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Preparation and characterization of polymer film for the release of drugs

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Dedication

First, I thank God for giving me the health, patience, willand for providing me with his blessing.

I dedicate this work to those who mean the most to me,

My father and mother who supported and encouraged me during my years of study.

My sisters Meriem, Karima, Houria, Saliha and Asmaa

My niece Kenza for her moral support,

My dear friend Imane for her presence and her encouragement.

Anyone who wises me happiness in this life.

Abstract

This work present a preparation and characterization of polymer films based on methylene blue, alginate, agar, starch, glycerol and citric acid for the local treatment of wounds.

In the first part we addresse traditional and current wound and treatment issues, highlighting challenges in chronic wound treatment and advanced film systems for wound treatment.

In the second part we intrdoduce polymer-based pharmaceutical films with methylene blue as effective alternatives for administering wound treatment.

In the result we have films formation confirmed by IR spectroscopy(C=O, 1602cm^{-1}), the crosslinking percentage (27⁄ for film 1, 80⁄for film2). For mthylene blue release, results indicate the controlled release with (8.9 10^{-3} mg/ml) at (240 min) and (8.6 10^{-3} mg/ml) at (150 min) for film1 and film2 respectively.

Keywords: Film, Polymer, Methylene blue, Wounds.

Résumé

Ce travail présente la préparation et la caractérisation de films polymères à base de bleu de méthylène, d'alginate, d'agar, d'amidon, de glycérine et d'acide citrique pour le traitement local des plaies.

Dans la première partie, nous abordons les problématiques traditionnelles et actuelles liées aux plaies et à leur traitement, en mettant en lumière les défis du traitement des plaies chroniques et les systèmes de films avancés pour le traitement des plaies.

Dans la deuxième partie, nous présentons des films pharmaceutiques à base de polymères avec du bleu de méthylène comme alternatives efficaces pour l'administration du traitement des plaies.

Dans le résultat nous avons la formation de films confirmée par spectroscopie IR (C=O, 1602cm^{-1}), le pourcentage de réticulation (27⁄ pour le film 1et 80⁄ pour film2). Pour la libération de bleu de méthylène, les résultats indiquent la libération contrôlée avec (8,9 *10^{-3} mg/ml) à (240 min) et (8,6 *10^{-3} mg/ml) à (150 min) respectivement pour film1 et film2.

Mots-clés : Film, Polymère, Bleu de méthylène, Plaies.

ملخص

يقدم هذا العمل إعدادًا وتوصيفًا لأفلام البوليمر استنادًا إلى الأزرق الميثيلين، الألجينات، الأغار، النشا، الجلسرين، وحمض الستريك للعلاج المحلي للجروح

في الجزء الأول، نتناول قضايا الجروح التقليدية والحالية وطرق العلاج، مسلطين الضوء على التحديات في علاج الجروح المزمنة وأنظمة الأفلام المتقدمة لعلاج الجروح

في الجزء الثاني، نقدم أفلامًا صيدلانية قائمة على البوليمر مع الأزرق الميثيلين كبدائل فعّالة لإدارة علاج الجروح

في النتيجة ، لدينا تشكيل أفلام أكده التحليل الطيفي (الاشعة تحت الحمراء ($1602\ cm^{-1}$، C = O)،

نسبة التشابك (للفيلم 1 ٪27و للفيلم 2 ٪80). بالنسبة لإطلاق المثيلين الأزرق، تشير النتائج إلى الإطلاق المتحكم به (8.9 10^{-3} ملغم/مل) عند (240 دقيقة) و (8.6 3-10 ملغم/مل) عند (150 دقيقة) للفيلم 1 وفيلم 2 على التوالي.

الكلمات الرئيسية: أفلام، بوليمر، الأزرق الميثيلين، الجروح

Summary

CHAPTER II: Pharmaceutical films based on polymers

CHAPTER III: Expérimental study

CHAPTER IV: Results and discussion

AFM	Force electron microscopy Atomic
AIBN	2,2-azobis(isobutyronitrile)
AV	*Aloe vera*
BCP	Biodegradable and compostable polymers
BDPs	Biodegradable polymers
DEA	Differential enthalpy analysis
DM	Dry matter
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DP	Degree of polymerization
ECM	Extracellular matrix
EGF	Epidermal growth factor
FFS	Film forming system
G6PD	Glucose-6-phosphate dehydrogenase
MB	Methylene Blue
MMA	Methyl methacrylate
MSCs	Mesenchymal stem cells
PDGF	Platelet-derived growth factor
PHBV	Poly hydroxybutyrate co vel
PMMA	Poly(methyl methacrylate)
PVA	Poly(vinyl alcohol)
PVC	Polyvinyl chloride
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
SM	Sesamol
TEM	Transmission electron microscopy
TG	The gelatinization temperature
TGF-β	Transforming growth factor-β
XRD	X-ray diffraction

Chapter I : Antiseptics for wounds treatments

Chapter II: Pharmaceutical films based on polymers

Chapter III: Experimental study

Chapter IV: Results and discussion

Chapter I : Antiseptics for wounds treatments

Chapter II: Pharmaceutical films based on polymers

Chapter III: Experimental study

GENERAL INTRODUCTION

General Introduction

Transdermal drug delivery systems (TDDS) have gained significant attention in recent years as an alternative to oral and parenteral routes of administration. TDDS offer several advantages such as improved patient compliance, reduced side effects, and avoidance of first-pass metabolism. Among the various TDDS, transdermal films have emerged as a promising approach for controlled and targeted delivery of drugs to the skin.Transdermal films are thin, flexible, and transparent polymeric formulations designed to be applied onto the skin surface. They form an intact film that acts as a physical barrier and enables sustained drug release. Transdermal films can be prepared using a variety of polymers, plasticizers, and penetration enhancers to optimize drug permeation and film properties [1]. Compared to conventional topical formulations, transdermal films provide several benefits, including:

Improved drug permeation and bioavailability;

Reduced dosing frequency and improved patient compliance;

Avoidance of hepatic first-pass metabolism;

Reduced gastrointestinal side effects associated with oral administration;

Ability to deliver drugs with short biological half-lives;

Transdermal films have been developed for various therapeutic categories, such as antiemetics, antidepressants, antifungals, and anti-inflammatory drugs. These films have shown promising results in terms of drug release kinetics, skin permeation, and therapeutic efficacy [2].

The main objective of this work is to develop and characterize films based on methylene blue for the local treatment of wounds.

Chapter 1 addresses the issues of wounds and traditional and current treatments, highlighting challenges in chronic wounds treatment and advanced film systems for wound treatment. *Chapter 2* explores pharmaceutical films based on polymers with methylene blue as effective alternatives for wound treatment administration. *Chapter 3* focuses on the materials and experimental procedures used to develop new treatments. Finally, *Chapter 4* encompasses the discussion of the obtained results, essential for evaluating the effectiveness of treatments and guiding future research in the field of wounds.

CHAPTER I

Antiseptics for wounds treatments

I-1-Introduction

Skin is considered as an important route of administration of drugs for both local and systemic effects. The effectiveness of topical therapy depends on the physicochemical properties of the drug and adherence of the patient to the treatment regimen as well as the system's ability to adhere to skin during the therapy so as to promote drug penetration through the skin barrier. Conventional formulations for topical and dermatological administration of drugs have certain limitations like poor adherence to skin, poor permeability and compromised patient compliance. For the treatment of diseases of body tissues and wounds, the drug has to be maintained at the site of treatment for an effective period of time [3].

I-2-Wounds

I-2-1-Definition

A wound is a type of injury that damages the skin or underlying tissues, often resulting from physical trauma or surgical incisions. Wounds can vary in severity, from minor cuts and scrapes to more serious injuries such as puncture wounds, lacerations, or deep tissue damage. Wounds can be classified based on their cause, depth, size, and whether they are open or closed. They can also be categorized as acute (healing within a predictable timeframe) or chronic (taking longer to heal or recurring). Proper wound care is essential to prevent infection, promote healing, and minimize scarring [4].

I-2-2-Types of Wounds

Wounds can be classified in several ways depending on the healing time and the necessity to consult with Wound Care Specialists depending on the severity of a particular wound. People are likely to suffer from different types of wounds throughout life while performing daily activities. Depending on the cause, site, and depth, a wound can lead from simple to severe one. Here, we have explained different types of wounds. Let's have a look:

I-2-2-1-Open or Closed

Wounds can be open or closed. Open wounds are the wounds with exposed underlying tissue/ organs and open to the outside environment, for example, penetrating wounds. On the other hand, closed wounds are the wounds that occur without any exposure to the underlying tissue and organs.

I-2-2-2-Acute or Chronic

A wound can be classified as acute or chronic depending on the healing time. Acute wounds are those that heal without any complications in a predicted amount of time. While chronic wounds, on the other hand, are those that take a relatively long time to heal with some complications.

- **Types of Chronic Wounds**

1. Pressure Injuries

Also known as bedsores, pressure sores, or decubitus ulcers, these wounds cause when there is a pressure and/or shearing force on the skin. The people who are more prone to these chronic wounds are with limited mobility due to any medical illness or unable to walk, move all or part of their body to a different position.

2. Diabetic Ulcers

These ulcers generally occur on the feet and are a result of changes to nerves and circulation in the body caused by diabetes. It includes Neuropathic, Ischaemic, and Neuro-ischaemic.

I-2-2-3-Clean or Contaminated

Wounds can also be classified on the basis if they are clean or contaminated. Clean wounds are those that do not have any foreign material or debris inside whereas contaminated wounds or infected wounds are those that might have some dirt, bacteria, or other foreign markets. Pressure wounds can be used as an example of an open or closed wound depending on its current stage.

I-2-2-4 Internal or External

Wounds can also be internal or external. Internal wounds can be due to impaired circulation, nervous system functions, neuropathy or medical illness, or decreased supply of blood, oxygen, or other nutrients while the external wounds can be due to an outside force or trauma caused by penetrating objects or non-penetrating trauma.

I-2-2-5-Non-penetrating Wounds

These wounds are the result of blunt trauma or friction with other surfaces. It includes:

- Abrasions

- Lacerations
- Bruises
- Concussions

I-2-2-6-Penetrating Wounds: They are the result of trauma and break through the full thickness of the skin. It includes:

- Stab wounds
- Cuts
- Surgical wounds etc [5].

I-3-Overview of the wound-healing process

Wound healing is a complex process that occurs in almost all tissues after damage, aiming at repairing a lost or injured tissue. The first phase of the healing process, the hemostasis, starts immediately after injury and aims to control the bleeding and to limit the spread of microorganisms within the body. Hemostasis involves several events, such as vascular constriction, platelet aggregation, and fibrin clot formation, with subsequent development of a scab that provides strength, protection, and support to the damaged tissue. [7-9] During this process, platelets release several growth factors, including the transforming growth factor-β (TGF-β), epidermal growth factor (EGF), insulin-like growth factor-1, and platelet-derived growth factor (PDGF), which are responsible for the activation of fibroblasts, endothelial cells, and macrophages in the surrounding environment [10,11]. The inflammatory phase, occurring simultaneously with the hemostasis, is characterized by the release of several proinflammatory cytokines, cationic peptides, proteases, reactive oxygen species, and growth factors, allowing the wound cleaning. [12,10] Growth factors like TGF-β, PDGF, fibroblast growth factor, and EGF play an important role in the communication between cells and their ECM, stimulating cell recruitment, proliferation, morphogenesis, and differentiation. [9, 11] After bleeding, the healing process involves the migration and infiltration of inflammatory cells into the wound. At this phase, neutrophils, macrophages, and lymphocytes are responsible for multiple functions, including the promotion of the inflammatory response, inhibition of the penetration of exogenous microorganisms, elimination of microbes, and stimulation of keratinocytes, fibroblasts, and angiogenesis [9]. Once the bleeding and inflammation are controlled, epithelial cells and fibroblasts migrate to the damaged region, supporting capillary growth, collagen synthesis, and new tissue formation. At this stage, epithelial cells replace dead cells, while fibroblasts are responsible for the production of

collagen, fibronectin, hyaluronan, glycosaminoglycans, and proteoglycans, which are the major constituents of the ECM and confer strength to the skin [12,7,11]. A granulation tissue is produced as a result of the growth of capillaries and lymphatic vessels from existing vessels present at the site of injury (neovascularization). Finally, in the maturation or remodeling phase, the new tissue is continuously remodeled until its composition and properties are close to those of the healthy tissue [9]. The ultimate goal of the wound-healing process is the regeneration of the injured skin without scar formation [6].

I-4-Wounds treatment conventionnel

Although the human skin has a natural ability to promote the self-regeneration after damage, this capacity can be compromised under specific conditions, like extensive skin loss, deep burns, chronic wounds, nonhealing ulcers, and diabetes [9,13]. In the next sections, the most commonly used traditional therapies for skin wound healing are described and the scientific evidence of their use is discussed. An inappropriate healing process can lead the wound to enter in a chronic state, which increases the risk of infection and affects the patient health and his/her quality of life (Figure I-1).

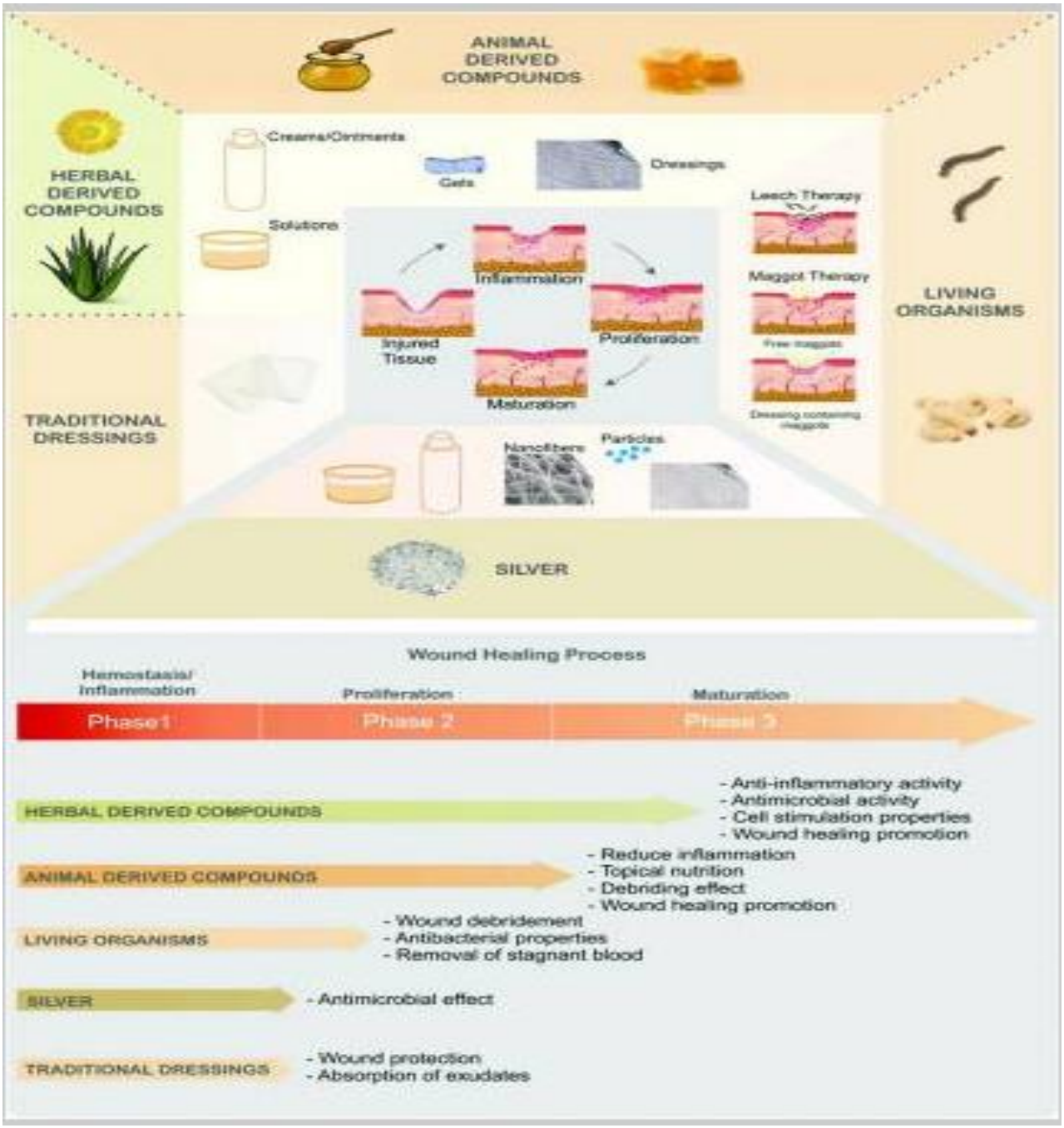


Figure I-1: Classification of traditional therapies for skin wound healing [6].

Traditional therapies and compounds are used in different phases of the healing process in a great variety of physical forms, either commercially available or under investigation, stimulating the skin regeneration process [6].

I-4-1-Herbal-derived compounds

Herbal-derived compounds are the most commonly used traditional therapies for the treatment of skin lesions. They include the application of herbs, herbal preparations, and finished herbal products, containing biologically active compounds that stimulate the healing process. Today, a great variety of plants, native from different regions of the world, are investigated and used for the treatment of skin lesions [13-15]. Herbal-based products are applied as extracts, emulsions, creams, and ointments, being commonly administrated through topical, systemic, and oral routes. Table 1 presents an overview of some plants under investigation for wound-healing applications [16-18].

Table I-1: Examples of some plants currently investigated for wound-healing applications [6]

Herb	***Main Constituents***	***Physical Forms and Administration Routes***	***Laboratorial and Clinical Evidence***	***References***
Aloe vera	Soluble sugars, nonstarch polysaccharides, lignin, polysaccharides, glycoproteins, and antiseptic agents	Forms: solutions, creams, mucilage, gels, and dressings Routes: topical and oral	Anti-inflammatory and antimicrobial activities; stimulate cell proliferation, collagen synthesis and angiogenesis; promote wound contraction	35, 36, 20–22
Hippophae rhamnoides (sea buckthorn)	Flavonoids (*e.g.*, quercetin, isorhamnetin), carotenoids (*e.g.*, α-, β-carotene, lycopene), vitamins (C, E, K), tannins, organic acids, triterpenes, glycerides of palmitic, stearic, oleic acids and, amino acids	Forms: aqueous leaf extract, seed oil Routes: topical and oral	Antioxidant and anti-inflammatory activities; stimulate the healing process; improve wound contraction and epithelialization; increase the hydroxyproline and protein content in the wound	23, 24
Angelica sinensis	Essential oils and water-soluble	Forms: ethanol extracts,	Stimulate the proliferation of human skin fibroblasts, the secretion of collagen, and	25

	ingredients; ferulic acid is the main active constituent	ferulic acid dissolved in DMSO Routes: n.a. (*in vitro* tests)	the expression of TGF-β in *in vitro* conditions	
Catharanthus roseus (*Vinca rosea*)	Contain two major classes of active compounds: alkaloids (*e.g.*, vincamine) and tannins	Forms: leaf ethanol extract Routes: topical	Antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococ cus aureus*; increase wound strength, epithelialization, and wound contraction	26
Calendula officinalis (marigold)	Triterpenoids and flavonoids	Forms: gels, aqueous extracts, hexane, and ethanolic extracts dissolved in DMSO Routes: topical	Anti-inflammatory and antibacterial activities; stimulate the proliferation and migration of fibroblasts *in vitro*; stimulate the collagen production and angiogenesis	26, 27, 28
Sesamum indicum	SM is the main antioxidant constituent, others include sesamolin and sesaminol	Forms: SM (purity>98%) and SM containing dexamethasoe Routes: intraperitonel and intramuscular routes	Improve the wound tensile strength, wound contraction, and the hydroxyproline levels in both normal and delayed wound models in rats	29
Morinda citrifolia (non)	Acids, alcohols, phenols, esters, anthraquinones, sterols, flavonoids, triterpenoids, saccharides, carotenoids, esters, ketones, lactones, lignans, and nucleosides	Forms: ethanol extract of plant leaves mixed with water Routes: oral	Improve the hydroxyproline content and reduce both the wound area and the epithelialization time in excision wounds in rats	30
Camellia sinensis	Polyphenols, flavonoids, tannins, caffeine, and amino acids	Forms: pure vaseline and ethanolic plant extract (0.6%) ointment	Reduce the healing time and the wound length of incision wounds created in Wistar rats	31,32

		Routes: topical		
Rosmarinus officinalis L. (rosemary)	Most bioactive constituents include terpenoids and polyphenols, such as carnosol, carnosic acid, and rosmarinic acid	Forms: aqueous extract and essential oil Routes: topical and intraperitoneal injection	Reduce the inflammation and improve the wound contraction, re-epithelialization, angiogenesis, and collagen deposition on full-thickness wounds in diabetic mice	33

I-4-2-Aloe vera

Aloe vera (AV), a popular herb in wound healing, is derived from the processing of fresh plant leaves, yielding two main products: aloe juice and gel. The gel is composed of biologically active compounds, including sugars, polysaccharides, vitamins, and enzymes, which exhibit anti-inflammatory, antiseptic, and antimicrobial properties. The gel stimulates fibroblast proliferation, collagen synthesis, and angiogenesis, and is used topically to treat skin lesions. In vivo trials confirm its effectiveness in wound healing by increasing collagen synthesis, growth factor expression, and fibroblast proliferation. Randomized clinical trials have shown that aloe vera gel accelerates wound healing and reduces healing time. Aloe vera gel has also been combined with natural polymers to produce blend films for wound-healing applications, which exhibit improved transparency, hydrophilicity, and degradation rate. These films can promote an optimized healing process and release aloe vera compounds directly to the wound site [37, 38] (Figure I- 2).

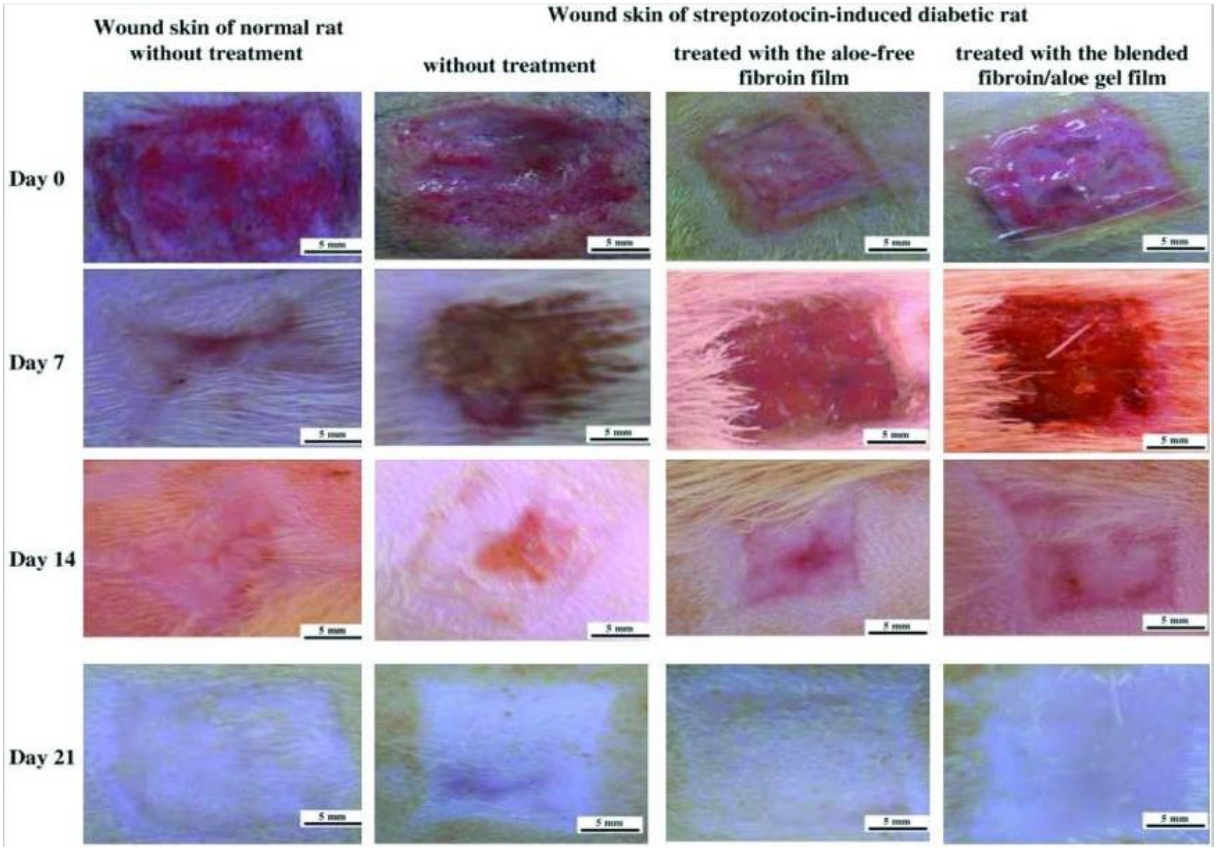


Figure I-2: Influence of fibroin/aloe gel film dressings on the wound healing of normal rat and streptozotocin-induced diabetic rat [10.6]

Topical and oral AV preparations are considered safe, but some patients experience adverse reactions. Topical preparations can cause skin itching, irritation, and photodermatitis, while oral administration may lead to diarrhea and vomiting. Clinical evidence demonstrates AV's ability to stimulate healing, but many studies have methodological flaws. There's a need for high-level evidence and large, randomized control trials to support AV-derived products for skin lesions. AV's physicochemical properties vary depending on species, climate, and processing, making standardization and quality control crucial [39].

I-4-3-Calendula officinalis

Calendula officinalis, also known as marigold, has been used for centuries to treat skin conditions due to its anti-inflammatory and antibacterial properties. Its chemical composition includes phenolic compounds, steroids, and terpenoids. The herb has been shown to promote wound healing by reducing epithelialization time, increasing collagen content, and stimulating angiogenesis. Topical application of Calendula officinalis gel improved collagen production in wound incisions in rats. Clinical trials have also demonstrated its effectiveness in treating ulcers and acute dermatitis during breast cancer irradiation. However, its mechanisms of

action and side effects are not yet fully understood, requiring further research to support its clinical application [40].

I-4-4-Animal-derived products

Animal-origin products, like honey and propolis, have been used in wound care since ancient times due to their therapeutic properties. Honey has been applied as a natural bioactive dressing material that fills and covers either superficial or deep wounds, providing a moist environment and topical nutrition. Propolis has also been employed as a result of its antioxidant, anti-inflammatory, and antibacterial properties. Frog skin and its secretions have also been explored in traditional medicine as ointment or temporary dressing that cover the wound, preventing the penetration of pathogens and the dehydration [41, 34]

I-4-5-Honey

Honey is a highly viscous and superconcentrated acidic sugar solution derived from nectar gathered and modified by honeybees. It has various therapeutic properties, including antibacterial, anti-inflammatory, and wound-healing abilities. The antibacterial activity of honey is attributed to its high sugar concentration, acidity, low water content, and presence of antimicrobial substances. Honey-based products have been shown to accelerate the healing process in diabetic ulcers, malignant wounds, and burns compared to traditional dressings. Clinical trials have demonstrated the effectiveness of honey in promoting wound healing, reducing scar formation, and inhibiting bacterial growth. While honey treatment is considered safe, it may result in itching and pain at the wound site due to its acidic nature. Further research is needed to fully understand the healing efficacy of honey in different types of wounds [42].

I-4-6-Propolis

Propolis is a substance collected by honeybees from trees and has been used in folk medicine due to its various biological properties and low toxicity. It contains polyphenols, flavonoids, and other compounds that contribute to its therapeutic activities, such as antimicrobial, antioxidative, anti-inflammatory, and healing properties. Propolis has been found to have anti-inflammatory effects by inhibiting the production of nitric oxide and can also exhibit antibacterial activity against a broad spectrum of bacteria. It can synergize with synthetic antibiotics to enhance their antimicrobial effects. In terms of healing properties, propolis has been shown to promote wound healing by stimulating collagen synthesis and

reducing inflammation. Clinical trials have demonstrated the beneficial effects of propolis in treating burns and chronic nonhealing venous leg ulcers. However, further research is needed to improve the manufacturing and quality control of propolis products, determine therapeutic levels and cytotoxic concentrations, and investigate possible side effects such as contact dermatitis [43].

I-4-7-Living organisms

The interest in the use of living organisms for wound healing has been significantly increasing in last years, providing alternative approaches for skin repair. Maggots have a remarkable antimicrobial activity and ability to stimulate the wound debridement, while leeches are very useful in the treatment of venously congested wounds.

I-4-8- Maggot debridement therapy

The use of fly larvae in wound care, known as maggot debridement therapy, is growing due to its effectiveness, safety, and simplicity. Medicinal maggots help clean wounds by digesting and removing dead tissue, as well as decomposing organic matter and pathogens. Maggot therapy is used in chronic wounds that have not healed with other treatments. Maggots can be applied directly to the wound or in bags or dressings that allow their secretions to reach the wound. The number of maggots used depends on their size and the size of the wound, with an average of 5-10 maggots per square centimeter of wound area (Figure I-3).

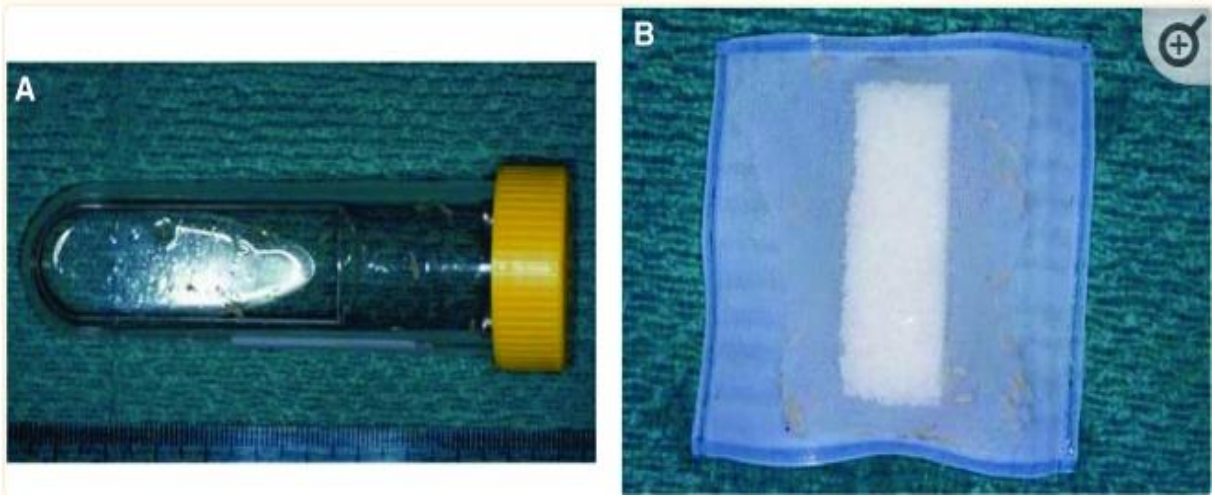


Figure I-3: (A) Free maggots suspended in isotonic saline solution before application onto the wound. **(B)** Biobag that contains maggots inside and a sponge to prevent the net to collapse [6]

Maggot therapy has gained attention for its therapeutic effects in modern wound care. Medicinal maggots provide wound debridement, inhibit biofilm formation, exhibit antimicrobial activity, and stimulate the healing process. Their proteolytic enzymes break down necrotic tissue, solubilize fibrin clots, and degrade extracellular matrix molecules, facilitating digestion and healing. Maggots also eliminate bacteria, including antibiotic-resistant strains, through bacterial ingestion, digestion, and secretion of natural bactericidal agents. The therapy enhances tissue oxygenation, fibroblast proliferation, angiogenesis, and granulation tissue formation. Studies have identified biologically active constituents in maggot secretions, such as amino acids, that stimulate endothelial cell growth and angiogenesis [44] (Figure I-4).

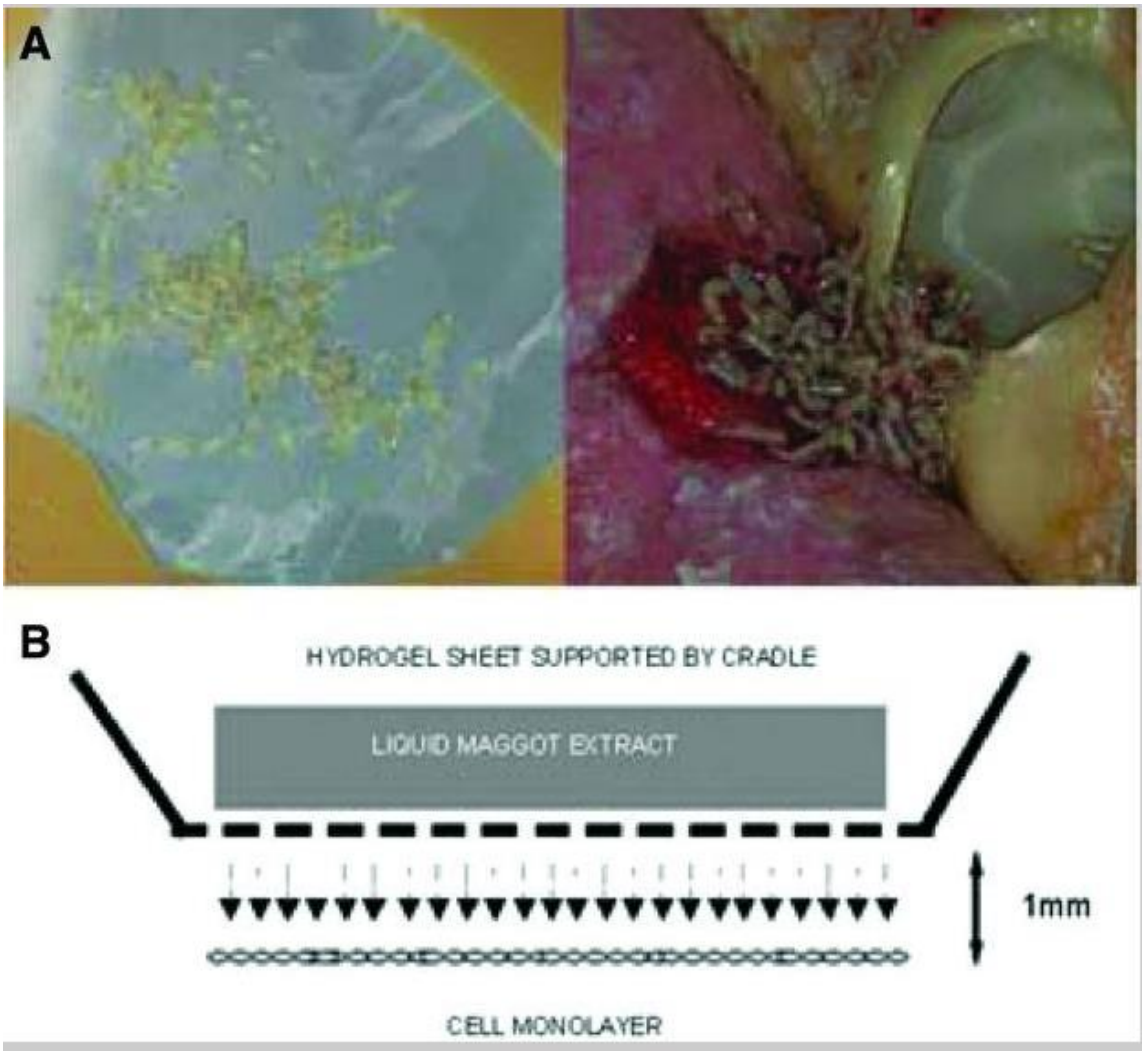


Figure I- 4: (A) Maggots before the application into a chronic wound, and maggots in direct contact with the wound at the end of the treatment, during the removal, **(B)** The experimental scheme used to test the effect of the delivery of maggot extract from a hydrogel wound dressing onto model wounds in monolayer cell culture [6, 45].

I-4-9-Leech therapy

Leech therapy, also known as hirudotherapy, is an alternative treatment for various skin disorders. Medicinal leeches inject compounds like anticoagulants, anesthetics, and analgesics into the wound, promoting healing. Hirudin, the main constituent of leech saliva, inhibits blood coagulation and acts as a bacteriostatic agent. Leech therapy has been used to remove stagnant blood from wounds and restore blood flow, with an overall success rate of 77.98% in a systematic review. Complications include bacterial infections, bleeding, and itching. Further studies are needed to establish standardized treatment protocols [46].

I-4-10- Silver and traditional dressings

Silver is a broad-spectrum antimicrobial agent used in skin lesion treatment, particularly wounds and burns. It's widely available in various products, including solutions, creams, gauze dressings, foams, and dressings. Silver-based products show excellent antimicrobial properties against microorganisms, but their use is limited by cytotoxic effects in mammalian cells. Cytotoxic effects are dose-dependent and vary according to silver ion concentration and dressing type. Additionally, silver may delay wound-healing processes. Alternative formulations, such as hydrogel dressings, nanoparticles, and nanofibers, have been developed to overcome these limitations, delivering low concentrations of silver ions to avoid toxic effects and promote healing [47] (Figure I-5).

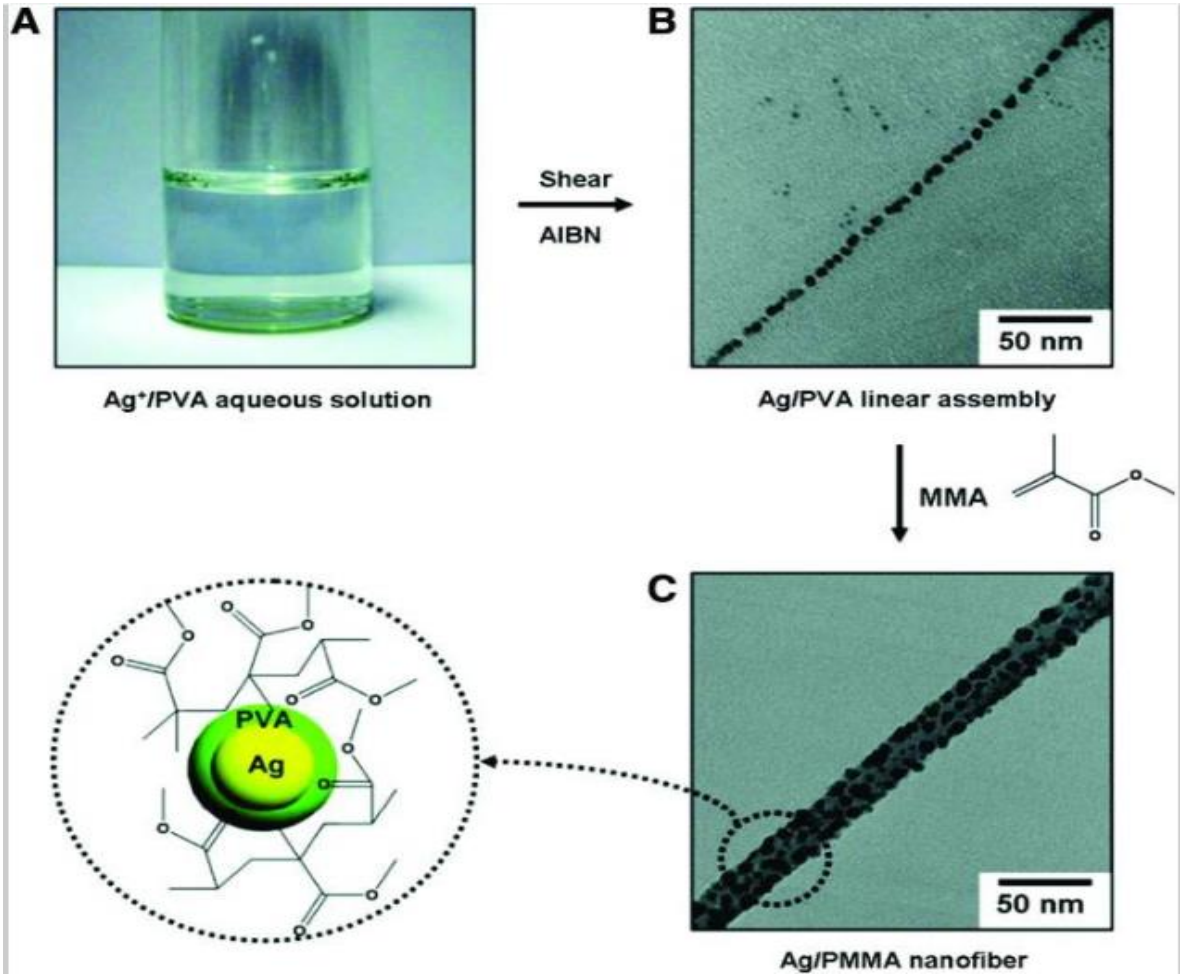


Figure I-5: Processing steps in the fabrication of PMMA nanofibers that contain silver nanoparticles through radical-mediated dispersion polymerization. Macroscopic image of Ag^+/PVA aqueous solution **(A)** and transmission electron microscopy images of Ag/PVA linear assembly **(B)** and Ag/PMMA nanofiber **(C)** [48].

Laboratorial studies showed successful regeneration of skin wounds using silver-containing materials. Silver nanoparticles increased fibroblasts, reduced inflammation, and promoted wound healing. Silver-based products demonstrated efficacy in treating various skin injuries, with benefits including accelerated healing, pain reduction, and easy removal. However, potential side effects include local maceration, cytotoxicity, bacterial resistance, and organ toxicity. Traditional dressings, such as gauzes and hydrocolloid, have limitations, including drying out the wound bed and causing trauma upon removal, prompting their use as secondary dressings or combined with other products [6].

I-5-Challenges in chronic wounds treatment

Chronic wounds are defined as wounds that fail to proceed through the normal phases of wound healing in an orderly and timely manner. Often, chronic wounds stall in the inflammation phase of healing. Despite differences in etiology at the molecular level, chronic wounds share certain common features, including excessive levels of proinflammatory cytokines, proteases, ROS, and senescent cells, as well as the existence of persistent infection, and a deficiency of stem cells that are often also dysfunctional due to repeated tissue injury, microorganisms and platelet-derived factors, such as transforming growth factor-β (TGF-β) or ECM fragment molecules, stimulate the constant influx of immune cells; the proinflammatory cytokine cascade therefore becomes amplified and persists for a prolonged time, leading to elevated levels of proteases. In acute wounds, proteases are tightly regulated by their inhibitors. In chronic wounds, protease levels exceed that of their respective inhibitors, leading to destruction of ECM and degradation of growth factors and their receptors. The proteolytic destruction of ECM not only prevents the wound from moving forward into the proliferative phase but also attracts more inflammatory cells, thus amplifying the inflammation cycle [49].

Immune cells produce ROS, which in low concentrations provides defense against microorganisms. In chronic wounds, however, the predominant hypoxic and inflammatory environment increases ROS production, which damages ECM proteins and causes cell damage. This sequence of events leads to an enhanced stimulation of proteases and inflammatory cytokines [50]. It has been suggested in an animal model that application of strong antioxidants reduces ROS to normal levels, which results in the reverse of the chronicity of wounds and improves healing [51].

Furthermore, chronic wounds are characterized by senescent cell populations with impaired proliferative and secretory capacities, rendering them unresponsive to typical wound healing signals [52]. It has been reported that fibroblasts from venous and PUs are senescent and have a diminished ability to proliferate. This diminished proliferative capacity is directly correlated with the failure of a wound to heal [53,54]. Accumulated data also indicate that chronic wounds contain senescent keratinocytes, endothelial cells, fibroblasts, and macrophages [55,56]. The senescent phenotype of cells in chronic wounds is attributed to oxidative stress that leads to DNA damage-related cell cycle arrest or to abnormal metabolic changes in diabetic patients, which results in defects in intracellular biochemical pathways such as the GSK-3β/Fyn/Nrf2 pathway [57, 58].

In recent years, mesenchymal stem cells (MSCs) have been shown to play an important role in wound healing . These cells can be recruited into the circulation in response

to injury. Subsequently, they are found to engraft into the remodeling microvasculature. Nonetheless, it has also been shown that stem cells in animals and patients with diabetes or chronic wounds are both deficient and defective. [59-60] Thus, these patients may require a direct delivery of healthy donor-derived functional MSCs to overcome this deficiency and achieve wound healing [61].

Nonhealing ulcers and wounds represent a failure to achieve complete reepithelialization in the appropriate temporal sequence of tissue repair [62]. Understanding the underlying molecular and physiologic perturbations of nonhealing wounds, one can appreciate the necessity to modify these wounds toward the characteristics of an acute healing wound. The need to restore the proper balance of cytokines, growth factors, proteases, and metabolically competent cells is illustrated in (Figure I-6) [63, 52].

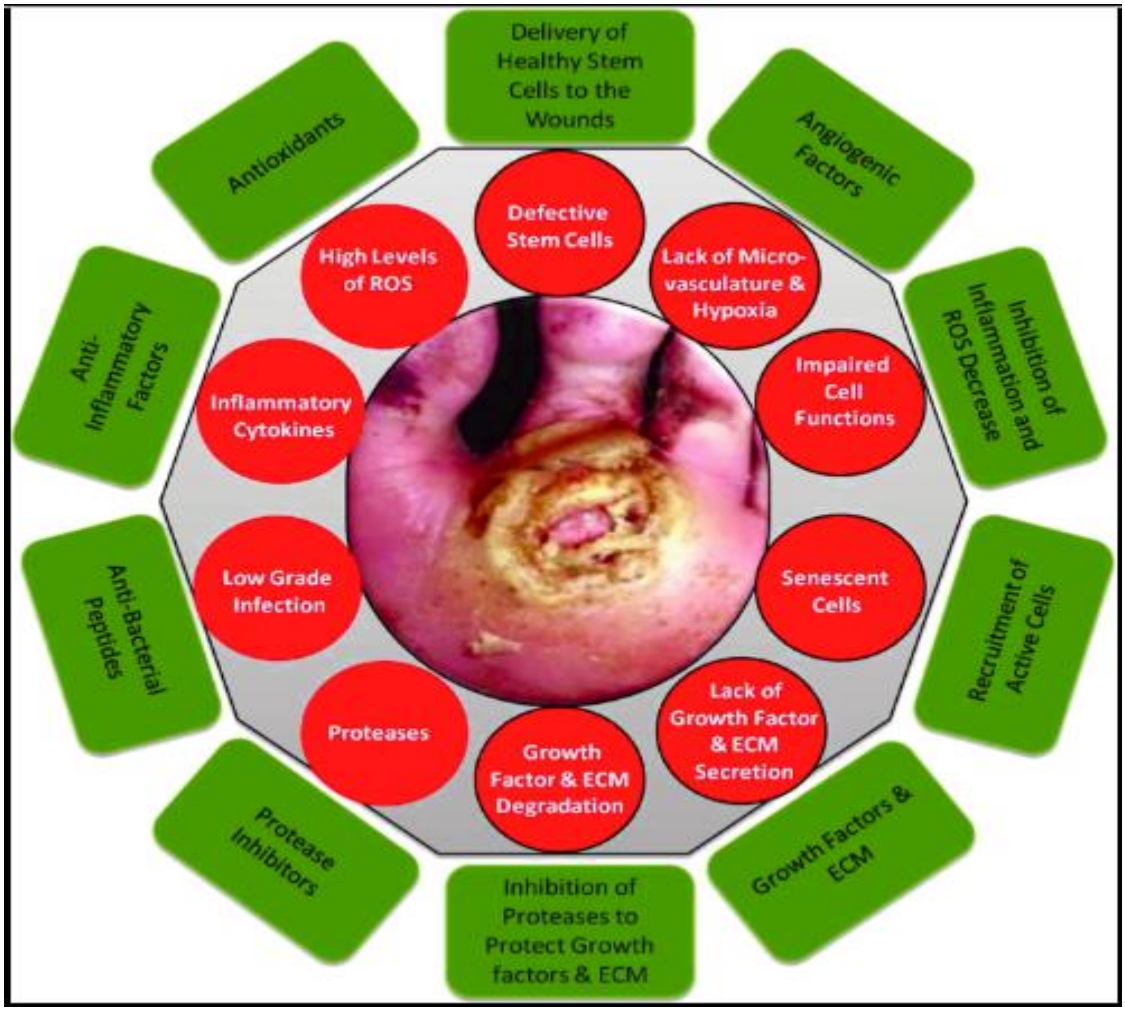


Figure I-6: Molecular and cellular deficiencies in chronic wounds (*red circles*) and factors required to overcome them (*green rectangles*) [63].

Nonhealing ulcers and wounds represent a failure to achieve complete reepithelialization in the appropriate temporal sequence of tissue repair. Such wounds are characterized by excessive inflammation (including elevated levels of proteases, ROS, and inflammatory cytokines), by senescent cell populations with impaired proliferative and secretory capacities, and by defective MSCs. Excessive inflammation leads to degradation of newly synthesized growth factors and ECM. There is a need to restore the proper balance of cytokines, growth factors, and proteases, to recruit functional cells (epithelial cells, fibroblasts, and endothelial cells) to the wound area, and to deliver healthy functional MSCs directly to the wound to compensate for the patient's own dysfunctional stem cells. ECM, extracellular matrix; MSCs, mesenchymal stem cells; ROS, reactive oxygen species [63].

I-6-Advanced film systems for wounds treatment

Film forming system (FFS) is a novel approach which can be used as an alternative to conventional topical and transdermal formulations. It is defined as non-solid dosage form that produces a film in situ, i.e. after application on the skin or any other body surface. These systems contain the drug and film forming excipients in a vehicle which, upon contact with the skin, leaves behind a film of excipients along with the drug upon solvent evaporation. The formed film can either be a solid polymeric material that acts as matrix for sustained release of drug to the skin or a residual liquid film which is rapidly absorbed in the stratum corneum.

After application of the formulation to the skin, the composition of the film forming system changes significantly due to the loss of the volatile components of the vehicle which results in formation of residual film on the skin surface. In this process the concentration of drug increases, reaching saturation level and with the possibility of reaching supersaturation level on the skin surface. Supersaturation results in the enhanced drug flux through the skin by increasing the thermodynamic activity of the formulation without affecting the skin’s barrier, thereby reducing the side effects or irritation [64, 65].

Film forming system is applied directly to the skin and it forms a thin, transparent film in situ upon solvent evaporation as shown in (FigureI-**7)** [3].

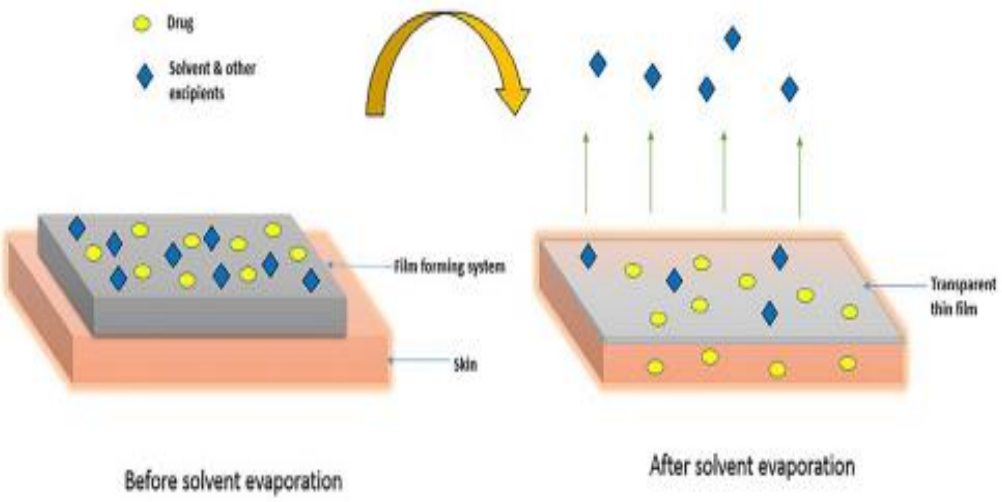


Figure I-7: Mechanism of film formation [3]

CHAPTER II

Pharmaceutical films based on polymers

II-Healing With Methylene Blue

II-1-Introduction

Numerous factors may alter wound healing a normally ordered process mediated by overlapping and interconnected biochemical interactions and cells. When healing stalls, one of the wound bed preparation players is off-balance. Whether the problem is compromised tissue needing debridement, infection (from colonization to actual infection), moisture balance, or an unhealthy wound edge, the cause(s) have to be addressed. Bacterial colonization and infection always come to mind (especially in immunocompromised populations), but other factors to consider include ischemia, lack of extracellular matrix, poor nutrition (eg, compromised protein and essential nutrient uptake that commonly affect premature neonates and chronically challenged, oncologically affected immunocompromised children), medication that interferes with healing, biologic inflammatory milieu imbalance, edema, and moisture imbalance.

In this these I discuss methylene blue (MB) and his topical antimicrobial and drying utility [66].

Figure II-1: Solution of Methylene Blue [67]

Methylene Blue is an organic antimicrobial cationic dye with a strong affinity for dead cells. MB is especially potent for gram-negative bacteria and fungi and attaches to protein-rich exudate and infectious debris. MB affects redox potential in many electron transport components of oxidative metabolism, “short circuiting” the bacterial electron transport

pathway. MB reduces antimicrobial burden, decreases hypergranulation, and has a drying effect without harming healthy cells. Also it is easy to apply, water-soluble, and dries quickly; its effect is potentiated with light application [66].

MB solution can be used in conjunction with enzymatic debriders, hydrogels, and advanced dressings. Original research studies have shown MB has the ability to inactivate antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, leading to decreased extracellular matrix degradation. MB decreases nitric oxide-mediated vasodilation, thereby decreasing tissue ischemia, edema, and weepy/macerated wounds [66].

II-1-2-Methylene Bleu contraindications:

Use of MB is contraindicted in persons with a history of hypersensitivity, renal insufficiency, glucose-6-phosphate dehydrogenase (G6PD) deficiency, and/or hemolytic anemia with Heinz bodies. It is not recommended for use in pregnant women or neonates due to increased hyperbilirubinemia in G6PD-deficient neonates and those with hemolytic anemia. In general, MB is used in various systemic conditions in large doses and its safety record is excellent [66].

II-2-Polymers:

II-2-1-Definition

A polymer is a large molecule or a macromolecule, which essentially is a combination of many subunits. The term polymer in Greek means ‘many parts’. Polymers can be found all around us, from the strand of our DNA, which is a naturally occurring biopolymer, to polypropylene which is used throughout the world as plastic.

Polymers can be naturally found in plants and animals (natural polymers) or can be human-made (synthetic polymers). Different polymers have a number of unique physical and chemical properties, due to which they find usage in everyday life.

Polymers are created by the process of polymerization, wherein their constituent elements, called monomers, are reacted together to form polymer chains, i.e., 3-dimensional networks forming the polymer bonds.

The type of polymerization mechanism used depends on the type of functional groups attached to the reactants. In the biological context, almost all macromolecules are either completely polymeric or are made up of large polymeric chains [68].

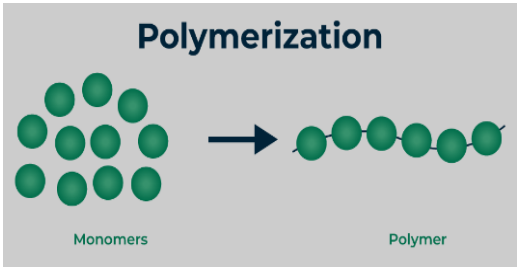


Figure II-2: Polymerization [69]

II-2-2-Classification of Polymers

Polymers cannot be classified under one category because of their complex structures, different behaviours and vast applications. We can, therefore, classify polymers based on the following norms:

II-2-2-1-Classification of Polymers Based on the Source of Availability

There are three types of classification under this category, namely, natural, synthetic, and semi-synthetic polymers.

- **Natural Polymers**

They occur naturally and are found in plants and animals. For example, proteins, starch, cellulose and rubber. To add up, we also have biodegradable polymers called biopolymers.

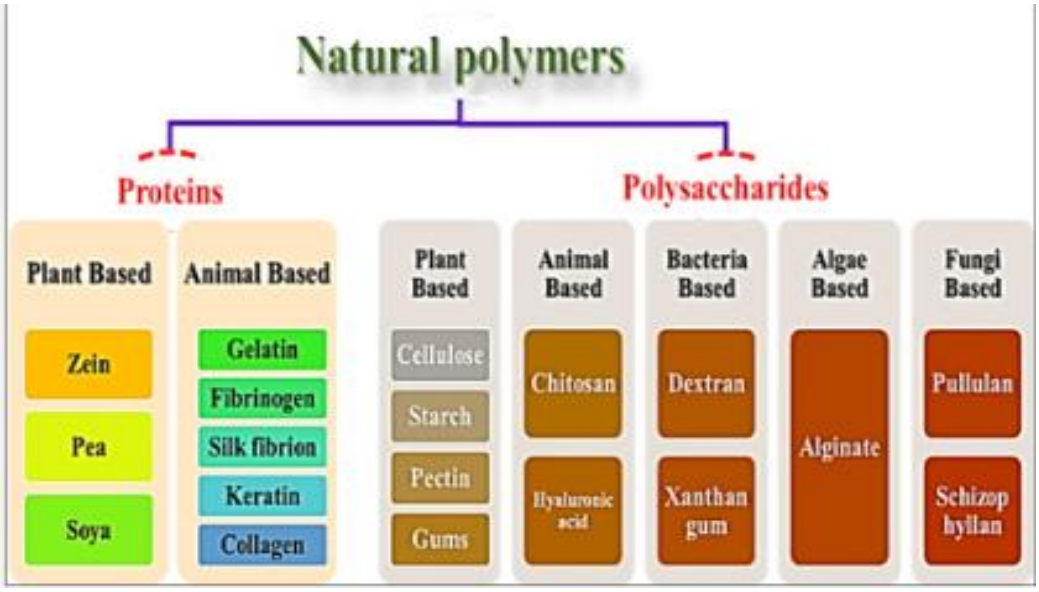


Figure II- 3: Natural polymers [70]

- **Semi-synthetic Polymers**

They are derived from naturally occurring polymers and undergo further chemical modification. For example, cellulose nitrate and cellulose acetate.

- **Synthetic Polymers**

These are human-made polymers. Plastic is the most common and widely used synthetic polymer. It is used in industries and various dairy products. For example, nylon-6, 6, polyether, etc.

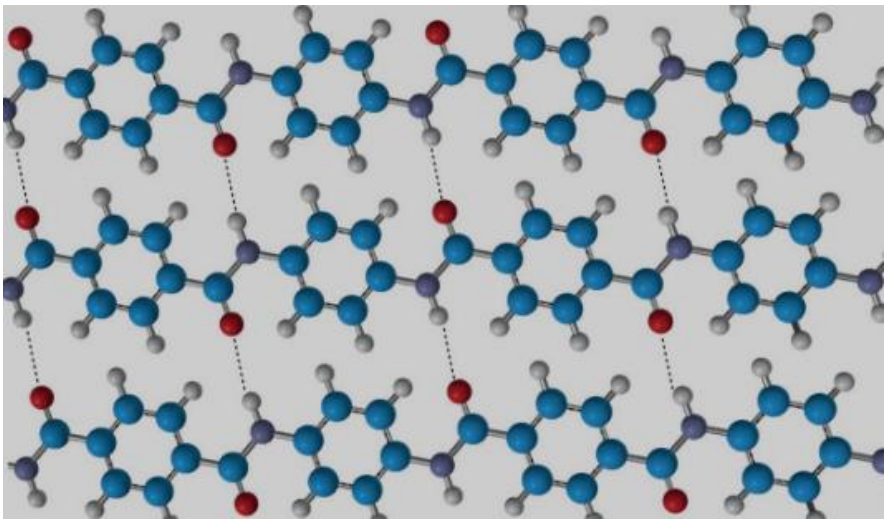


Figure II- 4: synthetic polymer [71]

II-2-2-2-Classification of Polymers Based on the Structure of the Monomer Chain

This category has the following classifications:

- **Linear Polymers**

The structure of polymers containing long and straight chains falls into this category. PVC, i.e., polyvinyl chloride, is largely used for making pipes, and an electric cable is an example of a linear polymer.

- **Branched-chain Polymers**

When linear chains of a polymer form branches, then such polymers are categorised as branched chain polymers. For example, low-density polythene.

- **Cross-linked Polymers**

They are composed of bifunctional and trifunctional monomers. They have a stronger covalent bond in comparison to other linear polymers. Bakelite and melamine are examples of cross-linked polymers.

II-2-2-3- Other Ways to Classify Polymers

1. Classification Based on Polymerization

- **Addition Polymerization:** For example, poly ethane, Teflon, polyvinyl chloride (PVC), etc.
- **Condensation Polymerization:** Examples include nylon -6, 6, perylene, polyesters, etc.

2. Classification Based on Monomers

- **Homomer:** In this type, a single type of monomer unit is present. For example, polyethene.
- **Heteropolymer or co-polymer:** It consists of different types of monomer units. For example, nylon -6, 6.

3. Classification Based on Molecular Forces

- **Elastomers:** These are rubber-like solids, and weak interaction forces are present in them. For example, rubber.
- **Fibres:** Strong, tough, high tensile strength and strong forces of interaction are present. For example, nylon -6, 6.
- **Thermoplastics:** These have intermediate forces of attraction. For example, polyvinyl chloride.
- **Thermosetting polymers:** These polymers greatly improve the material's mechanical properties. It provides enhanced chemical and heat resistance. For example, phenolics, epoxies and silicones.

II-2-3-Structure of Polymers

Most of the polymers around us are made up of a hydrocarbon backbone. A hydrocarbon backbone is a long chain of linked carbon and hydrogen atoms, possibly due to the tetravalent nature of carbon.

A few examples of hydrocarbon backbone polymers are polypropylene, polybutylene and polystyrene. Also, there are polymers which, instead of carbon, have other elements in their backbone. For example, nylon contains nitrogen atoms in the repeated unit backbone.

II-2-4-Types of Polymers

On the basis of the type of backbone chain, polymers can be divided into

- **Organic Polymers**: Carbon backbone

- **Inorganic Polymers**: Backbone constituted by elements other than carbon

Figure II-5: the basis of polymers synthesis (natural polymers and synthetic polymers [68]

On the basis of their synthesis:

- Natural Polymers
- Synthetic Polymers
    - **Biodegradable Polymers**

Polymers which are degraded and decayed by microorganisms, like bacteria, are known as biodegradable polymers. These types of polymers are used in surgical bandages, capsule coatings, etc. For example, poly hydroxybutyrate co vel [PHBV]

- **High-temperature Polymers**

These polymers are stable at high temperatures. Due to their high molecular weight, these are not destroyed even at very high temperatures. They are extensively used in the healthcare industries, for making sterilisation equipment and in the manufacturing of heat and shock-resistant objects.

II-2-5- A few of the important polymers are:

- **Polypropylene**: It is a type of polymer that softens beyond a specific temperature allowing it to be moulded, and on cooling, it solidifies. Due to its ability to be easily moulded into various shapes, it has a lot of applications.

A few of which are in stationary equipment, automotive components, reusable container speakers and much more. Due to its relatively low energy surface, the polymer is fused with the welding process and not using glue.

- **Polyethene**: It is the most common type of plastic found around us. Mostly used in packaging ,from plastic bags to plastic bottles. There are different types of polyethene, but their common formula is $(C_2H_4)_n$.

II-2-6-Properties of Polymers

II-2-6-1-Physical Properties

- As chain length and cross-linking increase, the tensile strength of the polymer increases.
- Polymers do not melt, and they change state from crystalline to semi-crystalline.

II-2-6-2-Chemical Properties

- Compared to conventional molecules with different side molecules, the polymer is enabled by hydrogen bonding and ionic bonding resulting in better cross-linking strength.
- Dipole-dipole bonding side chains enable the polymer for high flexibility.
- Polymers with Van der Waals forces linking chains are known to be weak but give the polymer a low melting point.

II-2-7-Some Polymers and Their Monomers

- Polypropene, also known as polypropylene, is made up of monomer propene.
- Polystyrene is an aromatic polymer, naturally transparent, made up of monomer styrene.
- Polyvinyl chloride (PVC) is a plastic polymer made of monomer vinyl chloride.
- The urea-formaldehyde resin is a non-transparent plastic obtained by heating formaldehyde and urea.
- Glyptal is made up of monomers ethylene glycol and phthalic acid.
- Bakelite or polyoxybenzylmethylenglycolanhydride is a plastic which is made up of monomers phenol and aldehyde.

II-2-8-Types of Polymerization Reactions

II-2-8-1-Addition Polymerization

This is also called chain growth polymerization. In this, small monomer units join to form a giant polymer. In each step, the length of the chain increases. For example, polymerization of ethane in the presence of peroxides.

II-2-8-2-Condensation Polymerization

In this type, small molecules like H_2O, CO, NH_3, are eliminated during polymerization (step growth polymerization). Generally, organic compounds containing bifunctional groups, such as idols, dials, diamines and dicarboxylic acids, undergo this type of polymerization reaction for example, preparation of nylon -6, 6.

II-2-9-Uses of Polymers

Here, we will list some of the important uses of polymers in our everyday life.

- Polypropene finds usage in a broad range of industries, such as textiles, packaging, stationery, plastics, aircraft, construction, rope, toys, etc.
- Polystyrene is one of the most common plastic actively used in the packaging industry. Bottles, toys, containers, trays, disposable glasses and plates, TV cabinets and lids are some of the daily-used products made up of polystyrene. It is also used as an insulator.
- The most important use of polyvinyl chloride is the manufacture of sewage pipes. It is also used as an insulator in electric cables.
- Polyvinyl chloride is used in clothing and furniture and has recently become popular for the construction of doors and windows as well. It is also used in vinyl flooring.
- Urea-formaldehyde resins are used for making adhesives, moulds, laminated sheets, unbreakable containers, etc.
- Glyptal is used for making paints, coatings and lacquers.
- Bakelite is used for making electrical switches, kitchen products, toys, jewellery, firearms, insulators, computer discs, etc.[68].

II-3-Starch

Starch is a polysaccharide derived from plants. This is the most important carbohydrate reserve in higher plants. It accounts for a significant proportion by weight of agricultural raw materials. It is found in the reserve organs of plants such as cereals (30-80% dry matter (DM)), tubers (60-90% DM), and legumes (20-25% DM). Starch is actively researched worldwide for various applications; in the composites and nanocomposites industry, starch can be used as a reinforcing filler and/or resin. Starch is biodegradable and renewable, making it a solution to the problem of plastic packaging. In the food industry, starch is the main energy source for human and animal nutrition and is an abundant, renewable and inexpensive nutritional component.

Starches with desirable functional properties play a role in improving the quality of a variety of foods, and chemically modified starches are commonly used as thickeners, gelling agents, binders, and sweeteners in many products may be replaced. In the agricultural industry, starch research results enable the cultivation of species that produce starch with desirable properties. Starch is also used in many other industrial sectors, such as paper manufacturing, pharmaceuticals, cosmetics, and textiles. In recent years, it has also emerged as a potential candidate for biofuel production [72].

II-3-1-Composition and primary structure of starch

The composition and structure of starch varies depending on the plant origin has been the subject of numerous bibliographic reviews. Starch is a carbohydrate with the crude formula $C_6H_{10}O_5$ 98-99% a mixture of two natural polymers: amylose and amylopectin. A molecule of α-D-glucopyranose (or α-D-glucose or anhydroglucose) is shown. In a circular form amylose is a nearly linear polymer, and amylopectin is it is a highly branched polymer. The remaining ingredient (1-2%) is starch lipids, proteins, minerals, and phosphorus on the surface. Strength is only on the inside. Although these ingredients are in trace amounts, it can change the physical and chemical properties of starch. In terms of the incidence of amyloidosis (depending on the origin of the plant), there are three types Starch: regular starch (20-35% amyloidosis), waxy starch (less than 15%) amyloidosis) and mutated starch or amylo (amyloidosis greater than 40%). Some types Mutated genotypes have either very low or very high levels of amyloidosis. this is the fact Waxy corn (amyloidosis <1%) and amyloma corn (almost 80%) amyloidosis).

II-3-1-1-amyloidosis

Amylose is an essentially linear polymer composed of multiple units. D-Anhydroglucopyranose is mainly bonded through α-type bonds (1-4) (Figure II-6). There are several branching points (link α (1 to 6)). Number of branches the larger the molecular weight. Have both ends various functions. The C4 position is the non-reducing end; C1 is the reducing end due to the hemic function of the terminal secondary alcohol. Depending on the plant origin, natural amylose contains 500 to 6000 glucosyl units, divided into: Several chains with average degree of polymerization (DP) >500 the average molecular weight is between 105 and 106, which is 10 times that of conventional synthetic polymers.

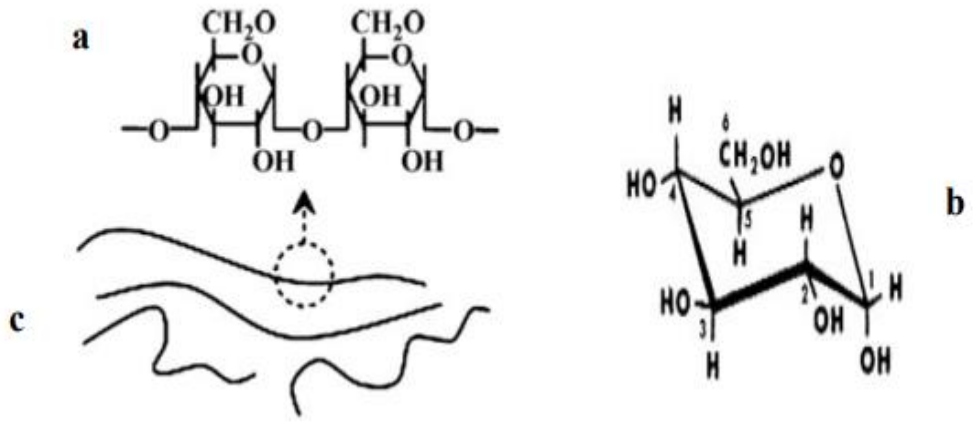


Figure II-6: Chemical structures of: (a) Amylose, (b) D-glucopyranose and (c) Amyloidosis [72]

Amylose has the specificity of complexer power of hydrophobic molecules such as iodine, fatty acids and alcohols. Its conformation and binding mode allow it to adopt helical forms with 6 units of glucose per turn, stabilized by bonds intramolecular hydrogen. Amylose can be extracted from dispersed starch grains in water by complexation with certain alcohols. Amyloidosis can also be synthesized «in vitro» by enzimatic means.

II-3-1-2-Amylopectin

Amylopectin is branched amyloidosis (Figure II-7). It consists of 10,000 to 100,000 repetitive units of glucose. In addition to the α (1 4) bonds of amyloidosis, 4 to 5% of points of branching are present in amylopectin through α acetal bonds (1 6). Its degree of polymerization and its molar mass are respectively in the 9600-15900 and 107 intervals-109 depending on the botanical origin of the starch. The mass high molar of amylopectin and its branched structure reduce chain mobility polymeric.

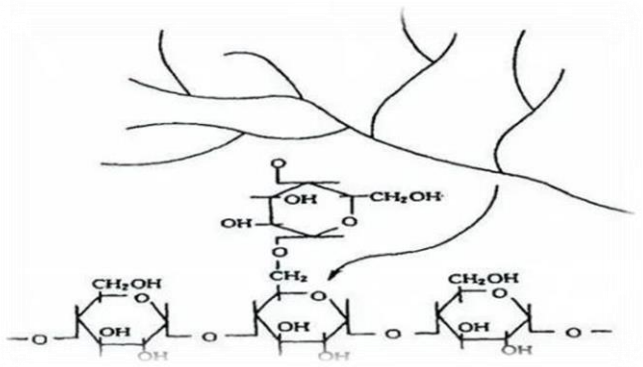


Figure II-7: Chemical structure and schematic representation of amylopectin [72]

Several models have been proposed to schematize the macromolecule amylopectin. Currently, the accepted model is the cluster model .This model consists of chains long interior (chains B or chains L) of DP 45 – 55, which form the framework of the macromolecule, short outer chains (chains A or chains S) of medium DP 14 – 18 bound to the rest of the molecule by their single reducing end and single chain C by molecule, of DP > 60 which carries the reducing end of the macromolecule. Chains B support "clusters" of chains A. differences in existing fine structure between amylopectin molecules of various botanical origins carry essentially on the ratio long chains L on short chains S. This ratio in number is 5 – 6 for tuber amylopectins and 8 – 10 for cereal amylopectins.

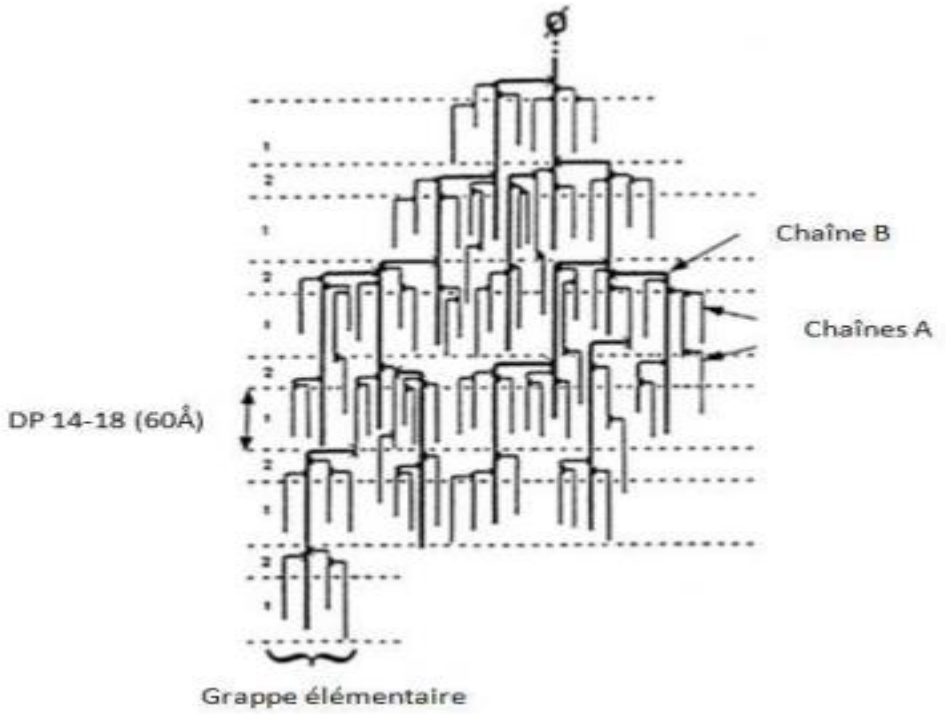


Figure II-8: Amylopectin cluster structure model [72]

II-3-2-Starch morphology and microstructure

II-3-2-1- Morphology of starch granules

After extraction and purification, starch becomes powder White, insoluble in cold water. This powder varies depending on its botanical origin consisting of dense microscopic units (1.5) of starch storage of different sizes from 1 to 100 μm. These units are called starch granules, and their shapes also differ. Functions derived from plants. Observing starch particles with a microscope using polarized light Maltese cross with branches meeting at the height of the cross Hile (starting point for the growth of starch particles) (Bio, 1844).

Birefringence is Positive, meaning that the polymer chains are organized radially within the structure grain.

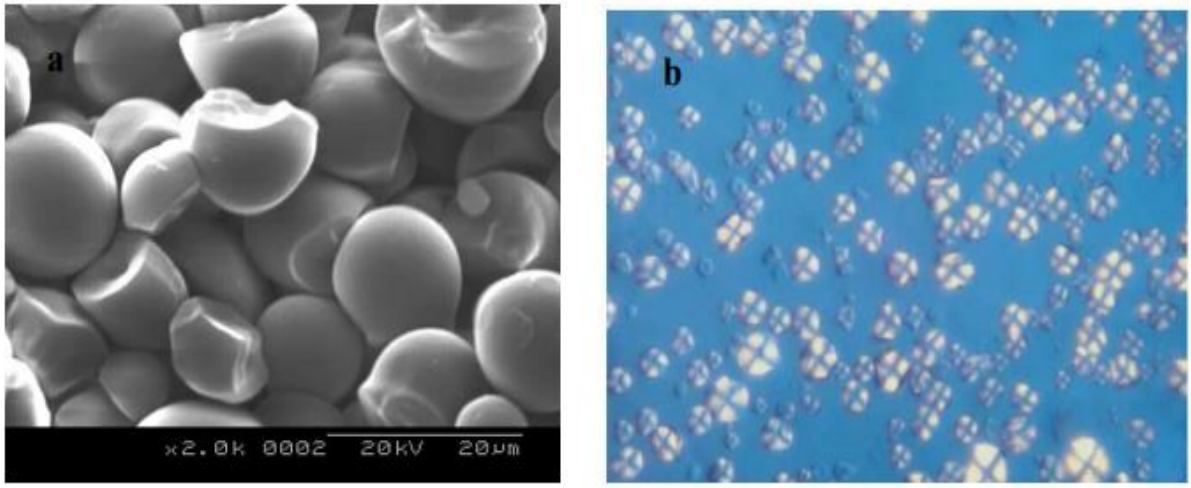


Figure II-9: Observations by scanning electron microscopy of cassava starch grains (a) and polarized light microscopy of waxy corn kernels (b) [72]

II-3-2-2-Microstructure of starch granules

The organization of starch particles is very complex and highly depends on their origin. . With the development of analytical techniques and the use of microscopes different levels of starch particle organization were revealed. Study of acid residues or enzymatic hydrolysis from starch granules. Observation of ultrafine sections using transmission electron microscopy (TEM) the particles exhibit an onion-like structure consisting of concentric layers. It consists of annual rings that are alternating between semi crystalline and amorphous (Figure II-10). The size and number of these annual rings vary depending on the origin of the plant, and its thickness varies between 120 and 400 nm.

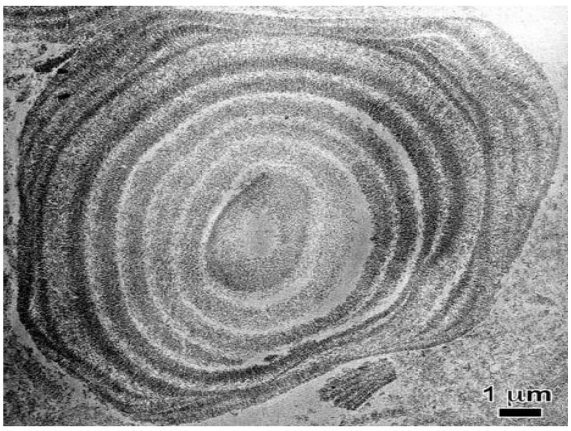


Figure II-10: MET image of an ultrafine cut of a grain of waxy corn starch partially

hydrolyzed to hydrochloric acid (inclusion in a resin, coloured by an aqueous solution of uranyl acetate and lead citrate) (micrograph: I. Paintrand, CERMAV) [72].

The existence of growth rings has been confirmed by X-ray diffraction at small angles (SAXS). Donald et al. (1997) proposed a starch grain organization model using different structural scales (Figure II-11).The thickness of the semi-crystalline repetitions (9 – 10 nm) would correspond to the structure in amylopectin cluster. Crystalline lamellae would consist of short chains amylopectin while amorphous lamellae would consist of points of branch.

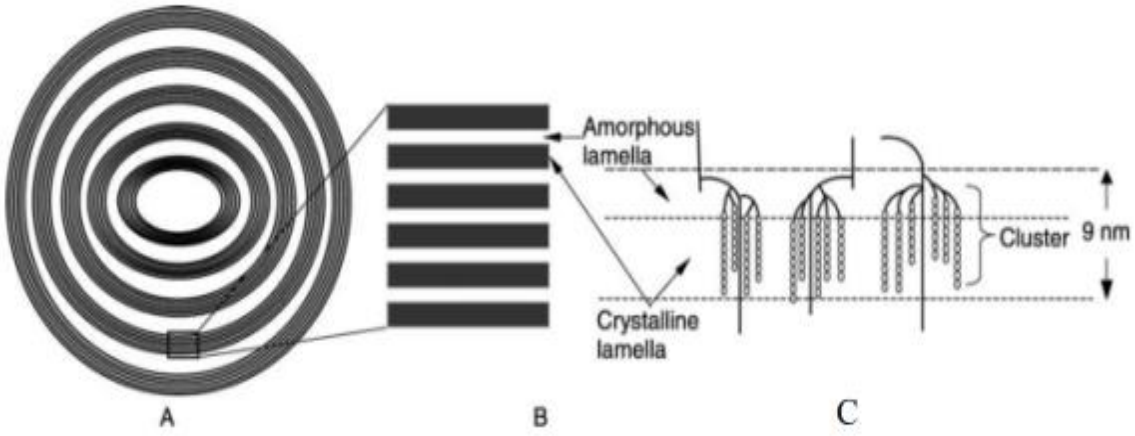


Figure II-11: Schematic representation of the lamellar structure of starch grains. (A): Microcrystalline lamella bundles separated by amorphous growth rings. (B): Enlarged views amorphous and crystalline regions. (C): Double helix structures formed by adjacent chains of amylopectin which give rise to crystalline lamellae [72]

Does the partially crystalline layer itself have a lamellar structure? The lamellae run tangentially to the growth rings and the grain surface. Proposed a model in which lamellae are internally organized. Spherical blocks (blocklets) with diameters from 20 to 500 nm depending on origin Botanical and their location within starch granules. This model is based on observations scanning electron microscopy (SEM), transmission electron microscopy (TEM), and force electron microscopy Atomic (AFM). In recent years, research using force microscopy has been conducted. On the surface of starch granules and within their structure. Proposed concept of blocklets size The size of wheat starch blocks (crystalline type A) is 10–50 nm. On the other hand, smooth pea starch (crystalline type B) forms more blocks. Sizes between 200 and 300 nm are important.

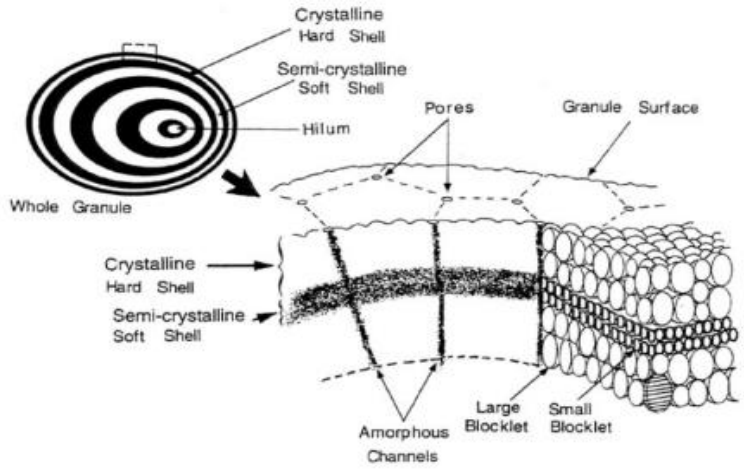


Figure II-12: Organization of the starch grain into "blocklets" [72]

Amylopectin is thought to support the crystal structure. Short chain Amylopectin has enough DP to form a self-bonded double helix Form clusters in parallel. Clusters are organized into lamellae It has a thickness of 9–10 nm and is characterized by an alternating arrangement of crystalline regions (double helix) and amorphous region (branch point). Amylose would be allowed. It connects blocks to each other and increases the strength of starch particles. His AFM local hardness study of starch particle cross section Wetting and swelling of these areas made it possible to locate the material. There are crystalline and amorphous starch particles. Detailed analysis of the internal structure of pea starch granules by AFM showed that the blocks were uniformly distributed and the amorphous growth rings originated from defects localized to particle growth.

II-3-2-3-Crystalline structure of starch grains

X-ray diffraction (XRD) analysis shows that starch is a semicrystalline polymer. The branch points of amylose and amylopectin form an amorphous region, and the short branches of amylopectin form a crystalline phase.

Natural starch can be classified into three crystal types based on its diffraction pattern: types A, B, and C (Figure II-13). Type A is characteristic of cereal starches (wheat, corn, rice) and is characterized by short side chains and dense branch points. Type B is characteristic of amylose-rich tuber and grain starches and is favored by long side chains and distant branch points. Type C is characteristic of legumes, but also of grains grown under certain temperature conditions. It is a mixture of types A and B In addition to the three crystalline types observed in natural starches, type V (from the German "verkleitung") is also characteristic.

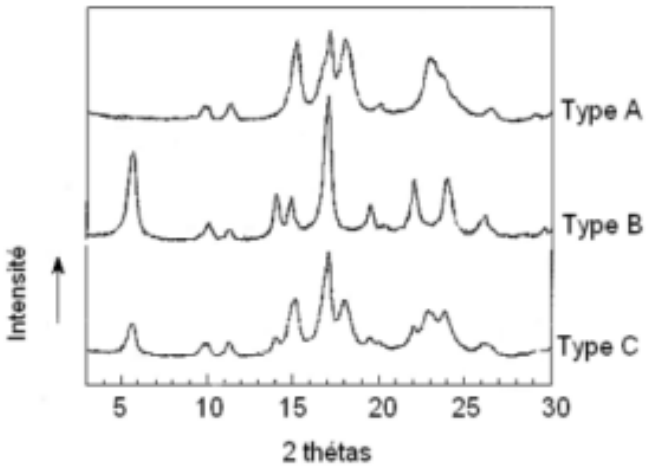


Figure II-13: X-ray diffractograms of crystal-type starches A, B and C [72]

Polymorphs A and B are normally distinguished by strong reflections at certain angles and additional reflections at other angles. The table below shows the characteristic diffraction angles of some starches.

Table II-1: Characteristic diffraction angles for polymorphs A and B

Nature	Radiation	2θ (°)	Crystalline type	Reference
recrystallized cassava	Cu Kα (λ = 0,154 nm)	10, 11, 15, 17, 18, 20, 23, 26, 30, 33	A + B	[72]
wheat	Cu Kα (λ = 0,15418 nm)	17,6 ; 19,9 ; 20,8 ; 26,7	A	
but	Cu Kα (λ = 0,1542 nm)	15 ; 23	A	
peas	Cu Kα (λ = 0,15406 nm)	6 ; 15 ; 17 ; 22 ; 24	B	
peas	Cu Kα (λ = 0,154 nm)	5,7 ; 15,1 ; 19,9 ; 22,1	A + B	
Polygonatum multiflorum ; Angelica dahurica	Co Kα (λ = 0,178901 nm)	5,6 ; 17 ; 15 ; 22 ; 24	B	
Trichosanthes kirilowii	Co Kα (λ = 0,178901 nm)	14,8 ; 16,9 ; 23,1	C	
Potato	Cu Kα (λ = 0,154 nm)	15 ; 17 ; 20 ; 22 ; 24	B	
Fritillaria	Co Kα (λ = 0,178901 nm)	6,6 ; 17 ; 23 ; 26 ; 28 ; 30	B	
Discorea opposita, Thunb	Co Kα (λ = 0,178901 nm)	6,5 ; 17,8 ; 20,1 ; 27,4	C	

Yam	n.i.	5,5 ; 17 ; 22 ; 24	B	
Innala	n.i.	5,5 ; 17 ; 18 ; 20 ; 23,5	C	
Native pea	Cu Kα (λ = 0,154 nm)	5,4 ; 14,8 ; 17,8 ; 19,4 ; 21,2	B	
Pea starch nanocrystals	Cu Kα (λ = 0,154 nm)	9,7 ; 11,4 ; 17,8 ; 22,9 ; 23,8	A	

The appearance of the X-ray diffractogram of starch depends on the moisture content of the grain being measured (Figure II-14). The higher the degree of hydration of the starch, the thinner the line becomes to a certain limit. Therefore, water is an essential part of the crystalline structure of type A and type B starches. The crystallinity of starch varies between 15% and 45% depending on the plant origin (0 is the criterion for completely amorphous material and 100 is the criterion for completely crystalline material).

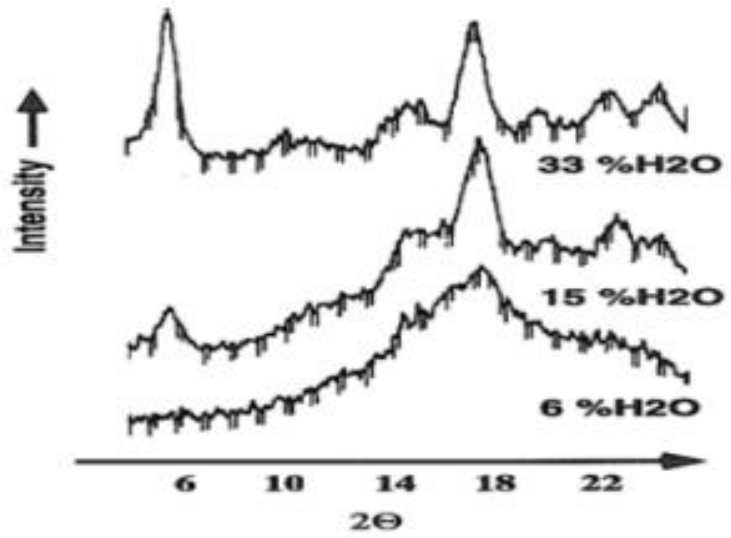


Figure II-14: Influence of water content on the resolution of the X-ray diffraction spectrum of a potato starch [72]

The diffraction peak intensity and diffraction pattern of type B starch increase from 10% moisture content up to 33%. In both the A and B polymorphs, the linear α-glucose chains are organized into a double helix with 2x6 glycosyl units per turn (Figure II-15). Each double helix is placed parallel to its neighboring double helix, offset by half a step along the axis of the double helix. This structure is stabilized by van der Waals interactions and hydrogen bonds.

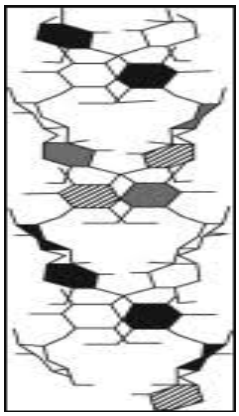


Figure II-15: Amylose double helix model [72]

The main difference between these two main polymorphs lies in the stacking of the double helices in the crystal lattice and the amount of water present between the double helices. The type a polymorph is relatively more compact with a low water content, while the type B polymorph has a more open structure containing a hydrated helical core. Starch grain structure can vary from the hilum (center) to the periphery. Some peas with mutant genotypes show different proportions of type A and type B polymorphs within the starch grain, and serve as useful models for understanding the evolution of different polymorphs. Indeed, pea starch granule centers are rich in B-type polymorphs, while peripheral regions are rich in A-type polymorphs. Cheetham and Tao (1998) observed a transition from polymorph A to polymorph B in maize grains, accompanied by a decrease in crystallinity and an increase in apparent amylose content.

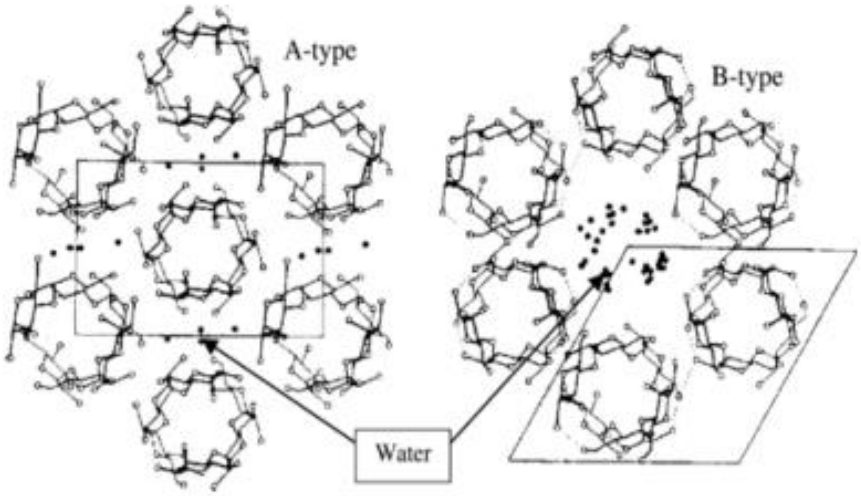


Figure II-16: Amyloidosis type A and B polymorphs [72]

The A-type polymorph is characterized by a monoclique mesh (a = 2.124 nm; b = 1.172 nm and c = 1.069 nm). Each double helix has six neighbors, forming a denser structure. In contrast, polymorph B is characterized by a hexagonal mesh (a = b = 1.85 nm and γ = 120°). Each double helix has three neighbors. The helices are arranged around a central cavity containing water molecules.

II-3-3-Hydrothermal behavior of starch granules

When heated in the presence of excess water at temperatures above 60°C, starch grains pass through three successive states: swelling, gelatinization and solubilization. A physical gel is formed during cooling: this is retrogradation.

II-3-3-1Gelatinization

Gelatinization or empeshing corresponds to the irreversible swelling and partial solubilization of starch grains in the presence of excess water and at temperatures above 60°C. During heating, starch grains absorb water in the amorphous zones. This swelling leads to the destructuring of the grain (rupture of hydrogen bonds in the crystalline zones, disappearance of the Maltese Cross). As the hydrogen bonds break down, the amylose diffuses out of the grain and solubilizes in the medium: this is the starching phase, leading to the formation of a starch starch. This starch paste is a suspension in which the swollen starch grains form the dispersed phase, and the solubilized amylose macromolecules form the continuous phase.

In highly hydrated media (water content over 70%), differential enthalpy analysis (DEA or DSC) reveals a melting endotherm corresponding to a first-order thermal transition. This is a transition from order (semicrystalline structure) to disorder (amorphous structure). The peak of the endotherm corresponds to the gelatinization temperature (TG). It depends on botanical origin and water content.

In a low-hydration environment (water content below 70%), starch transformation mechanisms become complex, and a second endotherm (M1) is identified by AED. The two endotherms (G and M1) have been observed for many starches (potato, normal corn, waxy corn, amylomaïs, smooth pea, wrinkled pea, etc.) but their origin is interpreted differently. For Donovan (1979) and Blanshard (1987), the two endotherms correspond respectively to gelatinization in excess of water and melting in a low-hydration environment. Biliaderis et al (1986) associate the second endotherm with non-equilibrium melting.

II-3-3-2-Retrogradation

Retrogradation refers to the structural reorganization (or recrystallization) that takes place during cooling of a destructured starch suspension. During cooling, when the polymer concentration is sufficient (> 1%), an opaque white gel is formed: this is gelling. This takes place in two stages, characterized by a statistical ball/double helix transition at the polymer chain segments, followed by crystallization by chain stacking. Amylose gels rapidly, while amylopectin gels more slowly and to a limited extent due to its branched structure. Starch gels can be likened to composite materials, with an amylose gel as matrix and amylopectin-rich grain ghosts as reinforcement.

Gels are made up of two phases, and the composition of each depends mainly on the degree of gelatinization and the amylose/amylopectin ratio of the starch grain Recrystallized amylose melts at around 120°C, while retrograded amylopectin crystals melt at low temperatures. Retrograded crystalline structures are of type B, whatever the macromolecule. It is retrogradation that governs the formation of starch-based films [73].

II-4-Films based on natural polymers

Natural polymers, such as polysaccharides, proteins, and nucleic acids, are components of biological systems responsible for performing a wide range of essential functions. For instance, certain natural polymers play a key role in the maintenance of the structural integrity of cells in plants and animals (e.g., cellulose and chitin), while others offer biological protection against surrounding environments (e.g., lysozyme). The diversity in terms of provenance and composition provides these natural polymers with distinct physicochemical and biological properties that can be of interest in various fields. In fact, natural polymers and their derivatives already find application in numerous sectors, e.g., in the manufacture of paper goods and textiles, as additives in food products, in the formulation of nutraceuticals and functional foods, and in the biomedical field (e.g., in cosmetic treatments and drug delivery). Owing to the natural abundance, renewability, and intrinsically negative carbon footprint of polymers derived from renewable resources, their exploitation is favourable and can play a pivotal role in the development of advanced materials in the shape of films, membranes, coatings, hydrogels, and micro- and nanoparticle systems [74].

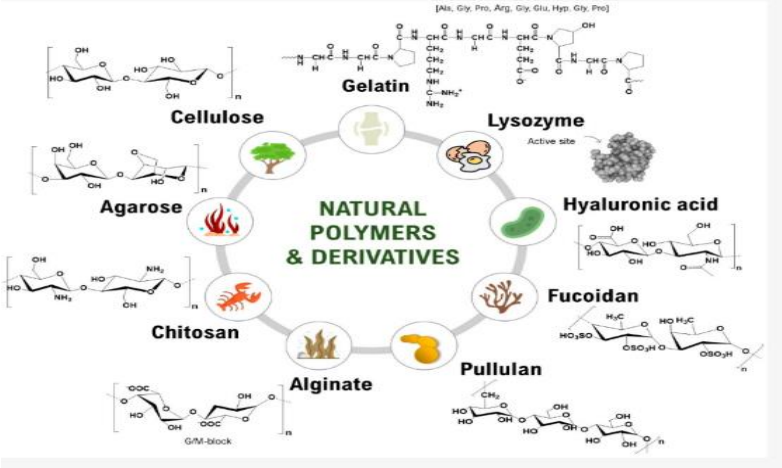


Figure II-17: Examples of the natural polymers and derivatives used for materials design by the BioPol4fun research group [74]

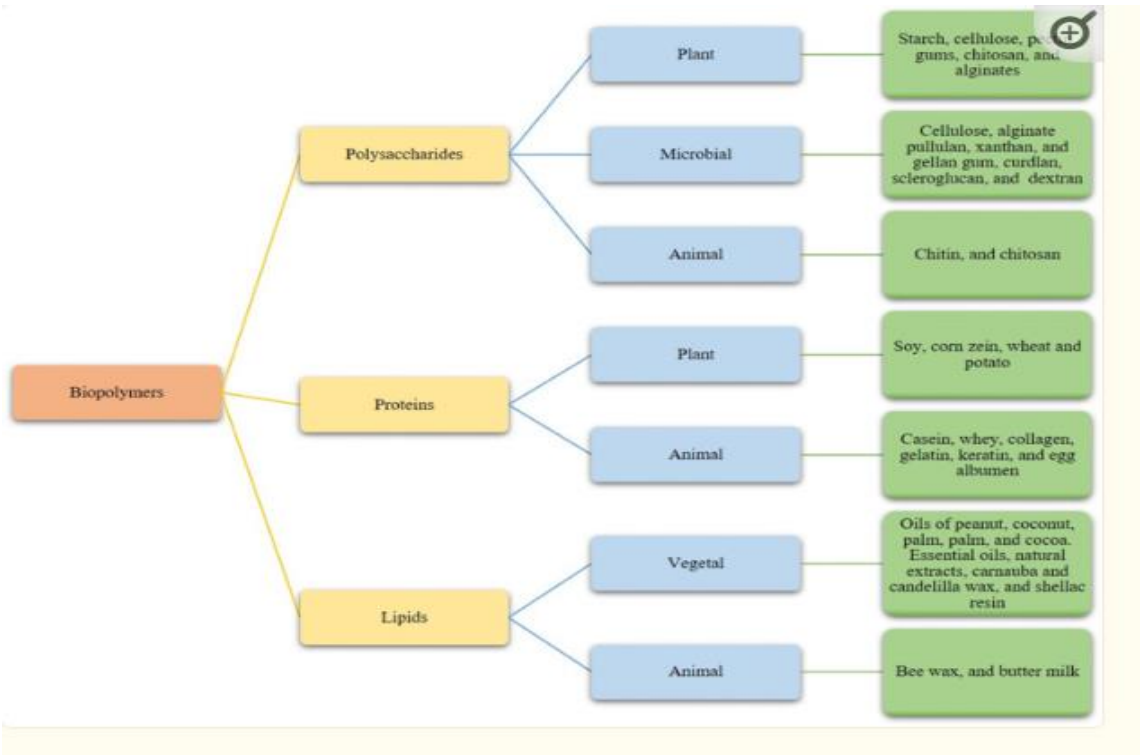


Figure II-18: Biopolymers used as natural edible films and coatings and their sources [74]

Natural polymers can be derived from various resources, such as plants, seafood, and algae, which can be applied to manufacturing sustainable food packaging. Despite this, earlier ancient civilizations employed different varieties of natural materials for food packaging, including ceramics, animal and plant parts, and shells. Nowadays, various forms of packaging have been developed in the markets based on marketing needs, technical requirements, easy opening, visual appeal, and portability. Moreover, in 2014, packaging accounted for 39.5% of

total plastic output. A recent study by Rydz et al. (2018) mentioned that 25.8 million tons of post-consumer plastic garbage were generated in Switzerland, Norway and 28 European countries. However, 69.2% of plastics were recovered via recycling and energy recovery technologies, and the remaining 30.8% ended up in landfills. A similar result was also shown by Souza et al. (2022) mentioned that around 30% post consumed plastics are still not recyclable because of multilayered materials, shredded plastics, and incompatible polymers.

Generally, municipal solid trash, including low-density plastic, is dumped on the ground or sent to a landfill, and that trash pollutes the soil and contributes to environmental contamination.

So, alternative strategies for reducing the hazards associated with plastic waste disposal are constantly being considered and developing new sustainable packaging. This problem can be solved by recycling, biodegradation under composting, and eliminating plastic waste by bioreactor land filling. As a replacement for traditional plastics, biodegradable and compostable polymers (BCP) have gained popularity, and BCP is especially recommended for the short-term packaging of food. Nowadays, packaging industries use three types of polymers: natural, modified natural and artificial polymers, and their classification is based on their origin.

Apart from this, polymeric nanocomposites have gained interest in recent years due to improvements in their mechanical, optical, electrical, and thermal properties, as well as their enormous potential as precious materials.

The application of biodegradable packaging materials has been employed in various fields such as food packaging sectors, biomedical systems, pharmaceutical and textile industries etc. Biodegradable packaging materials have been extensively studied for food packaging in the past 20 years.

Therefore, various types of packaging film have been developed using biodegradable polymers, such as edible film, smart and active packaging, intelligent packaging, modified atmospheric packaging, biodegradable film, antimicrobial packaging etc. Moreover, the application of these films is used in food packaging to protect the quality and enhance the shelf life of products in the food industries, such as fish freshness, milk freshness, fruits and vegetable freshness, meat freshness etc. Besides the packaging sectors, natural biodegradable polymers (BDPs) have been employed in many sectors. Such as, BDPs have many advantages that help ophthalmic formulations deliver drugs more effectively to the eye and achieve better therapeutic results. A report suggests that natural BDPs like gelatine and chitosan have been used effectively in ocular formulations to increase viscosity.

Apart from this, BDPs employed in various sectors such as microfluidic membranes, digital light processing additive manufacturing, microencapsulation systems, tissue engineering, electrophysiological signals, mulch film for agricultural crop protection. From the review of the literature, we found that no articles have provided comprehensive coverage of natural polymers, cold plasma technology intervention and nanotechnology applications in food packaging. Therefore, based on all the gaps, this current review focused on an updated account of natural polymers and the application of cold plasma technology and nano technology in the food packaging systems [75].

II-4-1-Films based on Polysaccharide

Polysaccharide-based films, also known as carbohydrate-based films, are hydrophilic polymers and therefore exhibit very low moisture barrier properties. A variety of polysaccharides and their derivatives have been used as biodegradable film-forming matrices. They include starch and its derivatives, cellulose and its derivatives, alginates, pectins, carrageenan, chitosan and various gums. Most efforts to improve the properties of these films were originally devoted to cellulose and starch. As biopolymers, these polysaccharides are of prime interest due to their availability and relatively low cost, but the low elasticity of these materials is a major drawback that limits their application. The major mechanism for the formation of polysaccharide-based films is the breaking of polymer segments and the reformation of polymer chains in the film-forming matrix or gel by solvent evaporation and hydrogen bonding [72].

II-4-4-Composite films

Protein- and polysaccharide-based films have excellent barrier properties to oxygen and carbon dioxide, while their resistance to water vapor transmission is limited due to their hydrophilic nature. Most of these films also have desirable mechanical properties, making them useful for improving the structural integrity of fragile products. In contrast, lipid films are resistant to moisture, but their mechanical properties are inferior to those of protein and polysaccharide films. Composite films contain both lipids and hydrocolloid components (proteins or polysaccharides) to exploit the advantages of both lipids and hydrocolloid components. When a barrier to water vapour is required, the lipid component can fulfil this function, while the hydrocolloid component provides the necessary mechanical strength.

II-4-5-Additives

Plasticizers and other ingredients are combined with film-forming biopolymers to modify physical properties or add functionality to films. Plasticizers are low-molecular-weight agents incorporated into film-forming polymer materials to increase film flexibility and processability. They cause an increase in the free volume of the polymer structure or the molecular mobility of a polymer matrix, as well as a decrease in the ratio of crystalline to amorphous regions and a lowering of the glass transition temperature. Sometimes, other ingredients such as antioxidants, antimicrobials, nutraceuticals, flavorings and colorants are added to film-forming solutions to create active packaging [72].

These natural raw materials are abundant, renewable and biodegradable, making them attractive raw materials for eco-friendly plastics. However, the application of plastics based on natural biopolymers is limited by their inferior mechanical and water vapour barrier properties compared with synthetic plastics derived from petrochemicals.

II-4-6-Film shaping

Two basic technologies, dry and wet processes, are used to prepare biopolymer-based films.

II-4-6-1- Dry processes

These processes are based on the thermoplastic properties of certain biopolymers. Indeed, some biopolymers exhibit thermoplastic behavior, i.e. like thermoplastic synthetic polymers, they can be reversibly melted. Thermoplastic biopolymers under low-moisture conditions are heated above their glass transition temperature by extrusion or thermo-compression methods. Heating amorphous polymers above their glass transition temperature transforms them into a soft, rubbery state, enabling films to be formed after cooling. Mainly starch- and protein-based films have been dry-processed. Some proteins with thermoplastic behavior can be processed by this method without further treatment, but other proteins and starch need to be plasticized before processing [72].

Dry processes have mainly been applied to the preparation of packaging biomaterials with plasticized starch and proteins using conventional processing techniques such as extrusion molding, compression and injection. Extrusion of biopolymers is the preferred method for high-throughput production for applications such as packaging. The thermo-compression method is also useful as a processing method due to its simplicity and ability to produce films without solubilization. Thermo-compression is a technology that enables

heterogeneous materials to be bonded under the simultaneous action of temperature and pressure, without any liquid phase forming during the bonding process, all in an atmosphere that varies from case to case. In these processes, specific mechanical energy, shear impact, pressure, plasticizer, time and temperature are important parameters in determining film properties.

Although dry processes require more equipment, they have some major advantages over wet processes: they are closer to industrial implementation, and they considerably reduce the solubility of the films obtained by creating a highly cross-linked film network.

II-4-6-2-Wet processes

A number of different processes are available for wet film production. The "casting" or solvent evaporation method is based on drying the film-forming solution. It comprises solubilization, casting and drying. The first step involves preparing a film-forming solution by dissolving a biopolymer in a suitable solvent such as water, alcohol or an organic solvent. The solution is then poured into a non-stick mold and evaporated at room temperature or elevated temperatures. The actual formation of a cohesive film depends on the nature, type and extent of the interactions of the polymers involved, as well as on film-forming conditions such as temperature and drying rate, moisture content, solvent type, plasticizer concentration and pH. The casting method has been commonly used for the preparation of biopolymer-based films.

Other methods, such as dipping and spraying, enable biopolymer-based films to be produced by the wet process. Dipping is used to apply a protective film to certain foods, or to technical products such as ceramics. This technique is also used to manufacture hard pharmaceutical capsules. The object to be coated is dipped into a solution to form a uniform gel layer on the surface of the object. Once the desired thickness has been reached, the object is removed from the solution and dried. Spraying involves spraying a polymer solution onto a surface in the form of droplets to produce a very thin film [72]

II-5-Conclusion

In conclusion, this chapter has shed light on the growing importance of pharmaceutical films based on polymers in drug formulation. We discussed various polymers, manufacturing methods, as well as the properties and applications of the resulting films. These films offer significant advantages such as drug protection, controlled release, and ease of administration, making them promising candidates for a diverse range of pharmaceutical products.

However, despite the progress made, challenges persist, particularly concerning the stability, biodegradability, and biocompatibility of the films. Further research is needed to optimize formulations, improve manufacturing processes, and assess the long-term safety of these systems.

Ultimately, pharmaceutical films based on polymers represent an exciting avenue for innovation in drug formulation, offering potential solutions to complex drug delivery issues. By continuing research efforts in this field, we can hope to see the emergence of new, more effective, safe, and accessible therapies to address the growing healthcare needs of the global population [73].

CHAPTER III

Experimental study

III-1-Introduction

Transdermal delivery might be the preferred method for administering drugs in certain scenarios. This approach maximizes the systemic or topical impact, enhances therapeutic efficacy, minimizes adverse effects, and sidesteps the first-pass effect in systemic action cases.

In general, transdermal systems are forms intended to allow the passage of active ingredients, incorporated in a reservoir, through the skin, either to have a systemic action, or to obtain a more local action, by fixation in sub-tissueunderlying skin [77].

III-2-Objective

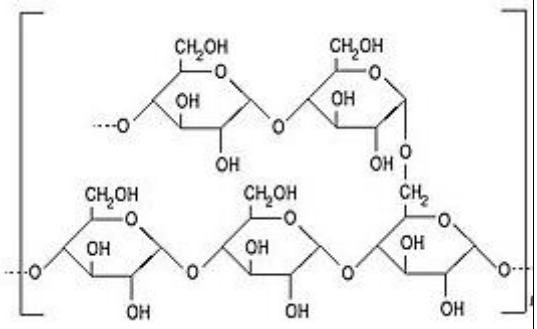
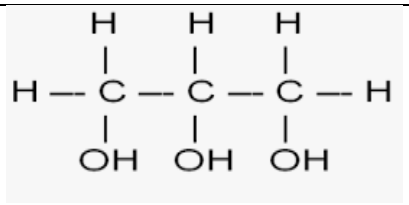
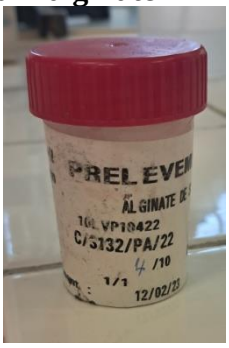
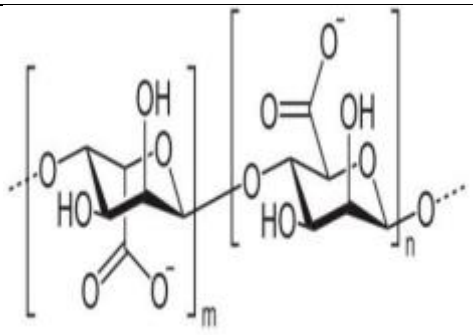
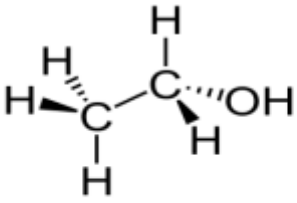
This study aims to achieve a controlled release of the active ingredient, methylene blue, with a delayed release profile. The release of methylene blue will be analyzed in two environments: one simulating the physiological environment with a pH of 5,6 and another with a pH of 6,7.

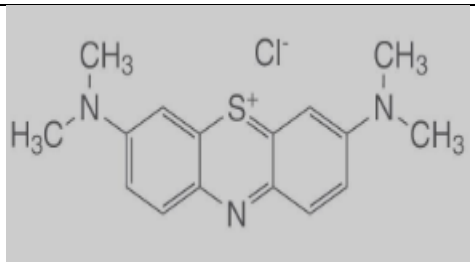
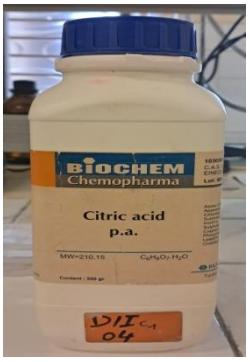
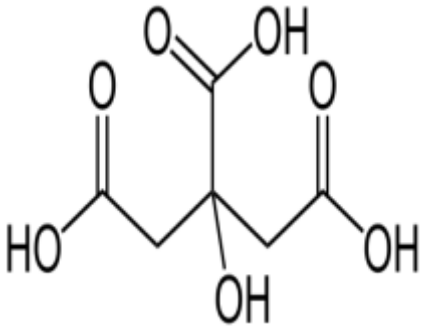
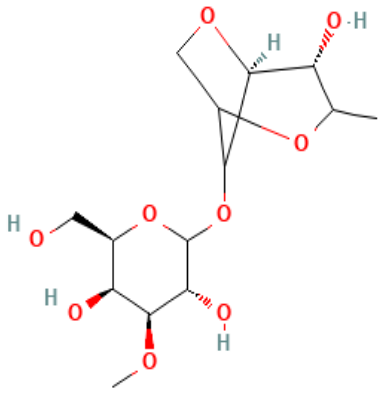
III-3- Materials and reagents used in the laboratory

Table III-1: List of materials used

Materials	
Beakers (50, 100, 250, 1000) ml	Graduated pipette (10 ml)
Graduated cylinders (10, 100) ml	Hot plate + magnetic stirrer
Thermometer	Spatula
Magnetic bar + magnetic rod	Laboratory bottles
Balance	Erlenmeyer flask
Micropipette	Pitri dishes
Ph testing paper	Volumetric flask
Microscope	Incubator
Spectroscopy UV-visible	Ph meter
Micro plaque + ampoules	

Table III-2: List of product used

Chemical product	**Formula bully**	**Mass Molaire g/mol**	**Chemical structure**
Starch soluble 	$C_6H_{10}O_5$	162	
Glycerol 	$C_3H_8O_3$	92.05	
Sodium alginate 	$C_6H_7NaO_6$	198,10	
Ethanol 96⁄ 	C_2H_6O	46,08	

Methylene blue 	$C_{16}H_{18}ClN_3S$	799,8	
Citric acid 	$C_6H_8O_7.H_2O$	210,15	
Agar-agar 	$C_{14}H_{24}O_9$	336.33 g/mol	

III-4- Physico-chemical techniques and characterization

III-4-1- UV-visible spectroscopy

Spectroscopy methods are physical study processes involving exchange phenomena between matter and electronic radiation. It could be absorption or emission of light and can be in the visible part of the spectrum or outside.

These exchanges involve well-defined energy levels of matter and therefore its quantification. By absorption of a photon having its own energy the molecule is carried in various excited states, the lowest energy level is called the fundamental state.

In a molecule, electronic transitions take place in the ultraviolet region (approximately 400-10 nm) and visible (800-400 nm).

• Visible: 800 nm (red) - 400 nm (indigo).

• Near-UV: 400 nm - 200 nm.

• Far-UV: 200 nm - 10 nm.

The range of the ultraviolet spectrum usable in analysis extends from approximately 190 to 400 nm. The visible spectrum range extends from approximately 400 to 800 nm [78].

Figure III-1: UV-Visible Spectrophotometer (micro plaque reeder)

- **Principle**

The spectrophotometer passes monochromatic radiation (light) wavelength) through a length L (length of the tank) of solution and measurement the absorbance A (quantity linked to the quantity of light absorbed by the solution).

The absorbance depends on the color of the radiation, its wavelength λ, i.e. I0 the intensity of the incident light and I the intensity of the transmitted light [79].

The absorbance A is proportional to the concentration of the solution according to Beer Lambert's Law.

$$A = \varepsilon \times L \times C \qquad \text{(III-1)}$$

With

A: absorbance of the solution (unitless).

L: length of the solution crossed by the light (in cm).

C: concentration of the solution (in $mol.l^{-1}$).

ε: molar extinction coefficient (in $L.mol\text{-}1.cm^{-1}$).

ε depends on the nature of the solution and the wavelength [79].

We will simply remember that:

$A = k \times C$ (III-2)

III-4-2- Infrared spectroscopy (I.R)

Infrared spectroscopy (sometimes referred to as IR spectroscopy) is a class of spectroscopy which deals with the infrared region of the electromagnetic spectrum. It covers a wide range of techniques, the most common being a type of absorption spectroscopy.

As with all spectroscopy techniques, it can be used to the identification of compounds or to determine the composition of a sample. The tables of infrared spectroscopy correlation are widely present in the literature scientist.

Infrared radiation can induce transitions in vibrational states and reactions associated with the fundamental electronic state of the molecule. [80]

The movements of the atoms of a molecule can be classified into three categories:

- translations.
- rotations.
- vibrations.

Infrared (IR) spectroscopy studies the vibrations of molecules when they are irradiated by an electromagnetic wave of frequency included in the range of infrared: approximately 0.8 and 1000 μm. This spectral zone is divided into:

- Near infrared: (0.8 to 2.5 μm) or 12500-4000 cm^{-1}).
- Medium infrared: (2.5 to 25 μm or 4000-400 cm^{-1}).
- Far infrared: (25 to 1000 μm or 400-10cm^{-1}) [81].

- **Principle**

IR spectroscopy is based on the interaction of IR light with the electron cloud chemical bonds [82]

When the molecule absorbs the energy provided by infrared electromagnetic radiation, it passes from its initial energetic state to an excited state, knowing that the internal energy of a molecule is composed of transition, rotation and vibration energies and the energy electronic, this absorption results in the appearance of characteristic bands of each organic compound at a given frequency.

The normal region of an infrared spectrum is located between 4000 and 400 cm, this is the region most generally exploited by chemists organicists for functional analysis [81]

III-5-Calibration cruve

III-5-1- Preparation of methylene blue solution:

The analysis involves dissolving 500 mg of methylene blue in 100 ml of distilled water, then adjusting the volume to 500 ml with the same solvent. Next, for the preparation of dilute solutions, we determined the initial concentration, C0, using the formula: C0 = m/v, yielding C0 = 1 mg/ml. To compute the volume of the dilute solutions (figure A and B),

(A) **(B)**

Figure III-2: Dissolving of methylene blue (A and B)

We applied the equation (C0*V0 = C1*V1), selecting the concentrations as shown in the table.

Table III-3: Concentration and volume for the law of dillution

Concentration (mg/ml)	Volume calculer (ml)	Volume complete up to 100 ml
0,8	80	20
0,7	87,5	12,5
0,6	85,7	14,3
0,5	83,3	16,7
0,4	80	20
0,3	75	25
0,2	66,6	33,4
0,1	50	50
0,04	40	60
0,01	25	75
0,003	30	70
0,001	33,3	66,67
0,0008	80	20
0,0005	62,5	37,5
0,0001	20	80
0,00002	20	80

The analysis was in λ_{max} =630nm, we possess the subsequent calibration data.

III-6- Film preparation protocol:

- **Preparation of film 1 (sodium alginate + starch + glycerol):**

We began by adding 20ml of warm distilled water into a beaker, followed by the gradual introduction of 0.5g of sodium alginate while stirring continuously for 40 minutes at a temperature of 50°C. Concurrently, we poured 40ml of lukewarm distilled water into another beaker, combining it with 1.37g of starch while stirring for the same duration at a temperature of 80°C.

Subsequently, we merged the two solutions in a separate beaker at 50°C, integrating 0.8g of glycerol while stirring for an additional 2 hours until complete gelatinization is achieved. (Figure III-3)

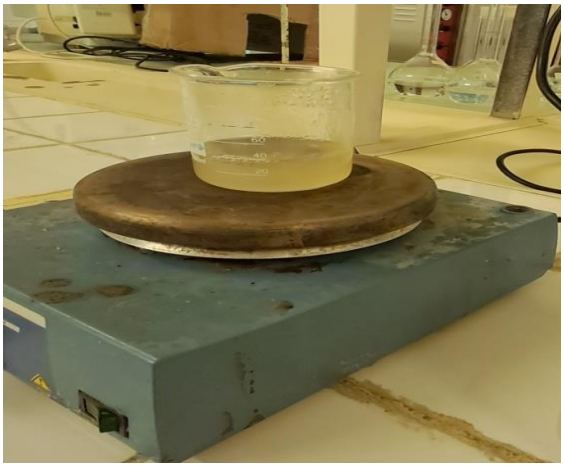


Figure III-3: The gélatinise solution

Following this mixing phase, we transferred the resultant solution into two kneaded dishes and allowed it to air-dry for 24 hours, followed by 48 hours of oven drying. (Figure III-4)

Figure III-4: Oven drying of the film

❖ **Preparation of film 2 (agar-agar + starch +citric acid):**

We initiated the process by pouring 20ml of warm distilled water into a beaker. Subsequently, we introduced 0.5g of agar-agar gradually, maintaining continuous stirring for 40 minutes at a temperature of 50°C. Meanwhile, in another beaker, we combined 25ml of lukewarm distilled water with 1.5g of starch, stirring for the same duration at a temperature of 80°C. Additionally, in a separate beaker, we dissolved 1g of citric acid in 25ml of warm distilled water, stirring for 40 minutes at 50°C.

Subsequently, we merged the three solutions in a separate beaker at 50°C, while stirring for an additional 2 hours until complete gelatinization is achieved.

Following this mixing phase, we transferred the resultant solution into two kneaded dishes and allowed it to air-dry for 24 hours, followed by 48 hours of oven drying.

III-7-Tests:

III-7-1-The degree of crosslinking

The degree of crosslinking of a polymer film refers to the density of crosslinking chemical bonds between polymer chains, which contributes to strengthening the structure and mechanical properties of the film. A crosslinking agent is a chemical substance added to the polymer to form these crosslinks.

In our case, citric acid acts as the crosslinking agent for the polymers (agar and starch) in our film. To calculate the degree of crosslinking, we need to know the quantity of crosslinking agent used and the total quantity of polymers.

Therefore: the quantity of crosslinking agent:

$$q_1 \text{ citric acid} = \frac{\text{the mass used}}{\text{its molar mass}} \quad \text{(III-3)}$$

The total quantity of polymers:

$$q_2 \text{ (agar-agar + starch)} = \frac{\text{the mass used of agar-agar}}{\text{its molar mass}} + \frac{\text{the mass used of starch}}{\text{its molar mass}} \quad \text{(III-4)}$$

The general formula to calculate the degree of crosslinking, usually expressed as a percentage, is [83]:

$$\text{Degree of Crosslinking (\%)} = \frac{\text{number of crosslinks formed}}{\text{total number of available reactive sites}} \times 100 \quad \text{(III-5)}$$

III-7-2- Swelling test:

The study of swelling allows establishing the kinetics of penetration of the dissolution medium into the film. This study allows determining its absorption rate and its volume increase over time using the equation:

$$\text{Swelling rate (\%)} = \frac{\text{Mass in the state inflate} - \text{Mass in the dry state}}{\text{Mass in dry state}} \times 100 \quad \text{(III-6)}$$

The prepared films are placed inside petri dishes after cutting into small pieces (1cm×2cm) and weighed. Then they are immersed in different media (distilled water for pH=7, acidic medium for pH=5.6, and pH=6.7) for 24 hours at room temperature. Afterward, the swollen films were wiped with filter paper and weighed again [84].

III-7-3- Active Ingredient Loading Test

The loading of the drug into the film typically involves two methods. In the first approach, the polymer to be used for the hydrogel is mixed with the drug; an initiator and a crosslinking agent, if necessary, can polymerize while incorporating the drug into the matrix [85].

However, in the second approach, a pre-formed film can swell in a drug solution until equilibrium is reached. In both cases, that is, after drug loading, the film is dried. The drug loading in the film is affected by several factors, namely the interaction between the polymer and solvent, the density of polymer network crosslinking, the presence of a solvent, etc. All these parameters significantly influence the extent of swelling. The drug loading per unit mass of a polymer can be calculated from the following expression [86].

$$\text{Active Principle Loading (\%)} = \frac{\text{Mass in the state inflate} - \text{Mass in the dry state}}{\text{Mass in dry state}} \times 100 \qquad \text{(III-7)}$$

Samples (1cm x 2cm) were cut and weighed, then immersed in a methylene blue solution for 24 hours at room temperature. Afterward, the PA-loaded films swelled and were weighed again.

III-8-The thickness of the films

Measured using an electronic micrometer (DIGITAL CALIPER) with a sensitivity of 0.001mm. Ten thickness measurements were taken for each sample at different points. The mean value is used to determine the film thickness [87].

Figure III-5: Electronic micrometer (DIGITAL CALIPER)

III-9- Preparation of buffer solutions

- **Preparation of pH=5.6 buffer solution:**

2,8 g of dibasic sodium phosphate are dissolved in 400 ml of distilled water, followed by the addition of 2,4 mg of citric acid in another 400 ml of distilled water.

The pH is then adjusted to 5.6 using a pH meter (Adwa).

- **Preparation of pH=6.7 buffer solution :**

Solution 1: 4 g of dibasic phosphate is dispersed in 500ml of distilled water;

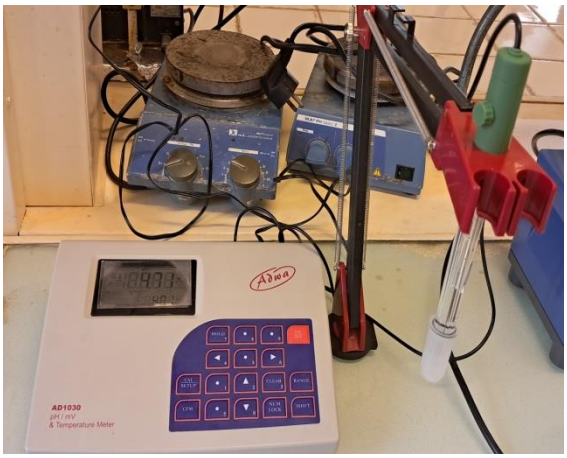
Solution 2: 3,94 g of monobasic phosphate are dissolved in 500ml of distilled water;

Solution 3: 1 g of glycerol is combined with 108ml of distilled water;

Solution 4: 239ml of acetic acid solution;

Solution 5: 100ml of sodium hydroxide.

To prepare the buffer, we took: 150ml of solution 1 + 150ml of solution 2 + 100ml of solution 3 + 300ml of ethanol + 1ml of solution 4. Then, the pH was adjusted using solutions 4 and 5 along with a pH meter until the pH reached 6,7 (figure III-6 A and B).



(A) **(B)**

Figure III-6: (A) pH meter (Adwa), (B) buffer solution

III-10- Methylene blue release study

We dissolve 1 gram of methylene blue in 100 ml of ethanol. Next, we cut the film into 1 cm squares and place them in a petri dish. We then apply the methylene blue solution onto the squares, cover the dish, and let them sit for an hour (Figure III-7).

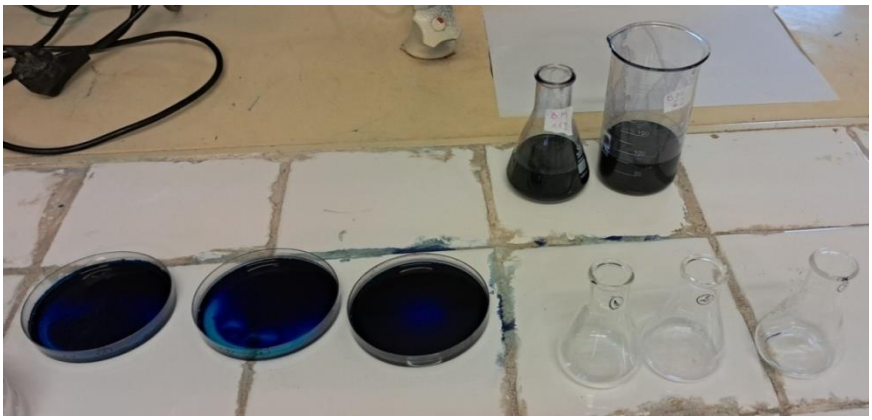


Figure III-7: Films paced into methylene blue solution

After one hour, take the film out of the solution, and transfer it into the Arlam Meyer apparatus. Add 10 ml of the buffer solution (pH= 5.6 and pH=6.7), and shake gently.

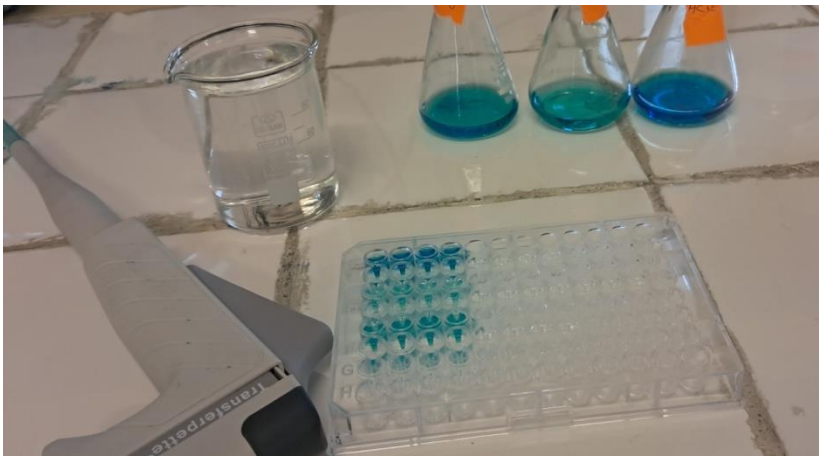


Figure III-8: the films in buffer solution

Samples of 200 microliters were withdrawn at predetermined intervals (30 minutes) and promptly replaced with an equivalent volume of fresh medium. After suitable dilution, the concentrations were analyzed using spectrophotometry at 630 nm.

Figure III-9: Reading abserbances in spectophotometry

III-11- Mathematical modeling of release profiles

The transfer of matter that occurs between the controlled release form and the surrounding aqueous milieu is complex due to the various simultaneous and successive phenomena that take place:

- Transfer of aqueous phase into form.
- Dissolution of the active substance in the aqueous Phase having penetrated into the form.
- Transfer of the active substance from the form to the external aqueous solution.

In order to simplify these problems, the following hypotheses should be considered:

- The forms are films and the active substance is uniformly dispersed in the polymer.
- The kinetics of release of the active substance from the polymer base matrix is controlled by diffusion.
- A simple process of release is considered assuming that there is only transfer of the product to the aqueous solution.

Diffusion phenomena are characterized by mathematical models most used by pharmacists:
The four kinetic models that were used: first order, Elovich equation, Huguchi and the power function.

The first order model has been decreed for diffusion through the polymer matrix. The Elovich equation is one of the diffusion expressions for slow chemical reactions. The Higuchi model could explain the mechanism of diffusion of the active principle through the porous network created by the solvent in the matrix. The power function was used to describe the exchange mechanism of the penetrated water proton.

First order:

$$\log C = \log C_0 + K \qquad \text{(III-8)}$$

Elovich's equation

$$C = a + K_E \ln t \qquad \text{(III-9)}$$

Higuchi's Higuchi

$$C = K_H t^{1/2} \qquad \text{(III-10)}$$

Fonction power

$$\ln C = \ln a + K_F \ln t \qquad \text{(III-11)}$$

Where: C: is concentration of the active ingredient released at time t, C_0: is the initial concentration of the active ingredient released at time t_0, a: represents the initial speed of the reaction and K, K_E, K_H and K_F are the first-order constants, Elovich, Huguchi and the power function respectively [88].

III-12-Optical Microscope (MOP):

The morphological study of synthesized materials was examined by optical microscope (OPTIKA). This part of the work allows the homogeneity of the film surface [89].

Figure III-10: Light microscope

III-13-antibacterial activity :

III-13-1 Determination of DI (Inhibition Diameter)

Evaluation of the antibacterial activity of films according to the diffusion method in agar medium (antibiogram). This technique is based on the appearance of an inhibition zone in the culture medium four bacterial strains: Gram positive bacilli, Pseudomonas aeruginosa gram negative, about the surroundings of the film disc.

III-13-2 Disc preparation

The discs of the films are prepared with a diameter of 10 mm. Then they are put in a test tube, and sterilized in autoclave and store until use.

1. Preparation of the culture medium

Sterilization and culture media (Muller Hinton) are autoclaved for 15 min at 120°C, then poured into the Petri dishes 4 mm high and left for a few minutes until solidified.

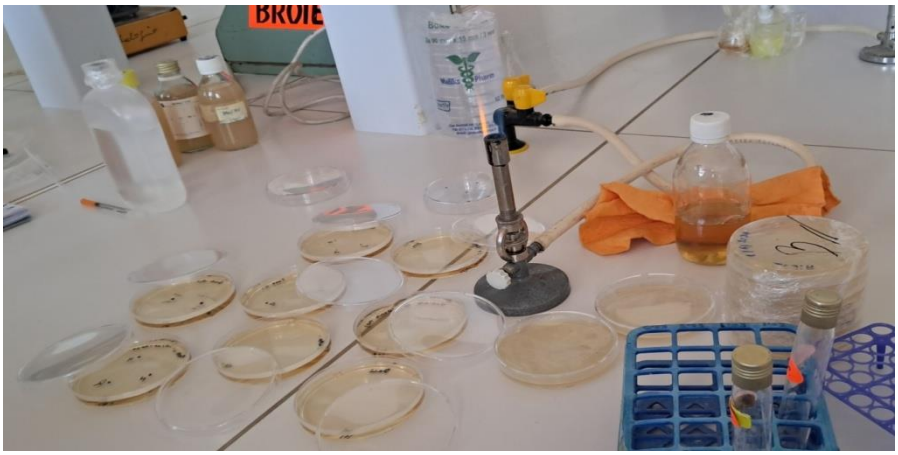


Figure III-11: Muller Hinton in Petri dishes.

2. **Inoculum**

- From a pure culture of bacteria to be tested on isolation medium, scrape by a platinum handle, some colonies well isolated and perfectly identical.
- Discharges the loop in 5 ml of 0.9% sterile physiological water, well homogenized the bacterial suspension.
- Seeding should be done in a few minutes after inoculum preparation.

3. Seeding of culture medium

- Culture is done in a sterile medium in the presence of benzene beak.
- Soak a sterile swab in the bacterial suspension (it avoids contamination of manipulator and bench).
- Wring it out by pressing it firmly, turning on the inner wall of the tube, in order to discharge to the maximum.
- Rub the swab over the entire gel surface, dry, from top to bottom.
- Repeat twice, turning the kneading dish 60°C each time, not forgetting to rotate the swab on itself. Finish seeding by passing the swab on the periphery of the agar.
- The discs are arranged and pressed onto the surface of the agar using a sterile clamp with a benzene nozzle, and then incubated at 37°C [90].

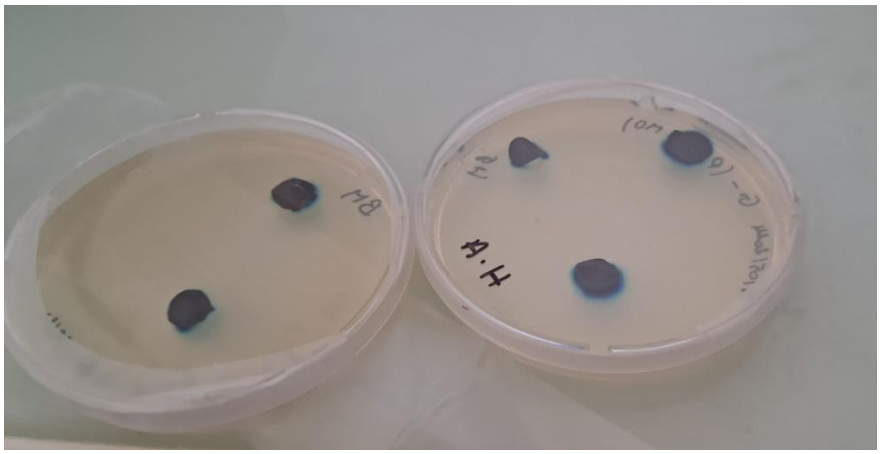


Figure III-12: The discs onto the surface of the agar.

CHAPTER IV

Results and Discussion

In this chapter, we will discuss the results obtained in the experimental section.

IV-1-Tests

IV-1-1-Crosslinking percentage

Crosslinking plays a crucial role in controlling drug release from polymers. The density and type of crosslinks significantly impact drug release rates, with higher densities typically leading to slower release rates due to increased microstructural tortuosity and decreased space between macromolecular chains. Additionally, the choice of crosslinking agents can affect drug distribution within the hydrogel, and nanoparticles can influence crosslinking density, which in turn affects drug release rates. By controlling crosslinking, researchers can tailor drug release profiles to specific applications, enhancing the efficacy and safety of various treatments [91].

	Film 1	Film 2
Result	27%	80%

In this case, the degree of crosslinking (0.27) suggests that there is a moderate level of crosslinking within the film composed of alginate (0.5g), starch (1.37g), and glycerol (0.8g). A degree of crosslinking of 0.27 implies that approximately 27% of available reactive sites on the polymer chains have participated in crosslinking reactions.

A degree of crosslinking of 0.27 can result in a film with desirable properties. It's likely to have good mechanical strength and flexibility while still allowing for some permeability, which could be suitable for applications where controlled release or barrier properties are important.

A degree of crosslinking of 0.8034 indicates a high level of crosslinking within the film composed of agar (1.5g), starch (0.5g), and citric acid (1g). This high degree of crosslinking suggests that a significant portion of available reactive sites on the polymer chains have participated in crosslinking reactions, resulting in a densely interconnected network.

Such a high degree of crosslinking can have several implications for the properties of the film. Firstly, it is likely to lead to excellent mechanical strength and stability due to the strong intermolecular bonds formed between the polymer chains. This can result in a film that is resistant to tearing and deformation.

Additionally, a high degree of crosslinking can also impact the permeability of the film. It may reduce the ability of molecules to diffuse through the film, making it potentially

useful as a barrier material in applications where controlling the passage of substances is important.

IV-1-2- Swelling test:

Film swelling is a crucial factor in drug release from polymer-based systems, as it increases the free volume within the matrix, allowing solutes to diffuse more easily. This enhanced free volume, along with changes in polymer structure and solvent penetration, influences the release rate and kinetics of the drug. Additionally, swelling can alter the interactions between the drug and polymer, affecting solubility and release rates. Overall, the swelling behavior of the polymer film plays a critical role in controlling the release rate and diffusion of drugs, making it a key consideration in the design of these systems for various therapeutic applications [92].

	Film 1	Film 2
Result	89,77%	96,83%

A swelling test result of 89.77% suggests that the film has absorbed water to a significant degree.

A swelling test result of 96.83% indicates that the film has a high capacity for absorbing solvent or liquid, likely influenced by its composition, crosslinking density, and hydrophilicity. Understanding the swelling behavior is crucial for optimizing the film's performance in various applications and for tailoring its properties to specific requirements.

IV-1-3- Active Ingredient Loading Test:

Drug loading is an important parameter for nanomedicines, as it determines the mass ratio of drugs to the carrier material and reflects the utilization of drugs during the nanomedicine preparation process. High drug-loading content is desirable as it can improve therapeutic effects and reduce side effects. The loading capacity of a drug carrier is determined by intermolecular interactions between drugs and carrier materials, such as hydrophobic interactions, electrostatic interactions, hydrogen bonding, π-π stacking, and van der Waals forces. Modifying drug-loading sites on carrier materials with interacting groups can help tailor drug-carrier interactions to improve loading capacity, release behavior, and stability. However, increasing drug content can decrease the drug loading rate, as the carrier material has a limited space capacity. For most nanomedicines, inert carrier materials are responsible for low drug-loading content (generally less than 10%). Achieving high drug-

loading content (over 50%) often compromises controlled release and pharmacokinetic properties [93].

	Film 1	Film 2
Result	9,24%	10,4%

A loading test result of 9.24% provides valuable information about the concentration of the active pharmaceutical ingredient in the transdermal film and its implications for drug delivery performance, dosing accuracy, and regulatory compliance.

The Active Ingredient Loading Test result of 10.4% indicates the efficient incorporation of active ingredients into the film, which can have significant implications for its performance and application suitability. Optimization of loading percentage and uniform distribution are essential considerations in the formulation and characterization of functional polymer films.

IV-2- The thickness of the films

Film thickness plays a crucial role in controlling the release of drugs from coated tablets. Thicker coatings tend to release the drug more slowly, while thinner coatings can result in faster release. The thickness of the coating layer directly impacts the drug release rate, and the choice of coating material significantly affects the drug release profile. In addition, the dissolution rate of the coating affects the drug release profile, with coatings that dissolve rapidly releasing the drug quickly and those with slower dissolution rates leading to sustained release. In the context of polymeric films, the thickness of the film can influence the release mechanism. For example, thicker films (1000 μm and above) can achieve almost perfect zero-order release, whereas thinner films (100 μm) exhibit Fickian diffusion. The aspect ratio of the film samples, which is the ratio of the disk diameter to the disk thickness, also affects the release mechanism. For films with a higher aspect ratio, the release is predominantly three-dimensional and is linearly related to the surface area. Overall, controlling the thickness of the coating or film is essential for achieving the desired drug release profile, which can be critical for optimal therapeutic outcomes [94].

	Film1	Film 2
Result	0.26mm	0.22mm

A thickness measurement of 0.26mm indicates a thin and flexible transdermal film suitable for drug delivery applications.

The thickness of 0.22mm provides additional context for interpreting the swelling test result, highlighting the film's high absorption capacity relative to its size. Consideration of the film's thickness is crucial for understanding its swelling behavior and optimizing its performance for diverse applications.

IV-3- Optical Microscope (MOP):

The presence of crystallites in film 1 and networks in film 2, observed by optical microscopy, is a crucial element to consider in the analysis of these materials. The crystallites in film 1 suggest a more ordered structural organization, which can influence its physical and chemical properties. They may also indicate a controlled crystallization process or specific polymerization occurring during the film formation.

On the other hand, the presence of networks in film 2 may indicate a more complex structure and potentially greater connectivity between the constituent molecules. This can have significant implications for mechanical strength, flexibility, or even the permeability of the film to various substances.

In discussing these observations, it is essential to analyze how these structural differences could affect the performance of the films in their intended applications. For example, film 1 with a more pronounced crystalline structure might be more suitable for applications requiring rigidity or dimensional stability, while film 2 with networks might be more flexible and adaptable, perhaps for applications requiring some elasticity or a response to variable external stresses.

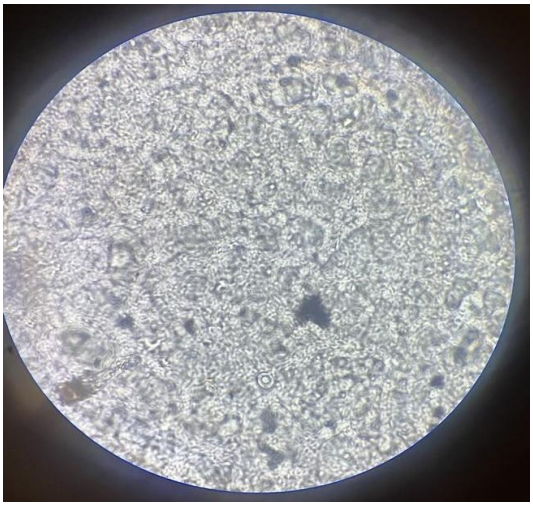


Figure IV-1: Film 1 on the optical microscope

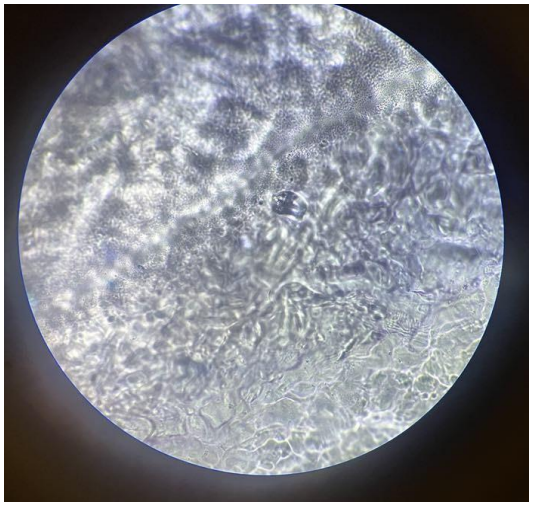


Figure IV-2:Film2 on the optical microscope

IV-4-Infrared spectroscopy:

- **For sodium alginate**:

The IR spectrum shown in (Figure IV-3) is used to determine the characteristic bands of the sodium alginate:

This absorption band **3600 cm^{-1}** typically corresponds to the stretching vibrations of hydroxyl (-OH) groups, which are commonly found in alcohols and carboxylic acids. In sodium alginate, it could be attributed to the hydroxyl groups present in the alginate polymer structure.

This absorption band **1650 cm^{-1}** often corresponds to the stretching vibrations of carbonyl (C=O) groups, indicating the presence of carboxylate or ester groups. In sodium alginate, it could indicate the presence of carboxylate groups from the uronic acid residues.

This absorption band **1419 cm^{-1}** is often associated with asymmetric stretching vibrations of carboxylate (COO-) groups. In sodium alginate, it could indicate the presence of carboxylate groups from the uronic acid residues.

This absorption band **1029 cm^{-1}** may correspond to the stretching vibrations of C-O bonds, indicating the presence of ether or ester linkages. In sodium alginate, it could be attributed to the glycosidic linkages between the monomeric units of the alginate polymer.

These absorption bands provide valuable information about the molecular structure and functional groups present in sodium alginate, aiding in its characterization and analysis [95].

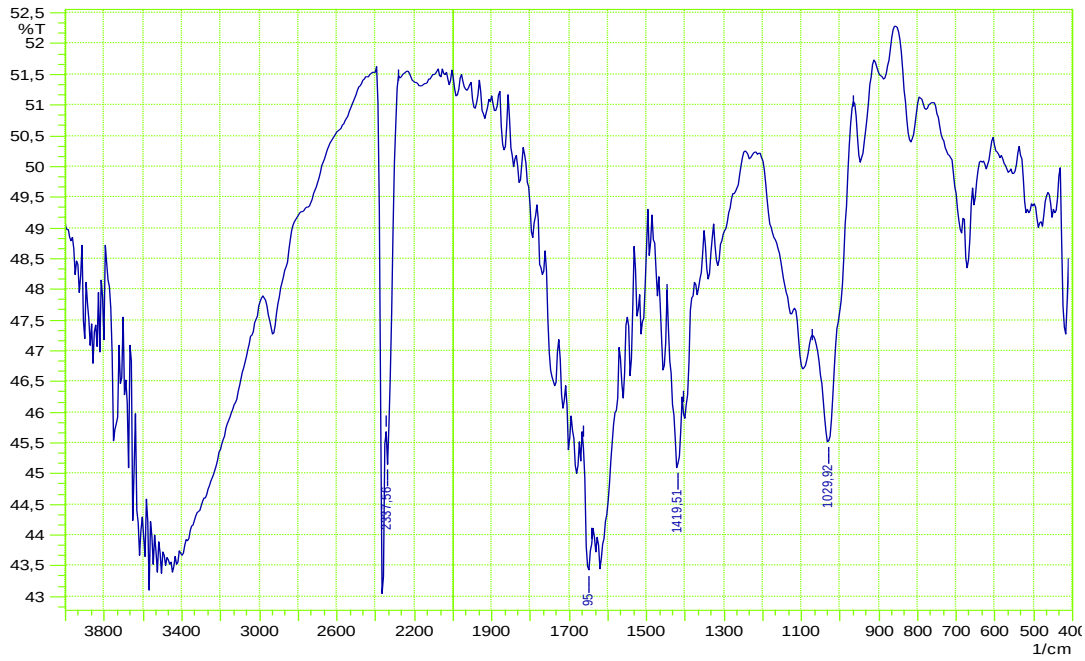


Figure IV-3: Sodium Alginate IR Spectrum.

- **For citric acid**:

The IR spectrum shown in (Figure IV-4) is used to determine the characteristic bands of the Citric acid:

This absorption band **3371 cm^{-1}** corresponds to the stretching vibrations of O-H bonds in carboxylic acids and/or hydroxyl groups.

This absorption band **1757 cm^{-1}** corresponds to the stretching vibrations of the carbonyl (C=O) group in the carboxylic acid functional group of citric acid.

This absorption band **1213 cm^{-1}** could correspond to the stretching vibrations of the C-O bonds in carboxylic acids or ester groups.

This absorption band **1110 cm^{-1}** could be attributed to the stretching vibrations of C-O bonds in carboxylic acids or ester groups.

This absorption band **894 cm^{-1}** may correspond to the bending vibrations of the C-H bonds in the methyl groups present in citric acid.

This absorption band **781 cm^{-1}** could be associated with the out-of-plane bending vibrations of the C-H bonds in aromatic rings, which are present in the structure of citric acid.

This absorption band **503 cm^{-1}** could correspond to the bending vibrations of O-H bonds or C-O bonds in citric acid.

These absorption bands provide valuable information about the molecular structure and functional groups present in citric acid, aiding in its characterization and analysis [96].

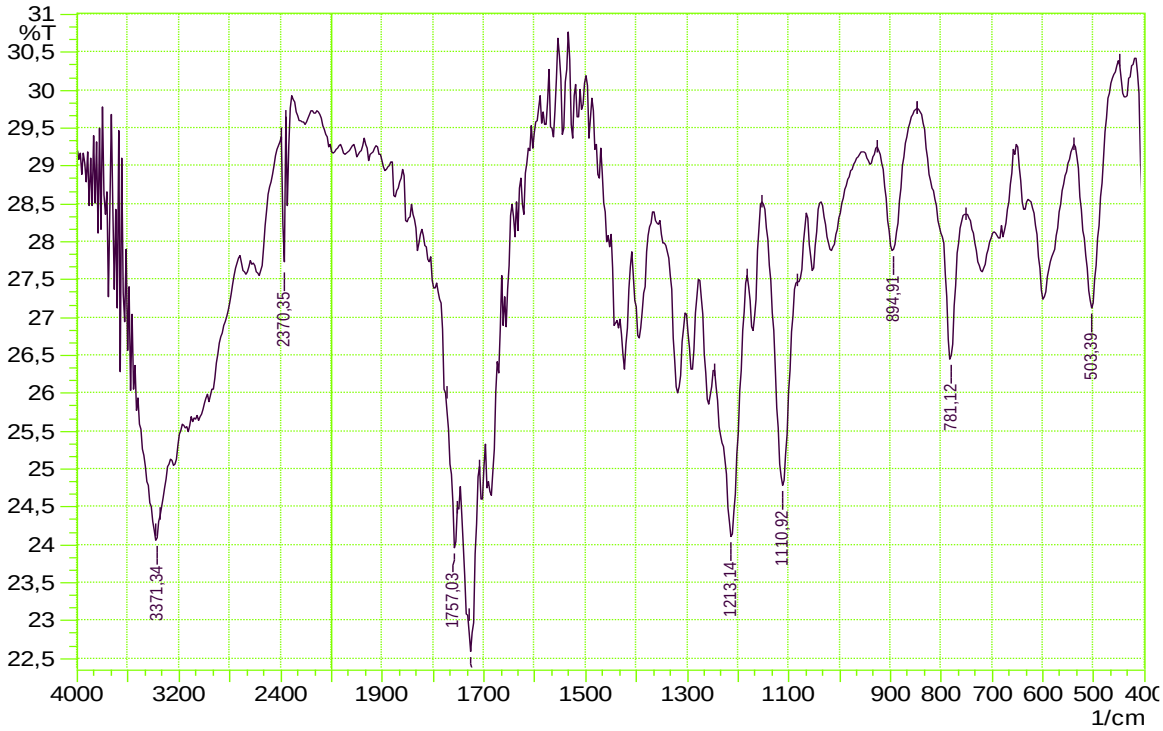


Figure IV-4: Citric acid IR Spectrum.

➢ **For agar-agar**:

The IR spectrum shown in (Figure IV-5) is used to determine the characteristic bands of the agar-agar:

This absorption ban **2937 cm^{-1}** d corresponds to the stretching vibrations of C-H bonds in the methyl (CH_3) and methylene (CH_2) groups present in agarose and agaropectin, which are the main components of agar-agar.

This absorption band **1839 cm^{-1}** could correspond to the stretching vibrations of carbonyl (C=O) groups, indicating the presence of ester linkages or possibly carboxylate groups.

This absorption band **1650 cm^{-1}** often corresponds to the stretching vibrations of carbonyl (C=O) groups, indicating the presence of ester linkages or possibly carboxylate groups. It may also indicate the presence of water molecules.

This absorption band **1469 cm^{-1}** could correspond to the bending vibrations of CH_2 groups or C-H bending vibrations in the agar-agar molecule.

This absorption band **1080 cm^{-1}** may correspond to the stretching vibrations of C-O bonds, indicating the presence of ether linkages in the agar-agar molecule.

This absorption band **931 cm^{-1}** could correspond to the bending vibrations of C-H bonds in the agar-agar molecule, particularly in the methyl (CH_3) groups.

: This absorption band **702 cm^{-1}** could correspond to the bending vibrations of C-H bonds or skeletal vibrations in the agar-agar molecule.

These absorption bands provide valuable information about the molecular structure and functional groups present in agar-agar, aiding in its characterization and analysis, particularly in applications such as food science, microbiology, and biotechnology [97].

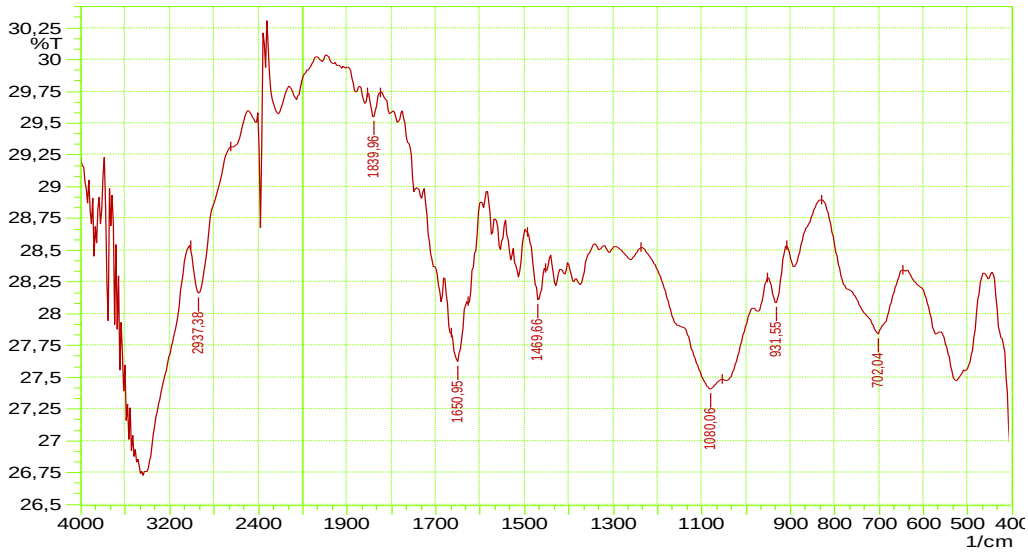


Figure IV-5: Agar-agar IR Spectrum.

- **For methylen blue**:

The FTIR spectrum shown in (Figure IV-5) is used to determine the characteristic bands of methylene blue (MB):

The peaks at **3331.5 cm^{-1}** represent O-H extending vibrations, and the spectra bands observed at **2917.7 cm^{-1}** signpost vibrations of CH_n, mostly due to C-CH_2 and C-CH bonds. The peak at **1733.3 cm^{-1}** describes C=O and the vibration of C=C. The peaks of **1030.4 cm^{-1}** are likely assigned to the -C- C- stretching [98].

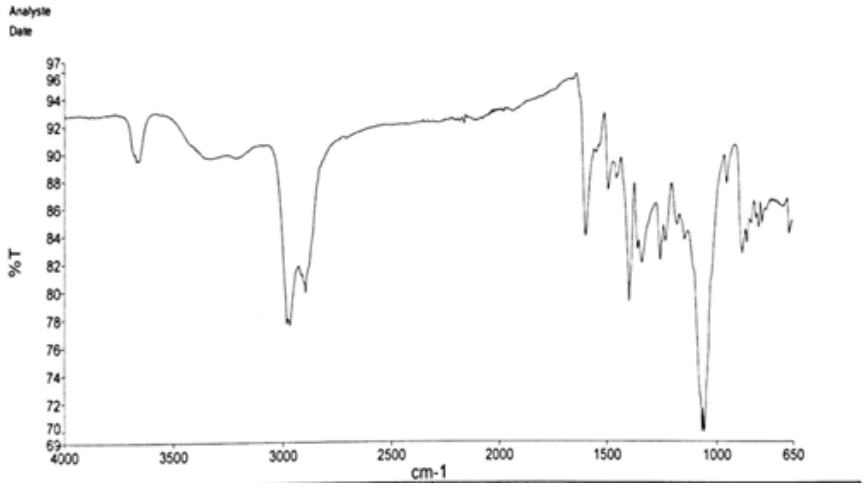


Figure IV-6: Methylene blue IR spectrum

- **For this film 1(sodium alginat+ starch+ glycerol)**:

To interpret the FTIR (Fouriertransform infrared spectroscopy) spectrum, we look at the peaks corresponding to specific functional groups in the molecule. Here's a rough breakdown:

3395 cm^{-1} : This is typically associated with the O-H stretch in alcohols or phenols.

1709 cm^{-1}: This corresponds to the C=O stretch, which is common in carbonyl compounds like ketones, aldehydes, or carboxylic acids.

1395 cm^{-1}: This peak could be due to the C-H bending in alkanes or the CH_3 bending in alkyl groups.

1199 cm^{-1}: This could be attributed to C-O stretching in ethers or esters.

1023 cm^{-1}: This might represent C-N stretching in amines or amides.

928 cm^{-1}: This peak could correspond to C-H bending in aromatic compounds or substituted aromatics.

879 cm^{-1}: This could be due to C-H bending in out-of-plane bending in aromatic compounds.

786 cm^{-1}: This peak may represent the bending mode of C-H in-plane deformation, often seen in aromatic compounds [99].

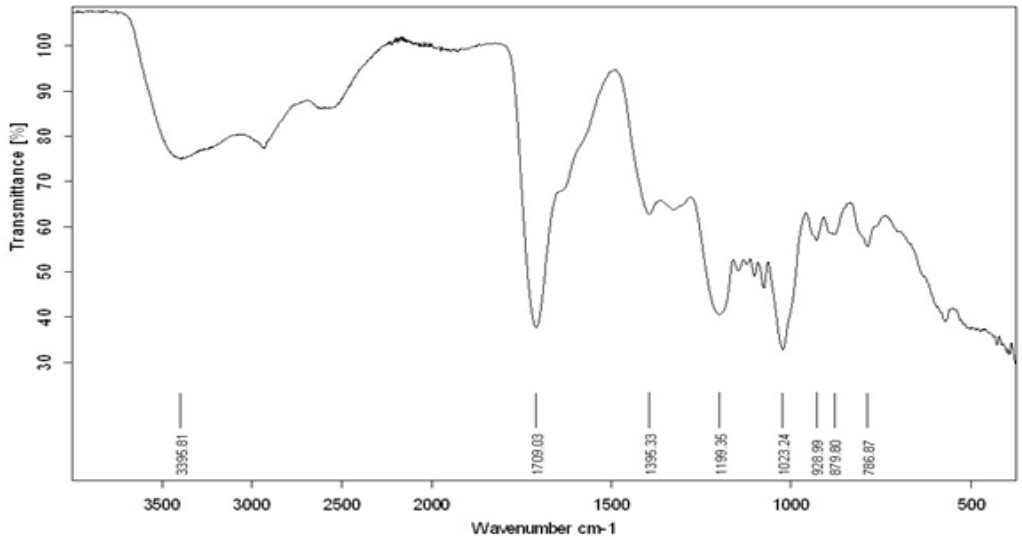


Figure IV-7: IR Spectrum of film1

- **For this film 2**:

Let's break down the potential assignments for the given FTIR bands:

3296 cm^{-1}: This could correspond to the N-H stretch in primary amines or amides.

2936 cm^{-1}, 2886 cm-1: These peaks are indicative of C-H stretching in alkanes or saturated hydrocarbons.

1602 cm^{-1}: This is typically associated with C=O carbonyl groups.

1410 cm^{-1}: This peak could represent C-H bending in alkanes or methyl groups.

1150 cm^{-1}: This might correspond to C-O stretching in ethers.

1077 cm^{-1}: This peak could be due to C-C stretching in alkanes [100].

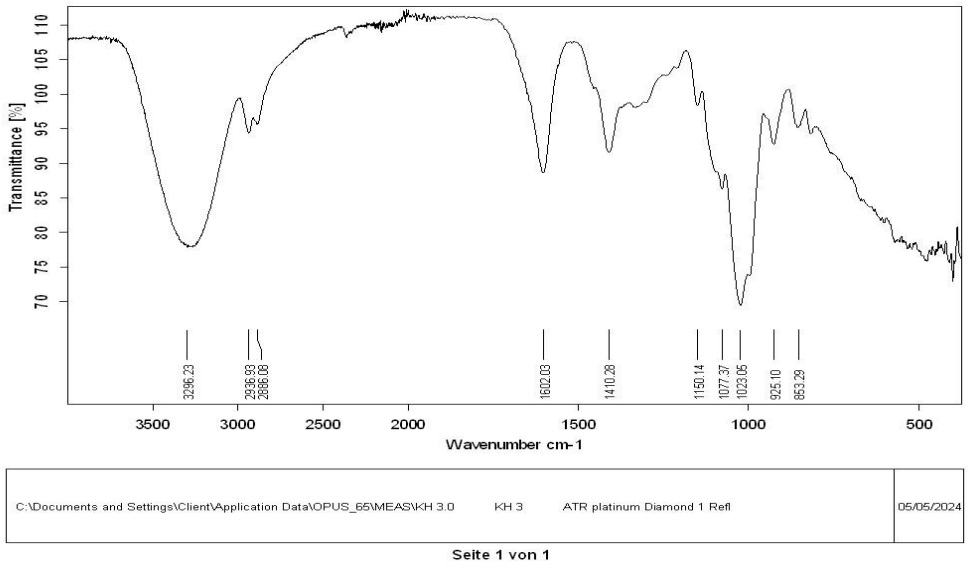


Figure IV-8: Film 2 IR spectrum

IV-5-Calibration cruve

This curve represents the calibration line, illustrating the direct relationship between the absorbance of the solute (methylene blue) under experimental conditions and its mass concentration.

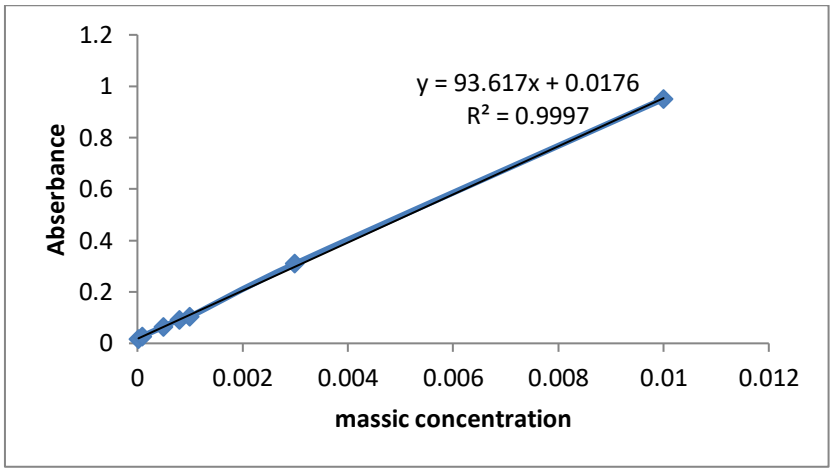


Figure IV-9: Calibration line

IV-6-Methylene blue release study in vitro (film 1 and film 2):

According to Figure IV-10:

In film 1, an increase in the concentration of methylene blue is observed up to a value of (8.9 10^{-3} mg/ml) at (240 min), after which it stabilizes.

In film 2, it can be noted that the released concentration increases over time until it reaches a maximum value of (8.6 10^{-3} mg/ml) at (150 min).

The graphs demonstrate the slow release of methylene blue (MB) from both films. The release profiles show that the MB exhibited a slow and steady release over time, with no significant changes in the release rate over the experiment period at 25°C.

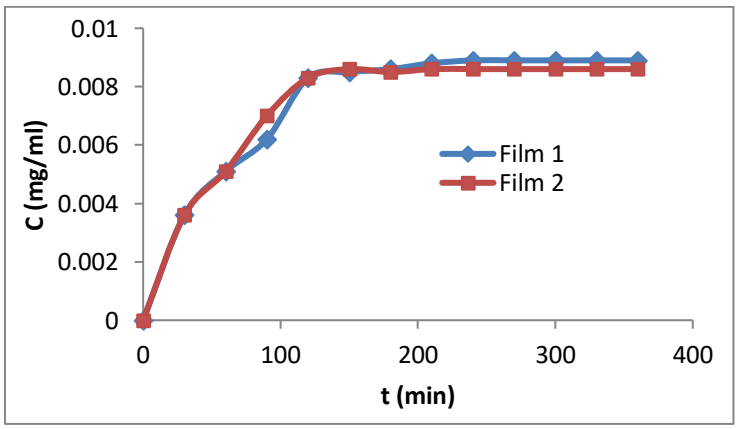
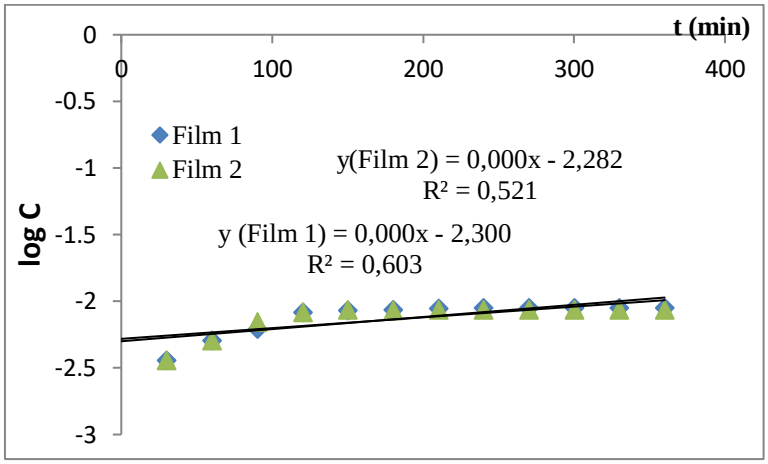


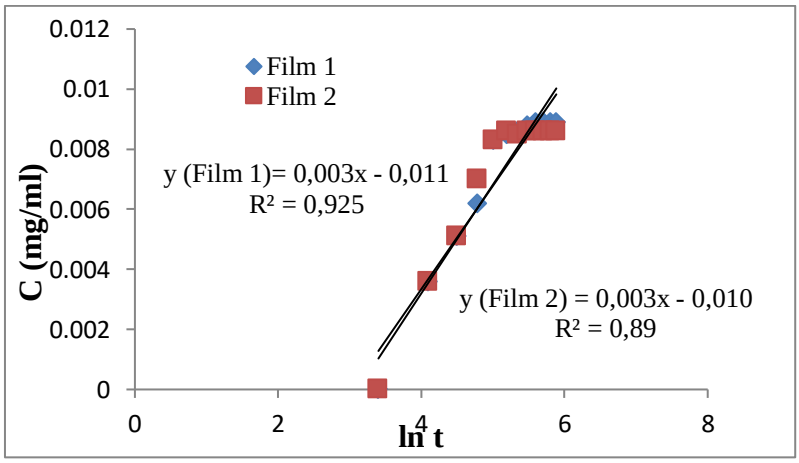
Figure IV-10: In vitro release profiles of methylene blue in film (film1 and film2)

IV-7- Mathematical modeling of release profiles

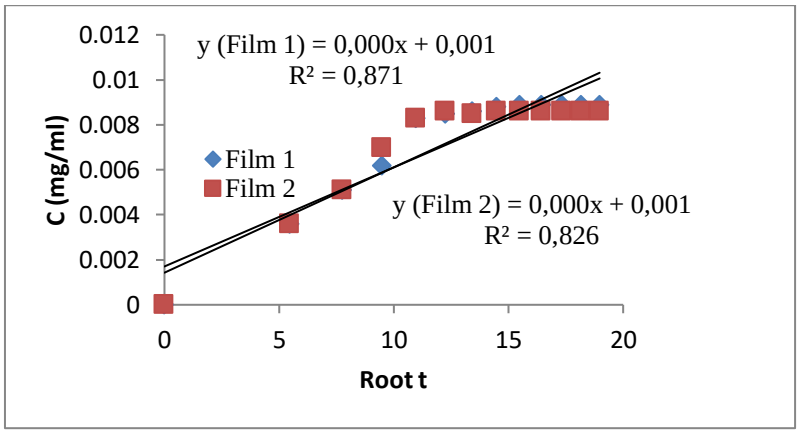
Before applying the four mathematical models (Pseudo-first-order, Elovich model, Huguchi model, power function) to the two curves (film 1 and film 2) of methylene blue liberation, we obtained the following curves (A, B, C, and D):



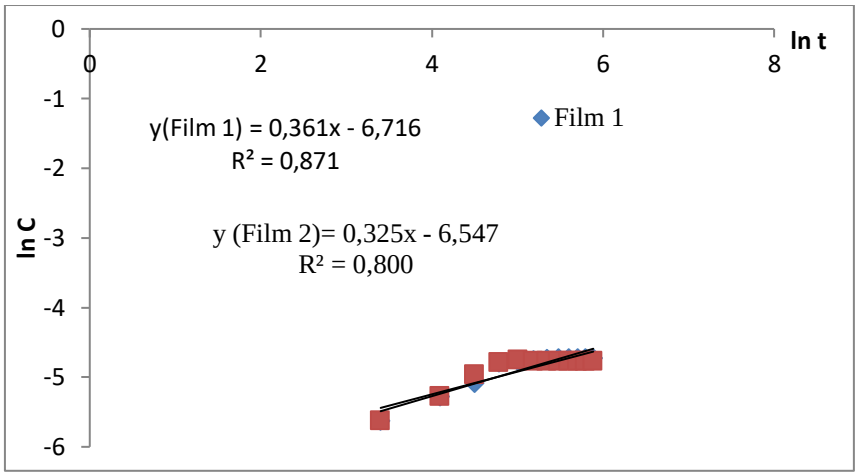
(A)



(B)



(C)



(D)

Figure IV-11: Release kinetics of formulations film 1 and film 2, (A) Pseudo-first -order, (B) Elovich model, (C) Huguchi model, (D) power function.

The Elovich's equation model for formulations (film 1 and film 2) where the correlation coefficients R^2 are between 0.925 and 0.890 is the best (figure IV-11 (B)).

IV-8- Antibacterial activity

The diameter of the film disc containing methylene blue used is 1cm, and the diameter obtained after the test with gram-negative bacteria is 1.8cm (figure IV-12).

Therefore, our medication is effective against this type of bacteria.

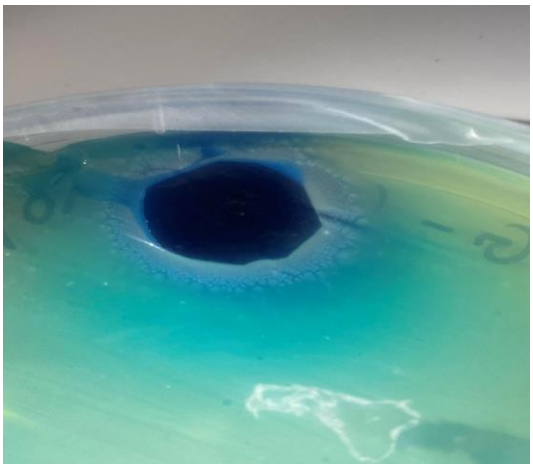


Figure IV-12: The film disc containing methylene bleu with gram-negative bacteria

However, with gram-positive bacteria, it is observed that the bacteria diffuse across the entire surface of the petri dish. (Figure IV-13)

Therefore, the medication is not effective against this type of bacteria.

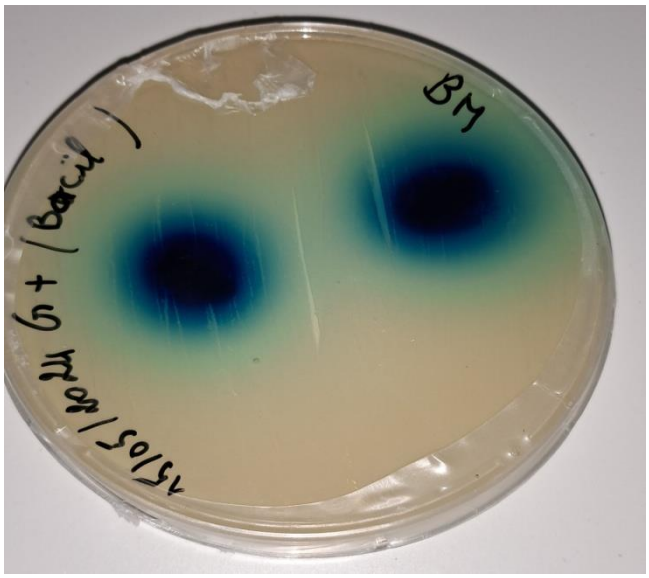


Figure IV-13: The film disc containing methylene with gram-positive bacteria.

IV-9- Conclusion

Based on the results obtained in this section, we conclude that:

- Moderate crosslinking allows for good mechanical strength, flexibility, and some permeability, suitable for applications needing controlled release or barrier properties. High crosslinking results in excellent mechanical strength, stability, and reduced permeability, potentially useful as a barrier material. The swelling test indicated that both films had significant water absorption capacity, likely influenced by their composition, crosslinking density, and hydrophilicity.

- According to the in vitro methylene blue release study for the different films 1 and 2, we achieved extended release, in film 1, an increase in the concentration of methylene blue is observed up to a value of ($8.9\ 10^{-3}$ mg/ml) at (240 min), after which it stabilizes.

In film 2, it can be noted that the released concentration increases over time until it reaches a maximum value of ($8.6\ 10^{-3}$ mg/ml) at (150 min).

- The Elovich's equation model for formulations (film 1 and film 2) where the correlation coefficients R^2 are between 0.925 and 0.890 is the best.

- For the antibacterial activity: Both films showed excellent activity against negative gram bacteria. This could be attributed to the antimicrobial properties of alginate and agar, which have been reported in previous studies.

General Conclusion

To further explore the properties and applications of the new pharmaceutical films prepared from alginate, agar, starch, glycerol, citric acid:

- A degree of crosslinking of 0.27 can result in a film with desirable properties. It's likely to have good mechanical strength and flexibility while still allowing for some permeability, which could be suitable for applications where controlled release or barrier properties are important.
- A degree of crosslinking of 0.8034 indicates a high level of crosslinking within the film composed of agar (1.5g), starch (0.5g), and citric acid (1g). This high degree of crosslinking suggests that a significant portion of available reactive sites on the polymer chains have participated in crosslinking reactions, resulting in a densely interconnected network.

Such a high degree of crosslinking can have several implications for the properties of the film. Firstly, it is likely to lead to excellent mechanical strength and stability due to the strong intermolecular bonds formed between the polymer chains. This can result in a film that is resistant to tearing and deformation.

Additionally, a high degree of crosslinking can also impact the permeability of the film. It may reduce the ability of molecules to diffuse through the film, making it potentially useful as a barrier material in applications where controlling the passage of substances is important.

- A swelling test result of 89.77% suggests that the film has absorbed water to a significant degree.
- A swelling test result of 96.83% indicates that the film has a high capacity for absorbing solvent or liquid, likely influenced by its composition, crosslinking density, and hydrophilicity.
- A loading test result of 9.24% provides valuable information about the concentration of the active pharmaceutical ingredient in the transdermal film and its implications for drug delivery performance, dosing accuracy, and regulatory compliance.
- The Active Ingredient Loading Test result of 10.4% indicates the efficient incorporation of active ingredients into the film, which can have significant implications for its performance and application suitability. Optimization of loading percentage and uniform

distribution are essential considerations in the formulation and characterization of functional polymer films.

- A thickness measurement of 0.26mm indicates a thin and flexible transdermal film suitable for drug delivery applications.
- The thickness of 0.22mm provides additional context for interpreting the swelling test result, highlighting the film's high absorption capacity relative to its size. Consideration of the film's thickness is crucial for understanding its swelling behavior and optimizing its performance for diverse applications.

Characterization Methods:

Infrared spectroscopy (IR) and light microscopy were used to characterize the films. These methods can provide information about the chemical structure and morphology of the films, respectively.

Methylene Blue Release:

The slow release of methylene blue from both films was observed. This could be due to the hydrophilic nature of alginate and agar, which may affect the diffusion rate of the dye.

Antibacterial Activity:

Both films showed excellent activity against negative gram bacteria. This could be attributed to the antimicrobial properties of alginate and agar, which have been reported in previous studies.

Potential Applications:

The slow release of methylene blue and antimicrobial properties of these films make them suitable for applications in wound dressings, biomedical devices, or antimicrobial coatings.

Future Directions:

To further optimize the films, studies could focus on varying the composition of alginate, agar, starch, glycerol and citric acid, as well as exploring different methods for cross-linking and modifying the films. This could enhance their properties and expand their potential applications. By considering these points, researchers can build upon the existing knowledge and develop more effective and versatile pharmaceutical films for various medical applications.

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