**: dextran, xanthan gum.
- **Algal polysaccharides**: agar, alginate, carrageenan.

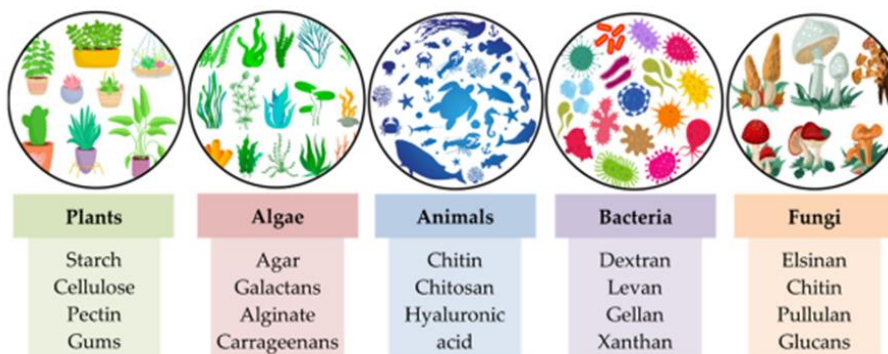


Figure I.4. Natural polysaccharides classified according to their origin [24]

1.3.1.3. Classification Based on Biological Function

- **Structural polysaccharides:** provide mechanical support and rigidity.
 - *Examples:* cellulose (plants), chitin (insects, fungi).
- **Storage polysaccharides:** act as energy reserves.
 - *Examples:* starch (plants), glycogen (animals).

I.3.2. Proteins

Proteins are macromolecules composed of amino acid chains linked by peptide bonds. They represent the final products of the genetic decoding process that begins with cellular DNA, functioning as essential molecular machines within the cell. Their roles encompass structural support, enzymatic activity, transport, and signaling [25, 26]. Typically, proteins are built from 20 standard amino acids encoded by DNA, although other amino acids, such as ornithine and citrulline, also contribute to critical biological processes [27]. Proteins can be categorized according to various criteria as illustrated in Figure I.5.

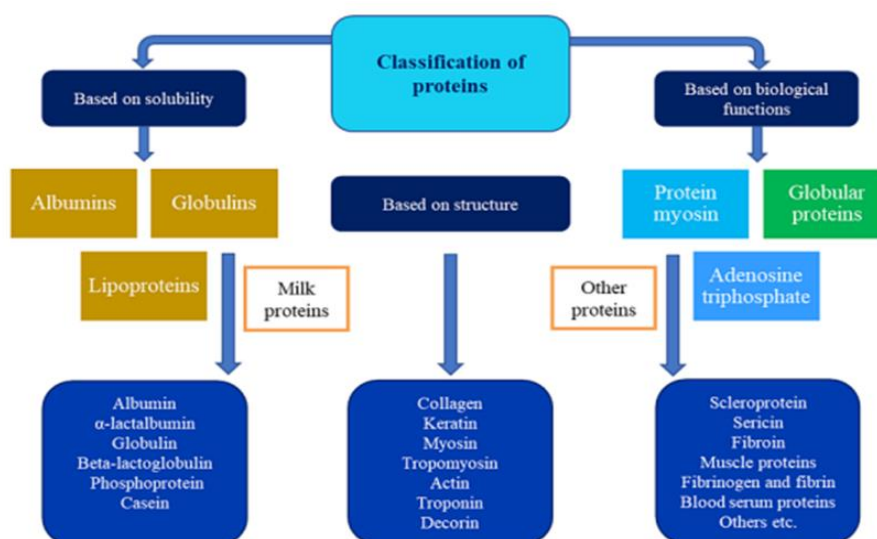


Figure I.5. Classification of some important proteins [28]

I.4. Synthetic Polymers:

Synthetic polymers are artificially synthesized materials produced in laboratories, commonly referred to as man-made polymers [29]. These polymers are primarily organic, featuring carbon-carbon bonds in their backbone structure [30]. They are biologically inert, meaning they do not cause adverse effects or harm to living organisms. Key properties of synthetic polymer biomaterials include non-carcinogenicity, non-immunogenicity, non-teratogenicity, and non-toxicity. In essence, they are inert, bioactive, and biodegradable [31]. Examples of synthetic polymers include poly(caprolactone) (PCL), poly(lactic acid) (PLA),

poly(lactide-co-glycolide) (PLGA), and other types such as poly(cyanoacrylates), poly(p-dioxanone) (PPDO), poly(amides) (PA), poly(anhydrides), poly(ethylene glycol) (PEG), and poly(vinyl alcohol) (PVA)[32].

I.4.1. Properties of Biopolymers

Biopolymers exhibit a wide range of physicochemical and functional properties that make them attractive for various industrial and scientific applications [33]. A key characteristic of biopolymers is their biodegradability, as they can be decomposed by microorganisms into environmentally harmless byproducts. Additionally, many biopolymers are biocompatible, which makes them suitable for use in biomedical and pharmaceutical fields [34, 35]. Their mechanical properties, thermal behavior, and barrier capabilities vary depending on their chemical composition and molecular structure. For instance, polysaccharide-based biopolymers such as cellulose and chitosan offer good mechanical strength and moisture absorption, while protein-based biopolymers like gelatin and zein are valued for their flexibility and film-forming ability. Furthermore, most biopolymers are derived from renewable resources and are non-toxic, positioning them as sustainable alternatives to conventional synthetic [36].

Table I.1. Different natural polymers with their source of origin and Properties [37-40]

Biopolymers	Source of origin	Properties
Cellulose	Plant tissue (Trees, cotton etc.), bacteria (<i>Acetobacter xylinum</i>).	Biocompatible, combine flexibility, stiffness, and tuneable properties.
Pectin	Plant cell walls, citrus Peels, apple pomace.	Biodegradability, biocompatibility, easy gelling capacity, and facile manipulation of pectin-based biomaterials.
Carrageenan	Cell wall matrix of red seaweeds	Biocompatibility, biodegradability, and bioactive features such as antibacterial, anticoagulant, antiviral, antioxidant, antitumor, antihyperlipidemic and immunomodulating characteristics.
Alginate	Brown algae of the Genera <i>Nacrocystis</i> , <i>Laminaria</i> , <i>Ascophyllum</i> , <i>Alario</i> , <i>Ecklonia</i> , <i>Eisenia</i> , <i>nercocystis</i> , <i>sargassum</i> ,	Biocompatible, biodegradable, and gel forming capacity

	cystoseria,fucu	
Chitin/ Chitosan	Animal (crustacean shells, exoskeletons of insects and other arthropods, e.g., crabs, shrimp, and krill etc.	Versatile, biodegradable, insoluble in water, rigid structure, and antimicrobial nature
Collagen	Connective tissue present in the skin of human beings	Biodegradable, biocompatible, non-toxic, bioactive. It has numerous surface adhering areas for cells, and it stimulates cell attachment, differentiation, and proliferation.
Starch	Grains, cereal and potatoes	Biocompatible, sustainable, and biodegradable.
Dextran	Leuconostoc mesenteroides, Streptococcus mutans, and Lactobacillus brevis	Wound-healing, angiogenesis-inducing capabilities, biodegradability, bio-adhesive biocompatibility as well as anti-inflammatory properties.

I.4.2. Advantages and Disadvantages of Natural and Synthetic Biopolymers

Biopolymers, whether natural or synthetic, offer numerous advantages that make them attractive for a wide range of applications, particularly in biomedical, environmental, and industrial fields. Natural biopolymers such as chitosan, alginate, gelatin, and cellulose are biodegradable, biocompatible, and derived from renewable sources, which makes them environmentally friendly and suitable for medical uses. Additionally, their chemical structures allow for easy functional modification to enhance their properties. However, they often suffer from poor mechanical strength, variability in quality due to natural origin, and limited processability, which restrict their use in applications requiring high stability and uniformity [41].

On the other hand, synthetic biopolymers such as polylactic acid (PLA), polycaprolactone (PCL), and polyethylene glycol (PEG) offer superior mechanical properties, tunable degradation rates, and consistent quality. Their production can be precisely controlled, enabling the development of customized materials for specific applications such as tissue engineering or drug delivery. Nevertheless, their main drawbacks include higher production costs and, in some cases, lower biodegradability compared to their natural counterparts. Overall, the choice between natural and synthetic biopolymers depends on the intended application, required material properties, and environmental considerations.

Table I.2. Principal advantages and disadvantages of biopolymers [42- 47]

Biopolymers	Advantages	Disadvantages
Natural Biopolymers	<ul style="list-style-type: none"> ○ Biocompatible and biodegradable, ○ Low toxicity, ○ Derived from renewable sources, ○ Often possess intrinsic biological activity (e.g., antimicrobial, mucoadhesive....) 	<ul style="list-style-type: none"> ○ Low thermal stability ○ Structural batch-to-batch variability ○ Limited mechanical strength ○ Sensitive to environmental conditions (pH, temperature, humidity)
Synthetic Biopolymers	<ul style="list-style-type: none"> ○ Biocompatibility, ○ Higher reproducibility, ○ Better mechanical, and chemical stability 	<ul style="list-style-type: none"> ○ Toxic, ○ Non-biodegradable, ○ Expensive synthesis procedure.

I.5. Biopolymers and their medical applications

Biopolymers are an important type of biomaterials that have many important applications in different fields of modern technology due to their distinctive properties and structures. One of these fields is the medical field, where biopolymers play a significant role due to their suitability for using in this field.

Biopolymers are a class of polymers that are derived from natural sources and are biodegradable, making them highly valuable for various biomedical applications [48]. Their biocompatibility, non-toxicity, versatility, and eco friendliness have led to a wide range of uses in the field of biomedicine [49].

Biomedical polymers have been extensively developed for promising applications in a lot of biomedical fields, such as drug delivery, disease detection/diagnosis, biosensing, regenerative medicine, and disease treatment. For example, polymer based carriers provide major advances in improving bioavailability and therapeutic outcomes at spatiotemporal drug delivery, greatly benefiting the treatment of diseases such as cancers, organ grafting, and infections.

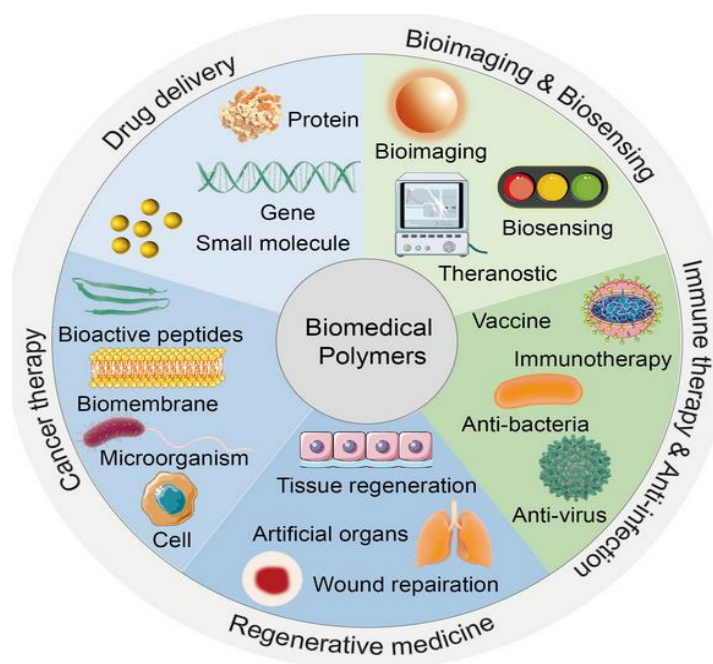


Figure I.6. Schematic illustration of smart polymers for biomedical applications [50]

Among the various biomedical applications of biopolymers, this study focuses specifically on their use in drug delivery systems, due to their ability to enhance therapeutic efficacy while minimizing side effects.

I.5.1. Application of biopolymers in drug delivery

The distinctive characteristics of biopolymer, such as biocompatibility, biodegradability, and non-toxicity, render them highly suitable for biomedical applications. Due to their potential to overcome various challenges in drug delivery, tissue engineering, wound healing, and medical implants, biopolymers have garnered considerable interest in the healthcare sector [51-53].

Polysaccharides have been used in biomedical sectors throughout the last century [54]. Polysaccharides have been evaluated as prospective candidates in various biomedical applications due to a variety of features such as biodegradability, biocompatibility, non-immunogenicity, and increased solubility and stability [55]. Furthermore, due to the abundance of polysaccharide sources and their inexpensive cost, they have been popular in a variety of biological and biotechnological applications [56]. Polysaccharides derived from algae, for example, have been widely used in biomedical applications such as wound treatment, regenerative medicine, and drug delivery control [57].

I.5.2. Drug delivery system

Drug delivery refers to the method or process by which pharmaceutical compounds are administered to achieve therapeutic effects in humans or animals. This field encompasses extensive research into the development of novel materials and carrier systems designed for efficient drug transport [58]. A primary focus within drug delivery research is the engineering and evaluation of delivery platforms composed of polymers, lipids, and ceramics, which encapsulate therapeutic agents. These include particle-based systems such as nanoparticles, micelles, liposomes, and dendrimers, which enhance drug targeting, as well as depot systems like microparticles, microrods, films, and meshes, which allow for localized drug release at a controlled rate over a defined period [59].

Such carrier systems exhibit high biocompatibility, low cytotoxicity, non-antigenicity, and tunable release mechanisms, making them valuable for applications in oncology, cartilage regeneration, and vascular grafts. Additionally, their ability to undergo emulsification, gel formation, foaming, and moisture absorption further enhances their efficacy in drug delivery [60, 61]. The unique mechanical properties, crosslinking capabilities, and biodegradable nature of polymeric materials contribute to their effectiveness as controlled drug delivery systems [62].

Controlled drug delivery aims to maintain a stable concentration of the therapeutic agent at the target site, often within the bloodstream, ensuring optimal therapeutic outcomes while minimizing dose-related toxicity and adverse effects. The most critical features of biomaterial-based drug delivery systems include controlled degradation and sustained release of the encapsulated drug [63]. This release is often governed by environmental triggers such as temperature, pH, or ion concentration [64]. For instance, the extracellular pH in normal tissues is approximately 7.4, whereas cancerous tissues typically exhibit a pH of around 6.5, allowing for the development of pH-sensitive polymeric carriers for targeted cancer therapy [65, 66].

Targeted drug delivery systems require precise activation of cellular sites for controlled drug release. Consequently, an optimized drug delivery approach must consider precursor synthesis, functionalization, composite fabrication, and drug encapsulation strategies to achieve the desired release kinetics [67, 68]. Parameters such as particle size, morphology, surface properties, bioavailability, and biodegradability must be carefully tailored to ensure site-specific drug delivery with controlled dosing [69, 70]. Biomimetic

polymeric nanoparticles of varying sizes have been synthesized to achieve efficient therapeutic agent loading, enabling molecular imaging of inflamed tissues while mitigating inflammation and immune responses [71, 72].

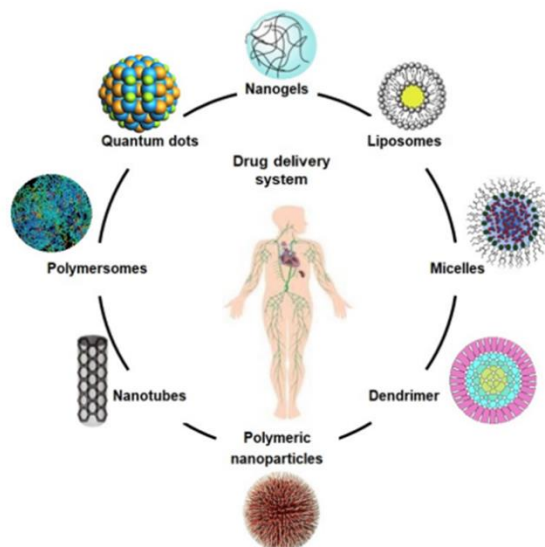


Figure I.7. The schematic diagram illustrates different drug carriers for the drug delivery system [73]

I.5.3. Polysaccharides-based DDSs

Polysaccharide based biopolymers have attracted significant interest in the development of drug delivery systems due to their numerous advantages as carriers for therapeutic agents. Compared to other biopolymers, polysaccharides offer several benefits: they are biodegradable, non-toxic, abundant, and biocompatible. Their degradation results in simple molecules such as sugars, which are easily metabolized by living organisms, making them an environmentally friendly option.

As the most abundant biopolymers on Earth, polysaccharides are naturally found in plants, animals, and microorganisms, where they serve key biological functions. Their wide availability and safe profile have led to their extensive use as food additives, thickeners, and stabilizers. Furthermore, polysaccharides possess a variety of functional groups (e.g., hydroxyl, carboxyl, and amino groups), allowing for mucoadhesive properties and non-covalent interactions with biological tissues. These chemical characteristics support their use in targeted and controlled drug delivery applications. Due to their renewability and sustainability, polysaccharides represent a green alternative to synthetic polymers derived from non-renewable sources, which can have negative environmental impacts.

In the context of therapeutic delivery, drug administration aims to maintain an optimal plasma concentration and direct the drug toward its specific site of action. Nanotechnology has emerged as a powerful tool in this field, enabling the development of nano- and micro-structured delivery systems that encapsulate active pharmaceutical ingredients. When embedded in a polysaccharide-based matrix, these drugs benefit from enhanced stability, protection from degradation, improved bioavailability, and targeted release. Among the most commonly used polysaccharides in biomedical applications are alginate, chitosan, and carrageenan, which are known for their versatility and therapeutic efficacy [74].

I.5.3.1. Chitosan Use for Drug Delivery:

Chitosan has gained increasing attention in the biomedical field, particularly in drug delivery applications, due to its unique structural and functional properties. It is the second most abundant natural biopolymer and is derived from the deacetylation of chitin—commonly extracted from the exoskeletons of crustaceans (e.g., shrimp and crabs), insects, and fungal cell walls [75]. Structurally, chitosan is a linear polysaccharide composed of $\beta(1\rightarrow4)$ -linked units of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) [76]. Thanks to its reactive amino ($-\text{NH}_3^+$) and hydroxyl ($-\text{OH}$) groups, chitosan exhibits excellent mucoadhesive, biodegradable, and biocompatible properties. It is readily soluble in acidic aqueous media and demonstrates pH-sensitive behavior, which is advantageous for the controlled and site-specific release of encapsulated drugs. Its cationic nature allows for effective interaction with negatively charged molecules and biological membranes, enhancing permeability, cellular uptake, and drug absorption.

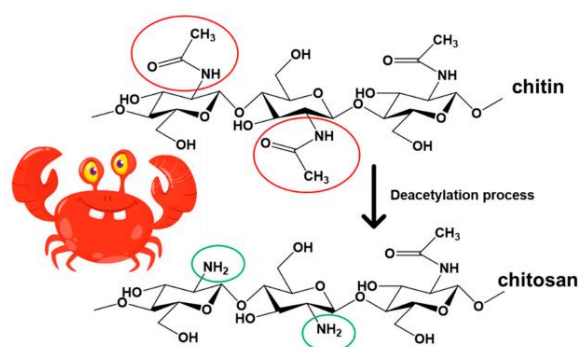


Figure I.8. Molecular structure of chitin and chitosan, the circles denote the amino groups of Chitosan [77]

As a result, chitosan has been widely employed in the development of nanocarriers, hydrogels, scaffolds, and membrane systems for pharmaceutical, dental, and tissue engineering applications, confirming its role as a key biopolymer in modern drug delivery technologies [78, 79].

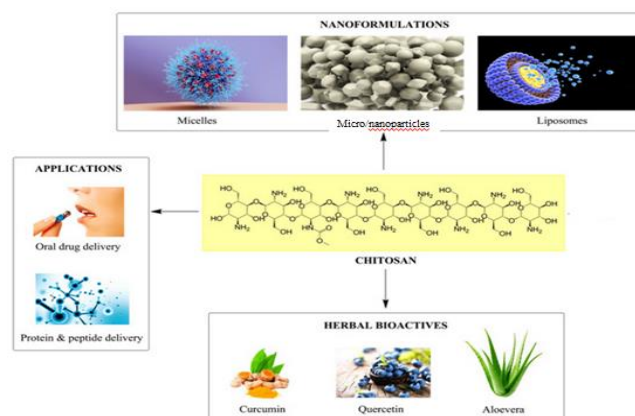


Figure I.9. Chitosan-based drug delivery systems and their biomedical applications

I.5.3.2. Pectin Use for Drug Delivery:

Pectin is a naturally occurring heteropolysaccharide primarily located in the primary cell walls of terrestrial plants, where it plays a crucial structural role. Commercial pectin is mainly extracted from citrus peels and apple pomace by products of the fruit juice industry making it a sustainable and cost-effective biopolymer [80]. Its hydrophilic nature, along with abundant carboxyl and hydroxyl groups, gives pectin notable biocompatibility, biodegradability, and low toxicity, making it particularly attractive for biomedical use. These properties also allow for easy chemical modifications and crosslinking, facilitating its application in controlled-release drug delivery systems.

Recent studies have highlighted the potential of pectin-based carriers for colon-targeted drug delivery, due to their ability to remain stable in the upper gastrointestinal tract and degrade specifically in the colon. This is especially relevant given findings by Saurabh et al. (2020), who reported that drug release from pectin matrices is significantly enhanced under colonic conditions, thus improving local drug concentration at the disease site. Furthermore, Liu et al. (2018) demonstrated that pectin-based nanoparticles enhance the bioavailability of anti-inflammatory and anticancer drugs by protecting them from premature degradation [81,82]. Given these advantages, pectin has become a promising material for the formulation

of oral, colon-targeted, and mucoadhesive drug delivery systems, often combined with other polymers or encapsulated into micro/nanoparticles for enhanced performance.

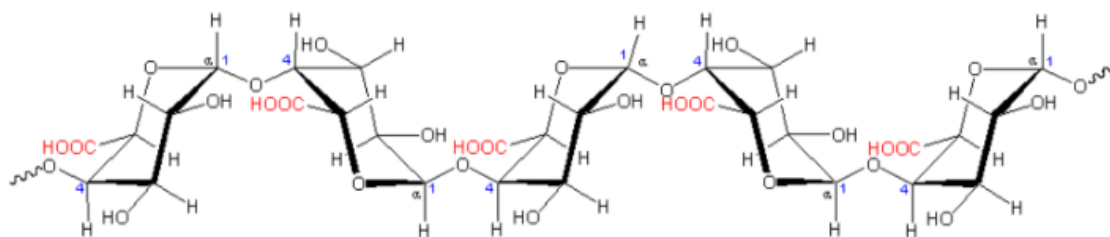


Figure I.10. Chemical structure of pectin.

Biopolymers, owing to their biocompatibility, biodegradability, and renewable nature, have emerged as essential materials in the development of environmentally friendly and biologically safe applications. Their diverse structures and properties enable their use in various biomedical fields, particularly in drug delivery, tissue engineering, and regenerative medicine. Polysaccharides, proteins, and synthetic biodegradable polymers have shown remarkable potential in creating innovative therapeutic systems.

Building on this general understanding, the next chapter focuses on a specific and highly promising application: the use of polymeric microparticles as advanced drug delivery systems.

Chapter II

In recent years, the design of advanced drug delivery systems (DDSs) has become a central objective in biomedical research, driven by the need for improved therapeutic efficacy, precision targeting, and reduced side effects. Among the various delivery platforms explored, polymeric microparticles have gained significant attention due to their versatility in transporting and releasing a broad range of bioactive compounds. These microscale carriers are particularly advantageous for the delivery of anti-inflammatory drugs, which often face challenges such as poor solubility, limited stability, and systemic side effects.

Following the previous chapter on biopolymers and their biomedical potential, this chapter focuses on one of their most impactful applications: the development of polymeric microparticles for drug delivery. It outlines their classification, key physicochemical properties, and commonly employed preparation methods. Particular emphasis is placed on drug encapsulation strategies, especially the use of polymeric microparticles for the targeted delivery of anti-inflammatory agents.

II.1. Definition of Microparticles

II.1.1 General definition

The term "microparticle" refers to spherical structures with diameters typically ranging from 1 μm to 1000 μm . Polymeric microparticles are composed of a polymeric matrix that facilitates the immobilization of a minor fraction of an active compound [83]. These microparticles can be synthesized using a variety of materials, including natural and synthetic polymers, ceramics, and glass, many of which are commercially available [84]. They serve as carriers for drugs or other active agents in controlled drug delivery systems.

II.2. Types of microparticles

Polymeric microparticles are classified as either microspheres (matrix systems) or microcapsules (core shell systems) based on their internal structure.

II.2.1. Microspheres:

These are homogeneous matrix systems in which the drug is either dissolved or uniformly dispersed throughout the polymer. Microspheres allow for a gradual release of the active compound as the polymer degrades or diffuses. As drug delivery carriers, microspheres represent a promising strategy for achieving sustained and controlled drug release. Various

fabrication techniques provide a wide range of possibilities for optimizing drug administration. This approach enables the precise delivery of small amounts of potent drugs, minimizes drug exposure at non-target sites, and ensures the protection of labile compounds both before and after administration, as well as during transit to the intended site of action [85].

II.2.2. Microcapsules:

These have a core shell structure, where the active principle is confined to a central core surrounded by a distinct polymeric membrane. This configuration enables more precise control over the release rate, often through diffusion or rupture of the outer shell. The core may exist in solid, liquid, or gaseous form, while the surrounding shell consists of a continuous polymeric layer that can be either porous or non-porous [86].

Microspheres consist of a polymeric matrix in which the active compound is uniformly distributed, either in a dissolved or suspended state. In contrast, microcapsules exhibit a heterogeneous structure, where the active agent is encapsulated within a distinct membranous shell. Both the matrix and shell systems serve to safeguard the encapsulated drug from environmental influences such as humidity, temperature fluctuations, pH variations, oxygen exposure, and enzymatic degradation [87].

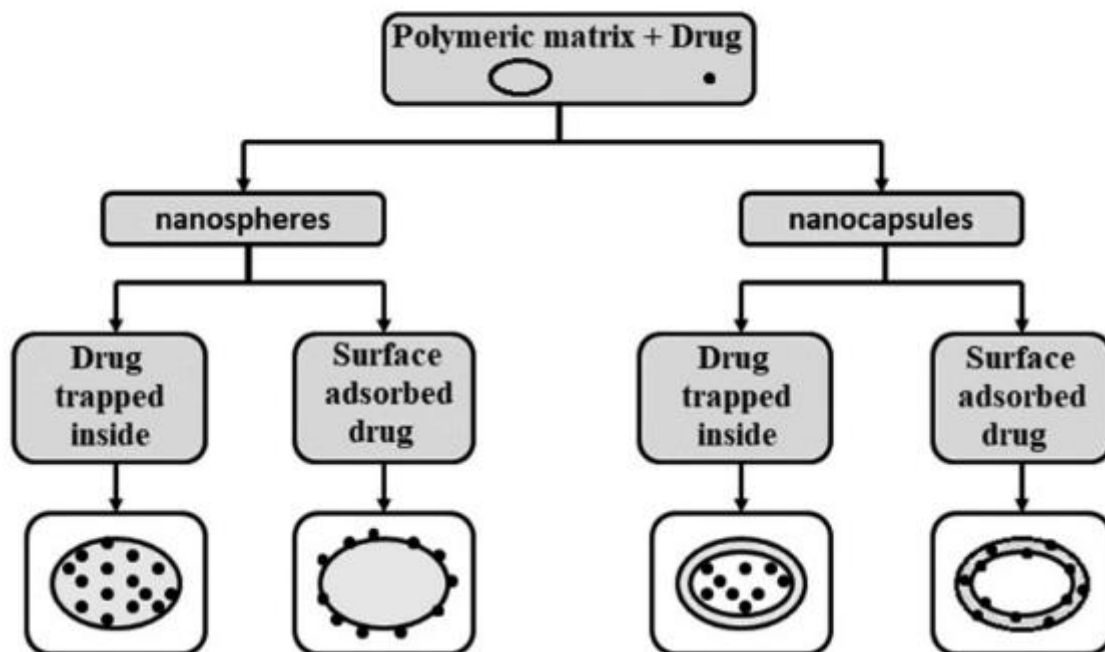


Figure II.1. Schematic representation of polymeric nanoparticles according to the structural organization [88].

II.3. Properties of microparticles

➤ **Size:** The size plays a role in the gastrointestinal performance: microparticles under 800 μm get through the pylorus without the influence of gastric emptying, thus eliminating the interpersonal and intrapersonal (nutrition-based) differences [89].

➤ **Surface Charge:** Surface charge plays a crucial role in particle stability and aggregation. Excessive aggregation can impair drug delivery efficiency by reducing content uniformity and potentially obstructing blood flow or target site accessibility [90].

➤ **Porosity:** Porosity affects the interaction of microparticles with surrounding tissues. Porous materials facilitate vascularization relative to non-porous biomaterials. Pores in the range of 30–40 μm can promote macrophage polarization, contributing to improved tissue repair and integration [91].

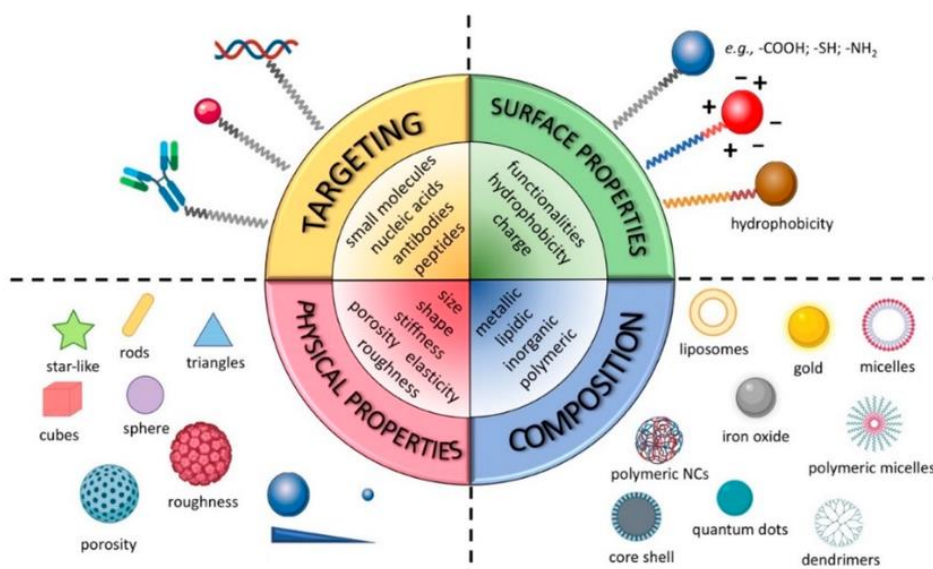


Figure II.2. Nanoparticle Properties for Drug Delivery [92].

II.4. Technique used for microparticle preparation

The preparation of microparticles is a rapidly advancing field with significant potential for various applications, particularly in drug delivery systems, materials science, and biotechnology. Several techniques are employed for the fabrication of microparticles, among which spray drying, coacervation (phase separation), emulsification with solvent evaporation, and microfluidic methods are the most commonly used. These approaches enable the encapsulation of active agents within polymer matrices, allowing for controlled release, protection, and targeted delivery

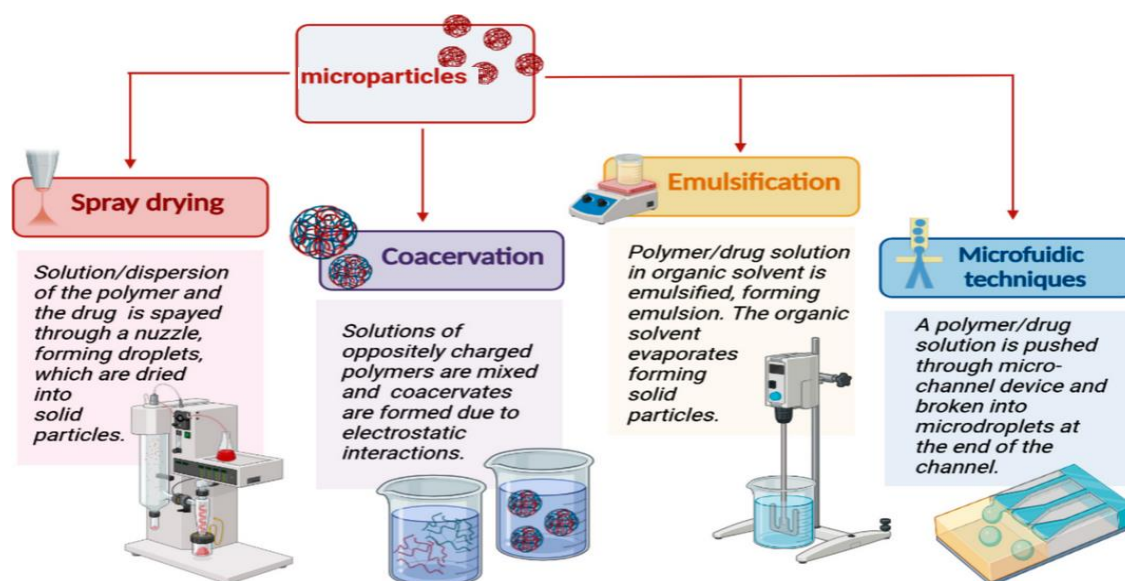


Figure II.3: Schematic Representation of Common Techniques for Microparticle Preparation [93].

Among the various microparticle preparation techniques, ionotropic gelation was selected in this project due to its simplicity, biocompatibility, and suitability for encapsulating sensitive bioactive compound.

II.4.1. Ionotropic Gelation

Ionotropic gelation is a widely used method for the preparation of microparticles, particularly when using natural polyelectrolytes such as polysaccharides. This technique relies on the ability of polymers bearing ionic groups to crosslink with oppositely charged counterions (e.g., calcium or sodium ions), resulting in the formation of solid microparticles through a sol–gel transition. The process typically involves dropping a polymer/drug solution into a solution containing multivalent ions, leading to the instantaneous formation of gelled microparticles. Key factors influencing particle size and morphology include polymer concentration, molecular weight, viscosity of the initial solution, surface tension, and the fluid dynamics during droplet formation (laminar or turbulent flow).

II.5. Mechanisms of Ionotropic Gelation

In general, ionotropic gelation occurs between oppositely charged molecules. Positively charged polymer chains react with negatively charged divalent or multivalent ions. The electrostatic reaction leads to forming of the microstructured particles with

interconnected nano-fibrillar networks. Such a structure can be achieved using three separate methods: internal, external, or inverse gelation. [94].

The process can be classified into three main approaches: internal, external, and inverse gelation

II.5.1. External gelation method:

The most frequently used method of ionotropic gelation is the external method. It is also known as a controlled diffusion method. The solution of a polysaccharide is instilled dropwise to the crosslinking solution. The bead matrix is obtained by the diffusion of crosslinking agents from the outer continuous phase into the inner structure of the polymer droplets. In the external layer of the formed hydrogel bead, the sol–gel transition process is fast, and gel formation is immediate. In the next steps, the counter-ions begin to penetrate through the next layers into the middle of the particle, creating a heterogeneous gelation profile in which the interaction between the ions and the polymer functional groups is maximum at the surface and zero at the core [95, 96].

II.5.2. Internal gelation method:

Internal gelling is also called in situ gelling. This approach is also widely described as a method for the preparation of polymer particles. In this case, the insoluble calcium salt (e.g., CaCO_3 and CaSO_4) is mixed with the polymer solution, and the obtained mixture is then extruded into an acid crosslinking bath. The altered conditions increase the solubility of the calcium salt, allowing it to be released, leading to the formation of a gel network of the polysaccharide. This allows the gelling mechanism to be controlled and the polymer to be uniformly exposed to cations, thereby creating a uniform gel network. The downside of this method is that the obtained matrices are characterized by lower density and larger pore sizes; therefore, they are more permeable than those obtained by external gelling, which results in lower entrapment efficiency and higher release rates. This is due to the increased permeability of the matrix, which is influenced by competition between Ca^{2+} and H^+ ions due to the added acid. It appears that although the acid in the gelling bath improves the solubility of calcium salts, it also competes with Ca^{2+} for interaction with the polymer. This problem can be solved by changing the pH of the gelling medium and the amount of calcium ions donors used [97].

II.5.3. Reverse gelation: Another approach is reverse gelation, which is based on the dropwise addition of a medium containing gelling agents to the polymer solution. Such a

method can be typically used in the case of emulsions for the preparation of polymer-based soft-shell microcapsules with oil content. This method uses small amounts of biopolymer, resulting in forming of a soft molecular shell.

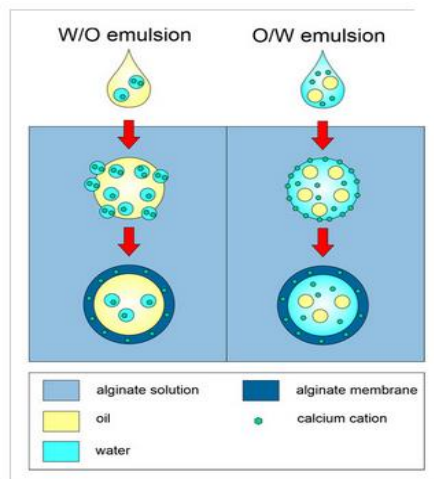


Figure II.4. Reverse gelation scheme depending on emulsion type used.

II.6. Encapsulation

Encapsulation is a process by which a core material whether in liquid, solid, or gaseous form is enclosed within a protective layer to enhance its stability against chemical degradation and environmental stress [98]. Inspired by the structure of natural cells, this technique was designed to preserve sensitive functional compounds under adverse conditions. The internal substance is commonly referred to as the core material, active agent, or internal phase, while the surrounding protective layer is known as the shell, carrier, encapsulant, or external phase [99].

Microencapsulation specifically involves enclosing bioactive compounds within a micro-scale shell to protect them from degradation during processing and storage [100].

In this approach, the core particles are coated with a suitable wall material that acts as a physical barrier, isolating them from ambient environmental factors. The choice of solvent and encapsulating material depends largely on the solubility characteristics of the core and shell substances. Moreover, the structural and chemical properties of the encapsulating matrix significantly influence the release profile of the active ingredients under defined conditions. In simple terms, microencapsulation allows for the controlled delivery and stabilization of sensitive compounds through their entrapment in a secondary protective layer.

Typically, the microencapsulation process involves four key stages: preparation of the core and shell materials, incorporation of the core into the encapsulant, and solidification. To improve solubility and reduce particle size, the core material may undergo milling, grinding, or melting. In the case of soluble substances, emulsifiers or surfactants are often added to enhance dispersion stability, while porous carriers are employed for encapsulating gases [101]. However, exposure to high temperatures during solidification can lead to degradation, diffusion, or loss of heat-sensitive actives. To mitigate this, techniques such as freeze drying, coacervation, spray chilling, or cross-linking are often employed [102, 103].

II.6.1. Historical

This technique has been employed in a diverse range of fields from chemicals and pharmaceuticals to cosmetics and printing. For this reason, widespread interest has developed in microencapsulation technology. Preparation of microcapsules dates back to 1950s when Green and Schleicher 2, 3 produced microencapsulated dyes by complex coacervation of gelatin and gum arabic, for the manufacture of carbonless copying paper. To this day, carbonless copy paper is one of the most significant products to utilize microencapsulation technology, and is still produced commercially. The technologies developed for carbonless copy paper have led to the development of various microcapsule products in later years.

In the 1960s, microencapsulation of cholesteric liquid crystal by complex coacervation of gelatin and acacia was reported to produce a thermosensitive display material. J. L. Ferguson developed nematic curvilinear aligned phase (NCAP), a liquid crystal display system by microencapsulation of nematic liquid crystal⁴.

Encapsulation technology has provided the enlargement of display areas and wider viewing angles [104].

II.6.2. Objective of encapsulation

- **Immobilization or entrapment:** involves restricting interactions between specific components of a system. When an ingredient requires separation, encapsulating it within microcapsules ensures controlled release upon rupture. This technique can enhance flavor persistence or regulate its release at precise moments, like during cooking. Additionally, immobilizing batteries or enzymes facilitates continuous processing while preventing unwanted dispersion.

- **Protection:** Encapsulation provides a protective barrier for fragile ingredients, shielding them from environmental factors such as oxidation, mechanical stress, and chemical degradation. For instance, vitamins and polyunsaturated fatty acids are highly susceptible to oxygen exposure, while biological cells can be damaged by shear forces. Similarly, certain drugs and probiotics face destruction during gastric transit. By incorporating these elements into microcapsules, their stability and functionality are preserved to a significant extent.
- **Controlled release:** ensures that an active ingredient is released in a precise manner for practical applications. For example, a drug must be delivered with specific kinetics to achieve its intended effect. In some cases, encapsulation does not release the main ingredient directly but instead facilitates the release of a by-product, such as when enzymes or catalysts are encapsulated.

Encapsulation can serve different purposes sometimes it is used to delay release, while in other situations, it accelerates availability. A common example is an instant powder, where fine insoluble particles are embedded within a highly soluble matrix, allowing rapid dissolution when mixed with liquid.

Structuration: through microencapsulation enables the transformation of a small liquid volume into a powder, simplifying the process of homogeneous mixing with a larger volume of dry material. This technique is particularly useful in pharmaceutical formulations, where precise dosage forms are required. Additionally, microencapsulation can enhance the flow properties of certain powders. For example, coating brown sugar a naturally clumpy substance with crystalline sugar results in a free-flowing powder, improving its handling and usability [105].

Understanding the physicochemical properties of drugs is essential before determining the appropriate method for the synthesis of the MPs because the wide range of pharmaceutical agents such as peptide, proteins, nucleic acids, antibiotics, and chemotherapeutics, have distinctive solubility and stability at different conditions (i.e., temperature, pH, and organic solvents) [106]. On the other hand, the fundamental properties of the polymers for the development of p-MPs involve their solubility and stability, their biodegradability and biocompatibility, and their physical (i.e., crystallinity and glass transition temperature) and mechanical properties (i.e., strength, elongation, and Young's modulus).

II.7. Microparticle based Drug Delivery Systems

The physicochemical properties of microparticles such as particle size, surface charge, porosity, and composition are critical parameters influencing their in vivo behavior and delivery performance. Microparticles offer numerous advantages in drug delivery, including protection of the active compound from environmental degradation, masking of undesirable taste, preservation of volatile substances, reduction of drug-related side effects, and enhancement of targeted drug delivery [107]. As a drug delivery system, microparticles can improve treatment efficacy due to their well-defined pharmacokinetic and physiological benefits, such as safeguarding bioactive agents, enabling controlled release, and facilitating precise drug transport to the target site [103]. Encapsulation technologies have led to the successful development of various pharmaceutical formulations.

❖ Controlled Drug Delivery:

Microparticles allow for the administration of drugs at a predetermined rate over an extended period. This controlled release improves drug stability, reduces dosing frequency, and enhances patient compliance.

❖ Sustained Drug Delivery:

Polymeric coatings on drug particles help maintain therapeutic drug levels in the bloodstream over time, minimizing fluctuations and reducing the risk of toxicity.

❖ Targeted Drug Delivery:

- Tissue-targeted delivery focuses drugs on specific organs or body cavities (e.g., anticancer drugs like doxorubicin and 5-FU).
- Intracellular delivery involves gene therapy, antisense oligonucleotides, and vaccine adjuvants for precise action at the cellular or organelle level.

❖ Cancer Therapy:

Microparticle systems offer site-specific delivery of chemotherapeutic agents, reducing systemic toxicity and increasing efficacy. For instance, 5-FU-loaded biodegradable microparticles have been used for the localized treatment of brain tumors.

❖ **Vaccine Delivery:**

Biodegradable microparticles improve antigen stability, enhance antigen presentation, and facilitate sustained immune responses, making them ideal for safe and effective vaccine formulations.

❖ **Oral Drug Delivery:**

Microparticles enhance the solubility and absorption of poorly water-soluble drugs and provide controlled or sustained release in the gastrointestinal tract, improving therapeutic outcomes.

❖ **Ocular Drug Delivery:**

Microspheres increase ocular bioavailability and prolong drug retention on the eye surface. Formulations using polymers such as Eudragit demonstrate slower drug release compared to unencapsulated drugs.

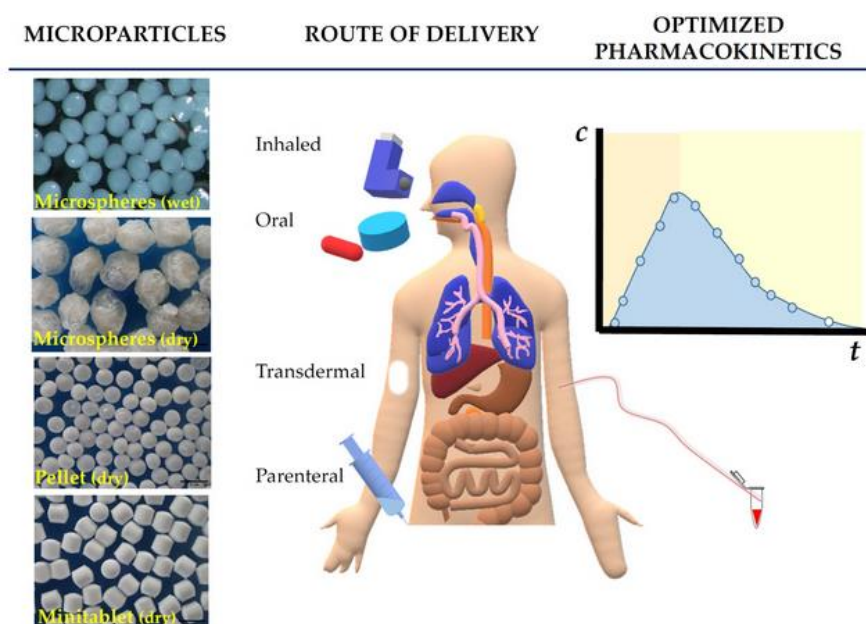


Figure II.5. Microparticles and Routes of Drug Delivery [89].

II.8. Mechanism of Drug Release from Microparticulate System

The basic object of drug release from any targeting drug delivery system is to achieve the drug concentration at therapeutic level at the site of action of which must be in therapeutic window [108]. Drug release from system is depend upon different factors that effect on the

drug release from the carrier that comprise composition of the drug delivery system, their ratio, physicochemical interaction among the components and their method of preparation [109]. Drug release from the drug delivery system is categorized into four categories on the basis of mechanism of drug escape from the carrier.

II.8.1. Diffusion Controlled Drug Delivery System

For the targeted drug delivery of drug, diffusion plays an important role for the release of drug in a controlled manner. It is a dominant mechanism of drug release pattern from the system as compared to other mechanism of drug release [110]. Diffusion controlled system releases the drug from a reservoir by infiltration from inner to outer medium on the basis of concentration gradient across the membrane. Diffusion of drug from system is the rate limiting step. Drug Diffusion from reservoir are typically either reservoir diffusion or matrix-based systems. By following the reservoir system, first drug dissolves in the core of the system and then diffuses out from the polymeric shell. In the matrix system, drug is homogeneously dispersed in the polymer matrix without any outer membrane barrier: therefore initially drug is released rapidly, followed by slow release as a function of time and distance for drug molecules present in the inner carrier of the system [111].

II.8.2. Osmosis

Osmosis is a fundamental physicochemical phenomenon widely utilized in drug delivery systems as a controlled release mechanism. It involves the movement of solvent molecules across a semipermeable membrane from a region of low solute concentration to a region of high solute concentration. This osmotic flow aims to balance the concentration of impermeable solutes on both sides of the membrane, generating a hydrostatic pressure difference. This pressure drives the solvent and with it, the dissolved drug through the membrane in a regulated manner. Osmotic pumps and osmotically controlled drug delivery devices take advantage of this mechanism to achieve sustained and predictable drug release profiles, independent of gastrointestinal pH or motility [112, 113]. This makes osmosis-based systems particularly valuable for delivering drugs that require consistent plasma concentrations over extended periods.

II.8.3. Dissolution

The dissolution process involves the detachment of the active pharmaceutical ingredient (API) from the surface of its solid form, followed by its diffusion into the

surrounding liquid medium. This mechanism is central to drug release, particularly for solid dosage forms, and can be strategically exploited to achieve a controlled and predictable release profile. In dissolution-controlled systems, both matrix based and membrane based formulations can be engineered to modulate the drug's release rate. When water soluble polymers are employed in these systems, the dissolution of the matrix itself often becomes the rate limiting step, thereby governing the overall kinetics of drug release. Such systems are especially useful for drugs with low aqueous solubility or requiring sustained therapeutic levels over time.

II.8.4. Degradation and Erosion

Polymer degradation and erosion represent another important mechanism for controlled drug release, especially in systems based on biodegradable polymers. Polymers such as polyesters, polyamides, and polysaccharides undergo degradation through enzymatic or hydrolytic cleavage of labile bonds, including amide, ester, and hydrazone linkages [114]. The degradation rate directly influences drug release kinetics and is affected by various factors such as polymer molecular weight, crystallinity, end-group functionality, and monomer composition. These polymers are particularly advantageous because they break down into biocompatible and non-toxic byproducts that can be easily metabolized or excreted from the body, eliminating the need for surgical removal and reducing long-term toxicity risks.

Together, these mechanisms illustrate the versatility of modern drug delivery strategies, enabling the development of systems tailored to specific therapeutic needs and patient conditions.

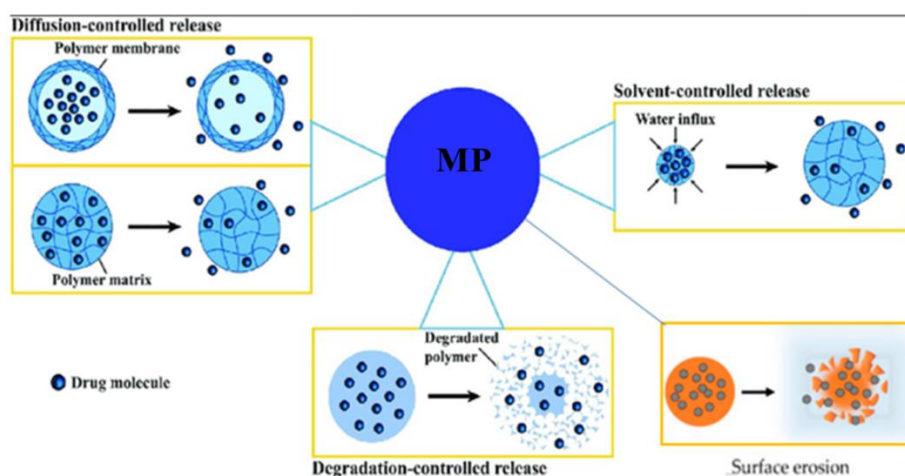


Figure II.6: Mechanisms of Drug Release from Microparticulate Delivery Systems

II.9. Chitosan Microparticles for Anti-Inflammatory Drug Delivery

Chitosan microparticles are widely investigated as carriers for the delivery of anti-inflammatory drugs, particularly non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac. Thanks to its natural origin, biocompatibility, biodegradability, and mucoadhesive properties, chitosan offers several advantages for controlled drug delivery [115]. When used as an encapsulation matrix, chitosan can enhance the bioavailability of poorly soluble drugs, prolong their residence time at the site of absorption, and reduce drug-related side effects. Diclofenac, a widely used NSAID, is associated with gastrointestinal irritation and a short plasma half-life. Encapsulation of diclofenac into chitosan microparticles allows for sustained and targeted release, particularly in the intestinal tract, minimizing systemic exposure and improving therapeutic efficacy [116]. Common techniques employed for the preparation of diclofenac-loaded chitosan microparticles include ionotropic gelation, emulsion cross-linking, and spray drying. These systems provide not only protection of the drug from premature degradation but also modulation of its release kinetics, making chitosan an excellent platform for anti-inflammatory drug delivery.

Table II.1. Current work on: encapsulation of anti-inflammatory drug in chitosan microparticle

Polymer	Drug	Method	Reference
Chitosan	Meloxicam	Ionic gelation	[117]
Chitosan	Diclofenac sodium	Thin-film hydration	[118]
Chitosan	Flurbiprofen	Spray-drying	[119]
Chitosan Montmorillonite	Ibuprofen	Emulsification	[120]
Chitosan	Ibuprofen	Ionic gelation	[121]

II.10. Encapsulation of essential oils

This technique is characterized by the formation of a functional barrier between the core material, which contains the oil, and the surrounding membrane. This barrier serves to prevent chemical and physical interactions, thereby preserving the biological, functional, and physicochemical properties of the core material [122].

Due to their small size and high surface-to-volume ratio, micro- and nanoparticles enhance the bioavailability of essential oils (EOs) and facilitate their diffusion to target sites, thereby ensuring prolonged and efficient activity. Moreover, encapsulation requires lower concentrations of EOs, which helps mitigate sensory impacts on food products, reduce the likelihood of microbial resistance, minimize potential toxic effects, and lower economic costs [123].

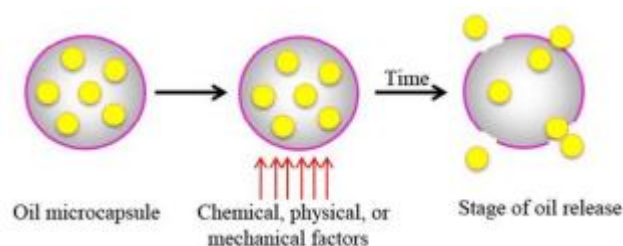


Figure II.7. Schematic diagram of controlled oil protection and release

In summary, polymeric microparticles represent a promising and versatile platform for advanced drug delivery systems. Their ability to encapsulate therapeutic agents and provide controlled, sustained, or targeted release enhances drug bioavailability, minimizes side effects, and improves patient compliance. Various applications, including cancer therapy, vaccine delivery, and oral and ocular drug administration, demonstrate the potential of these systems to overcome the limitations of conventional drug delivery methods. With continuous advances in polymer science and formulation techniques, microparticle-based drug delivery systems are paving the way toward more effective and personalized therapies.

The next chapters will focus on the experimental work conducted in this study, beginning with the materials and methods used for the development and characterization of polymeric microparticles.

Experimental Part

Chapter III

III.1. Introduction

Following the theoretical section, which provided a comprehensive overview of polymer based drug delivery systems and the properties of natural polysaccharides, this chapter, presents the experimental methodology adopted in this study. The research focuses on the formulation and characterization of polymeric microparticles designed for drug delivery applications, a topic of considerable interest in the pharmaceutical field.

The first part of the experimental work involves the preparation of chitosan/pectin microparticles incorporating diclofenac as a model anti-inflammatory drug. The microparticles were obtained using the ionotropic gelation technique, with sodium tripolyphosphate (TPP) serving as the ionic crosslinking agent. Several formulation parameters were optimized to evaluate their influence on the resulting microparticles. These particles were characterized in terms of their physicochemical properties, including Fourier-transform infrared spectroscopy (FTIR), particle size, swelling behavior at different pH values, and drug release profile.

In the second part of the study, essential oil of ginger was extracted and subsequently encapsulated within chitosan/ pectin microparticles, following a procedure similar to the one developed for diclofenac. The aim was to evaluate the potential of these biopolymer based systems for the encapsulation and delivery of natural bioactive compound.

This chapter details the materials used, the preparation techniques, and the experimental protocols implemented in both parts of the study.

III.2. Materials

To conduct this study, various chemicals, materials, and laboratory equipment were employed. The materials used are detailed below.

III.2.1. Equipment

All equipment and apparatus used throughout the formulation and evaluation procedures are presented in the following table.

Table III.1. Equipment used in the study

Equipments	Marque
Magnetic stirrer	IKA C-MAG HS10
Precision electronic balance (0.001 g)	Item PA213
Micropipettes	PI240683
Vortex mixer	V150
Centrifugeuse	5417R
pH meter	Ph 211
UV-Visible spectrophotometer	4251/50

III.2.2. Chemical reagents

All the chemical reagents used in this work are listed in the table.

Table III.2. List of chemical reagents used

Reagent	Formula	Molar Mass (g/mol)	Supplier
Trisodium citrate dihydrate	$C_6H_5Na_3O_7 \cdot 2H_2O$	294.10	Merck
Sodium phosphate dibasic	Na_2HPO_4	141.96	Merck
Sodium phosphate monobasic dihydrate	$NaH_2PO_4 \cdot 2H_2O$	156.01	Merck
Tween 80 (Polysorbate 80)	$C_{64}H_{124}O_{26}$	~1310.00	Sigma-Aldrich
Citric acid (monohydrate)	$C_6H_8O_7 \cdot H_2O$	210.15	Sigma-Aldrich
Acetic acid	CH_3COOH	60.05	Merck
Sodium hydroxide	$NaOH$	40.00	Sigma-Aldrich

III.2.3. Biopolymers and natural materials

In addition to conventional reagents, this study made use of biocompatible natural polymers and plant based materials due to their functional properties in drug delivery systems.

➤ Chitosan

Chitosan is a cationic polysaccharide obtained by the deacetylation of chitin, was selected as the primary polymer for microparticle formulation. It is widely recognized for its biodegradability, biocompatibility, and mucoadhesive properties, making it a valuable material in pharmaceutical applications. Its amino and hydroxyl functional groups enable chemical interactions and encapsulation of various bioactive agents. Although chitosan is insoluble in water, it dissolves in acidic solutions ($\text{pH} < 6.5$) due to protonation of amino groups [118]. In this study, low molecular weight chitosan (50,000–190,000 Da, degree of deacetylation 91%) from Merck was used without further modification. Its chemical structure is shown in Figure III.1.

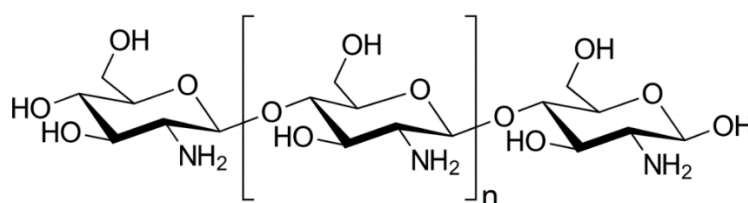


Figure III.1. Chemical structure of chitosan (CS).

➤ Pectin

Pectin is a natural anionic polysaccharide derived from plant cell walls, mainly composed of galacturonic acid units. Known for its gelling ability and compatibility with cationic polymers, it was used in combination with chitosan to form polyelectrolyte complexes. Apple-derived pectin with a degree of esterification of 60% (Sigma-Aldrich) was employed in this study. Its average molecular weight ranges from 60,000 to 130,000 Da. The structure of pectin is illustrated in Figure III.2.

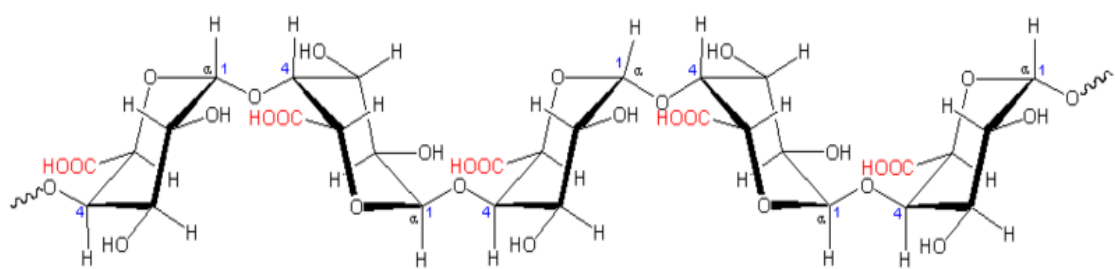


Figure III.2. Simplified chemical structure of pectin

➤ Tripolyphosphate (TPP)

($\text{Na}_5\text{P}_3\text{O}_{10}$, CAS No. 7758-29-4, $M = 367.86$ g/mol), supplied by Sigma-Aldrich, was used as an ionic crosslinker in the preparation of microparticles via ionotropic gelation. Its structure is shown in Figure III.3.

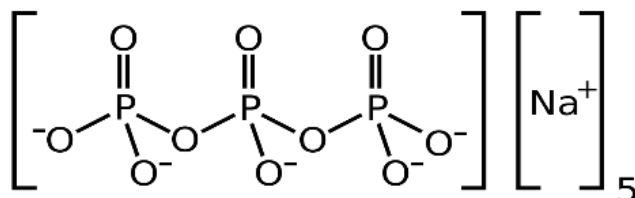


Figure III.3. Chemical structure of sodium tripolyphosphate (TPP)

III.2.4. Active Pharmaceutical Ingredient

Diclofenac sodium (2-[(2,6-dichlorophenyl)amino]benzene acetic acid) is a widely used nonsteroidal anti-inflammatory drug (NSAID) with high permeability but low solubility in water, placing it in Biopharmaceutics Classification System (BCS) Class II [119]. It was selected as a model drug for encapsulation due to its therapeutic relevance and narrow therapeutic window. The compound used in this study was supplied by Sigma-Aldrich.

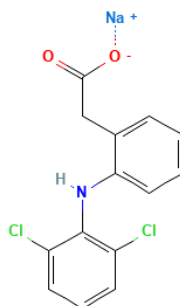


Figure III.4. Chemical structure of sodium Diclofenac

III.2.5. Plant Material

Ginger, *Zingiber officinale*, commonly known as ginger, is a herbaceous plant from the *Zingiberaceae* family [120]. It is well known for its anti-inflammatory, antioxidant, and antimicrobial properties, primarily attributed to its bioactive constituents such as gingerols and shogaols [121]. In this study, dried ginger rhizomes were used as a natural source of essential oil for encapsulation purposes. The plant material was locally purchased in wilaya de Chlef, Algeria, and used without any further processing.



Figure III.5. Dried ginger

III.3. Methods

III.3.1. Preparation Method of Microparticules

Microparticules based on Chitosan (CS), Pectin (PEC) were performed by ionic gelation method described by Calvo [122], with some essential modifications. The experiment begins with the preparation of polymers solutions.

III.3.1.1. Preparation of Solutions

The chitosan solution was prepared by dissolving of specific amount of Cs (depending on the desired concentration) (Table III.3) in 15ml of 1% acetic acid solution (1% w/w) at 60°C, under magnetic stirring. After complete dissolution the obtained solution was filtered and brought to room temperature.

Separately, a pectin solution was prepared by dissolving a defined amount of pectin (PEC) (at different concentrations) in a volume of distilled water under magnetic stirring at 50°C until a homogeneous solution was achieved.

In parallel, an aqueous solution of tripolyphosphate (TPP) was prepared by dissolving a precise quantity of TPP in distilled water to obtain a known concentration.

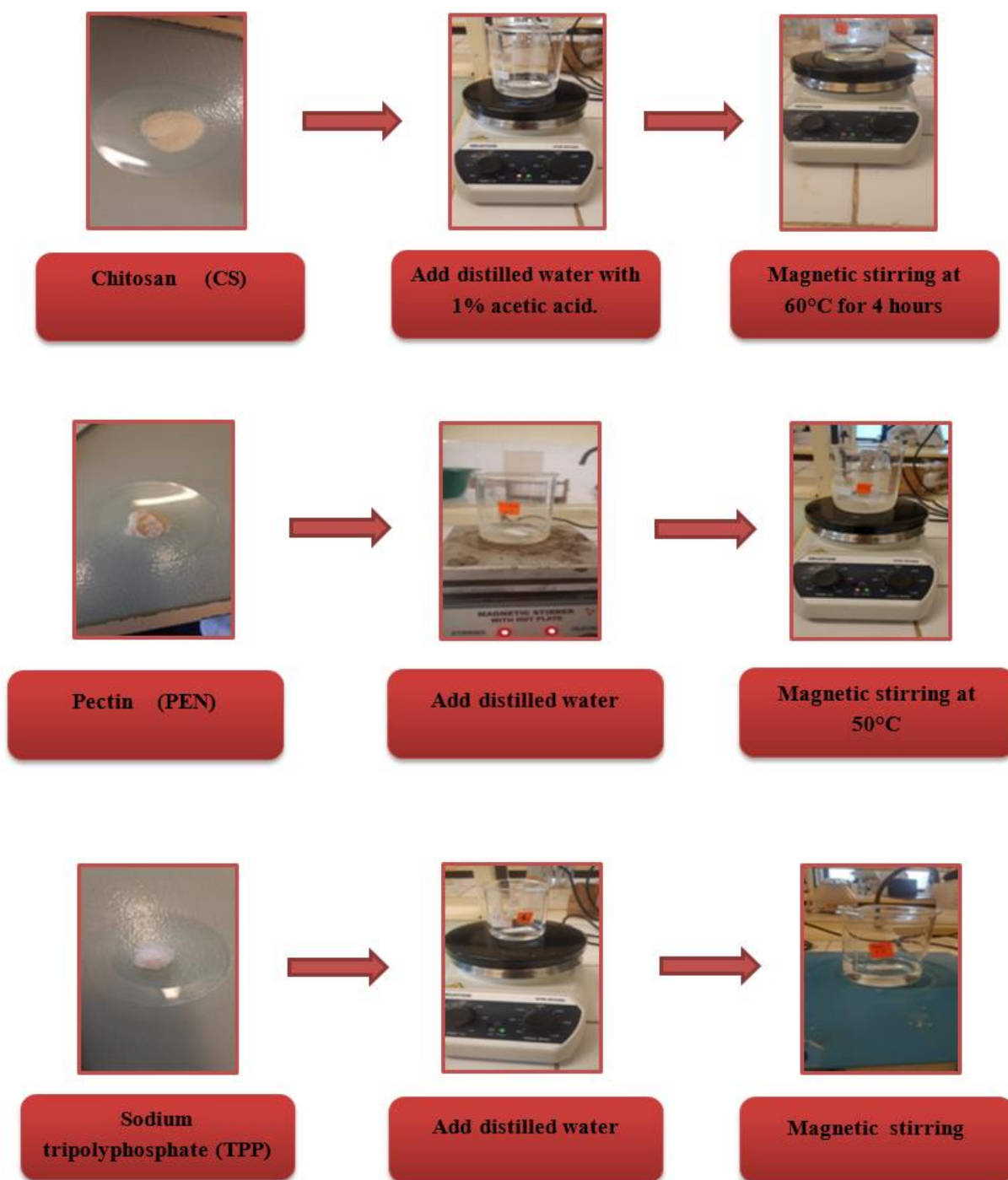


Figure III.6. Preparation of CS, PENC, and TPP solutions.

III.3.1.2. Preparation of CS/PEC Microparticles

Microparticles were prepared by ionotropic gelation. First, 10 mL of pectin solution was added dropwise into 10 mL of TPP solution under constant magnetic stirring using a syringe. Stirring was maintained for an additional 5 minutes to ensure complete mixing. Subsequently, a precise volume of chitosan solution was gently added dropwise into the

TPP/PEC mixture under gentle magnetic stirring, leading to the immediate formation of microspheres. After 2 hours at room temperature, the crosslinking process was considered complete.

The resulting particles were then purified through several successive washing cycles with distilled water to remove excess TPP and unreacted polymers. Finally, they were dried at room temperature until a constant weight was achieved, and stored in Eppendorf tubes for further characterization, ensuring protection from humidity and external contaminants.

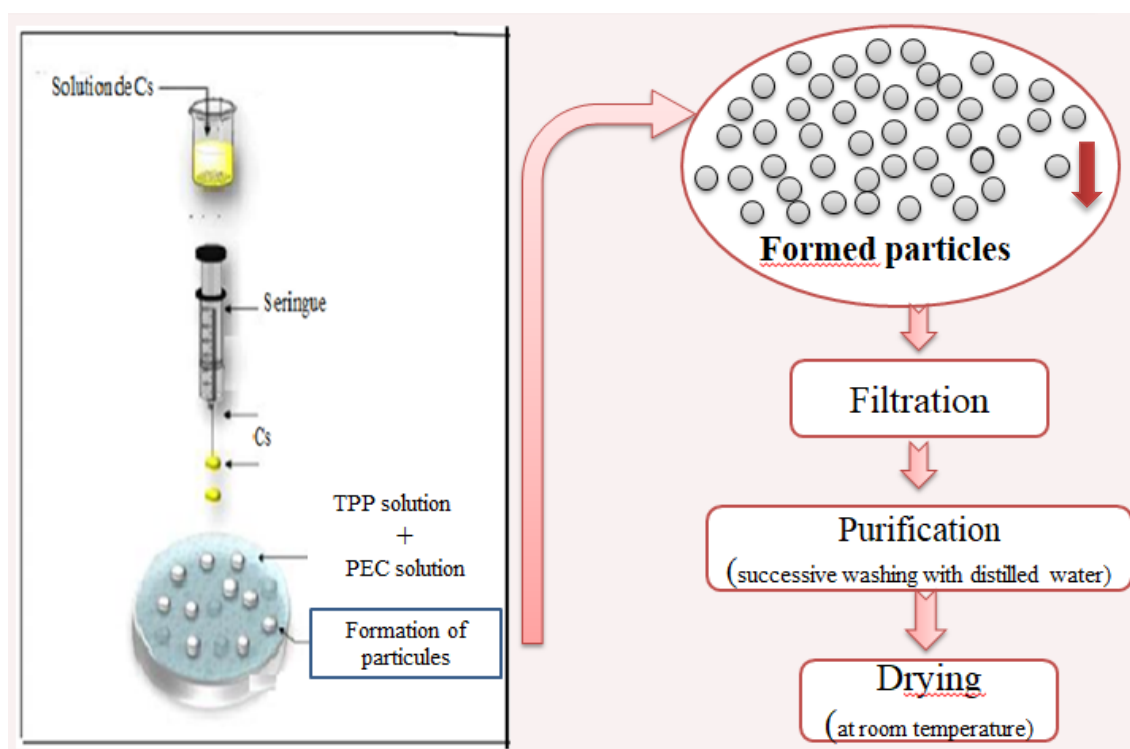


Figure III.7. Preparation of CS/PEN microparticles via ionotropic gelation

III.3.1.3. Preparation of Microparticles Containing Sodium Diclofenac (Cs/PEC/SD)

The encapsulation of sodium diclofenac (SD) within chitosan/pectin microparticles was carried out using the ionic gelation technique. The most stable and well-formed Cs/PEC microparticles, previously characterized in terms of diameter, sphericity, and swelling degree were selected for this formulation.

The experimental procedure followed the previously described method, with slight modifications. Diclofenac sodium solutions of varying concentrations were prepared in a pH 6.8 aqueous medium. Then, 2 mL of this solution was added to the chitosan solution,

previously adjusted to pH 5. To enhance dispersion, 1% Tween 80 was incorporated, and the mixture was stirred magnetically for 10 minutes.

The resulting chitosan/diclofenac mixture was then added dropwise into the TPP/pectin solution under gentle agitation for 2 hours to ensure complete crosslinking. The formed microparticles were filtered and thoroughly washed with pH 6.8 buffer solution to eliminate residual TPP and unencapsulated diclofenac.

III.3.2. Preparation of Microparticles Containing Ginger Essential Oil

III.3.2.1. Experimental Protocol for Essential Oil Extraction (GEO)

The essential oil was extracted from dried ginger rhizomes using hydrodistillation with a Clevenger type apparatus. A specific amount of finely powdered dry ginger (e.g., 80 g) was placed in a 1 L round-bottom flask containing 800 mL of distilled water. The mixture was heated to boiling, and the distillation was continued for 4 hours at 100°C. The condensed vapors were collected in the graduated arm of the Clevenger apparatus, where the essential oil was separated from the aqueous phase due to its immiscibility.

The extracted essential oil was collected, dried over anhydrous sodium sulfate to remove any remaining moisture, and then stored in amber vials at 4 °C until further use.



Figure III.8: Clevenger apparatus used for hydrodistillation of ginger essential oil.

III.3.2.2. Formation of Microparticles Containing Ginger Essential Oil

The following experimental work represents a preliminary phase of this research project. As part of an exploratory study, only two types of ginger essential oil loaded microparticles were prepared in order to evaluate the feasibility and efficiency of the ionotropic gelation process for encapsulating volatile active compounds.

An efficient microencapsulation system based on chitosan/pectin was developed for the encapsulation of ginger essential oil using ionotropic gelation.

Chitosan, pectin and TPP solutions were prepared following the previously established protocol. Ginger essential oil (400 μ L) was first emulsified into the chitosan solution (adjusted to pH 5 using 1 N NaOH). Tween 80 (0.2%) was added as a non-ionic emulsifier, to ensure a homogeneous dispersion of the oil, the mixture was vortexed for 5 minutes.

Separately, the pectin solution was slowly added dropwise into the TPP solution under continuous magnetic stirring. The chitosan/oil emulsion was then gradually introduced into the TPP/pectin mixture while maintaining gentle stirring for 2 hours.

The resulting microparticles were purified by three successive washes with pH 6.8 phosphate buffer to remove unreacted components. Finally, the microparticles were dried at room temperature until a constant weight was achieved.

To summarize the experimental conditions and facilitate the identification of the prepared formulations, Table III.3 presents the design and codification of the synthesized microparticles. This table compiles the key parameters investigated as part of the present research work.

To better highlight the formulation strategy, the compositions are expressed as mass ratios between chitosan (CS), pectin (PEC), and sodium tripolyphosphate (TPP). These normalized ratios (CS/PEC and CS/TPP) provide a clearer understanding of the relative contributions of each component to microparticle formation and structural integrity.

In addition, the incorporated bioactive compound sodium diclofenac is expressed as a mass ratio relative to chitosan, ensuring a consistent basis for comparison across all drug loaded formulations. The amount of ginger essential oil, used in the preliminary phase, is expressed in volume (mL) for each formulation.

Table III.3. Experimental program and codification of synthesized samples.

sample code	Cs (%) (w/v)	Cs/PEC (w/w)	Cs/TPP (w/w)	DS	GEO (mL)
CsP1	1	1:1	1:1	//	//
CsP2	1.5	1/1.5	1.5:1		
CsP3	2	1:2	2:1		
CsP4	1	1:1	1:1.5		
CsP5			1: 2		
CsP6	1.5	1.5:1	1:1		
CsPD1	1	1:1	1:1	1:0.2	
CsPD2			1:1,5		
CsPD3			1:2		
CsPH1	1.5	1.5:1	1.5:1	//	0.4
CsPH2			1:1		

III.4. Characterization of microparticles

III.4.1. Synthesis Yield

III.4.1.1. Microparticle Production Yield

The production yield of the microparticles was calculated to evaluate the efficiency of the formulation process. It corresponds to the ratio of the dry mass of microparticles recovered after air drying to the total mass of polymers initially used in the formulation. The yield was calculated as follows:

$$\text{MPs Yield (\%)} = \frac{\text{amount of recovered microparticles}}{\text{total amount of polymers used}} \times 100 \dots\dots\dots (1)$$

III.4.1.2. Ginger Essential Oil Extraction Yield

The yield of ginger essential oil (GEO) was calculated based on the dry weight of the plant material used for the hydrodistillation process. The following equation was applied:

$$\text{GEO Yield (\%)} = \frac{\text{Mass of essential oil obtained (g)}}{\text{mass of dry matter used (g)}} \times 100 \dots\dots\dots (2)$$

This calculation reflects the percentage of essential oil extracted relative to the dry weight of the ginger rhizomes. It serves as an important parameter to assess the efficiency of the extraction process and the availability of bioactive compounds for encapsulation.

III.4.2. Structural Characterization by FTIR

The structural characterization of the microparticles (MPs) was carried out using Fourier Transform Infrared Spectroscopy (FTIR). All samples including raw materials (chitosan, pectin, TPP and diclofenac sodium) and drug-loaded microparticles were prepared as potassium bromide (KBr) pellets. Spectra were recorded in the range of 4000 to 400 cm^{-1} with a resolution of 4.0 cm^{-1} , using an FTIR spectrometer (Shimadzu Corporation, Kyoto, Japan).

The resulting spectra allowed for the identification of characteristic absorption bands corresponding to specific functional groups. Comparisons between the spectra of the microparticles and those of the individual components enabled the detection of potential molecular interactions, such as hydrogen bonding and electrostatic interactions, which may occur between the biopolymers and the encapsulated bioactive compounds. These spectral variations provided insight into the structural organization of the microparticles and confirmed the successful encapsulation of diclofenac sodium and ginger essential oil.

III.4.3. Characterization of Microparticles by UV-Visible Spectrophotometry

UV-Visible spectrophotometry was employed to quantify the amount of active compounds encapsulated or released from the microparticles. This technique provides valuable complementary information regarding the encapsulation efficiency and release kinetics of both diclofenac sodium and ginger essential oil.

➤ **Principle**

UV-Visible spectrophotometry is based on the absorption of light by molecules, causing electronic transitions from a ground state to an excited state. When a sample is exposed to light in the ultraviolet (UV) or visible (Vis) range, specific wavelengths are absorbed depending on the electronic structure of the molecules present. The UV-Vis spectrophotometer used in this study (**EVOLUTION1280**, Thermo Scientific) is equipped with:

- A **deuterium lamp** for UV radiation (180–400 nm),
- A **tungsten lamp** for the visible range (400–800 nm).

The absorbance measurements were interpreted using Beer–Lambert’s Law, defined by the equation:

$$A = \varepsilon \times l \times C \dots\dots\dots (3)$$

Where:

- **A**: Absorbance,
- **ε** : the Molar Absorption Coefficient ($\text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$),
- ***l***: the Path Length of the cuvette (cm),
- **C**: the Concentration of the sample ($\text{mol} \cdot \text{L}^{-1}$).

This method allowed the determination of the residual amount of unencapsulated compounds (from the supernatant) and the drug released at different pH levels, through previously established calibration curves.



Figure III.9 : UV–Visible Spectrophotometer used for quantitative analysis

III.4.4. Morphological and Size Analysis of Microparticles by Optical Microscopy

The size and surface morphology of the synthesized microparticles were examined using optical microscopy. A small quantity of dried microparticles was dispersed on a glass slide and observed under a light microscope at various magnifications. Images were captured to qualitatively assess the particle shape and surface characteristics.

The particle diameter was estimated by measuring at least 100 individual microparticles using image analysis software. The average particle size and standard deviation were calculated and used to evaluate the influence of formulation parameters on microparticle formation.

III.4.5. Swelling Behaviour in Aqueous Solutions

The swelling behavior of the microparticles was investigated to assess their potential as drug carriers, as this property directly influences both drug loading and release capacities. The swelling study was performed gravimetrically using different buffer solutions to simulate various physiological environments.

A constant amount (30 mg) of dried microparticles was placed in Eppendorf tubes containing 2 mL of buffer solution at different pH values: Acetate buffer (pH 3.6) to simulate the acidic conditions of the stomach, and Phosphate-buffered saline (PBS, pH 7.4) to mimic physiological (blood) conditions. The obtained suspension was maintained at $37^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$ under magnetic stirring at 120 rpm. At specific time's intervals the suspension was centrifugated, the supernatant was removed, the excess of liquid was removed by carefully

blotting with filter paper, and the swollen sample was weighed. The microparticules (dry and swollen) were weighed with an accuracy of ± 0.0001 g on an electronic microbalance. All experiments were performed in triplicat. The percentage of swelling ratio (Q%) was determined with equation (4)

$$Q(\%) = \frac{W-W_0}{W_0} * 100.....(4) [1,3]$$

Where: **W**: the weight of swollen sample (mg)

W₀: the initial weight of dry sample (mg)

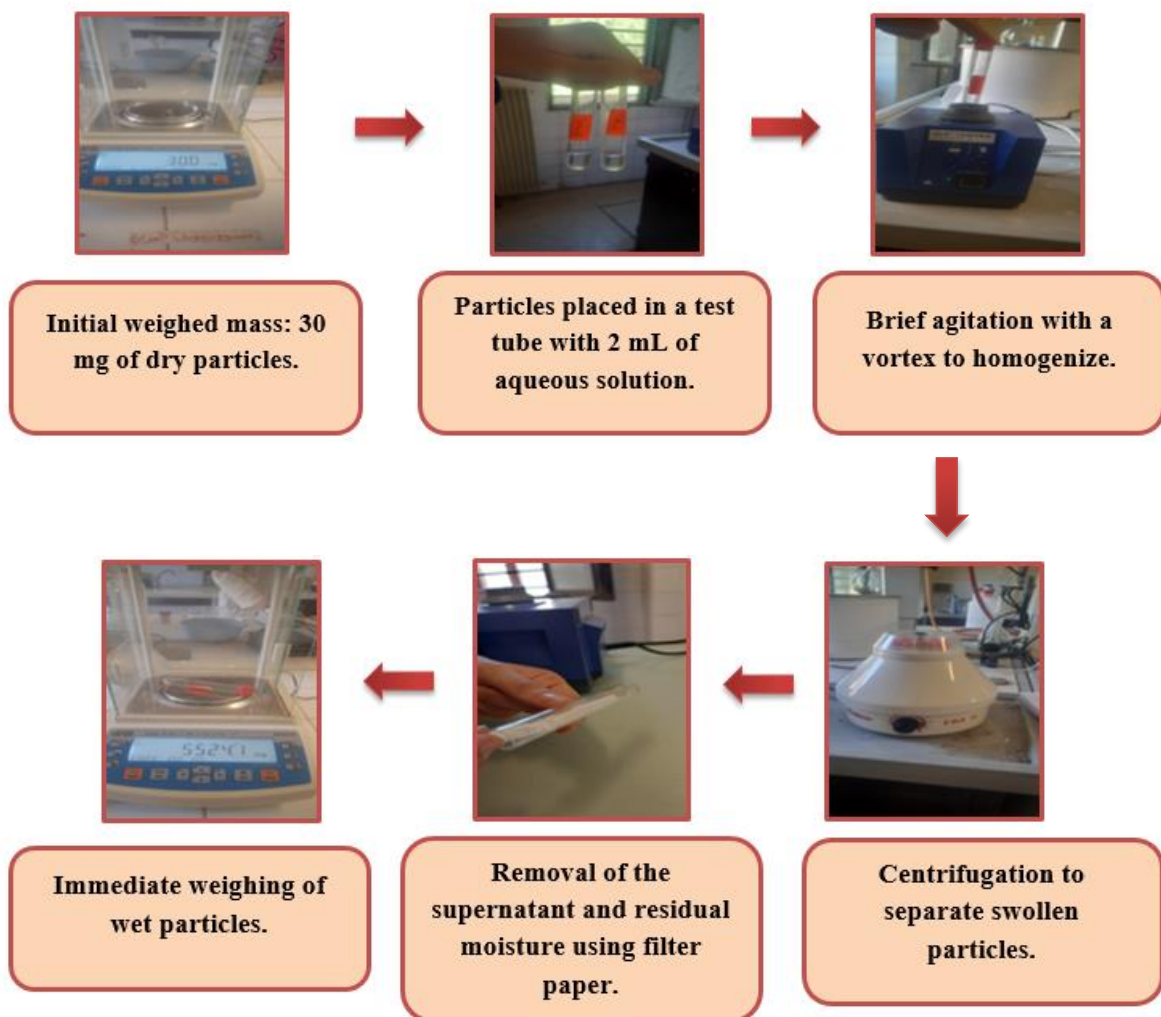


Figure III.10. Experimental Setup for Swelling Study

III.4.6. Drug loading studies

The diclofenac loading capacity is a critical parameter that reflects the amount of drug successfully incorporated into the microparticles relative to the total weight of the particles. It provides essential information about the efficiency of the encapsulation process and the potential therapeutic dosage the delivery system can provide.

To evaluate the encapsulation efficiency of diclofenac sodium in the microparticles prepared by the ionotropic gelation method, the amount of free (non-encapsulated) drug remaining in the supernatant was quantified after centrifugation. In this process, diclofenac was incorporated directly into the initial polymer mixture during microparticle formation with chitosan, pectin, and TPP. After completion of the ionic crosslinking and particle formation, the dispersions were centrifuged to separate the formed microparticles from the supernatant. The absorbance of the recovered supernatant was measured using a UV-Visible spectrophotometer at the maximum absorbance wavelength of diclofenac (typically around 276 nm), in order to determine the concentration of unencapsulated drug. A standard calibration curve of diclofenac sodium in distilled water was previously established and used for this quantification.

The encapsulation efficiency ($E_{ef}\%$) was calculated by comparing the amount of drug initially added to the formulation with the amount recovered in the supernatant, according to the following equation [5]:

$$m_l = m_i - m_s \quad \dots\dots\dots(5)$$

$$E_{ef}\% = \frac{m_i - m_s}{m_i} \times 100 \quad \dots\dots\dots(6)$$

Where, m_l is the amount of loaded Diclofenac (mg), m_i is the initial amount of DS (mg) and m_s is the amount of DS found in supernatant (mg).

III.4.7. Drug Release Profile

The in vitro release behavior of diclofenac sodium (DS) from the microparticles was investigated under simulated gastrointestinal conditions to assess their potential for oral drug delivery. The release study was conducted in two consecutive phases, mimicking the pH variations encountered in the digestive tract.

Initially, Each sample of DS-loaded microparticles (30mg) was suspended in 6 mL of hydrochloric acid buffer solution at pH 3.4 to simulate gastric conditions. The suspension was maintained under gentle agitation (100 tr/min) at 37 ± 0.5 °C for 2 hours. At predetermined time intervals, 1 mL of the release medium was withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The collected samples were filtered, and the concentration of DS was determined by measuring the absorbance using a UV-Vis spectrophotometer at 276 nm, based on a previously established calibration curve.

After 2 hours, the microparticles were separated, rinsed, and transferred to a phosphate buffer solution at pH 7.4. The release monitoring continued under the same conditions (temperature and agitation). Sampling was performed at regular intervals, and the DS concentration in the samples was similarly measured.

The cumulative percentage of drug released at each pH was plotted as a function of time to evaluate the release kinetics and the pH-sensitivity of the formulation. This two-step release study allowed the assessment of the protective ability of the biopolymeric matrix in acidic environments and its capacity to ensure drug release under intestinal conditions.

The efficiency of DS releasing ($R_{ef}\%$) was calculated using the Eq. (7) [3]:

$$Ref(\%) = \frac{m_r}{m_l} * 100 \dots\dots\dots(7)$$

Where: m_r : the amount of drug released from microparticles (mg)

m_l : the amount of the drug loaded in the particles (mg).

III.4.7. Loading and Release Studies of Ginger Essential Oil (GEO)

Following the encapsulation and release evaluation of diclofenac sodium, similar investigations were conducted to assess the entrapment and release behavior of ginger essential oil (GEO) within CS/pectin-based microparticles.

Due to the volatile and hydrophobic nature of essential oils, evaluating their encapsulation efficiency and release profiles is crucial to ensure their stability and enable controlled delivery in aqueous environments. The encapsulation of GEO was achieved during microparticle formation via the ionic gelation technique. After air drying, the supernatant was collected to quantify the non-encapsulated oil using UV–Visible spectrophotometry at the characteristic wavelength of GEO, based on a previously established calibration curve.

The in vitro release of GEO was studied in phosphate-buffered saline (PBS, pH = 7.4), simulating physiological blood conditions. The microparticles were suspended in PBS and incubated at 37 ± 0.5 °C under continuous stirring (120 rpm). At regular time intervals, 1 mL of the medium was withdrawn and replaced with fresh PBS. The amount of released GEO was measured spectrophotometrically at 280 nm using a Nanodrop One spectrophotometer (Thermo Scientific). The release efficiency (Ref%) was calculated using the same equation employed for diclofenac sodium (Eq. 6).

This release study provides insight into the release kinetics of GEO from the biopolymeric matrix and helps assess the potential of the system for the pH-sensitive delivery of volatile bioactives.

III.5. Conclusion

This chapter has detailed the materials, experimental protocols, and analytical techniques employed throughout this research. The synthesis of chitosan/pectin based microparticles, incorporating either diclofenac sodium or ginger essential oil, was successfully achieved using the ionotropic gelation method. The various characterization techniques including yield evaluation, FTIR spectroscopy, optical microscopy, swelling behavior analysis and UV–Visible spectrophotometry were selected to assess the physicochemical properties, encapsulation efficiency, and release profiles of the developed systems. These well-defined methods lay a solid foundation for the interpretation of the experimental results presented in the next chapter, where we discuss the performance, interactions, and release behavior of the formulated microparticles in different pH environments.

Chapter IV

IV. I. Introduction

This chapter presents and analyzes the experimental findings obtained during the development of polymer based microparticulate systems composed of chitosan and pectin, ionically crosslinked using sodium tripolyphosphate (TPP). The primary objective of this research was to design an effective encapsulation platform for the anti-inflammatory drug diclofenac sodium, aimed at achieving controlled drug delivery. In addition, preliminary investigations were conducted to evaluate the encapsulation potential of ginger essential oil, a natural compound known for its anti-inflammatory and antioxidant properties.

The chapter begins with the characterization of the prepared microparticles, including particle size analysis, encapsulation efficiency, and morphological evaluation. Subsequently, in vitro release profiles of diclofenac sodium and ginger essential oil are discussed, with particular attention to the influence of formulation composition and processing parameters. Finally, a critical interpretation of the results is provided, supported by comparisons with relevant literature, in order to identify the key factors affecting the performance of the developed delivery systems.

IV.2. Microparticle Formation

Chitosan based microparticles were developed using the ionic gelation technique, a widely adopted, mild, and solvent free method for the fabrication of polymeric micro/nanoparticles. This approach is particularly advantageous in pharmaceutical and biomedical contexts, as it occurs entirely in aqueous media, thus preserving the stability of sensitive bioactive compounds.

The formation of the microparticles was driven by electrostatic interactions between the protonated amino groups of chitosan and the carboxylate groups of pectin. Ionic crosslinking was further facilitated by the addition of sodium tripolyphosphate (TPP), a multivalent anionic agent, which reinforced the polymer matrix by promoting intermolecular bridges. The combination of chitosan and pectin two biocompatible and biodegradable polysaccharides allowed for the generation of well structured microparticles capable of encapsulating therapeutic agents under physiologically compatible conditions.

IV.2.1. Ionic Gelation mechanism of CS/Pectin Microparticles

The microparticles were obtained via a controlled ionic gelation process performed entirely in aqueous solution. The procedure began with the dissolution of pectin in a TPP solution, enabling both the activation of pectin's carboxyl functions and the establishment of a polyanionic environment. Subsequently, chitosan solution was added dropwise under magnetic stirring, initiating the immediate formation of microparticles.

This process relied on the ionic complexation between the positively charged $-\text{NH}_3^+$ groups of chitosan and the negatively charged $-\text{COO}^-$ groups of pectin, with additional crosslinking provided by the phosphate groups of TPP. These synergistic interactions led to the formation of a cohesive, three dimensional polymer network, resulting in the precipitation of stable, spherical microparticles.

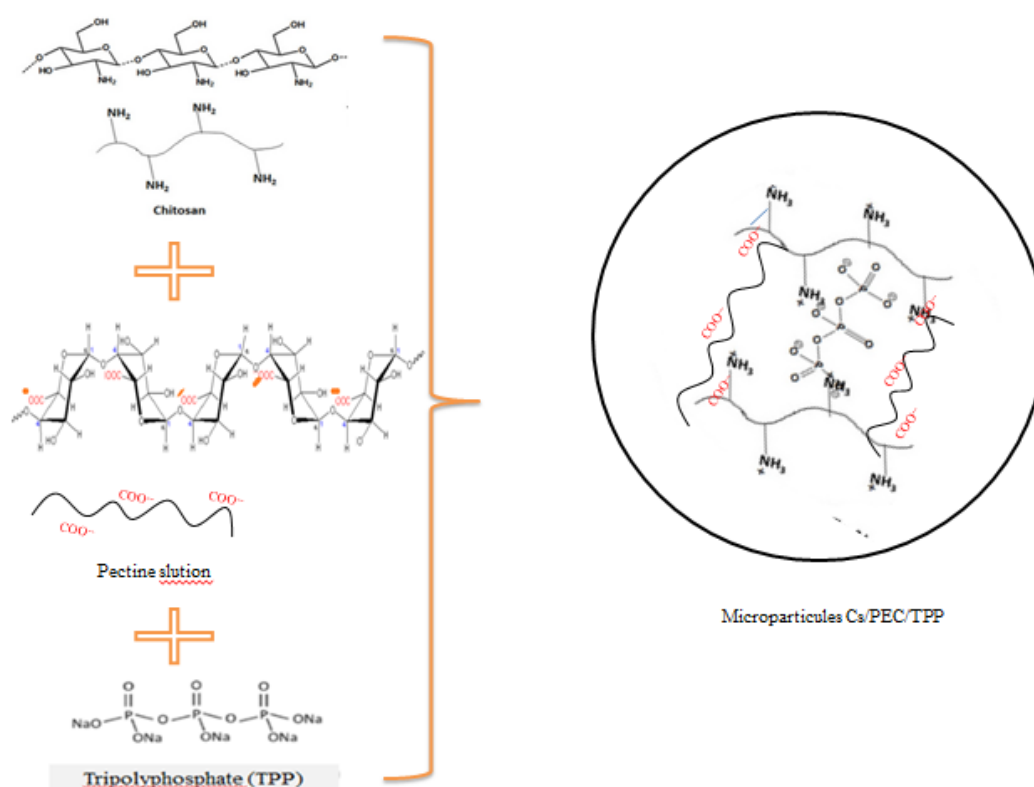


Figure IV. 1. Mechanism of CS/Pectin Microparticles

Similar ionic crosslinking strategies have been reported in the literature for the development of polymeric matrices based on chitosan and pectin. For instance, Kouchak et al. (2014) successfully formulated chitosan/pectin films using TPP as a crosslinker, highlighting the role of phosphate groups in enhancing mechanical strength and network stability [124].

Moreover, Mekhail et al. (2021) developed CS/pectin nanoparticles for oral drug delivery, demonstrating the applicability of this method to encapsulate bioactives with improved release behavior [125]. These examples confirm the relevance and reproducibility of the ionic gelation method in forming polymeric microparticles and hydrogels from natural biopolymers under mild, biocompatible conditions.

IV.2.2. FTIR Analysis of Cs/PEC/TPP Microparticles

Fourier Transform Infrared Spectroscopy (FTIR) was performed to confirm the chemical interactions between chitosan (CS), pectin (PEC), and the crosslinking agent TPP in the microparticles.

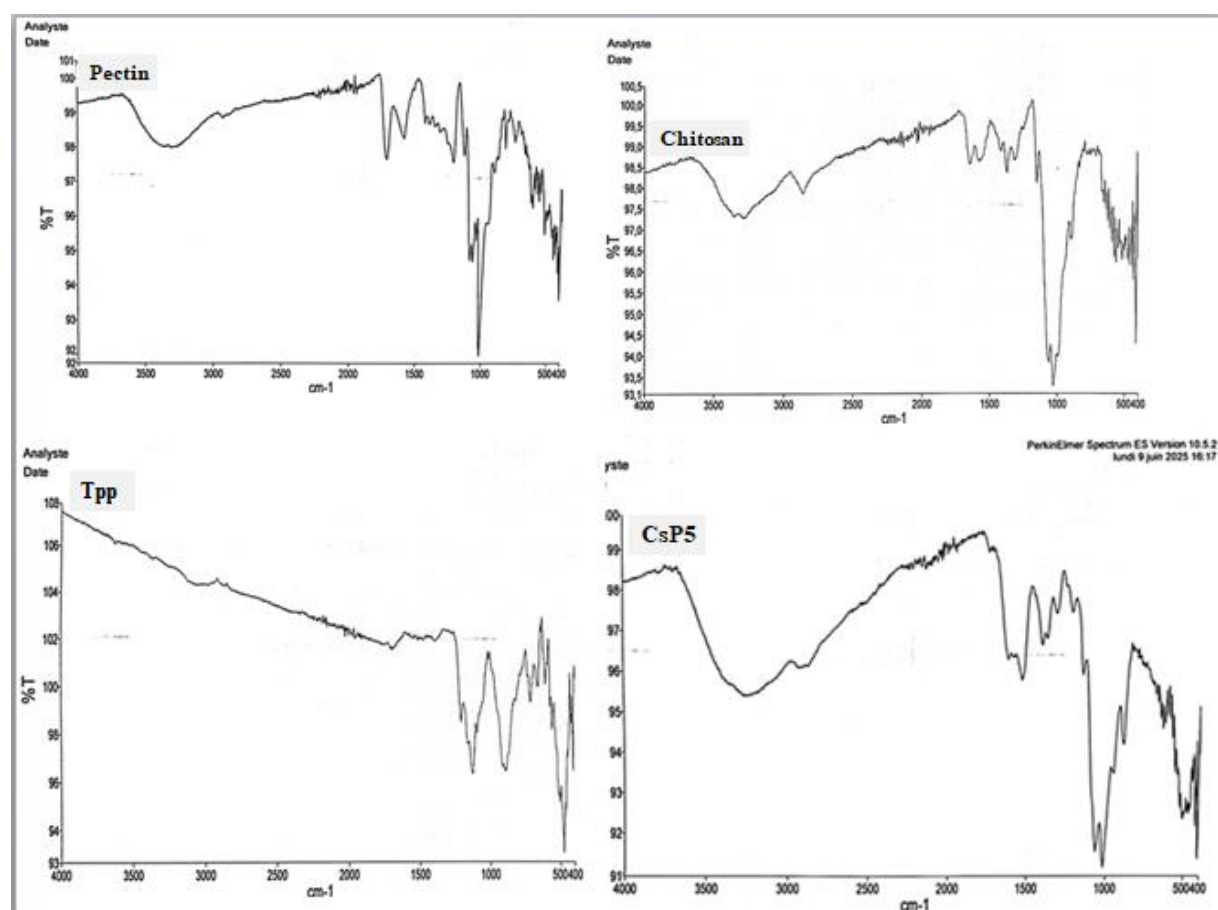


Figure IV.2. FTIR spectra for Cs, PEC, TPP and Cs/PEC microparticles

The FTIR spectra revealed characteristic peaks of both biopolymers and highlighted possible intermolecular interactions:

- The broad band around 3200–3400 cm^{-1} corresponds to the O–H and N–H stretching vibrations, indicating hydrogen bonding between CS and PEC.

- The peak near 1640 cm^{-1} is attributed to the amide I (C=O) stretching of chitosan and may shift slightly due to crosslinking.
- The absorption band around $1420\text{--}1450\text{ cm}^{-1}$ corresponds to symmetric stretching of COO^- groups in pectin.
- A **new or intensified peak near $1200\text{--}1250\text{ cm}^{-1}$** is often associated with P=O stretching of TPP, confirming successful ionic crosslinking with chitosan's NH_3^+ groups.
- These spectral changes suggest the formation of a polyelectrolyte complex structure stabilized by TPP, confirming the successful crosslinking and interaction between the components.

IV.2.3. Production Yield of CS/Pectin Microparticles

One of the key performance indicators in microparticle preparation is the production yield, which reflects the efficiency and material recovery of the synthesis process. High yields are desirable to ensure reproducibility and minimize material loss. Table IV.1 presents the synthesis yields of the CS/Pectin microparticles prepared under different formulation conditions.

Table IV.1. Synthesis Yield of CS/Pectin Microparticles

Formulation Code	CsP1	CsP2	CsP3	CsP4	CsP5	CsP6
Yield (%)	70,45	75	80.5	77	79	82

The synthesis yields of the prepared chitosan-based microparticles ranged from 70.45% to 82%, with the highest value obtained for formulation CsP6. These results indicate an overall good production efficiency of the ionotropic gelation method, with all formulations exceeding 70%, a threshold commonly considered acceptable in similar works involving natural polymer-based microparticles. Previous studies (Calvo et al., 1997; Gan & Wang, 2007) have reported comparable yields when using chitosan and TPP under controlled conditions, typically ranging from 65% to 85%, depending on factors such as polymer concentration, crosslinker ratio, and process parameters [126,127]. Accordingly, the yields

obtained in this study confirm the practicality and reliability of the selected synthesis approach.

IV.2.4. Microscopic and Morphological Analysis of Cs/PEC/TPP Microparticles

The particles obtained are characterized on a macroscopic and microscopic level (assessment of the shape and size of the particles). Following this, a margin of respective concentrations of the components is chosen, according to selection criteria (spherical shape and micrometric size). The aim is to determine the experimental domain in which we can consider launching our formulations in order to obtain the optimal formula.



Figure IV.3. Macroscopic and Microscopic Characterization of Ionically Crosslinked Microparticles

The figure shows the visual and microscopic appearance of chitosan/pectin microparticles crosslinked with TPP. In their wet state, the particles appear spherical, smooth, and uniformly dispersed, indicating successful ionic gelation and strong crosslinking interactions between the polycationic chitosan, polyanionic pectin, and TPP as a multivalent crosslinker. After drying, the particles exhibit a noticeable reduction in size and a slightly yellowish coloration. This shrinkage is characteristic of hydrogel based microparticles undergoing water loss and matrix densification.

Microscopic observation confirms the spherical morphology and homogeneity of the microparticles. Their regular shape and smooth surface reflect an efficient encapsulation process and a stable polymeric network. The presence of the active compound within the microparticles can also influence their optical properties, resulting in a slightly translucent or

colored appearance under the microscope. Overall, the morphology and microscopic characteristics of the particles suggest their suitability for controlled drug delivery applications.

IV.2.5. Influence of Chitosan and TPP Concentration on Microparticle Size

Microparticulate systems are widely valued in drug delivery due to their small size, which significantly influences parameters such as cellular uptake, biodistribution, and therapeutic efficiency. To assess the impact of formulation variables on particle morphology, the average diameters of the CS/Pectin microparticles were determined by optical microscopy. The results corresponding to each formulation are presented in Table IV.2.

Table IV.2. Average diameter of Cs/PEC microparticles obtained by ionic gelation.

Formulation Code	CsP1	CsP2	CsP3	CsP4	CsP5	CsP6
Average Diameter (μm)	310	425	610	280	235	405

The data indicate that the microparticles displayed average diameters ranging from 280 μm to 610 μm , with corresponding production yields between 75% and 93%. The particle size was influenced by several factors, including the chitosan/pectin ratio and the TPP concentration,

The experimental results revealed a significant increase in the average diameter of the microparticles, ranging from 210 μm to 615 μm as the chitosan concentration increased from 1% to 2% (w/v). This trend can be attributed to the rise in viscosity of the chitosan solution, which limits droplet fragmentation during the emulsification or dripping process. As a result, larger droplets are formed and subsequently solidified in the presence of the crosslinking agent (TPP).

This finding is supported by previous studies. Hu et al. (2017) reported that low polymer concentrations produce irregularly shaped beads with rough surfaces due to insufficient structural integrity [128]. In our case, concentrations below that of formulation CsP1 resulted in completely deformed particles. Similarly, Lin et al. (2016) investigated the effect of

alginate concentration on particle size and found that increasing the alginate content from 1% to 3% led to a particle size increase from 728.7 μm to 1053.4 μm [129]. This is explained by the formation of larger droplets in more viscous dispersions, leading to the production of larger microspheres upon gelation.

In addition to chitosan content, the chitosan/TPP ratio was found to significantly influence the final particle size. Low TPP concentrations resulted in poorly crosslinked and agglomerated microspheres due to insufficient network stabilization. On the other hand, a higher concentration of TPP enhanced the ionic crosslinking reaction, promoting faster and denser gel formation. This increased crosslinking density induces a greater contraction of the polymer network, resulting in smaller, more compact and rigid microparticles. Such behavior is typical of highly crosslinked systems, where reduced chain mobility limits particle expansion.

These findings are consistent with previous reports, which demonstrated that increasing chitosan concentration leads to larger microparticles due to higher viscosity [124], while elevated TPP levels enhance ionic crosslinking, resulting in denser and smaller particles due to network contraction.

Therefore, the optimal adjustment of both chitosan and TPP concentrations is critical for controlling microparticle morphology and size, which are key parameters in determining encapsulation efficiency and release kinetics in drug delivery applications.

IV.2.6. Results of Ginger Essential Oil Extraction

Ginger essential oil (GEO) was extracted from dried ginger rhizome powder by hydrodistillation using a Clevenger-type apparatus. The process was conducted over 4 hours, and the recovered oil was stored in amber bottles at 4°C until further use.

The extraction yielded 0.56% of essential oil, calculated relative to the dry weight of ginger used. This value is consistent with previous studies reporting essential oil yields between 0.3% and 3.0% for dried ginger, depending on factors such as the plant variety, drying method, and extraction time (Singh et al., 2008; Ghosh et al., 2019) [130,131].

Although moderate, this yield is acceptable for dried botanical material and confirms the effectiveness of the hydrodistillation process under laboratory conditions. The obtained oil exhibited a pale yellow color and a strong aromatic odor, characteristic of the presence of

volatile sesquiterpenes and phenolic compounds, making it suitable for encapsulation applications.

IV.3. Swelling Behavior of CS/PEC Microparticles

The swelling degree is a critical parameter that reflects the ability of polymeric microparticles to absorb water and expand in aqueous environments. It is directly related to the network structure, crosslinking density, and hydrophilic nature of the polymers used. In the context of drug delivery, the swelling behavior can influence the diffusion rate and release profile of encapsulated molecules.

The swelling behavior of the microparticles was evaluated in aqueous solutions at different pH values (pH 3.4 and 7.4) in order to determine the optimal conditions for obtaining biocompatible microspheres, given their potential application as carriers for anti-inflammatory drugs. The swelling kinetics were monitored over a 24 hour period to ensure equilibrium was reached for all samples.

IV.3.1. Swelling Behavior of CS/PEC Microparticles at pH 7.4

The swelling capacity of chitosan/pectin microparticles was investigated at physiological pH (7.4) to assess their suitability as drug delivery carriers. The results demonstrated that the swelling ratio is significantly influenced by both the chitosan concentration and the amount of TPP used as crosslinking agent. The results are presented in figure IV.4.

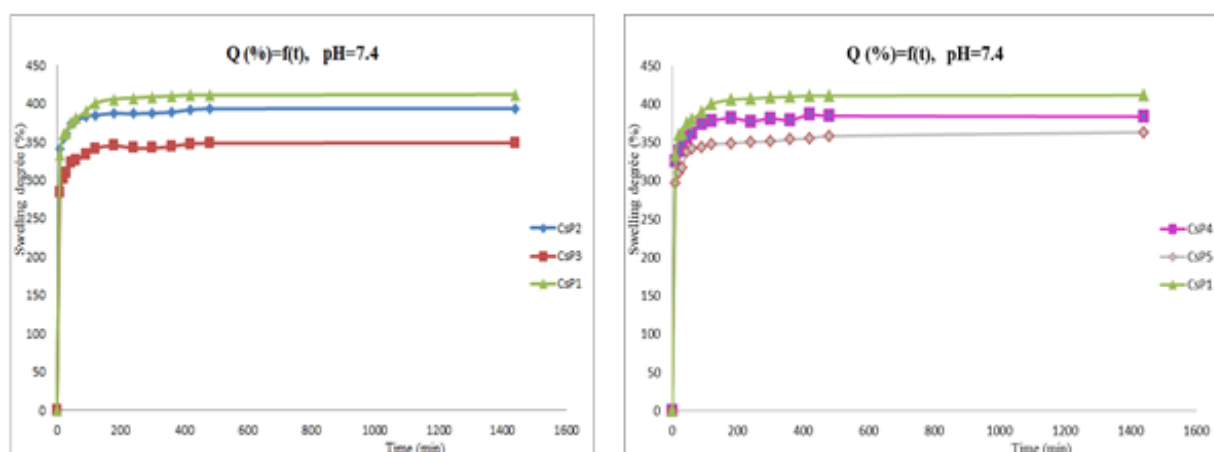


Figure IV.4. Swelling Behavior of CS/PEC Microparticles at pH 7.4

An increase in chitosan concentration from 1% to 2% (w/v), as observed in samples CsP1 to CsP3, led to a moderate increase in the swelling ratio, ranging from approximately 350% to 450%. This trend can be attributed to the higher availability of amine groups (-NH₂) in the polymer matrix, which enhances water uptake due to increased hydrophilicity and network expansion. However, further increase in polymer concentration may lead to increased chain entanglement and viscosity, which hinders water diffusion and limits swelling [128].

In contrast, increasing the TPP concentration while maintaining chitosan at 1% (w/v), as in samples CsP1, CsP4, and CsP5, resulted in a noticeable decrease in the swelling ratio from 350% to 240%. This inverse relationship is due to the denser ionic crosslinking network formed between the positively charged amino groups of chitosan and the negatively charged phosphate groups of TPP, which reduces the free volume available for water absorption. A higher crosslinking degree results in compact, rigid microspheres with limited swelling capacity.

These observations align with previous studies on chitosan-based hydrogels and microparticles, which have consistently shown that both polymer concentration and crosslinking density are critical factors in modulating the physicochemical properties of the system.

IV.3.2. Swelling Behavior of CS/PEC Microparticles at Acidic pH (pH = 3.4)

The swelling behavior of CS/PEC microparticles was also evaluated in acidic medium (pH = 3.4) to simulate gastric conditions. In this environment, the swelling ratio was significantly higher than at neutral or physiological pH, with values ranging from 500% to 700% depending on formulation parameters (figure IV.5). This pronounced swelling in acidic conditions can be explained by the protonation of chitosan's amino groups (-NH₂) under low pH. At pH 3.4, these groups become positively charged (-NH₃⁺), leading to increased electrostatic repulsion between polymer chains, thus favoring network expansion and greater water uptake. It is also important to note that when the TPP concentration was increased (as in CsP4 and CsP5), a reduction in the swelling degree was again observed, even at acidic pH. This reinforces the conclusion that ionic crosslinking with TPP restricts network flexibility, regardless of pH, by forming compact and rigid microparticles. These findings confirm that acidic pH promotes swelling in chitosan based systems due to enhanced protonation and chain expansion, in contrast to the more compact structures formed under stronger crosslinking conditions.

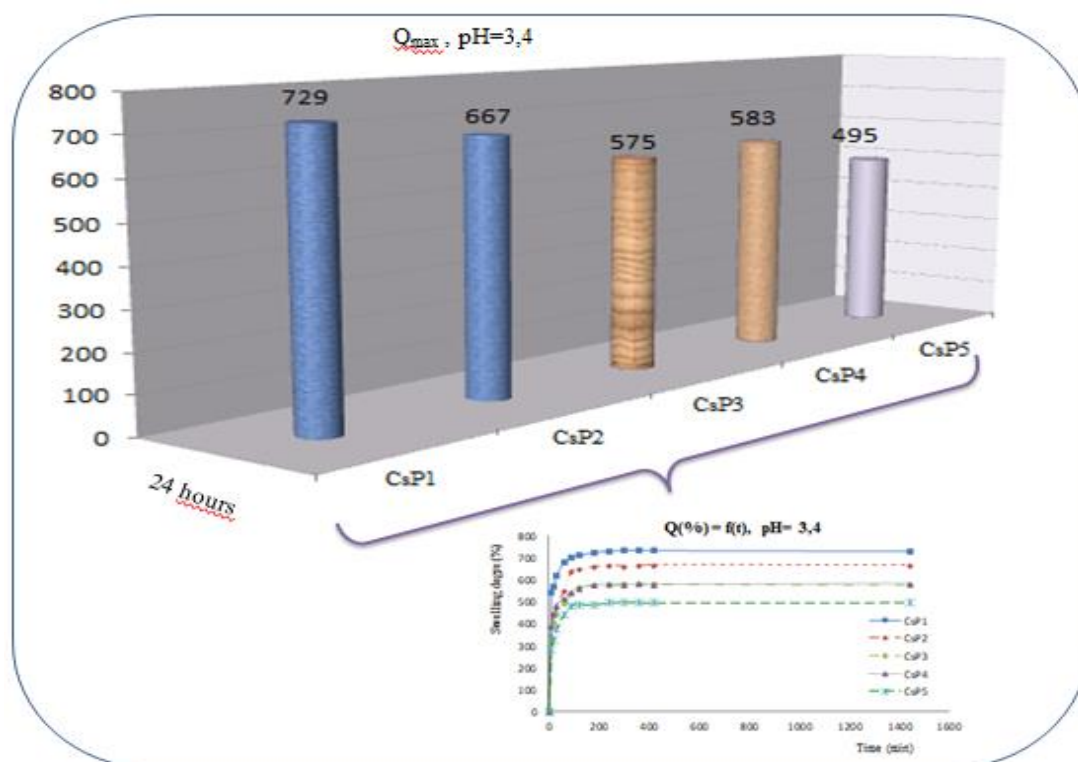


Figure IV.5. Swelling Behavior of CS/PEC Microparticles at Acidic pH (pH = 3.4)

IV.3.3. Effect of PEC Incorporation on the Swelling Behavior of Chitosan Microparticles

It is worth noting that the swelling degree observed in the present study for CS/PEC microparticles is markedly higher than that typically reported for microparticles made of chitosan alone. While chitosan exhibits moderate swelling in aqueous media due to its semi-crystalline structure and limited hydrophilicity, the incorporation of pectin into the polymer matrix significantly enhances the water absorption capacity.

This improvement is attributed to the hydrophilic nature and branched, amorphous structure of pectin, which contains abundant free carboxylic and hydroxyl groups. These functional groups interact with water molecules via hydrogen bonding, promoting greater water uptake and expansion of the polymeric network. Furthermore, the interpolymer complexation between the amino groups of chitosan and the carboxyl groups of pectin creates a more porous and less tightly packed structure, which facilitates water diffusion.

As a result, CS/PEC microparticles demonstrated higher swelling degrees, particularly in acidic medium (pH 3.4), where protonation of chitosan further supports network loosening

and water penetration. This synergistic behavior highlights the advantage of using polyelectrolyte complexes over single-polymer systems in the design of drug delivery

Based on the results obtained from the swelling studies, the formulation exhibiting the most significant swelling capacity at physiological pH was selected for drug encapsulation. This choice was made under the assumption that a greater swelling degree would facilitate drug diffusion by enhancing the porosity and hydrophilicity of the microparticles. Therefore, the optimal chitosan concentration was used in the subsequent encapsulation experiments involving diclofenac sodium (DS).

IV.4. Cs/PEC/DS Microparticles

IV.4.1. Loading Efficiency of Diclofenac (DS)

This type of polymeric microparticle system was developed with the objective of enabling the targeted delivery of anti-inflammatory drugs, particularly for localized inflammation treatment. Diclofenac sodium (DS), a model anti-inflammatory agent, was selected for encapsulation into chitosan/pectin-based microparticles via ionic gelation using sodium tripolyphosphate (TPP) as crosslinker.

The encapsulation efficiency (EE%) of DS was determined according to Equation III.6 (Chapter III), which is based on the quantification of the free, non-encapsulated drug remaining in the supernatant after the encapsulation process. To perform this quantification, a calibration curve was established using UV–Vis spectrophotometry in PBS. The resulting linear calibration curve is shown in Figure IV.5, and follows the equation:

$$\text{Abs} = 0.4123 \times [\text{DS}] \quad (R^2 = 0.977)$$

This equation was used to calculate the concentration of DS in the supernatant, and subsequently, the amount encapsulated within the microparticles. The results of encapsulation efficiency for the different formulations are summarized in Table IV.3.

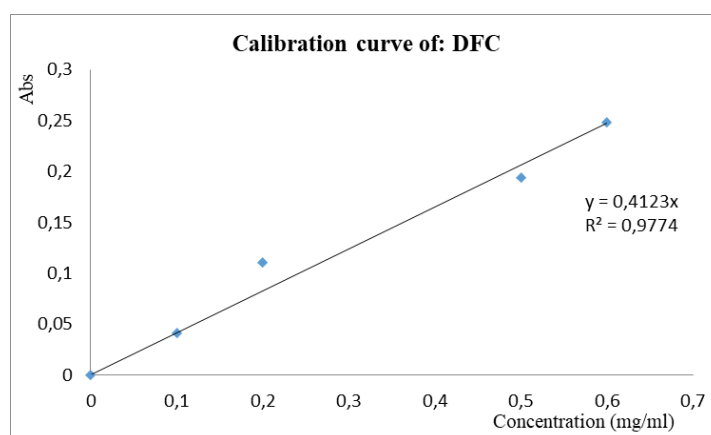


Figure IV.6. Calibration curve of diclofenac sodium in PBS ($\lambda = 276 \text{ nm}$)

This calibration curve was used to determine the concentration of free (non-encapsulated) DS in the supernatant after microparticle preparation. The results of DS loading efficiency are summarized in Table IV.3.

Table IV.3. The encapsulation efficiency results

Sample code	CsPD-1	CsPD-2	CsPD-3
The loading efficiency (%)	83	77	70

The values obtained demonstrate a relatively high encapsulation efficiency, indicating strong interactions between DS and the biopolymeric matrix. The combination of chitosan (a cationic polymer) and pectin (an anionic polymer) promotes electrostatic interactions and hydrogen bonding, which enhance drug entrapment within the microparticles.

Interestingly, although the chitosan concentration was kept constant across all formulations, a decrease in EE% was observed with increasing TPP concentrations: formulation CsPD-1, with the lowest TPP level, exhibited the highest encapsulation efficiency (83%), whereas CsPD-3, with the highest TPP concentration, showed the lowest (70%). This behavior can be explained by the effect of TPP induced crosslinking: higher TPP levels result in a denser and more rigid network, which may hinder drug diffusion and reduce the available free volume within the polymer matrix for effective entrapment. These findings are in good agreement with the swelling behavior discussed earlier (Section IV.3). The correlation between decreased swelling and lower encapsulation confirms that a tightly crosslinked matrix limits both hydration and drug loading.

Similar observations have been reported in recent studies. For instance, Silva et al. [132] showed that lower crosslinking degrees in pectin/chitosan nanoparticles led to improved diclofenac loading efficiency (up to 88%) due to enhanced matrix permeability. Likewise, Patil and Sawant [133] found that moderate crosslinking with TPP yielded higher EE in chitosan/alginate microparticles encapsulating non-steroidal anti-inflammatory drugs, whereas excessive TPP significantly reduced EE%. These results reinforce the idea that optimal crosslinking is crucial to balance structural integrity and drug encapsulation performance.

IV.4.2. In Vitro DS Release from Cs/PEC Polymeric Systems

The in vitro release of diclofenac sodium (DS) from Cs/PEC microparticles was evaluated under simulated gastrointestinal conditions. The study consisted of two sequential phases: an initial 2 hour incubation at acidic pH (3.4) to mimic the gastric environment, followed by 24 hours at physiological pH (7.4) representing intestinal conditions. The kinetics of DS release from Cs/PEC microparticles are shown in Figure IV.7.

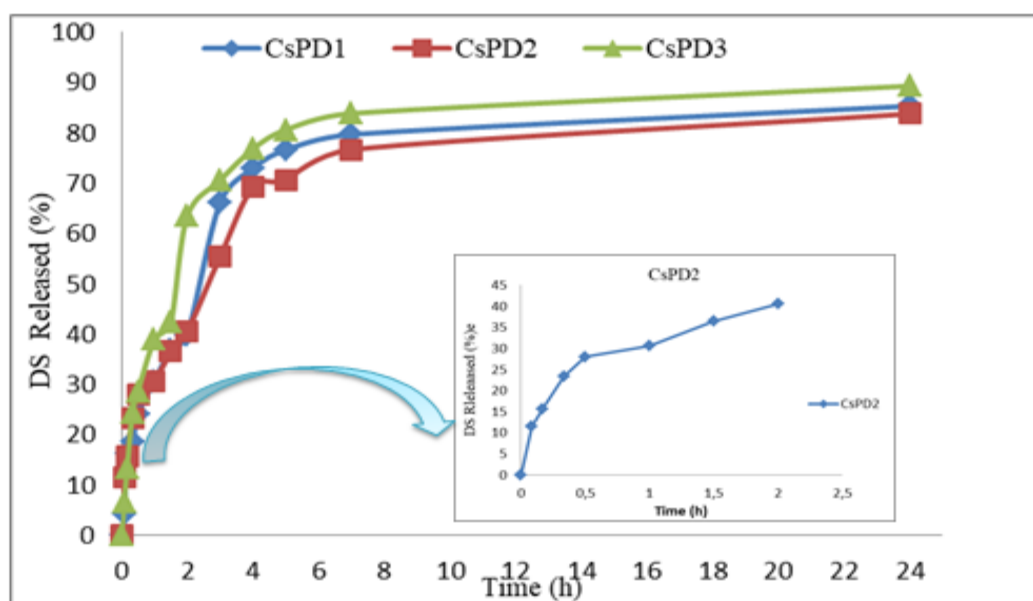


Figure IV.7. In vitro release kinetics of (DS) from Cs/PEC microparticles: initial 2 hour release in acidic medium (pH 3.4), followed by release in PBS, pH 7.4. An inset shows a zoomed in view of the release kinetics during the first 2 hours.

The release profile exhibited a biphasic behavior, characterized by a relatively rapid initial release followed by a more sustained release phase. During the first 2 hours at acidic pH, the cumulative release of DS reached 40 %, 38 %, and 35 % for formulations CsPD1, CsPD2, and CsPD3, respectively. This early release can be interpreted as a moderate burst

effect, particularly pronounced in the CsPD1 formulation. Such behavior is typically associated with low crosslinking density, which results in a more open polymeric network, facilitating early drug diffusion.

At acidic pH, chitosan exhibits increased swelling due to the protonation of its amino groups, which enhances hydration of the matrix and accelerates drug diffusion. In contrast, the CsPD3 formulation, prepared with a higher concentration of TPP, displayed a denser and more compact network that limited premature drug release and significantly reduced the burst effect. This behavior is consistent with the higher encapsulation efficiency observed for CsPD3 (EE% = 91%).

Upon transfer to physiological pH (7.4), a gradual and sustained release of DS was observed in all formulations, reaching up to 92 % at 24 hours for CsPD1. This extended release is attributed to the progressive swelling and relaxation of the Cs/PEC matrix under neutral conditions, which allows controlled drug diffusion from within the polymeric structure. A decrease in the release rate with increasing TPP content was also noted, confirming the influence of crosslinking density on the release kinetics.

These findings are consistent with results reported in the literature. For example, Moustafa et al. 2020 reported DS release values of 25–40 % at acidic pH from chitosan/pectin microparticles, followed by complete release under intestinal conditions [134]. Similarly, other studies have demonstrated that the burst effect can be minimized by increasing the ionic crosslinking level (e.g., with TPP) or by applying a gastro-resistant coating. In our system, despite the absence of such coating, the Cs/PEC microparticles effectively protected the drug under acidic conditions and enabled efficient release under physiological conditions.

In conclusion, the obtained release profile highlights the potential of Cs/PEC microparticles as a promising oral drug delivery system for anti-inflammatory agents. The combination of high encapsulation efficiency, pH-sensitive swelling behavior, and sustained drug release supports their application in controlled-release formulations, particularly for drugs such as diclofenac sodium.

Following the investigation of the release profile of diclofenac from Cs/PEC microparticles, particular attention was given to a preliminary study focused on the encapsulation of ginger essential oil (GEO). Although this part of the work was not explored in depth, it represents an innovative and highly relevant research avenue. The encapsulation of

natural bioactive compounds such as GEO well known for its anti-inflammatory and antioxidant properties into polymeric matrices of natural origin holds great promise for the development of multifunctional therapeutic systems. The combination of a biocompatible polymer and a natural active compound aligns with the principles of green chemistry and controlled drug delivery. The successful encapsulation of such a volatile and sensitive compound may pave the way for novel biomedical applications, particularly in the design of synergistic systems offering sustained release and enhanced therapeutic efficacy.

IV.5. Formation of Cs/PEC/GEO Microparticles

The preparation of GEO loaded microparticles was based on a two step encapsulation strategy. First, ginger essential oil was emulsified into the chitosan solution using magnetic stirring, with the addition of a small amount of Tween 80 to improve emulsion stability. The resulting emulsion was visually stable and homogeneous, with no visible phase separation, indicating a good dispersion of the oil droplets within the chitosan matrix. This step is critical for ensuring uniform encapsulation, as the chitosan not only serves as the polymeric carrier but also acts as an emulsifying agent due to its cationic nature and viscosity.

The chitosan-oil emulsion was then added dropwise into a gelling bath containing pectin and TPP, where ionic crosslinking occurred rapidly. The droplets instantly solidified into well-formed spherical microparticles, with no apparent deformation or flattening. This indicates that the emulsion was sufficiently stabilized, and that the gelation process was fast and effective, allowing for the preservation of the spherical morphology. Visual observation confirmed the formation of smooth, round, and uniform particles.

After drying, the microparticles retained their integrity and shape. It was also noted that the characteristic aroma of ginger essential oil was markedly reduced, suggesting that the encapsulation system helped retain volatile compounds by minimizing their evaporation. This is consistent with the expected barrier effect provided by the dense polymeric network, which can offer protection against degradation and volatilization during drying and storage.

IV. 5.1. Encapsulation and In Vitro Release of Ginger Essential Oil (GEO)

Among the various microparticle formulations developed, two were selected for the encapsulation of ginger essential oil: CsP2 and CsP6. These formulations contain relatively higher concentrations of chitosan, which was expected to promote stronger matrix formation, improved oil entrapment, and better morphological stability. The increased polymer content

may also contribute to a denser internal structure, which helps to retain volatile and hydrophobic compounds such as essential oils.

The encapsulation efficiency (EE%) of GEO was calculated according to Equation (III.7) described in Chapter III, based on the difference between the initial amount of oil used and the amount detected in the supernatant after microparticle recovery. The concentration of non encapsulated GEO was quantified using UV-visible spectrophotometry in ethanol, following a calibration curve established and presented in Figure IV.8.

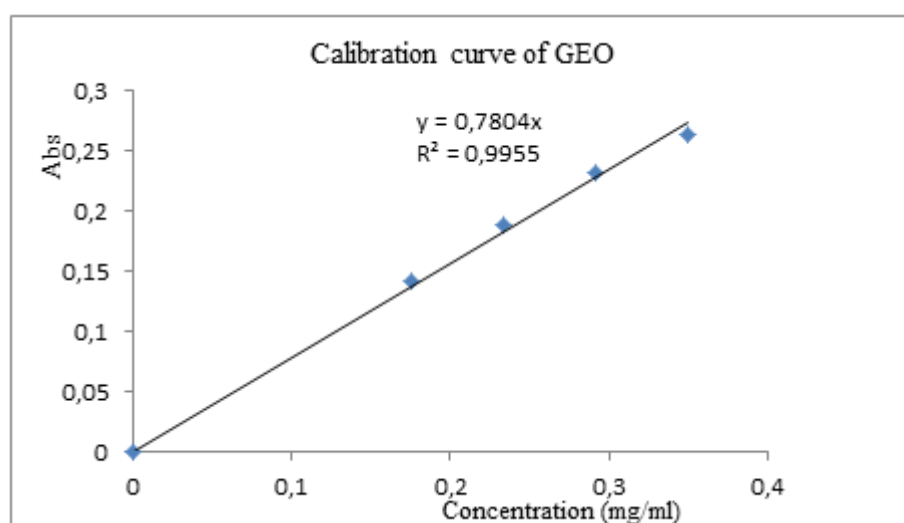


Figure IV.8. Calibration curve of GEO in ethanol ($\lambda = 285 \text{ nm}$)

Remarkably, the absorbance values of the recovered supernatants were found to be extremely low (close to 0.002 or 0.0012), indicating that almost all the oil was successfully entrapped within the microparticles. These values correspond to an encapsulation efficiency approaching 99% -100%, which is unusually high. While this result is surprising, it may be attributed to several factors, including the stability of the emulsion before gelation, the high viscosity and film-forming ability of the chitosan, and the rapid ionic crosslinking with the pectin/TPP system. The presence of Tween 80 likely contributed to the fine dispersion of GEO droplets, thereby facilitating efficient entrapment within the matrix.

Several recent studies have reported high encapsulation efficiencies for ginger essential oil using natural polymer based systems [135]. For instance, Goyal et al. (2020) achieved over 90% encapsulation of GEO using chitosan/alginate nanoparticles, highlighting the role of ionic gelation in stabilizing volatile compounds. Similarly, Nakhjavani et al. (2021) reported excellent encapsulation yields using nanoemulsified GEO within

biopolymeric carriers, attributing the high efficiency to the stabilization of oil droplets and the rapid solidification of the matrix [136].

These findings support the hypothesis that a chitosan based ionic gelation system can serve as an effective carrier for volatile bioactive compounds, such as essential oils. The observed encapsulation efficiency and sustained release behavior highlight the potential of this biopolymeric platform for the future development of bioactive loaded delivery systems designed for controlled release and protection against degradation and volatilization. This approach aligns with the principles of green chemistry and offers promising perspectives for biomedical and pharmaceutical applications involving sensitive natural actives.

These promising encapsulation results motivated further investigation into the release behavior of GEO from the polymeric microparticles. Understanding the *in vitro* release kinetics is essential to evaluate the system's potential for controlled delivery of bioactive compounds.

IV.5.6. In Vitro Release Profile of GEO

The release of ginger essential oil (GEO) from the Cs/PEC microparticles was evaluated under simulated intestinal conditions (pH 7.4) to assess the performance of the biopolymeric system in an aqueous environment. The release profile exhibited a gradual and sustained release, with cumulative release values ranging between 50% and 60% after 24 hours, depending on the formulation. This behavior reflects the ability of the chitosan /pectin matrix to retain the volatile oil and limit its rapid diffusion, thanks to its dense and crosslinked structure.

Such a release pattern is consistent with the hydrophobic nature of GEO, which slows down its migration through the hydrophilic polymer network. Additionally, the ionic gelation mechanism involving TPP and pectin contributes to the formation of a compact microparticle structure, which acts as a barrier to rapid oil diffusion. These factors collectively explain the incomplete release observed at 24 hours.

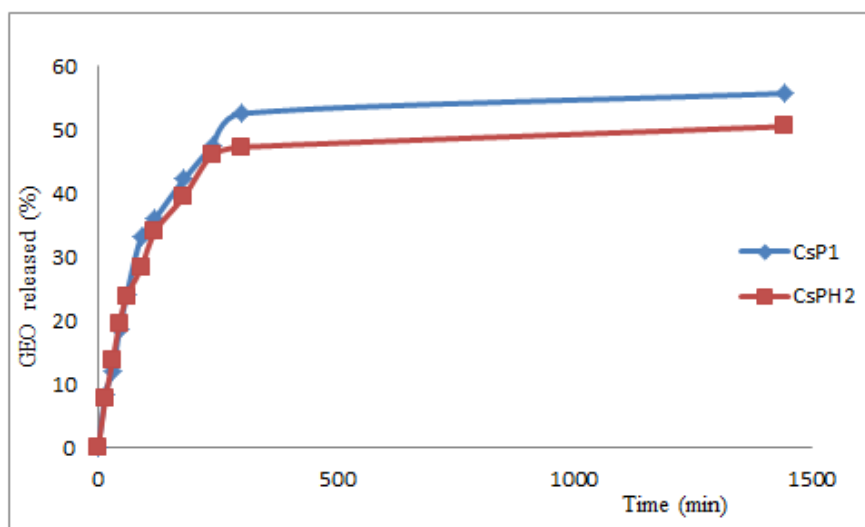


Figure IV.9. In vitro release kinetics of GEO from Mps in phosphate buffer solution (pH 7.4), for samples; CsPH1 and CsPH2

Importantly, this result is in agreement with several studies reported in the literature. For instance, Moghimi et al. (2021) observed approximately 55% release of GEO from chitosan–gelatin microcapsules after 24 h, while Azeem et al. (2022) reported a similar 60% release from alginate–pectin capsules under comparable conditions [137,138]. These findings confirm the reliability and reproducibility of our results, and further support the suitability of natural polymeric carriers for the controlled and prolonged release of sensitive and volatile bioactive compounds.

IV.6. Conclusion

The present experimental work demonstrated the successful formulation of polymeric microparticles based on chitosan (Cs) and pectin (PEC), ionically crosslinked with TPP, for the encapsulation and controlled release of two bioactive agents: diclofenac (DS), a model anti-inflammatory drug, and ginger essential oil (GEO), a natural compound with known antioxidant and anti-inflammatory properties.

Taken together, these findings support the potential application of natural polymer-based microparticles for the dual delivery of synthetic and natural anti-inflammatory agents, opening new avenues for multifunctional therapeutic systems based on green and biocompatible technologies.

General Conclusion

General Conclusion

This research work focused on the development and evaluation of polymeric microparticles based on chitosan (Cs) and pectin (PEC), ionically cross-linked with sodium tripolyphosphate (TPP), for the encapsulation and controlled release of two bioactive compounds with well known anti-inflammatory properties: diclofenac sodium (DS) and ginger essential oil (GEO).

During the initial stage, various formulations of Chitosan/pectin microparticles were successfully synthesized by ionotropic gelation, with diameters ranging from 280 to 610 μm . Their size and morphology were significantly influenced by the concentrations of chitosan and TPP, where higher polymer content increased particle size, while higher crosslinker concentration led to denser and more compact structures.

The microparticles exhibited a clear pH-responsive swelling behavior, evaluated at two key physiological pH levels relevant to oral delivery. At acidic pH 3.4 (simulating gastric conditions), a high swelling degree (500–700%) was observed due to the protonation of chitosan's amino groups. At intestinal pH 7.4 (PBS), swelling was more moderate (300–600%), reflecting stronger matrix compaction and ionic interactions. This dual pH-responsive behavior highlights the potential of these microparticles for controlled and targeted drug release in the gastrointestinal tract.

The encapsulation of diclofenac resulted in high encapsulation efficiencies and demonstrated a biphasic release profile. A limited release occurred in acidic medium, while a sustained and gradual release was observed at pH 7.4, confirming the gastro-resistant character of the system. These findings validate the potential of the Cs/PEC microparticles as efficient carriers for hydrophilic drugs intended for prolonged and site specific delivery.

A preliminary study was conducted on the encapsulation of GEO into Cs/PEC microparticles using ionotropic gelation. The resulting microparticles were smooth and spherical in shape, with a nearly complete encapsulation efficiency. In vitro release studies at pH 7.4 revealed a slow and partial release of GEO (around 50–60% over 24 hours), in line with previously reported data.

These findings demonstrate the ability of the Cs/PEC polymeric system to effectively encapsulate and protect volatile, sensitive compounds while enabling their controlled diffusion.

Overall, this study demonstrates the great potential of natural biopolymers as innovative materials for the encapsulation of both synthetic and natural active agents. The developed systems align with the principles of green chemistry and offer a promising platform for the design of biocompatible, multifunctional drug delivery systems with prospective biomedical and pharmaceutical applications.

This work provides a solid foundation for the future development of advanced polymeric carriers integrating natural materials and bioactive compounds for targeted and effective therapeutic deliver.

Perspectives

This work opens up promising perspectives for :

- Optimization of the GEO based system:

Evaluate the long term stability of GEO within the polymeric matrix.

Study the biodegradability of the carrier system under physiological conditions.

- Extension to other active compound:

Encapsulate other bioactive molecules, especially those that are: poorly soluble, unstable or sensitive to environmental conditions. Improve their stability, bioavailability, and controlled release profiles.

- Development of advanced delivery strategies:

Combine GEO with other active agents to achieve synergistic therapeutic effects.

Design targeted release systems, especially for: inflammatory diseases, or conditions related to oxidative stress.

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