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Graduation thesis for Master's Degree in Chemistry
Specialty: Pharmaceutical chemistry

Theme:

Deep learning for Modeling formulation and drug release of topical Patches Containing Vitamin C.

Before the jury composed of:

- Dr H. Douba Presidente
- Dr M. Hammoudi Supervisor
- Dr R. Moumen Co-promoter
- Dr L. Bouhouf Examinatrice

Presented by:

- Rahmoune Fatima Zahra
- Amrouche yassemine

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Thanks

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Dedication

I thank *ALLAH* for giving me the strength and the patience in my studies and to finish this modest work

With tremendous love and sincerity, i dedicate this effort to my grate *father* who provided me with his encouragement, love, support, he was and he stills the secret of my success.

My big thanks goes to the meaning of love and tenderness, to my smile and the mystery of my existence, my loving *mother*, she sincerely sacrificed herself to be who I am now thank you *mum*.

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Thanks to me, I really deserve this success and I'll keep working to fulfill all my dreams

FATIMA

Dedication

In honor of Islam and the blessing of our prophet Mohammad

Praying and Peace be upon him

First of all, I thank myself for all the efforts I made. I went through a very difficult stage, despite this. I was strong and I was able to overcome myself. I was very persistent and strong and loved to work with full merit, I really deserve all the appreciation and thanks.

I wish myself all success in my career, thanks to God, and thanks to me, my efforts have reached this decisive stage, God willing, more successes for me, as I am very proud of myself.

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I dedicate this humble work with great love, sincerity and pride: the one who gave me life, the symbol of tenderness, who sacrificed herself for my happiness and my success, to *my Mother.*

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Yassemin

ملخص

الهدف من هذه الدراسة هو اعداد رقع جلدية تحتوي على الفيتامين C بالاعتماد على تركيزات مختلفة من البوليميرات البيولوجية؛ الكي توزان و الجينات الصوديوم. واستخدم الغليسرول و التوين 80 كمليّنات لهاته الرقع، وجرى تقييمها بتحديد الخصائص الفيزيائية والكيميائية، اذ انها اظهرت خصائص جيدة :(سمكها الذي وجد في المعدل بين 0.44 ملم بالنسبة للتركيبة F2 و 0.12 بالنسبة للتركيبة F9 بالإضافة الى مقاومتها للانحناء وكذا محتوى الرطوبة...). بالإضافة الى التفاعل بين البوليميرات المستعملة والعنصر النشط(الفيتامينC) باستخدام تقنية FTIR التي اظهرت تفاعلا بين الكيتوزان والالجينات وعدم وجود اي تفاعل لهما مع العنصر النشط مما يدل ان الفيتامين C قدتم تغليفه خلال عملية تحظير الرقع الجلدية، فضلا عن هذا الوصف المورفولوجي بواسطة تحليل المجهر الالكتروني الماسح SEM الذي اظهر بدوره بعض التموجات على السطح الخارجي للرقع وبالتالي تدفق جيد وعالي للدواء. أيضا اختبرنا تحرير الفيتامين C في وسط مشابه للبشرة ؛حيث من بين كل الرقع المختبرة F5 اعطت اعلى معدل تحرير للعنصر النشط بنسبة 70% خلال 80 دقيقة ونسبة تغليف بلغت 31.79 % في المساحة المختبرة، هذه الاخيرة تم اختيارها كأفضل رقعة. تم تطبيق نموذج الشبكة العصبية الاصطناعية (RNA) لمحاكاة وتوقع نسب تحرير الفيتامين C؛ باستخدام MATLAB تم تحرير هيكلة هامة لنموذج الشبكة العصبية الاصطناعية حيت وجدنا 3 عناصر ادخال وطبقة مخفية بها 8 خلايا عصبية و عنصر اخراج واحد يمثل نسبة تحرير الدواء ونه فالنموذج المتحصل عليه فسر بدقة تحرير العنصر النشط في كل التركيبات، هذه الدقة تتمثل في المعامل R^2 الذي يساوي 0.99973 و كذلك الخطأ النسبي(MSE= 0, 244036)، والخطأ المتوسط المطلق يساوي (ERA= 2,441) و (RMSE= 0, 4940).

الكلمات المفتاحية: نموذج الشبكة العصبية الاصطناعية (RNA)، فيتامين C، تحرير الدواء، رقع جلدية، تغليف الدواء

Résumé

Dans ce travail, onze (11) patches transdermiques de F1 à F11 contenant la vitamine C ont été préparés à base de deux bio polymères ; le chitosan (Chi) et l'alginate de sodium(Alg) à des différentes concentrations. Le glycérol et la Tween 80 ont été utilisés comme plastifiants et amplificateurs de perméation. Tous les patches ont été caractérisées par des analyses physico-chimique et ils ont montré de bonnes propriétés physico-chimiques :l'épaisseur qui ont été trouvés en moyenne entre 0,13 mm et 0,44 mm pour la gamme de F2 jusqu'à F9, résistance au pliage, teneur en humidité…etc., en plus de l'interaction principes actif-polymères à l'aide la technique FTIR qui a démontré une interaction entre (Chi) et (Alg) et aucune interaction avec le principe actif (vitC) qui signifie que ce dernier a été encapsulée dans nos formulations, ainsi une caractérisation morphologique par une analyse de microscope électronique de balayage MEB qui a montré quelques ondulations sur la surface externe en résultant une meilleure libération, aussi un test de libération in vitro, parmi tous les patches étudiés ;F5 a présenté un taux élevée de libération est de 70% après 80 min et un pourcentage

d'encapsulation est de 31,79% dans la surface sélectionnée, et ceci a été choisi comme la meilleure formulation. Le modèle de réseau de neurones artificiel (RNA) a été appliqué pour simuler et de prédire la libération de la vitamine C. à l'aide de MATLAB, une architecture significative de notre modèle a été criée avec trois (3) paramètres d'entrés, une seule couche cachée avec huit (8) neurones, et une sortie qui représente DD% (libération des médicaments). Le model RNA a prédire avec précision la libération cinétique de la vitamine C de chaque formulation. Cette performance a été démontrée par le R^2 obtenue qui est égale à 0,99973 pour la modélisation de la cinétique de libération, et une erreur moyenne (MSE= 0,244036), ainsi l'erreur relative absolue (ERA= 2 ,441) et (RMSE= 0,4940).

Mots clé : Modélisation ARN, vitamine C, libération de PA, encapsulation, patches transdermique.

Abstract

In this work, eleven (11) transdermal patches containing vitamin C were formulated based on different rations of two biopolymers chitosan (Chi) and sodium-alginate (Alg), Glycerol and Tween 80% were used as plasticizers and permeation enhancer. All the patches were evaluated for their physicochemical characteristics and showed good physicochemical properties: (thickness which were found in average between 0.44 (mm) for F2 and 0.12 (mm) for F9, folding endurance, moisture content) besides to the drug polymers interaction using FTIR technique which demonstrated the interaction between (Chi) and (Alg) and absence of any interaction with drug (Vit C) that mean the vitamin C were encapsulated in our formulations, and a morphological characterization by SEM microscope analysis that showed some undulations on the external surface resulting high delivering, along with their in vitro released test, among all the studied patches, F5 presented high delivery (70%) after 80 minutes, with percentage of encapsulation close to 31,79% in the selected surface and this was selected as the best formulation. The artificial neural network (ANN) model was applied to simulate and predict the vitamin C release. Using MATLAB, a significant architecture of our model was cried with 3 inputs in the inputs layer, one hidden layer with 8 neurons, and one output which represents the DD% (drug delivering%). The ANN model accurately predicted the kinetic release of vitamin C from each formulation. This performance was demonstrated by the obtained R^2 = 0.99973 for release kinetics modeling, with root mean square error (MSE =0.244036) and absolute relative error (ARE_{max}=2.441), (RMSE= 0.4940)

Keywords: ANN modeling, vitamin C, drug delivering, encapsulation, transdermal patches.

SC: chitosan solution

Chi: chitosan

Alg: sodium-alginate

Vit C: vitamin C

Gly: glycerol

Tw 80: tween 80

KBr: Potassium bromide

FITR: Fourier transforms infrared spectroscopy

SEM: surface micro-structure and morphology (scanning electron microscopy)

MEB: microscope électronique de balayage

TDDS: Transdermal drug delivery system

DDS: drug delivery system

DD%: percentage of drug delivery

AI: artificial intelligence

ANN: artificial neural network

AGI: artificial general intelligence

ASI: artificial super intelligent

ANNI: artificial neural network intelligent

MATLAB: matrix-laboratory

FDA: Food and Drug Administration

DSC: differential scanning calorimetric

ML: machine learning

ADALINE: adaptive linear

MADALINE: multiple adaptive linear

LMS: Least Mean Square

ART: Adaptive Resonance Theory

SOM: self-organized maps

SVM: support vector machines

MLP: multi-layer Perceptron

RMSE: Root means squared error

R^2: coefficient of determination

ARE: absolute relative error

Lm: function Levenberg-Marquardt

F1: membrane of 100% chitosan

F2: membrane of (90% chitosan / 10% alginate)

F3: membrane of (80% chitosan / 20% alginate)

F4: membrane of (70% chitosan / 30% alginate)

F5: membrane of (60% chitosan /40% alginate)

F6: membrane of (50% chitosan / 50% alginate)

F7: membrane of (40% chitosan / 60% alginate)

F8: membrane of (30% chitosan / 70% alginate)

F9: membrane of (20% chitosan / 80% alginate)

F10: membrane of (10% chitosan / 90% alginate)

F11: membrane of 100% alginate

General Introduction

General introduction

The pharmaceutical industry has seen a significant surge in data digitization in recent years. However, the difficulty of evaluating, and utilizing knowledge to solve complicated healthcare problems arises with digitalization. This encourages the application of artificial intelligence (AI) in numerous areas of society especially in the pharmaceutical industry. AI is a technology-based system involving a variety of advanced tools and networks that can mimic human intelligence. Its applications in the pharmaceutical field are constantly being expanded, because the absence of new technology hinders healthcare and drug development and making it a time-consuming and costly; that can be solved by applying AI, allowing for faster therapeutic target validation and structural design optimization and it can help with the development of pharmaceutical products by providing rational drug design, including personalized medicines and managing clinical data for future drug discovery and development (1), AI may also be used to evaluating and predicting the performance of recently discovered galinic types.

In recent years, there has been a surge in interest in creating and developing new drug administration routs, transdermal system was presented to overcome the difficulties of drug delivery especially oral route that's why transdermal patches have become well-known in the field of drug delivery systems. According to FDA (Food and Drug Administration), patches are medicated adhesives placed on the skin to deliver a specific dose of medication from the dermal barrier into the bloodstream, and they are designed to control the release of the active substances (2), usually through a porous membrane covering a reservoir of medication, which is an advantage of transdermal drug delivery over other types of delivery systems such as oral, topical, *IV*, *IM*, and so on (3). Several types of transdermal patches may be recognized based on the positioning of the control components: reservoir system, matrix system, and micro reservoir system, the difference between them is in the rate and time of delivering the active ingredient.

Predicting the reliability and performance of pharmaceutical products in general, and patches in particular, becomes easier in the presence of artificial intelligence AI, which encompasses a wide range of branches, each with its own purpose and field of application. Because of its ability for simulating, making predictions, pattern recognition, and modeling, ANN models are regarded the most popular among AI models. Its application as a technology has been widespread in recent years in many parts of life and it has been used in many

scientific fields such as: in solar energy, solubility of solid drugs. One of the great advantages of ANN's models is their ability to learn (store experimental knowledge), generalize (make knowledge available) or extract rules automatically from complex data.

As part of our study, we aim to prepare transdermal patches containing vitamin C as active ingredient.

The work of our thesis was carried out in the field of pharmaceutical chemistry, we are interested in the problem of the preparing patches based on biopolymer (chitosan and alginate), and if the encapsulation of the vitamin C was done during the preparation, our main objective of this study is to use the artificial neural network ANN to simulate and predict the results of drug delivering, in order to obtain a mathematical model in the form of interface which must be reliable and generalized at all times according to the following assumptions:

- The biopolymers (Chitosan/Alginate) can encapsulate a large amount of the active ingredient without using a cross linking agent.
- Among the various branches of the artificial intelligence (AI), the ANN architecture created with a single hidden layer and a minimal number of neurons can simulate and transfer the results of drug delivering to a reliable mathematical model.

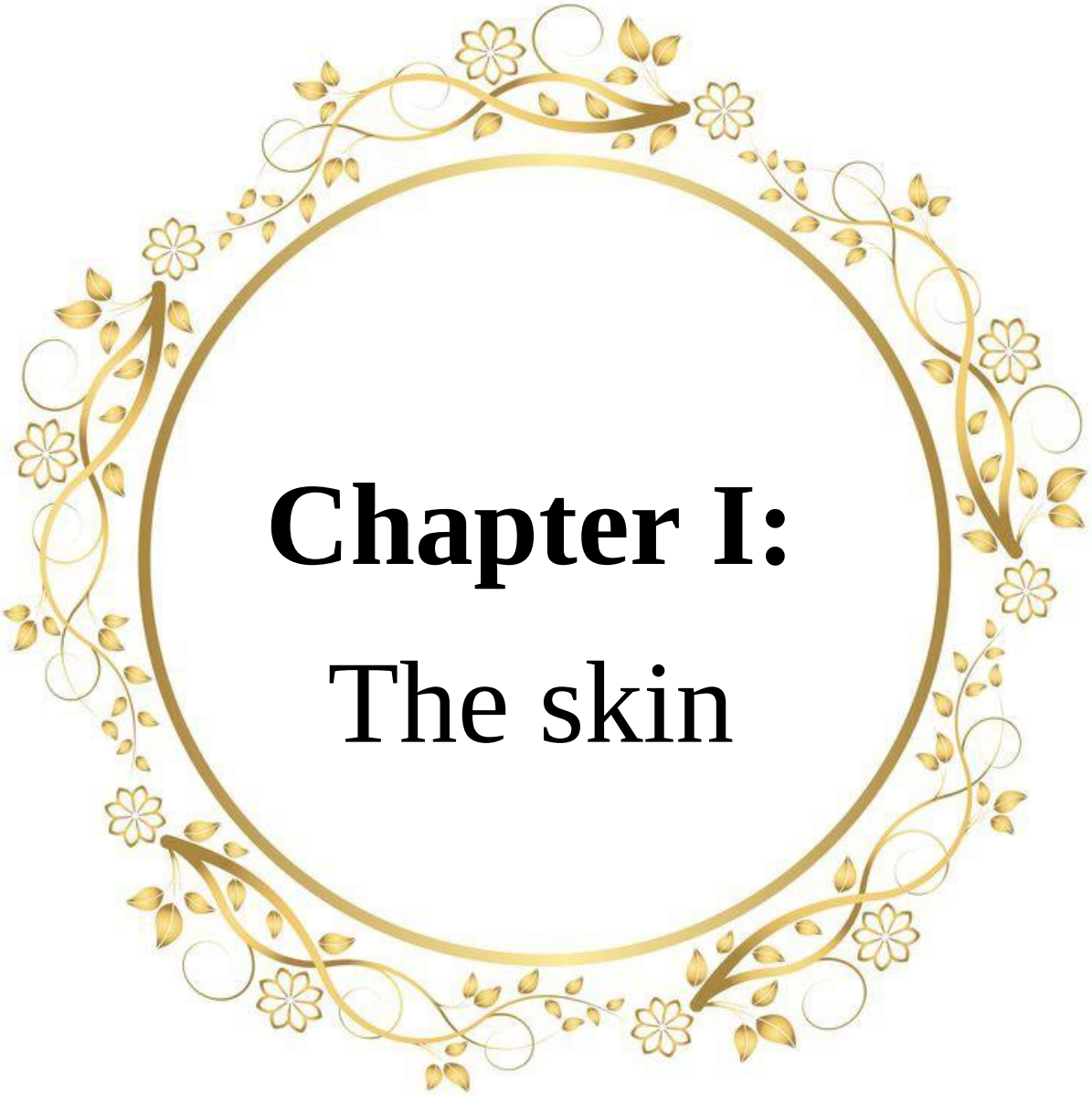
This thesis will be structured in three main sections: The first section consists of giving a bibliographic review in 3 chapters:

- Generalities on the skin and the vitamin C.
- Patches and transdermal drug delivery system.
- Artificial Neural Networks

The second part defined the experimental study which includes:

- The preparation of the transdermal patches.
- Physic-chemical characterization of the prepared membranes using FTIR technique.
- A morphological characterization using SEM technique.
- In vitro drug release

The third section consists of modeling the results of the active substance delivering using ANN model, and finally a general conclusion to conclude all what we have found



Chapter I:

The skin

I. Introduction

Located on the outermost layer covering a living body, skin is an organ which protects the underlying body from the external environment such as shocks, temperature, ultraviolet radiation, chemicals and other threats. **(1)**

It represents the largest organ in the human body, and is made up of many types of cells **(2)** and it reflects our origin, the lifestyle in which we live, and our state of health. The skin is composed of three layers, listing from the outside, which are the epidermis, the dermis, and the subcutaneous fat tissues, each layer is responsible of its own function but it occurred some changes in skin color or pigmentation, which indicate that the skin is not in a good conditions, and this change is due to external factors to which the skin is exposed, such as sunlight, fatigue and anxiety. That is why cosmetic labs offer several natural products in order to protect the skin. **(3)**

One of the most important of these preparations includes in its composition the vitamin C, which is existing in high levels in both the dermis and the epidermis, but with age, aging causes a decrease in the content of vitamin C. **(4)**

I.1.Definition

The skin is the largest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical, chemical, and biologic assailants, as well as prevention of excess water loss from the body and a role in thermoregulation. **(5)**

Also the skin is an ecosystem composed of $1.8m^2$ of diverse habitats with a large quantity of bends, invagination and specialized niches that support a wide range of microorganisms. The primary role of the skin is to serve as a physical barrier, protecting our bodies from potential assault by foreign organisms or toxic substances. The skin is also an interface with the outside environment. **(6)**

In other side skin is considered as a highly efficient barrier that limits molecular transport both from and into the body, preventing molecular permeation; witch is named as transdermal barriers delivers; especially for drugs; because The transdermal route of drug

delivery hence appears to be a good alternative approach instead of an oral route as it eliminates chances of drug loss by hepatic metabolism and even provides better patient compliance as opposed to other routes like parenteral route. **(7)**

Other functions include protection and sensation. To fulfill these functions, mechanical stability is as important as mechanical flexibility. However, the mechanical balance of skin can be threatened by disease, trauma, medical or cosmetic treatments. In order to understand the skin behaviour following the onset of these conditions, knowledge of the mechanical behaviour of healthy skin in normal conditions is essential. **(8)**

Our skin is a non-uniform organ in its structure; which explains the variety of its functions.

I.2.Composition of skin

Human skin is composed of several layers, each with a unique structure and function, but most research on its mechanical properties has ignored this non-uniform layered structure. **(9)** For many clinical and cosmetic applications, however, knowledge of the mechanical behaviour of the various skin layers is indispensable. For example, the benefit of transdermal drug delivery is that the micro needles exclusively damage the pain-free outer skin layer, the epidermis. Its mechanical response is therefore of particular interest. For needle insertion into the underlying dermal layer or for diseases such as pressure ulcers, the combined mechanical response of all individual skin layers is important. Although often not recognized, this is also the case during the removal of skin adhesives or the use of consumer products such as shavers. For all these applications, the subcutaneous fat layers contribute by attenuating or dispersing the external pressures, even when those are very small trial (see layers of the skin in figure I.1). **(10)**

The skin has three layers with different thickness, strength and function:

- Epidermis: Thin outer layer
- Dermis: Thick inner layer
- Hypodermis or sub cutis: A fatty layer of subcutaneous tissue.

I.2.1.Epidermis

The epidermis is the outermost layer of the skin and covered with epidermal cells, is thin and a vascular it varies in thickness from less than 0.1 mm on the eyelids to nearly 1 mm

on the palms and soles. As dead surface squares are shed (accounting for some of the dust in our houses), the thickness is kept constant by cells dividing in the deepest (basal or germinative) layer **(11).**The epidermis is normally regenerating every 4 to 6 weeks. Its functions are to maintain skin integrity, to provide a physical barrier against assault by microorganisms and the environment, and to maintain hydration by holding in moisture.

The epidermis may be divided into the following strata, or sub-layers (figure I.2):

- **Stratum corneum (horny layer)**

The epidermis it contains an essential and very important layer which is called the Horney layer « stratum corneum ». The stratum corneum has a thickness of only 10–20 µm **(12)**. It consists of 15 to 25 layers of flattened hexagonal cells. These cells are rich in keratin **(9)**. pH of the stratum corneum is between 4.5 to 5 (acid medium); this pH plays a role in the regulation of proteases which involves desquamation **(13)**.Also, the outer layer of the skin consists of several layers of dead cells that have lost their nuclei, and they are eliminated through the peeling process (desquamation), this process takes approximately 28 days. **(14)**

- **Stratum lucidum**

This layer is a translucent line of cells found only on the palms and soles.

- **Stratum granulosum (granular layer)**

This layer is composed of two to three cells thick, contains select keratinocytes.

- **Stratum spinosum:**

This layer is composed of keratinocytes that become larger, and contain less water as they travel to the surface of the skin.

- **Stratum germinativum–stratum basal (basal layer):**

This layer is made up of a single layer of cubic cells; these are the keratinocytes **(10)**. The stratum germinativum comprises since 10% of its cells divide every day**.** These cells, by migrating towards the most superficial layers of the epidermis, become polyhedral, thus losing their nucleus to become corneocytes. pH of the basal layer is close to that of the dermis about 7,2. **(15)**

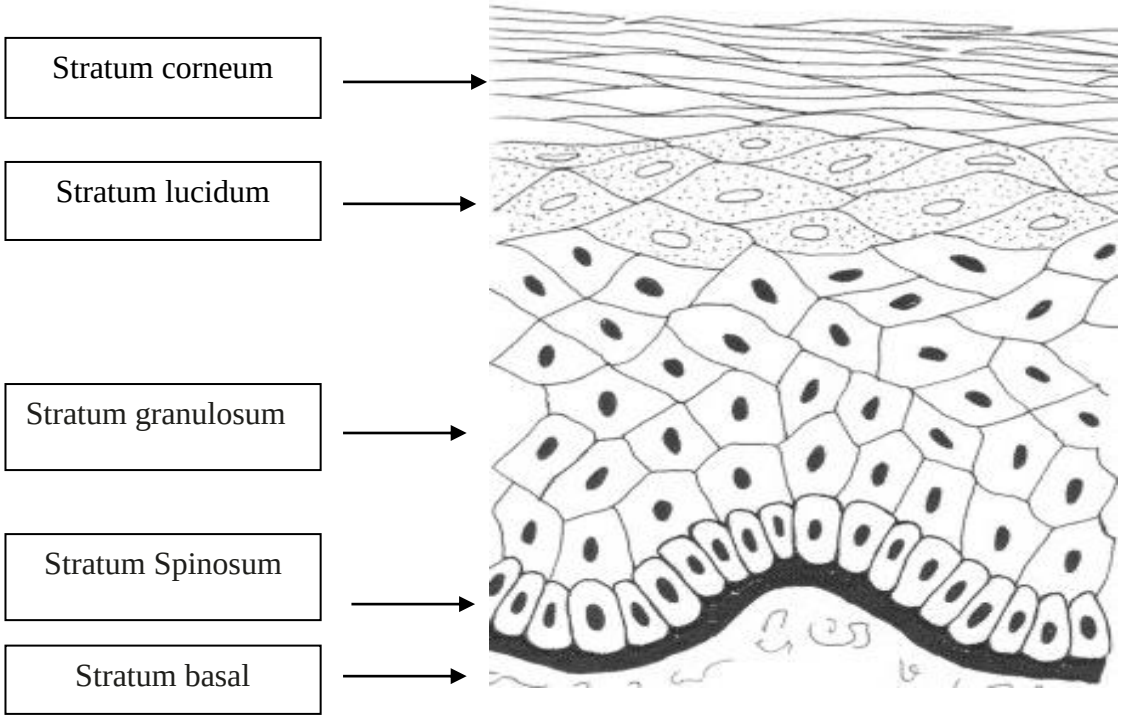


Figure I.1: structure of the epidermal barrier. **(16)**

Epidermal derivatives (appendages) are essential parts of the skin that have adapted to serve a variety of special functions and the principal derivatives of the epidermal layer are:

- Melanocyte
- Basket Cells
- Merkel Cells
- Blood Vessels
- Gland
- Hair and hair erector muscles
- Hair Follicle
- Sweat Gland.

I.2.2.Dermis

The dermis can be divided into two anatomical regions: the papillary and reticular dermis. The papillary dermis is the thinner outermost portion of the dermis, constituting approximately 10%of the 1-4 mm thick dermis. It contains relatively small and loose distribution of elastic and collagen fibrils within a significant amount of ground substance. Its content in water and vascular volume show physiological variations that can alter the mechanical behaviour of skin as whole. **(8)**

The dermis contains blood vessels, hair follicles, lymphatic vessels, sebaceous glands, and eccrine (sweat) and porcine (scent) glands. It's composed of fibroblasts, which form collagen, ground substance, elastin, and other extra cellular, matrix proteins. Ground substance, an amorphous substance composed of water, electrolytes, plasma proteins, and muco-polysaccharides, fills the space between cells and the fibrous components, making the dermis turgid. Collagen fibers, the major structural proteins of the body, give skin its strength. Elastin is responsible for skin recoil or resiliency. Thick bundles of collagen anchor the dermis to the subcutaneous tissue and underlying supporting structures, such as fascia, muscle and bone. **(10)**

I.2.3.Subcutaneous tissue (Hypodermis)

The hypodermis is defined as the adipose tissue layer found between the dermis and the aponeurosis and fasciae of the muscles. Its thickness varies with anatomical site, age, sex, race, endocrine and nutritional status of the individual **(8).** The subcutaneous tissue is composed as well as major blood vessels, nerves, and lymphatic vessels**. (10)**

The mechanical functions of the subcutaneous adipose tissue include allowing the overlying skin to move as a whole, both horizontally and vertically, and the attenuation and dispersion of externally applied pressure. **(17)**

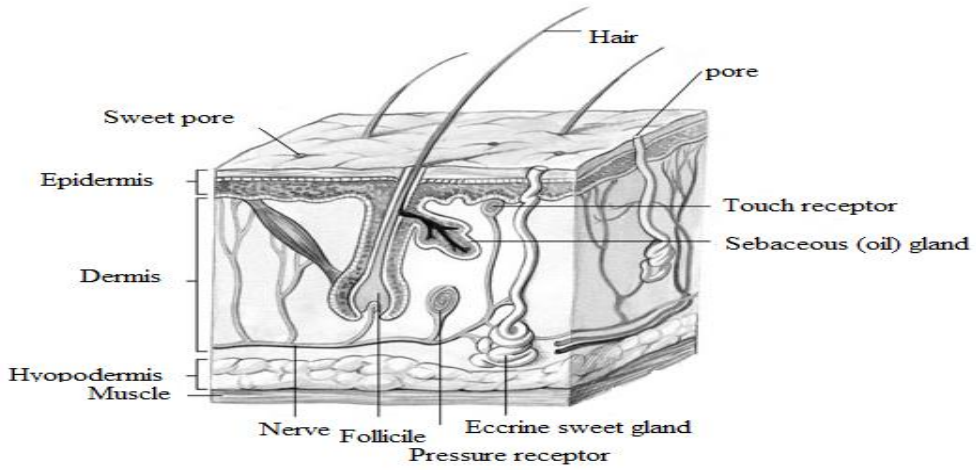


Figure I.2: principal layers of the skin from: the integumentary system "Dumies". **(18)**

I.3.Different types of skin

The type of skin is determined by genetics, although it will also be affected by other factors and can change with time. Based on these characteristics, there are five types of healthyskin: normal, dry, oily, combination (both oily and dry skin) and sensitive. Below, we describe the main characteristics of each type of skin. **(19)**

- **Normal skin:** is a term widely used to refer to well-balanced skin. Normal skin is well balanced: neither too oily nor too dry. A normal skin has in general: fine pores, good blood circulation, fresh and uniform in a pink color and transparency.
- **Dry skin:** is used to describe a skin type that produces less sebum than normal skin. Dry skin lacks the lipids that it needs to retain moisture and build a protective shield against external influences.
- **Oily skin**: It has a glossy shine and visible pores the causes of oily skin are: genetics, hormonal changes and medication, stress**.**
- **Combination skin**: A combination of skin is identified as having an oily zone and dry cheeks. The oiliness and the dryness are a response to weather conditions with summers causing extreme oiliness and winters causing dryness. **(20)**
- **Sensitive skin:** it is become irritated or inflamed easily. Sensitive skin is not determined by how much sebum a person's skin produce people with dry or oily skin can also have sensitive skin. In some cases, sensitivity is related to skin conditions, such as eczema. **(21)**

I.4.Skin function

Skin is much more than an outer covering so that it has many important functions, such as:

- ✓ **Protection:** Skin acts as a physical barrier to microorganisms and other foreign matter, protecting the body against infection. The outer layer (stratum corneum) is slightly acidic, creating resistance to pathogenic organisms; it also helps protect against excessive water loss, chemicals and other harmful substances, and ultraviolet radiation.
- ✓ **Thermoregulation:** skin regulates body temperature through vasoconstriction, vasodilation, sweating, and excretion of certain waste products, such as electrolytes and water.
- ✓ **Metabolism:** Synthesis of vitamin D in skin exposed to sunlight activates the metabolism of calcium and phosphate, minerals that play an important role in bone formation.
- ✓ **Synthesis of Vitamin D:** vitamin D is required to allow the body to absorb calcium and phosphorus. When the skin is exposed to ultraviolet light or sunlight, it converts a vitamin D precursor to vitamin D via the liver and kidneys. **(10)**

Each layer of skin is responsible about some skin's function as it shown in Table I.1.

Table I.1: layers and cells responsible on some skin function. **(11)**

Function	Structure: layers and cell involved
Protection against: chemicals, particles ultraviolet radiation antigens, haptens microbes.	Horny layer Melanocytes Langerhans cells
Preservation of a balanced internal environment.	Horny layer
Prevents loss of water, electrolytes and macromolecules Shock.	Horny layer
Shock absorber Strong, elastic.	Dermis and subcutaneous fat
Temperature regulation.	Blood vessels Eccrine sweat glands
Insulation.	Subcutaneous fat
Sensation.	Specialized nerve endings
Lubrication.	Sebaceous glands
Protection and prising.	Nails

Studying the acidity or the basicity of the skin is an important factor to know the right function of the skin layers.

I.5.Skin pH

The skin's pH is acidic, ranging from about 4.2 to 5.6, depending on the area of the body and whether or not the skin is occluded. Skin should be kept in the acidic pH range for several reasons. After an injury to the skin, its barrier function recovers faster when the skin pH is more acidic, rather than more alkaline (less acidic).

An acidic environment prevents premature desquamation, or shedding, of dead skin cells. Also, people with an acidic skin pH have less of a tendency toward sensitive skin, which is typically more alkaline. The pH of the skin helps regulates some of the functions of the stratum corneum, including its permeability, defense against bacteria and fungi, and the integrity and cohesion of skin cells. The microorganisms (skin flora) that live on or infect the skin grow differently based on the skin pH. Normal flora grows better at an acidic pH, whereas pathogenic organisms, such as staphylococci, streptococci, and yeast, grow better at a neutral pH. Skin products with a higher pH are thought to promote bacterial growth. **(10)**

The pH of the dermis is close to 7, and it's transformed into an acid pH of about **5** at surface of the skin. This considerable increase in the activity of H^+ ions is due to hydrolyse of the epidermis which generates several water soluble acids, especially the uronic acids, pyrrolidone carboxylic acid and lactic acid. These acids are not intervening in a way sensitive in establishing the pH of the skin. **(22)**

I.6.Vitamin C

In the presence of our oxygen-rich atmosphere, ultraviolet light generates reactive oxygen species in skin. In addition to sunlight, other inflammatory insults including smoking and pollution generate reactive oxygen species. Reactive oxygen species, in turn, cause oxidation of nucleic acids, proteins, and lipids. Reactive oxygen species alter DNA, as well as its repair, and trigger cytokine cascades that result in photo aging and photo carcinogenesis. The body protects itself naturally from reactive oxygen species by using antioxidants to neutralize them before they cause damage to the skin and its components. Vitamin C, or L-ascorbic acid, is the most abundant antioxidant in skin. Although most plants and animals synthesize L-ascorbic acid to protect themselves from free radical attack. **(23)**

I.6.1.Definition and structure

Vitamin C, or l-ascorbic acid, is a water-soluble organic compound, most common in the living world. It was discovered in the 18th century during a sea voyage.

Vitamin C has the general chemical formula $C_6H_8O_6$; it is belonging to the groups 6 atom sugars and is a derivative of D-Glucose. It is composed of ketone function, a lactone cycle and two alcohol functions: one primary and other secondary as it shown in figure I.3.Ascorbic acid has two optical forms: levogyro and dextrogyro but only the natural levogyro form (L-ascorbic acid) is biologically active. This vitamin can be extracted from nature or synthesized from D-Glucose**. (24)**

Physic-chemical Characteristics of vitamin C are shown in the table I.2.

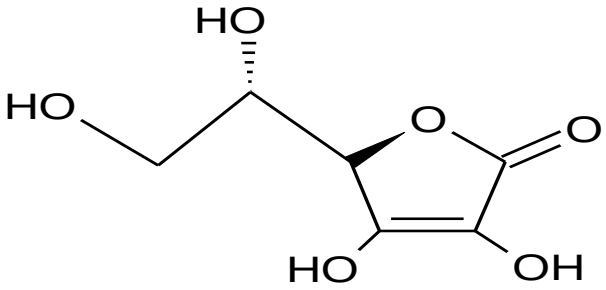


Figure I.3: chemical structure of the vitamin C. **(25)**

Table I.2: physicochemical properties of L-ascorbic acid. **(24)**

Aspect	**Molecular weight**	**Density**	**Boiling temperature**	**Water solubility**	**Dissociation constant**	**Rotation power**
White solid	176,124 g/mol	1,65 g/cm^3 at 20 C°	191C°	333,0 g/L at 20 c°	pka1=4,1 pka2=11,8	[αD]=+21° in water

I.6.2.Role of vitamin C in skin

Vitamin C is involved in the formation of the skin barrier and collagen in the dermis and plays a physiological role in the skin against skin oxidation, in anti-aging of wrinkles, and in cell signal pathways of cell growth and differentiation, which are related to the occurrence and development of various skin diseases, Vitamin C has a dual role of ant oxidation and pro-oxidation, and this role maintains the balance of the two reactions in the body. High levels of Vitamin C in the cells lead to oxygen-promoting reactions, which cause DNA damage, the

depletion of energy reserves, and failure of cellular metabolism. Vitamin C is also involved in resistance to UV-induced oxidative stress, inhibition of melanogenesis and promotion of the differentiation of keratinocytes and has been used for a long time as a clinical treatment reagent. **(26)**

I.7.Drug penetration routes

There are two possible routes of drug penetration across the intact skin **(27)**. The drug molecule is crossed through the healthy stratum corneum through the skin pathways, through the intercellular fat areas or through the cells **(28).** This allows transport of hydrophilic or polar materials. Transport through the intracellular spaces occurs by diffusion of lipophilic or no polar substances through the continuous lipid matrix. **(27)**

The figure I.4 below shows that surface of the skin touches the obstacle of the stratum corneum. This layer is moist as it contains water in the intercellular areas, and fat. These characteristics allow the drug molecule to pass through and spread upon contact with the skin and then reach the dermis. The path between cells is the fastest, even if it is longer. **(9)**

I.7.1.pathways involved in drug permeation

Drug permeation is percutaneous absorption or permeation which involves drug passage or transepidermal diffusion.

Transepidermal includes molecular intracellular and intercellular penetration:

Hydrophilic drugs penetrating through the intracellular pathway and substances penetrating through the intercellular pathway. These molecules diffuse into the non-aqueous lipid matrix. Drug molecules can pass through hair follicles or via the aqueous route to salty sweat glands.

Transdermal permeation is a series in sequence like:

- ✓ Adsorption of a penetrant molecule onto the surface layers of stratum corneum.
- ✓ Diffusion through stratum corneum and through viable epidermis.
- ✓ Finally through the papillary dermis into the microcirculation.

The viable tissue layer and the capillaries are relatively permeable and the peripheral circulation is sufficiently rapid. Hence diffusion through the stratum corneum is the rate limiting step. **(29)**

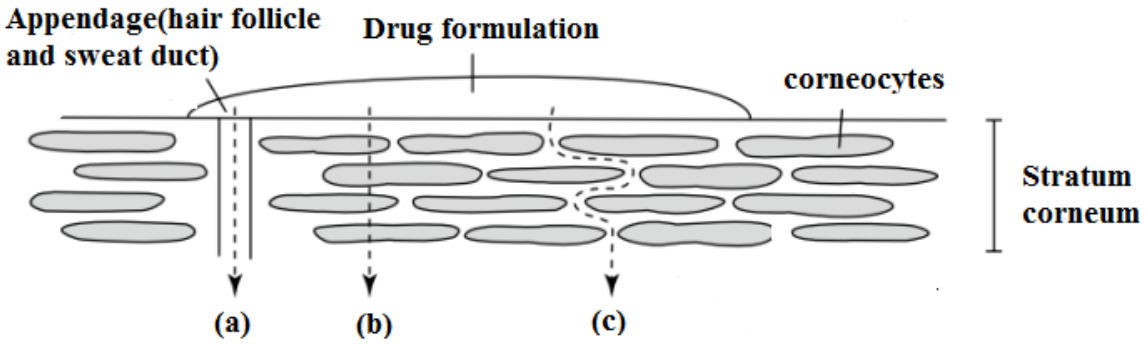


Figure I.4: Drug permeation pathways in the skin (stratum corneum shown): (a) the appendage route, (b) the transcellular route, and (c) the tortuous extracellular route. The transcellular and intercellular routes constitute the Trans epidermal pathway. **(30)**

I.8.Different mechanisms of absorption of molecules by skin

Absorption is a passive phenomenon for which the molecule from the surface of the sin must be transported to the blood. In the case of application of a cosmetic product such as, transdermal patches which contains vitamin C, the diffusion of the molecule will be deep but without exceeding certain limits; the action by the cosmetic must be only local. Among the different ways of passage we distinguish:

I.8.10.Passage through the stratum corneum

This layer made up of dead cells and they pass through the trans-cellular and inter-cellular pathway.

- **The trans-cellular way:** the molecule contained in the beauty products by the following conditions: that they are of small size or that they are hydrophilic; that is to say that they have an affinity for the water present in the well hydrated stratum corneum leading to making them soluble.
- **The inter-cellular pathway:** the lipophilic active ingredients which retain fat circulate in the inter-lipids by the cohesion of the cells of stratum corneum, then the interstitial liquid which fills the spaces between the cells of the deepest layers of the skin. Therefore trans-cellular permeation, where the passage is direct. **(31)**

I.9.Factors effect on skin permeability

The skin permeability is affected by several physical and chemical factors, including:

- **Temperature:** skin permeability can be changed by temperature. The conditions such as exposure to IR radiation of sun, fever, or higher vascularization (e.g., blood flow, or increased number of capillaries) increase skin temperature. Skin permeability followed by an increase in skin temperature can be related to the fluidization of inter-coenocytes lipid tails which lead to increased dermic clearance. Moderate skin heating to approximately 40°C was shown to increase transdermal delivery. On the other hand, it is reported that increasing the temperature of skin may reduce drug absorption by increased evaporation of a volatile penetrate and decrease defective concentration of compound on skin surface.
- **Water Balance:** the dry skin of humans is an essential component of their thermoregulation. In contrast, the high rate of evaporation from the skins limits the capacity to maintain body temperatures above ambient levels as well as the ability to be active during the day when evaporative stress high.
- **Anatomical Site Differences:** regional variation in skin permeability at different body sites may be related to skin thickness, number of cell layers, cell size of the epidermis and stratum corneum, and distribution of hair follicles and sweat pores. Because of thick layers of stratum corneum, permeability in palmar and plantar skin is expected to be less than that in the scalp or forearm**. (32)**

I.10.Physic-chemical characteristics of substances influencing absorption by the skin

The different active principles contained in drugs diffuse through a cell membrane according to a concentration gradient. The rate of diffusion is dependent on its molecule size and fat solubility, its degree of ionization. Drugs are of the non-ionized form are usually fat soluble (lipophilic) and readily diffuse across cell membranes. The ionized form has low lipid solubility; hydrophilic). **(33)**

- **Degree of ionization:** the permeation flux in the skin depends on the degree of ionization of the permeate because the ionization influences the solubility and the partition. An ionized species has a low permeability coefficient than a non-ionized species since the partition coefficient between the hydrophobic and hydrophilic medium is lower. Vitamin C deteriorates due to ionization and a neutral or alkaline pH; it only likes an acid pH. **(34)**

- **size and nature of the molecule that must cross the skin barrier :**
  - ✓ More than the molecules are small, more it easily will penetrate.
  - ✓ A hydrophilic molecule will tend to obstruct the trans-cellular pathway and will not be able to hydrate well.
  - ✓ Lipophilic molecule will pass in the intercellular spaces and the cutaneous annexes to diffuse in the deeper layers. **(15)**
  - ✓ By its hydrolipidic nature, the stratum corneum allows the absorption of amphiphilic molecules and moderately lipophilic molecules. **(35)**
  - ✓ More than the skin is thick and oilier, more permeable it is. Therefore it is necessary to clean the skin well before making a scrub or transdermal patches which contains an active principal in the cosmetology side to facilitate the penetration of cosmetic products and influence it well.
  - ✓ Irritated skin will be more permeable. Excessive hygiene, the use of too aggressive soaps can also alter the surface hydrolipidic film and make the skin more permeable. Therefore the more the skin is hydrated the more it is permeable. **(15)**

I.11.Skin care products

Our skin reflects our origin, lifestyle, age and state of health. Skin color, tone and evenness, pigmentation, as well as skin surface characteristics are signs of our skin's health. And to, protect and to treat our skin and hence to keep it in "good condition". Skin care products are readily available in daily life and they play a major role in health and nursing care. The promotion of skin care products including their claims are often based on an effect (e.g., moisturizing, antioxidant), evoked by an active (e.g., urea, tocopherol) that is delivered through a vehicle (e.g., lotion) that relies on a specific technology (e.g., nanotechnology). In addition, "without" claims (e.g., without parabens) often accompany nowadays promotions.

The concept of skin care is not well defined. It is a kind of umbrella term covering cleansing, perfuming, changing appearance, changing body odour, protecting and keeping the skin in a good condition. **(36)**

The skin care products can be used to protect and develop the skin condition; these products may have some categories such as:

- **Antifungal and antimicrobials (topical):** products that inhibit the growth of organisms that cause superficial skin infections; such as yeast.
- **Liquid skin protectants (also called skin sealants):** products that protect the skin by forming a transparent protective barrier.
- **Moisture barriers (also called protectants):** ointments, creams, or pastes that protect the skin from urinary and fecal incontinence by shielding the skin from irritants or moisture.
- **Skin cleansers:** pH-balanced products used to provide moisture and to effectively remove urine, feces, or both without patient discomfort.
- **Therapeutic moisturizing products:** lotions and creams used to replace lost lipids in skin. **(10)**

  Among the products used for skin care there is the vitamin C which is the most famous organic element protecting the humane skin.

I.12.Vitamin C absorption

As one of the water soluble vitamins, Vitamin C is a small enough molecule that it can readily pass (diffuse) through cell membranes to be absorbed through the epidermis and transported to the underlying dermis where it can directly fight oxidative stress that leads to wrinkling, hyperpigmentation and development of skin cancers. However, Vitamin C absorption through the skin greatly depends on the pH of the Vitamin C application. Because the skin is slightly acidic, so, too, must the Vitamin C delivery system be acidic. A pH below 4.0 is ideal. The concentration of Vitamin C also impacts absorption. You don't necessarily absorb more Vitamin C just because a product has a higher concentration of Vitamin C in it. Maximum absorption seems to happen with 20% Vitamin C. Higher concentrations will likely lead to excess Vitamin C being transported from the tissues into the blood where it will eventually be filtered out and excreted in urine. One major challenge to topical delivery is getting Vitamin C to live cells beneath the (dead) stratum corneum. The key to effective topical delivery of Vitamin C is exfoliation removing the uppermost layer of dead cells allows Vitamin C formulas to be applied directly to live cells. Chemical peels, microderm abrasion and other gentle mechanical exfoliants will all work. **(37)**

I.13.Topical application of vitamin C

In our daily life, our skin is frequently exposed to the sun and some external factors, and if human health is not in good condition, it reflects this damage to the skin. And with this, we

must provide glasses for our skin by providing vitamin C through topical application **(38)**. Some previous studies show that absorption of vitamin C by the skin is highly dependent on pH. Vitamin C does not settle in the skin in abundance, because it decomposes slowly, due to the exposure of the skin to air, heat and light. Thus, people using topically applied solutions have 0.6%-10% vitamin C, according to human studies. **(4)**

I.13.1.Application of vitamin C in cosmetology

Due to its biological and chemical properties, vitamin C can be used in the cosmetic industry, especially in topical care. Their applications are:

- **Anti-aging care, cutaneous aging (wrinkles, fine lines):** with age, the levels of vitamin C in the skin decrease to 60-70% and the skin loses its resistance to external aggressions (UV pollution) and its rigidity. As vitamin C is stimulating to the synthesis of collagen allowing a better elasticity of the skin and the filling of wrinkles, and to combat oxidative stress by increasing antioxidant levels is skin's cells. It can be used in anti-aging cosmetic products with concentration required to 5% and its action can be increased by combining it with other antioxidants such as vitamin E (the vitamin C being hydrophilic and vitamin E lipophilic, they act synergetically in the skin and other cosmetology used like:
- **Lightening and unifying care:** Vitamin C can be used as a whitening agent against brown tasks left by the sun and tasks due to tobaccos.
- **Antioxidant in cosmetic formulations:** vitamin C and its derivatives can be used as an antioxidant in cosmetic formulations, with concentrations between 0.5 to 3% from ascorbic acid and its water-soluble esters, and 0.1 to 0.5% for its lipophilic esters. **(24)**

I.14.Conclusion

Thus the skin is a heterogeneous organ, containing a number of cellular layers, divided into distinct regions. The epidermis is the outer region of embryonic ectodermal origin, which covers the connective tissue, it has several layers each with special function .Human skin is very sensitive, especially when exposed to many external factors, so that it must be protected by some products insults vitamin C. Which is a powerful antioxidant for the skin health; but if we mix it with water, it will rapidly degraded, which makes the formula unstable in a serum or in a cream **(39).**So why don't we think about giving up these products like creams and serums, and replacing them with transdermal patches that contain vitamin C.

Chapter II

Transdermal Drug Delivery system

Patches

II. Introduction

The most common routes of drug delivery are the oral and parenteral routes with the majority of small molecule drugs conventionally delivered orally but these conventional routs of medication delivery have many inherent limitations which could potentially be overcome by advanced drug delivery methodologies such as the transdermal drug delivery system **(1)**. This is due to the skin which forms the most important boundary of the body, and is chosen as the route of drug administration when local and systemic therapeutic effects are sought **(40).** Topical drug films that called patches are devices used to deliver active ingredients through the epithelial tissues of the body to treat systemic diseases. And have been designed to provide controlled continuous delivery of drugs via the skin to the systemic circulation, and is used to deliver a specific dose of medication through the skin and into blood stream. **(41)**

Nowadays the preparation of these patches are based on the biopolymers like polysaccharides which are the main constituents of the topical films due to their distinctive properties, and as we have done in this work, the formation of biofilms based on polymers such as chitosan and Alginate. The combinations of these two polymers are considered to be interesting pharmaceutical excipients in the production of transdermal patches. **(40)**

And in this chapter we talked about the transdermal patches and how does skin absorbs the active ingredient mentioned in the patches, and how does it react with this biofilm.

II.1.Transdermal drug delivery system (TDDS)

The transdermal drug delivery route has been recognized as an attractive alternative to oral and parenteral delivery due to its numerous advantages **(42)**. TDDS have been appointed as effective platforms to improve the pharmacological and therapeutic properties of drugs. These systems not only aid the handling and administration of drugs but also help the drug to overcome certain barriers such as the low solubility caused by drug's hydrophobicity reaching the targeted tissues without premature degradation **(43),** It is a non-invasive approach for the administration of drugs, and transdermal patches can be self-administered by the patient. In particular, they overcome the first-pass metabolism effect of the liver and provide maintenance of constant drug plasma concentration, offering a prolonged duration of pharmacological action. In addition, delivery of drugs can be easily checked by removal of the patch when immediate withdrawal of the treatment is required. **(42)**

The drug that deliver trough the patches are initially penetrates through the stratum corneum and then passes through the deeper epidermis and dermis without drug accumulation in the dermal layer. When drug reaches the dermal layer, it becomes available for systemic absorption via the dermal microcirculation **(27).** But there are several parameters that affect the delivery of the drug from the patch to the skin; Absorption, for instance, depends on the site of application, thickness and integrity of epidermis, size of drug molecule, permeability of the drug delivery membrane, degree of skin hydration, pH of the drug, drug degradation by skin flora and body conditions, such as body temperature, that are responsible for blood flow alteration. Skin thickness and blood flow are two parameters that vary with age and are responsible for different responses to the same transdermal device, a notable effect on pharmacokinetics of the drug **(43).**

II.2.Transdermal patches

A transdermal patch or skin patch is a medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and into the blood stream. Often; this promotes healing to an injured area of the body **(44).** The development of patches began in the early 1970 and the first patch, was approved by the FDA « Food and Drug Administration » in 1979. **(9)**

According to Prausnitz and Langer, transdermal delivery systems can be divided in three generations of development. As stated in European Pharmacopoeia, transdermal patches are designed to provide the controlled and sustained release of active substances to the systemic circulation after cross the skin barrier. The majority of the actual patches are within the first-generation class that carries specific types of drug sable to cross skin barriers at a therapeutic level but with little or no enhancement. Moreover, this type of patches is limited to drugs with suitable properties such as low-molecular weight, lipophilic and efficacious at low doses. The second generation is characterized by some improvements that allow the delivery of small molecules by increasing skin permeability. Third generation patches enable the transdermal delivery of small molecules of drugs or macromolecules such as proteins or DNA. **(45)**
Several polymers are the backbones of transdermal patches, and different types of either synthetic or natural polymers are used to produce transdermal patches. **(42)**

These biopolymers have been widely used in biomedicine such as wound dressing, skin substitutes and drug delivery patches based on biodegradable or non-biodegradable synthetic polymers. **(45)**

The benefits of administration (patches) are:

- The reduction of side effects by maintaining a constant rate of active ingredient released during the time of application,
- The decrease in the frequency of administration and the possibility of chronic therapy with active ingredient with a low half-life,
- Improved patient compliance and comfort,
- Rapid reduction of blood levels of active ingredient by taking off the device. **(31)**
- Minimization of fluctuation in peak plasma concentration as in case of the oral and parenteral route
- No issue of hepatic first-pass metabolism (presystemic metabolism),
- Reduced dosing frequency and a dose of drug and its bioavailability improvement. **(7)**

II.3.Composition of transdermal patches

A patch is constituted as a whole by the superposition of the different layers; so the development of this transdermal devise is linked to the good knowledge of the functionality of each layer.

The main components of transdermal patch are the following and shown in figure II.1:

II.3.1.Structural elements

- **A protective external support**

It must offer the best compromise between flexibility and conformability; it allows retaining moisture from the skin and moisturizing the area of application, in order to guide the active ingredient towards the skin. This is necessary when the active ingredient is coated some adhesive. **(31)**

- **A drug reservoir**

A layer containing the active ingredient in dissolved or dispersed form, and for successfully developing a transdermal drug delivery system, the drug should be chosen with great care for the desirable properties of a drug for transdermal delivery **(46).**Transdermal patches offer much to drugs which undergo extensive first pass metabolism, drugs with narrow therapeutic window, or drugs with short half-life.

- **An adhesive layer**

Is the most important part of the patch, it increases the permeability of stratum corneum so as to attain higher therapeutic levels of the drug. **(41)**

- **An internal protective layer**

To be removed before use and not involved in the administration of the active substance. Considered as a component of the primary packaging, it preserves the system outside its use. **(31)**

II.3.2.Functional elements

- **A polymer matrix**

Is the backbone of TDDS, which control the release of the drug. Polymer should be chemically non-reactive, should not decompose on storage, should be non-toxic, cost should not be high. **(41)**

- **Membrane of control:** to control the speed of release. **(31)**

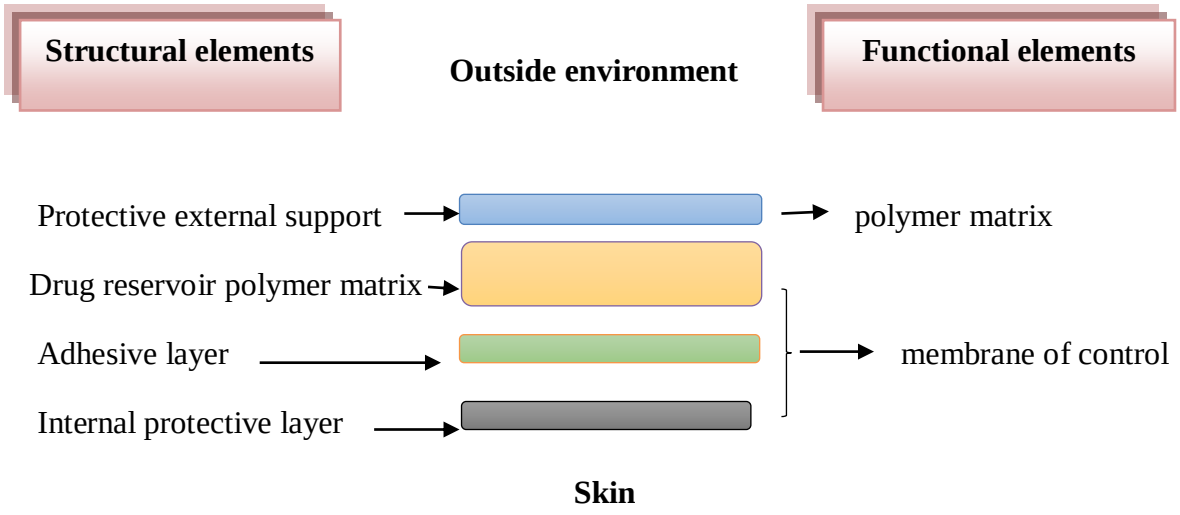


Figure II.1: General representation of a transdermal patch. **(47)**

II.4.Types of patches

All current transdermal patches consist of an impermeable external support, a compartment compressing the active ingredient and a release control element, besides to the adhesive element. Depending on the positioning of the control elements, several types of transdermal systems are distinguished:

- **Single layer drug in adhesive**

In this type the adhesive layer contains the drug. The adhesive layer not only serves to adhere the various layers together and also responsible for the releasing the drug to the skin. The adhesive layer is surrounded by a temporary liner and a backing **(44).** As shown in figure II.2

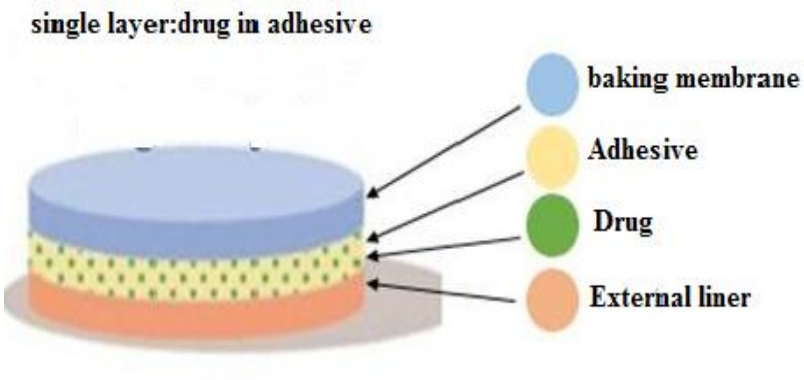


Figure II.2: drug in adhesive with single layer in a transdermal patch. **(46)**

- **Multi -layer drug in adhesive**

The Multi-layer Drug-in-Adhesive is similar to the Single layer Drug-in-Adhesive in that the drug is incorporated directly into the adhesive **(46).** This type contains an immediate drug-release-layer and other layer will be a controlled release along with the adhesive layer. The adhesive layer is responsible for the releasing of the drug. This patch also has a temporary liner-layer and a permanent backing **(44).** As indicated in figure II.3

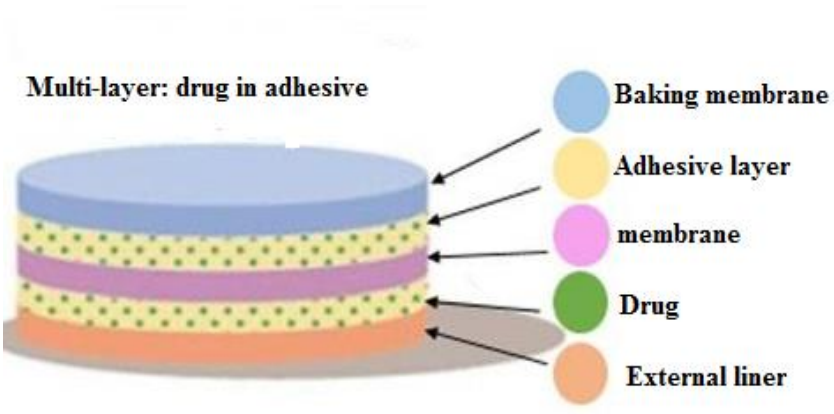


Figure II.3: drug in adhesive in a multi-layer patch. **(46)**

- **Reservoir system**

In this system the drug reservoir is embedded between an impervious backing layer and a rate controlling membrane (figureII.4). The drug releases only through the rate controlling membrane, which can be micro porous or non-porous. In the drug reservoir compartment, the drug can be in the form of a solution, suspension, gel or dispersed in a solid polymer matrix.

Hypoallergenic adhesive polymer can be applied as outer surface polymeric membrane which is compatible with drug **(44),** the active ingredient here is either completely or partially encapsulated in a compartment where the release surface is covered by a polymeric control membrane. **(31)**

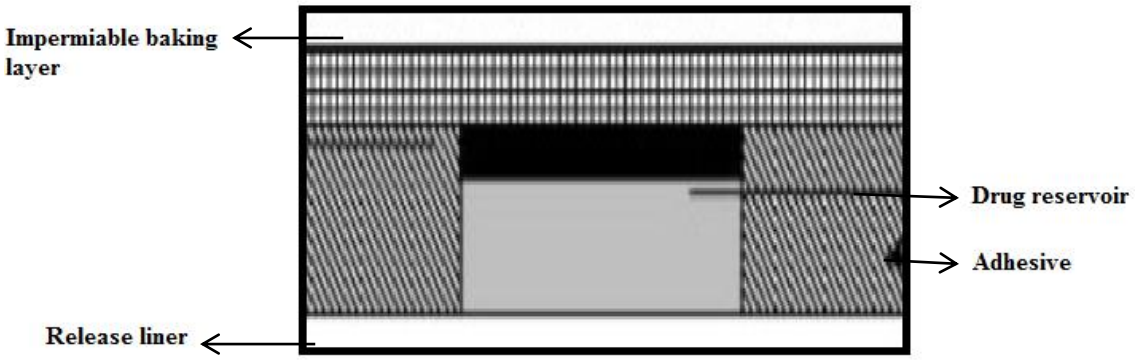


Figure II.4: drug in adhesive in a reservoir system. **(48)**

- **Matrix system**

a. Drug in adhesive: This type of patch is formulated by mixing the drug with adhesive polymer to form drug reservoir. It then followed by spreading on an impervious backing layer by solvent casting or melting method. The top of the reservoir is protected by an unmediated adhesive polymer layers. It may further be categorized into single-layer and multi-layer drug-in-adhesive. The system is considered to be compatible with a wide variety of drugs. Moreover the system is competent to deliver more than one drug in a single patch. It offers advantages in reduced size and thickness and improved conformability to the application site.**(44)**

b. Matrix-dispersion: In a matrix transdermal patch, the reservoir is a hydrophilic or lipophilic polymer matrix and the drug is homogenously dispersed in the matrix by placing the drug-polymer matrix over a plate with an impermeable laminate backing.**(49)**

II.5.Evaluation of transdermal patches

Transdermal patches have been developed to improve clinical efficacy of the drug and to enhance patient compliance by delivering smaller amount of drug at a predetermined rate. This makes evaluation studies even more important in order to ensure their desired performance and reproducibility under the specified environmental conditions **(46)**. These studies are predictive of transdermal dosage form sand can be classified into following types:

- Physicochemical evaluation
- Drug-polymer interaction studies
- In vitro drug release studies.

II.6.Physico-chemical characterization

Various tests have been reported in the literature with aim of evaluating transdermal patches as discussed below.

II.6.1.Patch thickness

The thickness of transdermal film is determined by traveling microscope, dial gauge, screw gauge or micrometer at different points of the film. **(46)**

II.6.2.Uniformity of weight

It is calculated by weighing ten different patches individually, and then the average weight and standard deviation were calculated. The acceptance criteria are that none of the weight should show a big deviation from mean weight.

II.6.3.Percentage Moisture content

The prepared films are to be weighed individually and to be kept in a desiccators containing fused calcium chloride at room temperature for 24 hours **(9).** Percentage of moisture in the patch is calculated using EQ II.1

$$\text{Moisture content (\%)} = (\text{initial mass} - \text{final mass}) / \text{initial mass} * 100 \qquad \textbf{EQ II.1}$$

II.7.Drug-polymer interaction studies

This parameter is used to evaluate any possible interaction between drug and polymer proposed to be used for transdermal drug delivery device preparation. This test uses differential scanning calorimetric (DSC), Fourier transform infrared spectroscopy (FTIR) techniques. **(7)**

II.7.1.In vitro drug release studies

Drug release mechanisms and kinetics are two characteristics of the dosage forms which play an important role in describing the drug dissolution profile from a controlled release dosage forms and hence there in vivo performance.

II.8.Polymers used to prepare a transdermal patch

Polymers are the backbone of TDDS, which control the release of the drug from the device **(48)**.The polymers employed in transdermal patch formulation have various functions, such as matrix formation, drug delivery rate control, pressure sensitive adhesives, backing laminates, and protective drug release liners. They should be biocompatible with the skin, and should render a constant and effectual supply of the drug throughout the delivery period advertised by the manufacturer **(49)**; the following criteria should be satisfied for a polymer to be used in transdermal formulations:

- Molecular weight, glass transition temperature and chemical functionality of the polymer should be such that the specific drug diffuses properly and gets released through it.
- It should be stable, non-reactive with drug, easily manufactured and fabricated into the desired product and inexpensive.
- The polymer and its degradation product must be non-toxic to the host.
- The mechanical properties of the polymer should not deteriorate excessively when large amount of active agent are incorporated.**(48)**

Among the polymers utilized for the preparation of transdermal patches are: Chitosan and sodium- alginate thus they particulate delivery system which is one of the most popular delivery systems investigated in recent years to encapsulate small molecule drugs.**(50)**

II.8.1.Chitosan

II.8.1.1.Definition and structure

Chitosan is a partially deacetylated polymer of N–acetyl glucosamine. Chitosan is generally prepared from chitin (figure II.5), (2 acetamido–2–deoxy–1, 4–D–glycan) and chitin may be found in a lot of natural sources **(51).** It is found in certain mushrooms and in the abdominal walls of ternite queens **(52).** European authorities approved chitosan as safe for consumption, and a monograph on chitosan hydrochloride was included in the fourth edition of the European Pharmacopoeia (2002). In addition, it is an approved food additive in Japan and has been widely used in the food industry. **(51)**

Chitosan is a polysaccharide composed of the random distribution; it is a macromolecule with chemical structure $C_{56}H_{103}N_9O_{39}$. Chitosan chemical structure is the chain of monomeric N-acetyl-β-D glucosamine units linked by a glycosidic bond (1→4); it has three types of reactive functional groups, one amine group and two hydroxyl groups at C2, C3, and C6 positions respectively. **(53)**

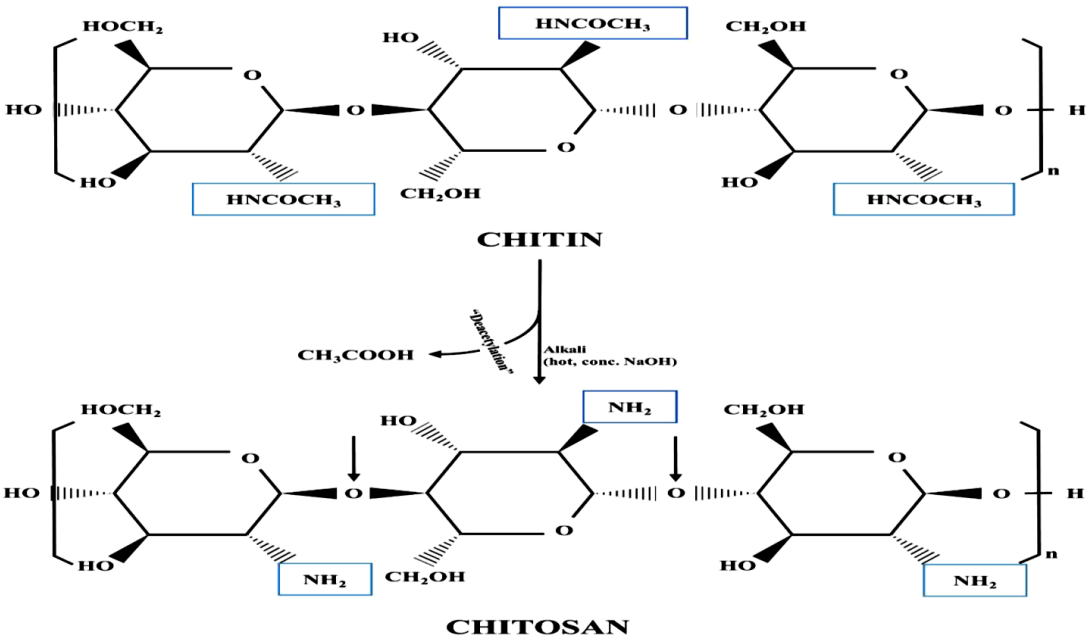


Figure II.5: Chemical structure of chitin and chitosan, chitosan product of chitin. **(51)**

The method of producing chitosan from the exoskeletons is shown figure II.6.

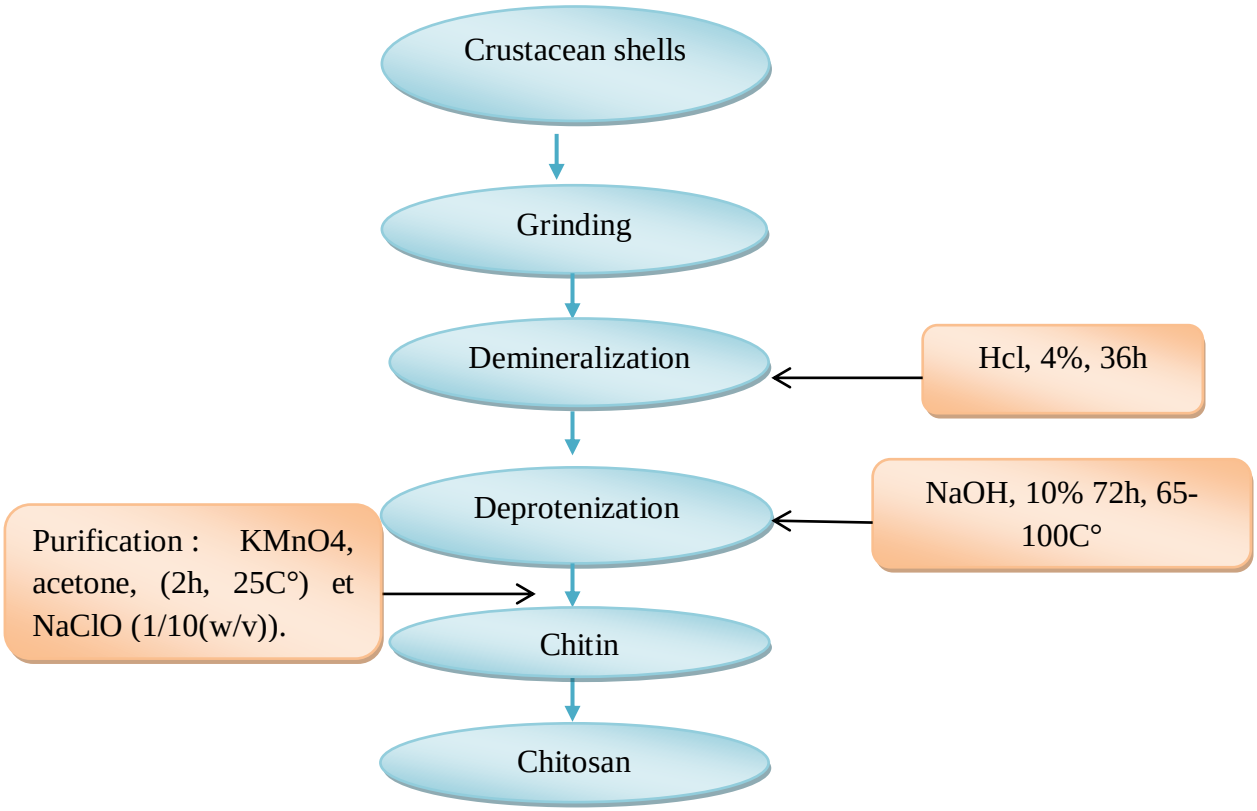


Figure II.6: method of making chitin and chitosan. **(53)**

II.8.1.2.Properties and Applications of Chitosan

Chitosan is widely used in a range of diverse fields, including food, agriculture, waste management, and medicine. Obviously, chitosan as a composite material has been extensively studied. Its various properties (antimicrobial properties; permeability and solubility; it decreases swelling and improves mechanical properties), make it very suitable for possible future applications in food and drug packaging, antimicrobial films, and coatings, such as applying coatings on fresh and cut fruits or vegetables **(51**), also it can be used in cosmetics because it has a chemical affinity with lipids, so it can be used on the skin **(54).** Table II.1.

Table II.1: some properties and applications of chitosan. **(55)**

Field of application	application	Properties
Pharmacy	Drug encapsulation	Absorbable material with possibility of control of release if active ingredient
Clinic	Dialysis membrane, bandage	Water and ion retention, stimulate regeneration of tissue
Cosmetic	Cream, shampoo, repellant	Moisture retention, anti-electrostatic, surfactant
Food industry	Restricting of fruits, vegetable or meat purees	Formation of thick film
Treatment of water	Cation flocculating agent	Polyelectrolytes, metal manufacture

II.8.2.Sodium Alginate

II.8.2.1.Definition and structure

Alginates are groups of linear anionic polysaccharides derived from kelp or Sargasso algae of brown algae and several bacterial strains **(56)**, the first isolated of alginates was by Stanford in 1881 and has since become a multifunctional ingredient in many applications. Alginates are included in a group of compounds that are generally considered safe by the FDA **(51)**. Since its discovery, alginates have been researched extensively on their physical and chemical properties. Because, they are non-toxic, rich in source and easy to obtain. It has been proved to be biocompatible and biodegradable in human body. **(56)**

Chemically, sodium-alginate's structure is ($NaC_6H_7O_6$) with a linear chain of D-mannuronic acid (M units) and L-guluronic acid (G units) linked via 1,4-glycosidic bond **(20),** as shown in figure II.7.This structure results flexibility in the formulations that contained alginate.

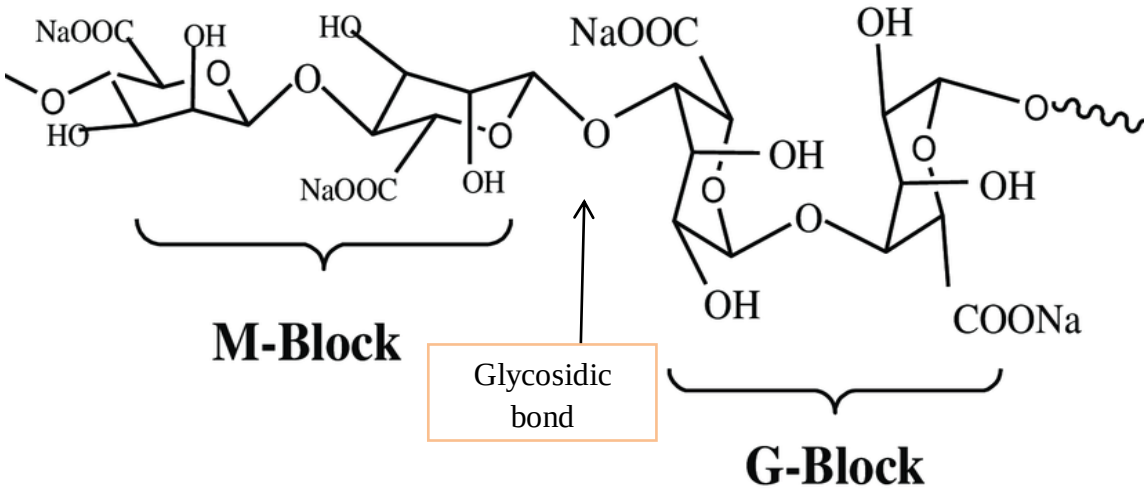


Figure II.7: structure of sodium alginate. **(57)**

II.8.2.2.Preparation of sodium alginate

Commercially available alginates are currently extracted from raw algae. Generally, the production consists of several steps, through which the water-insoluble mixed salts of alginic acid present in the brown seaweed cell walls are extracted and converted to soluble sodium alginates, as it expressed in the following figure II.8, Alginates can also be synthesized by bacteria, mainly from two strains, *Pseudomonas aeruginosa*, the pathogenic bacteria, and *Azotobacter vinelandii*, a nitrogen fixing soil-dwelling bacteria **(56).** The chemical composition of the alginate varies with different species of algae, different parts of the same plant and seasonal changes. Nevertheless by selection of raw materials with different properties, it is possible to prepare a variety of alginate with characteristics constant values **(55).** Due to its chemical structure alginates is soluble in aqueous solution forming viscous colloidal solutions with pseudo elastic behavior, but on the other hand, alginic acid is insoluble in aqueous solutions. It precipitates at pH is less than 3. **(58)**

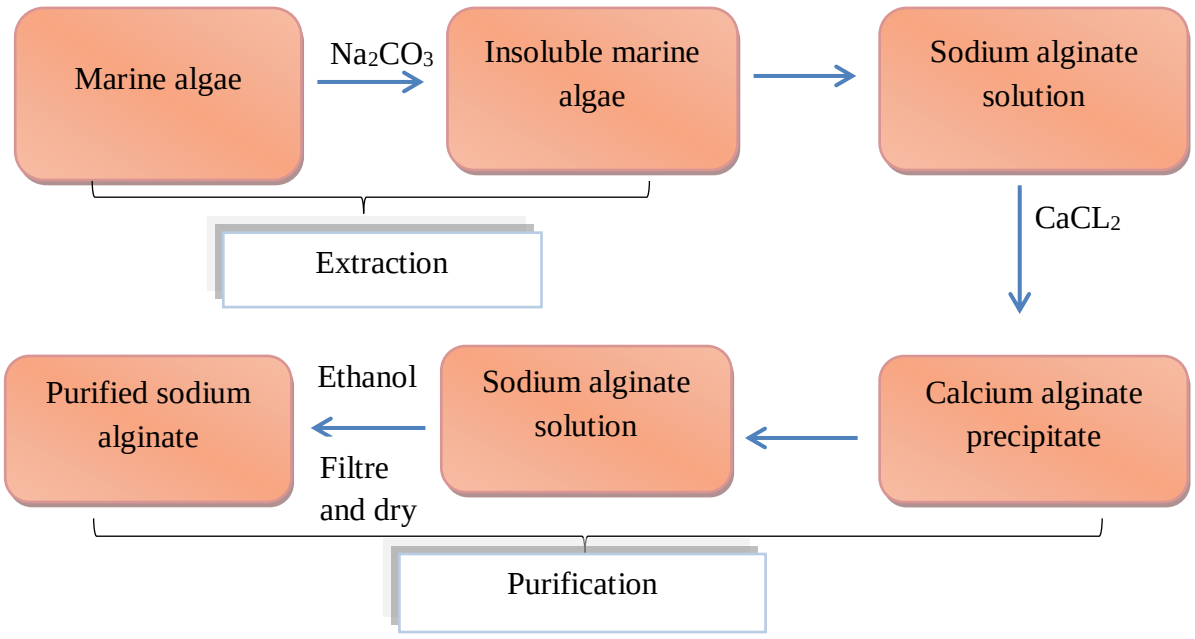


Figure II.8: industrial process of sodium alginate preparation. **(59)**

II.8.2.3.Application of alginate

Alginate is widely used in a range of diverse fields, including food, agriculture, and medicine due to its various properties which makes it suitable for possible future application in food and drug packaging, antimicrobial films, and coatings, for example applying coatings on fresh and cut fruits or vegetables. **(51)**

Currently, alginates have been widely used in wound healing due to their beneficial properties, such as biocompatibility, non-toxicity and high absorption capacity. Some wound dressings prepared from alginate can absorb excess wound fluid, provide a moist environment and minimize bacterial infections at the wound site **(56)**; also because it absorbs water quickly making it useful as an additive in dehydration products and in paper and textile manufacturing, it is also used for cosmetics and pharmaceutical preparations. **(55)**

II.9.Transdermal diffusion

Diffusion is a passive kinetic process that determines the movement of molecules under the influence of a concentration gradient; from a high concentration region to the less concentrated one. The drug is then spread from the surface of the skin to the deep layers until the concentrations are equilibrated. This passive diffusion mechanism is described by Fick's law. EQ II.2.

$$J = -A * D * (\frac{dC}{dx})$$ **EQ II.2**

This equation describes the rate of transfer (flux J) of a substance per unit of surface A of the membrane (PATCHE) which is proportional to the diffusion coefficient of the surface and the concentration gradient measured through a membrane (dC/dx).

With:

- **J:** rate of diffusion (flux) **mol/cm^2*S**
- **D:**drug diffusion coefficient **cm^2/S**
- **A:** lateral surface of the membrane **cm^2**
- **(dC/dx):** Concentration gradient over a distance x.

Diffusion through a transdermal system is influenced by several factors which can be grouped into: factors which effect on the efficiency of the skin barrier and factors which influence on the behavior of molecules that permeate towards this barrier; the first category is related to the patient (thickness of the corneal layer and the number of follicles that diffusion could change from person to another). The second category is related to the active ingredient; it is the physicochemical and biological properties of molecules that determined the diffusion rate even the stability of a drug product. Ideally, the molecules must be both lipophilic and hydrophilic to be able to defuse into the stratum corneum. **(9)**

II.10.Recommendation for use:

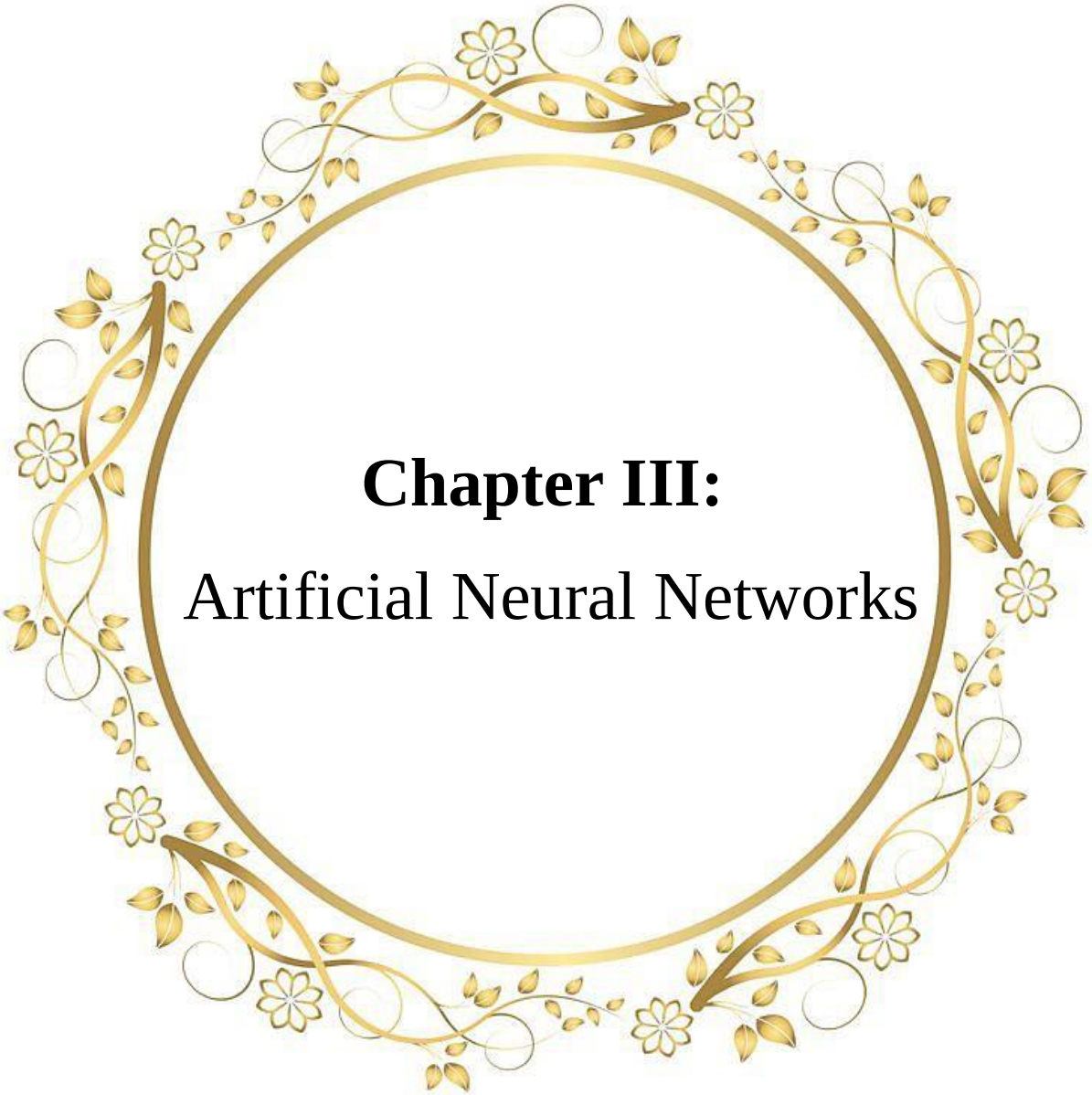
Always alternate the sites of application of the patch to avoid local irritation. In a transdermal therapeutic system, the kinetics of a patch release is independent of where the patch is administrated: either the semi-permeable membrane (reservoir patch) or the interaction between the matrix and the active ingredient regulates the absorption of the particles by the skin. A location that does not contains a lot of skin folds is usually chosen, and it is necessary to stick the patch on a clean and dry skin (press 30 seconds to glue the patch).

II.11.Conclusion:

The development of new and effective DDS has been greatly improving treatments efficacy, their administration through transdermal route has been attracting considerable attention due to its numerous advantages, such as less frequent, painless and flexible dosing, as it also generates less amount of dangerous waste. These factors have been stimulating the research and development of transdermal DDS to effective deliver therapeutic molecules to skin targets. Patches are the most common used transdermal DDS, mostly due to their simple design, application and low production cost. In order to solve the limitations associated with these passive transdermal DDS. Both biopolymers and synthetic polymers have been exploited to engineer robust devices able to deliver accurate amounts of drugs during a period of time with minimal side effects by different types of patches. Chitosan and alginate are the combination that leads to high levels of drug releasing on skin barriers according to their structures biocompatibility and biodegradability even their renewable capacity and low toxicity properties.

Although major achievements have been attained in the development of more sophisticated patches, some limitations still need to be overcome.

Particularly, physicochemical properties of drug continue to play an important role in its transport through the skin layers and low transcutaneous flux, drug incorporation and retention in the patch still limited their application. Further research should be developed in order to attain patches with ideal features from the simplest to most complex hierarchical patches with stimuli- and multiple-release of a large pool of drugs. Moreover, new patches designs could be developed with different geometry and arrangement (multi-layered and non-symmetric) to solve the wanted limitations such as poor adhesion, low drug flux, patch stability and storage. **(43)**



Chapter III:

Artificial Neural Networks

III. Introduction

Artificial Intelligence (AI) is the knowledge domain that targets the development of computer systems to solve problems by giving them cognitive powers for performing tasks that usually require human intelligence. Hence, simulation of human intelligence, with computer programming and technologies, is the main objective of AI. Whereas, machine learning is one of the branches of AI, in which computer systems are programmed based on the data and type of input. Machine learning (ML) gives the capability to AI for solving problems based on available data. Likewise, artificial neural network (ANN) is an evolved method of ML algorithms, developed on a concept of imitating the human brain A single neuron is considered as a cell, processing electrochemical signals or nerve.

Impulses and the human brain is a complicated network of neurons that transfers information, with the help of various interlinked neurons. ANN models are considered as most popular among AI models because of their architecture, which is the collection of neurons linked with other neurons in various layers. ANN is non-linear and complex systems of neurons and neuron is a mathematical unit. **(60)**

III.1.Artificial intelligent

In 1956, the term Artificial Intelligence was defined by John Mc Carthyas: the science and engineering of making intelligent machines. It can also be defined as the development of computer systems that are capable of performing tasks that require human intelligence, such as decision making, object detection, solving complex problems, (Figure III.1). **(61)**

Artificial Intelligence (AI) is a branch of Science which deals with helping machines finds solutions to complex problems in a more human. This generally involves borrowing characteristics from human intelligence, and applying them as algorithms in a computer friendly way. **(62)**

Artificial intelligent has been described as software that behaves in some limited ways like a human being, it has been defined in many ways: Winston [1984] suggests one definition of artificial intelligent as the study of ideas that enable computers to be intelligent, Rich and Knight [1991] defined artificial intelligent as the study of how to make computers do things which, at the moment, people do better. The most common definition or description of the artificial intelligent are:

- Artificial intelligent is intelligent because it learns; transforms data into knowledge.
- Artificial intelligent is about intelligent problem solving; and represents the ability to adapt to the environment and to deal with incomplete or incorrect knowledge. **(63)**

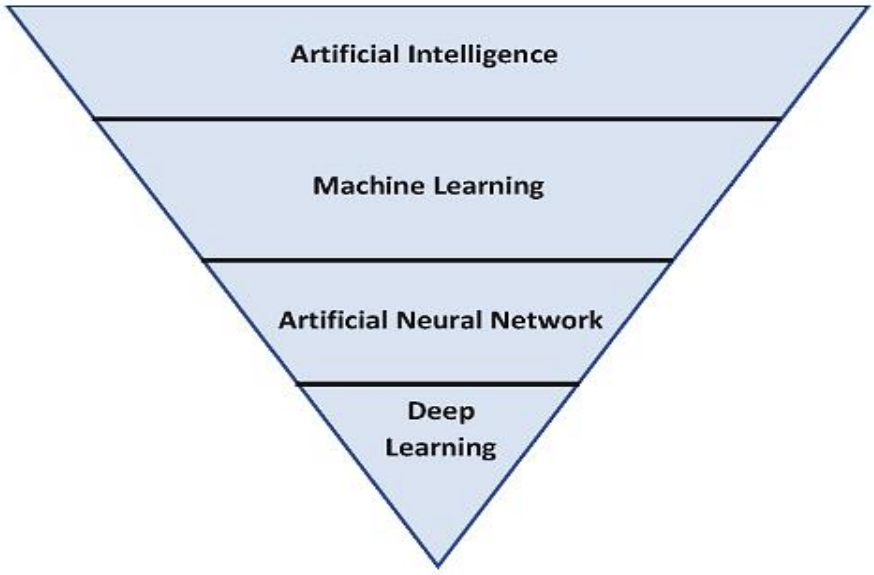


Figure III.1: pyramid of the different branches of AI. **(64)**

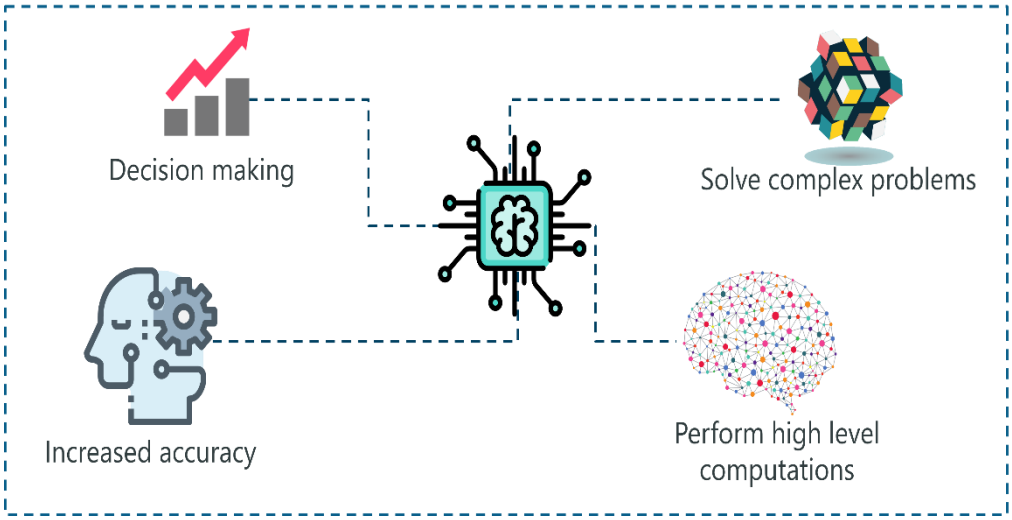


Figure III.2: artificial intelligent. **(65)**

Artificial intelligent has three principal types: Artificial Neural Network Intelligence, Artificial General Intelligence, and Artificial Super Intelligence.

III.1.1.Artificial neural network intelligent ANNI

Artificial neural networks are part of the area known as an intelligent system which is computational model inspired by the nervous system of living beings. They have the ability to acquire and maintain knowledge (information based) and can be defined as a set of processing units, represented by artificial neurons. **(66)**

III.1.2.Artificial General Intelligence AGI

Also known as Strong AI, AGI is the stage in the evolution of Artificial Intelligence wherein machines will possess the ability to think and make decisions just like us humans.

III.1.3.Artificial super intelligent ASI

Artificial Super Intelligence is the stage of Artificial Intelligence when the capability of computers will surpass human beings. ASI is currently a hypothetical situation as depicted in movies and science fiction books, where machines have taken over the world. **(61)**
The most common type of AI is the artificial neural network.

III.2.Artificial neural network

III.2.1.History of the artificial neural network

In the 1940s, Warren McCulloch and Walter Pitts explored the computational abilities of net- works made up of theoretic mathematical models of simple neurons. By combining these neurons, it is possible to construct networks that will compute any of the limited basic Boolean logical functions, including symbolic logic (EXP, “I am a man, and all men are mortal. Therefore, I am mortal”. **(67)**

The first publication related to neuro-computing dates was in the 1943, when McCulloch and Pitts composed the first mathematical model inspired by biological neurons, resulting in the first conception of the artificial neuron. In 1949, the first method for training artificial neural networks was proposed; it was named Hebb’s rule and was based on hypothesis and observations of neuron-physiologic nature (Hebb1949). Many other researchers have continued the development of mathematical models based on the biological neuron, consequently generating a large number of topologies (structures) and learning algorithms. Among the different branches that emerged, the work of Frank Rosenblatt stands out. Between 1957 and 1958, Rosenblatt developed the first neuro-computer called Mark I

Perceptron, making the basic model of the Perceptron. The Perceptron model stirred interest due to its capability of distinguishing simple patterns. **(66)**

In 1960 Windrow and Hoff developed a network called ADALINE, which is abbreviation of Adaptive Linear Element, later they proposed the MADALINE, the Multiple-ADALINE. It consisted on a network whose learning is based on the Delta rule, also known as LMS (Least Mean Square) learning method.

Following this earlier work, many researchers of that time were encouraged to conduct research in this area. However, in 1969, neuro-computing suffered a major setback with the publication of the classical book "Perceptron's: An Introduction to Computation Geometry" by Minsky and Papert (1969). The authors discussed emphatically the limitations of the neural networks of that time which were composed of a single layer, such as the Perceptron and the ADALINE on learning the relationship between inputs and outputs of very basic logical functions.

In the 1970s, Teuvo Kohonen developed the theory of associative memory in which pairs of pat- terns are stored so that the presentation of one of the patterns in a pair directly evokes the associated pattern without any serial search. Kohonen also developed a model of self-organizing topographical maps. Many areas of the brain demonstrate topographical organization, and he demonstrated how such maps could rise in a relatively undifferentiated neural network simply as a result of experience. **(67)**

Researches on neural networks were greatly reduced, and some of the few works thereafter were: the derivation of prediction algorithms using reverse gradients (Werbos 1974), the development of the ART (Adaptive Resonance Theory) network by Grossberg (1980), the formulation of the self-organized maps (SOM) by Kohonen (1982), and the recurrent network based on energy functions proposed by Hopfield (1982).

One of the fundamental works of that time was the publication of Rumelhart, Hinton and Williams' book "Parallel Distributed Processing" (Rumelhart et al. 1986), which brought to light one algorithm that allowed the adjustment of weight matrices of networks with more than a single layer. Consequently, solving the old problem of learning patterns. The proposal of this algorithm, called "back propagation," definitely revived and motivated researches in artificial neural networks.

Some interesting work, in particular, includes the learning algorithm based on Levenberg Marquardt method, which fostered efficiency improvement of artificial neural networks in diverse applications; the artificial neural networks based on support vector machines (SVM), which can also be used for pattern classification and linear regression; and the development of neural integrated circuits with several circuit configurations. **(66)**

III.2.2.Definition of artificial neural network ANN

Artificial Neural Network (ANN) models were inspired by the branch of biological sciences that studies how the neuro-anatomy of living animals has developed in solving problems. Which is a computational model inspired in the natural neurons.

Artificial Neural Network consists of many interconnected processors known as neurons. It simulates the human brain's biological neural network. The biological neural network is the mechanism through which a living organism's nervous system functions, enabling complex tasks to be performed instinctively. The central processing unit of that nervous system is known as a "neuron". The human brain has around 10 to 100 billion neurons, each connected to many others by "synapses". The human brain has around 100trillion synapses. These connections control the human body and its thought processes. Briefly, ANNs attempt to replicate the learning processes of the human brain. The first ANN theories were expounded by researchers attempting to explain human behaviour and the thinking process by modeling the human brain.

According to Nelson and Illingworth [1990], ANNs are also called:

- ✓ Parallel distributed processing models
- ✓ Connectives
- ✓ connectionism models
- ✓ Adaptive systems
- ✓ Self-organizing systems
- ✓ Neuro-computing
- ✓ Neuro-morphic systems. **(63)**

III.2.3.Biological neuron

The human brain consists of an estimated 10^{11} (100 billion) nerve cells or neurons, a highly stylized example of which is shown in Figure III.3.

A neuron is special biological cell that processes information, it is composed of a cell body, or soma and two types of out-reaching tree-like branches: the axon and the dendrites. The cell body has a nucleus that contains information about genetic characters and plasma that holds the molecular equipment for producing material needed by the neuron. **(67)**

Neurons communicate via electrical signals that are short-lived impulses or «spike" in the voltage of the cell wall or membrane, neuron receives signals from other neurons through its dendrites (receivers) and transmits signals generated by its cell body along the axon (transmitter). The interneuron connections are mediated by electrochemical junctions called synapses.

A synapse is an elementary structure and functional unit between two neurons (an axon strand of one neuron and a dendrite of another) which are located on branches of the cell referred to as dendrites. When the impulse reaches the synapse's terminal; certain chemicals called neurotransmitters are released. The neurotransmitters diffuse across the synaptic gap, to enhance or inhibit, depending on the type of the synapse, the receptor neurons own tendency to emit electrical impulses. The synapse's effectiveness can be adjusted by the signals pass in through it so that the synapses can learn from the activities in which they participate. This dependence on history acts as a memory, which is possibly responsible for human memory. Each neuron typically receives many thousands of connections from other neurons and is therefore constantly receiving a multitude of incoming signals, which eventually reach the cell body. This is then transmitted to other neurons via a branching fibre known as the axon.

The synapses are the connections which enable the transfer of electric axon impulses from a particular neuron to dendrites of other neurons, as illustrated in Figure III.3. It is important to note that there is no physical contact between the neurons forming the synaptic junction, so the neurotransmitter elements released on the junction are in charge of weighting the transmission from one neuron to another. **(68)**

In total, the human brain contains approximately10^{14} to 10^{15} interconnections. Neurons communicate through a very short train of pulses, typically mille seconds in duration. The message is modulated on the pulse-transmission frequency. This frequency can vary from a few to several hundred hertz, which is a million times lower than the fastest switching speed in electronic circuits. However, complex perceptual decisions such as face recognition are

typically made by humans within a few hundred milliseconds. These decisions are made by a network of neurons whose operational speed is only a few mille seconds. This implies that the computations cannot take more than about 100 serial stages. **(69)**

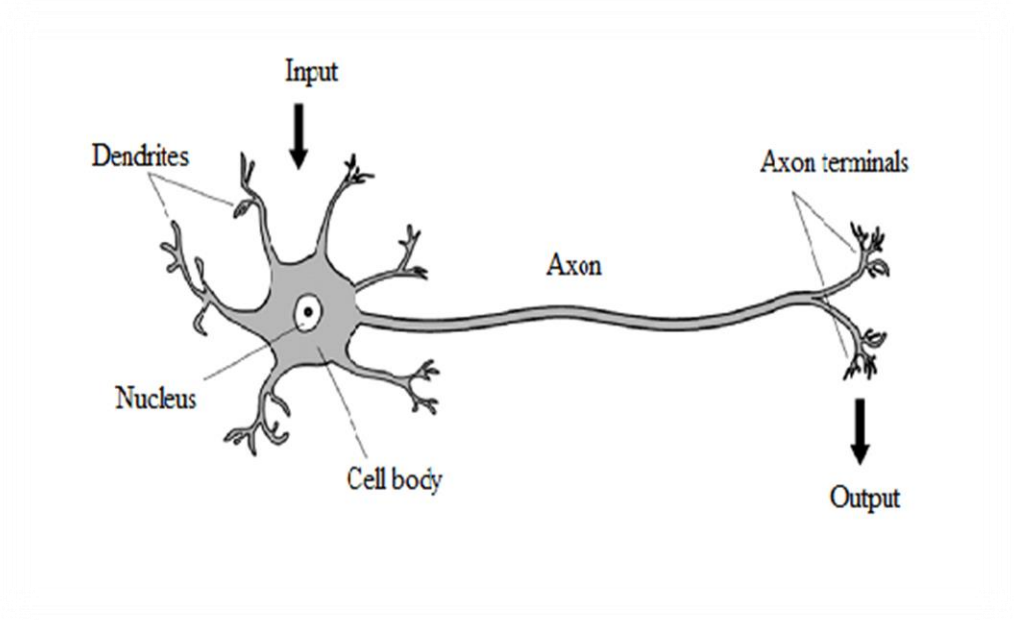


Figure III.3: structure of biological neuron.

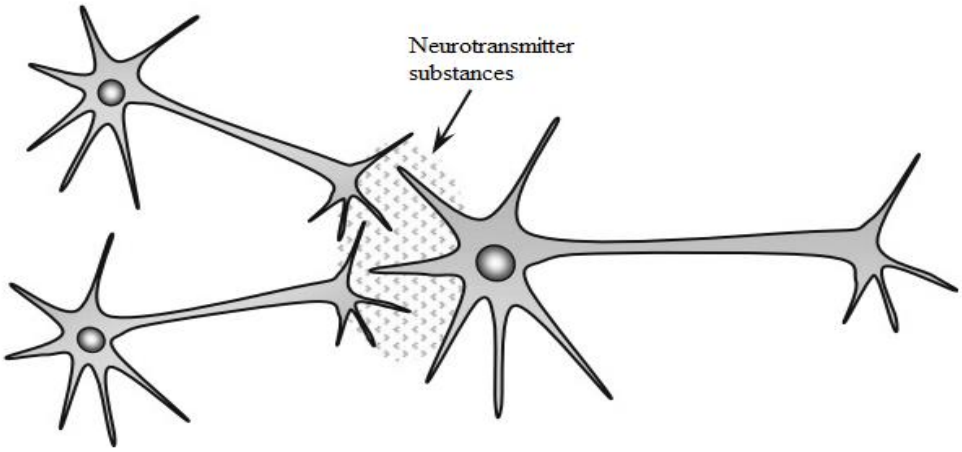


Figure III.4: synaptic connection between neurons. **(66)**

III.2.4.Artificial neuron

The artificial neural network structures were developed from known models of biological nervous systems and the human brain itself. The computational components or processing units, called artificial neurons, are simplified models of biological neurons. These models were inspired by the analysis of how a cell membrane of a neuron generates and propagates electrical impulses. **(66)**

The complexity of real neurons is highly abstracted when modelling artificial neurons. These basically consist of inputs (like synapses), which are multiplied by weights (strength of the respective signals), and then computed by a mathematical function which determines the activation of the neuron. Another function (which may be the identity) computes the output of the artificial neuron (sometimes in dependence of a certain there should). ANNs combine artificial neurons in order to process information. **(70)**

The architecture of an artificial neural network defines how its several neurons are arranged, or placed, in relation to each other. These arrangements are structured essentially by directing the synaptic connections of the neurons.

In general, an artificial neural network can be divided into three parts, named layers, which are known as:

a) Input layer

This layer is responsible for receiving information (data), signals, features, or measurements from the external environment. These inputs (samples or patterns) are usually normalized within the limit values produced by activation functions. This normalization results in better numerical precision for the mathematical operations performed by the network.

b) intermediate, or invisible layers

These layers are composed of neurons which are responsible for extracting patterns associated with the process or system being analyzed. These layers perform most of the internal processing from a network.

c) Output layer

This layer is also composed of neurons, and thus is responsible for producing and presenting the final network outputs, which result from the processing performed by the neurons in the previous layers. The main architectures of artificial neural networks, consider the neuron disposition. **(66)**

Generally, in an ANN, data is received through the input layer neurons and then transformed

To the neurons in the first hidden layer through the weighted connections established between the input layer and the first hidden layer. Here, the data in each layer are mathematically processed and then the result is transformed to the next layer. Below steps explain how the incoming data (x_i) is processed by (j_i) node in the next layer and Figure III.5 represents this process. **(71)**

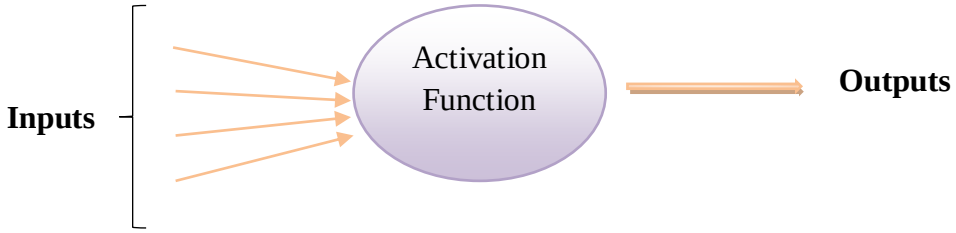


Figure III.5: representative scheme of the architecture of ANNs. **(72)**

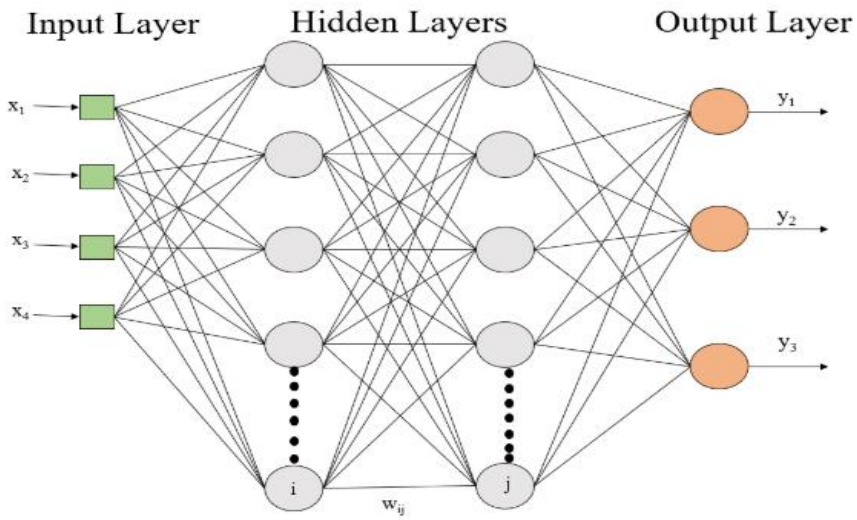


Figure III.6: General Structure of Artificial Neural Network with Two Hidden Layers. **(73)**

III.2.5.Application of ANNs

Windrow, Rumelhart and Lehr [1993] argue that most ANN applications fall into the following three categories: Pattern classification, Prediction and financial analysis, and Control and Optimization. In practice, their categorization is ambiguous since many financial and predictive. Applications involve pattern classification. A preferred classification that separates applications by method is the following:

- Classification,
- Time Series,
- Optimization.

Classification problems involve either binary decisions or multiple-class identification in which observations are separated into categories according to specified characteristics. They typically use cross sectional data. Obviously, the interdependence of the observations need to be considered as most real world problems may contain observations that do not fall directly into the defined categories. Solving these problems entails 'learning' patterns in a data set and constructing a model that can recognize these patterns. **(63)**

III.2.6.Artificial neural networks in medical uses

Artificial Neural Networks (ANNs) have been introduced in pharmaceutical applications as techniques to model data showing non-linear relationships, ANNs have been applied success fully to model controlled release drug delivery, ANN was used to predict dissolution profiles from matrix-controlled release, described a modelling and optimization study for dissolution from extended release tablets.**(74)**

III.3.Artificial neural network and training data

The network is trained with the data obtained via the mathematical formulation. In a real-life scenario, training samples consist of measured data of some kind combined with the "solutions" that will help the neural network to generalize all this information into a consistent input–output relationship.

For example, let's say that you want your neural network to predict the eating quality of a tomato based on color, shape, and density. You have no idea how exactly the color, shape, and density are correlated with overall deliciousness, but you can measure color, shape, and density, and you do have taste buds. Thus, all you need to do is gather thousands upon thousands of tomatoes, record their relevant physical characteristics, taste each one (the best part), and then put all this information into a table.

Each row is what I call one training sample, and there are four columns: three of these (color, shape, and density) are input columns, and the fourth is the target output.

The training of the ANN is accomplished through a learning process. While in the training process, weights are modified for attaining required results. In the training process, some sample data is processed to the network and weights are modified to attain better approximation of the desired output.

The learning process is mostly classified into two categories: supervised learning, and unsupervised learning.

III.3.1.Supervised learning:

In supervised learning, a training set is presented to the model. The training set constitutes of input examples and corresponding target outputs. The inputs are noted for the response of the network, and the weights between with networks are adjusted for error reduction, for the attainment of the desired output. The network follows successive iterations during this process until the computed result converges to the correct one. Construction of the training set requires special consideration. A training set is considered an ideal one, and it should be giving a better representation of the underlying model. Otherwise, a reliable model with desirable results cannot be achieved with an unrepresentative training set.

In the supervised learning process, the networks are trained first before its operation in a model for predictive outputs. Significantly, when the network starts computing the intended outputs with the series of inputs, with fixed weights, then the ANN model can be set for the required operation. Few of the well-known algorithms with a supervised learning method are the Adaline (used for binary data), the Perceptron (used for continuous data), and the Madaline (developed from the Adaline).

III.3.2.Unsupervised learning

Unsupervised learning does not follow a training set or a targeted output approach. Instead, it trails the input data pattern of the underlying model. In this process, the ANN model adjusts its weights, against the supplied inputs, thus producing outputs similar to inputs. The model, without any outer support, recognises the patterns and differences in the inputs. In this process, the clusters are formed; each cluster consists of a group of several weights, in such a way that related input path results in a similar output. If any new pattern is detected during the iteration process, it is classified as a new cluster.

Auto encoders, Hebbian Learning, Deep Belief Nets, Self-Organising Map, Generative Adversarial Networks, and Algebraic Reconstruction Technique (ART) are the few most renowned algorithms for unsupervised learning. Unsupervised ANN models are used in diagnosing diseases, image segmentation and many more. Unsupervised algorithms have become very useful and powerful tools in segmentation of magnetic resonance images for detection of anomalies in the body systems. **(60)**

III.4.Mapping by ANNs

The primary reason for ANN popularity is due to approximated data output. There are five main steps for the approximation function in the ANN model, as given below.

III.4.1.Data pre-processing

In data pre-processing, the appropriate predictors are selected as inputs before processing to a network for mapping. There are three general processes in data pre-processing, mentioned as follows:

- **Standardising:** The input values are rescaled to a uniformed scale.
- **Normalising:** It normalises a vector to have unity variance and zero mean value.
- **Principal component analysis:** This process replaces the groups of related variables by new unrelated variables by detecting linear dependencies between them. **(60)**

III.4.2.Selection of network architecture

Network architecture comprises several hidden neurons, the number of hidden layers, the flow of data, the way neurons are interconnected, and specific transfer functions. Recurrent neural networks, multi-layer Perceptron (MLP), probabilistic neural networks, radial basis function networks, generalised regression neural networks and time-delay neural networks are the few of the renowned architectures.**(60)**

III.4.3.Network training

About function mapping, the training process is known as the calibration of the network through input and out pairs. During the training process, ANN might suffer from the over fitting and under fitting. The overall performance of the network decreases because of these two mentioned factors. This unfitting of the network, during the training process, can be managed by increasing the number of epochs, but it may result in network over fitting if the

number of epochs is more significant than a required number. Epoch is defined as a process of providing one pass or iteration of input through the network and modifying the weights. The optimal number of epochs can be determined by the comparison of training error and model testing procedure.

III.4.4.Simulation

Simulation is the ultimate goal of applying ANN networks. It is the representation of predicted output data for an ANN model.

III.4.5.Post-processing

There are three types of sets in which sample data are distributed: (i) the training set, (ii) the validation set, and (iii) the testing set. The training set is used to train the ANN model; it is a set of sample data that is used to modify or adjust the weights in the ANN to produce the desired outcome. The validation set is used to inform the ANN when training is to be terminated (when the minimum error point is achieved). The test set provides an entirely independent way of examining the precision of the ANN. The test set is a set of sample data that is used for the evaluation of the ANN model. **(60)**

III.5.Conclusion

Operation of the ANN model is the simulation of the human brain, and they fall under the knowledge domain of AI. The popularity of ANN models was increased in the early 1990s, and many studies have been done since. The basic ANN model has three main layers, and the main process is performed in the middle layer known as the hidden layer. The output of the ANN model is very much dependent on the characteristics and function it carries under the hidden layer. Among the feed forward and feedback networks, the latter one propagates the error unless it became minimal for more effective results. The ANN models can perform supervised learning as well as unsupervised learning depending upon the task. In this era of automation, the AI plays an important role, and most of the daily use applications are based on the architecture of ANN models. This ANN technology, combined with other advanced and AI knowledge areas, is making life easier in almost.

Chapter IV

Materials and methods

IV. Introduction

In our work we prepared membranes based on chitosan sodium-alginate to study the encapsulation of vitamin C and the release at pH=7.4.

This chapter is intended for the description of the materials and the qualitative and quantitative analysis techniques used in this work, and the operating protocols for the synthesis and characterization of the encapsulation of vitamin C, as well as the release of active principle from the membranes which we have prepared.

Our graduation project was carried out at the process engineering laboratory of the University of Khemis-Miliana for the preparation of Chitosan Alginate membranes, and also we did our UV-Visible(delivering study) at national observation sustainable environment ONEDP Khemis-Miliana Ain Defla, also at the level of INCC/GN: National Institute of Criminalistics and Criminology of the National Gendarmerie Bouchaoui Algeria and in the Sub-direction of the Scientific and Technical Police Chevalier Algeria for the analysis of Surface microstructure and morphology scanning electron microscopy « SEM » and the infrared spectrum.

IV.1.Preparation of membranes

In this study, the materials used are as follows: Chitosan, sodium-Alginate, Ascorbic acid (vitamin C), glacial acetic acid, distilled water, Tween 80%, Glycerol.

IV.1.1.products used

- **Chitosan:**Chitosan used in this study was supplied by Sigma-Aldrich (China) with a medium molecular weight (Mw) varied between (190,000-310,000 $g.mol^{-1}$), and viscosity of 1% w/v acetic acid at 25°C between 200 and 800 cps and degree of solubility is 1% in acetic acid in temperature equivalent to 25°C.
- **Alginate:** the sodium-alginate used in the preparation of this membranes was purchased from BIOCHEM CHEMOPHARMA (French) which have a molecular weight (Mw) of 198.1 $g.mol^{-1}$, with viscosity of 1% w/v solution at 25°C is 5.5 ± 2 cps, it's concentration of chloride is 1%. And soluble in distilled water.
- **Ascorbic acid:** Ascorbic acid used as an active ingredient was from BIOCHEM CHEMOPHARMA (French), it has molecular weight 176.13 $g.mol^{-1}$, with melting point between 190 -1920 °C, and its solubility in water at 20°C is 20.50 to 21.50.

- **Acetic acid:** The acetic acid used is the glacial acetic acid was purchased from BIOCHEM CHEMOPHARMA, which have molecular weight of 60.05 $g.mol^{-1}$ and it is characterised by the freezing point which is not below then 16.2 °C, percentage of water is 0.2%.
- **Tween 80:** The tween used in our experience is also from BIOCHEM CHEMOPHARMA, it physical state at 20°C is liquid and boiling at temperature upper then 100°C, it has golden yellow color with density at 20°C 1080 $Kg.m^3$ and viscosity at 25°C is average 400-500 cSt, and pH value is 6.
- **Glycerol:** It was purchased from BIOCHEMOPHARMA, it is liquid at 20°C, clear, colorless, odorless, it boils at 182°C, it's density is 1.25 $g.cm^{-3}$.

All other reagents were used without addition al purification.

IV.1.2.Equipment and Glassware

- Beaker 50ml,
- Volumetric flask 100ml,
- Watch glass,
- Spatula,
- Petri dish,
- Filter paper,
- Magnetic stirring bars,
- Magnetic stirrer,
- Digital scale.

IV.1.3.Methods

IV.1.3.1.Preparation of Chitosan membrane

In a digital scale we weigh 1 g of the chitosan powder, then we put it in a beaker and adding 50 ml of acetic acid (2N) diluted, then we put the mixture under a magnetic stirrer for about 2 hours (Figure IV.1). After stirring we poured the chitosan solution into petri dish and Put them in the oven for drying at 37°C for 24 hours, after drying we unmold the membrane and store it away from moisture.

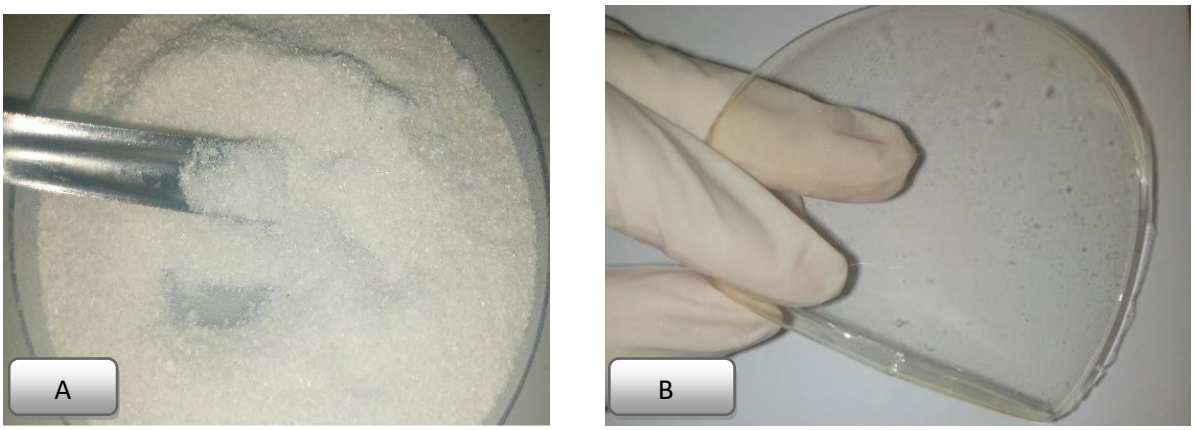


Figure IV.1: (A): chitosan powder of medium molecular weight. (B): final chitosan membrane after drying.

IV. 2.2.2. Preparation of Alginate membrane

All sodium alginate films were prepared by the casting method: for the 100 ml solution; 1.3 g of sodium alginate powder is dissolved in 100 ml of distilled water and stirred magnetically for 24 hours, 200 rpm. Then pour the result solution in Petri dish and dry it at 60°C for 24 hours. This method was modified according to the laboratory conditions which work has been carried out, as shown in the Figure IV.2.

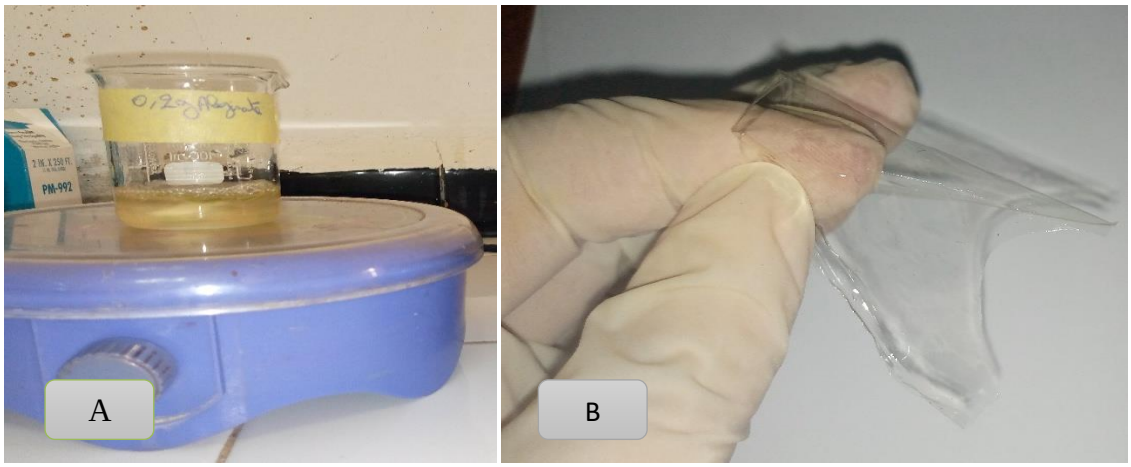


Figure IV.2: (A): sodium alginate powder dissolved in distilled water under magnetic stirrer, (B): flexible Na-alginate membrane after removed it from the Petri dish.

IV.1.3.3.Preparation of Chitosan/ sodium-alginate/ vitamin C membranes

The formulation of vitamin C topical patches was performed by the method explained below with different percentage of chitosan and sodium alginate, as indicated in Table IV.1.

The first step was by preparing each chitosan solution by adding the weights indicated in the table IV.1 of chitosan powder to the equivalent volume of acetic acid and stirred the solution about 2 hours, and simultaneously preparing the sodium-alginate solution by dissolved alginate powder in distilled water under magnetic stirrer for 2 hours, in the last half hour we add 0.02 g of the active ingredient which is the vitamin C and we still stirring the mixture (Alg-Vit C) until we obtained an homogenous mixture. Both (Alg-Vit C) and chitosan solutions were filtered to remove any undissolved solids and impurities and left to stand until air bubbles have disappeared.

The next step was by mixing the two solution: we poured the mixture (Alg-Vit C) by drops on the chitosan solution, then we late it stirred also for 2 hours, before the two hours expire we add both of (0.008 g Tween 80 and 0.001 g of Glycerol) and we stay stirred the mixture the finish the 2hours.

The last step was to pour equal volumes of the final solution into petri dishes and putting them in the oven at 37°C for about 24 hours; the method of preparing topical patches is showing in figure IV.3.

Finally, a gummy tape was then adhered to the support membrane keeping the matrix side up (Single layer drug in adhesive). The drug matrices were kept in desiccators until their characterization.

Table IV.1: Formulations used to make different composite membranes. The calculationis for the 100 ml solution.

	Percentage % SC / Alg	SC weight (g)	Alg weight (g)	Tween 80 (g)	Glycerol (g)	Vit C (g)
F1	100 /0	1	0	0.008	0.001	-
F2	90/10	0.9	0.1	0.008	0.001	0.02
F3	80/20	0.8	0.2	0.008	0.001	0.02
F4	70/30	0.7	0.3	0.008	0.001	0.02
F5	60/40	0.6	0.4	0.008	0.001	0.02
F6	50/50	0.5	0.5	0.008	0.001	0.02
F7	40/60	0.4	0.6	0.008	0.001	0.02
F8	30/70	0.3	0.7	0.008	0.001	0.02
F9	20/80	0.2	0.8	0.008	0.001	0.02
F10	10/90	0.1	0.9	0.008	0.001	0.02
F11	0 /100	0	1	0.008	0.001	0.02

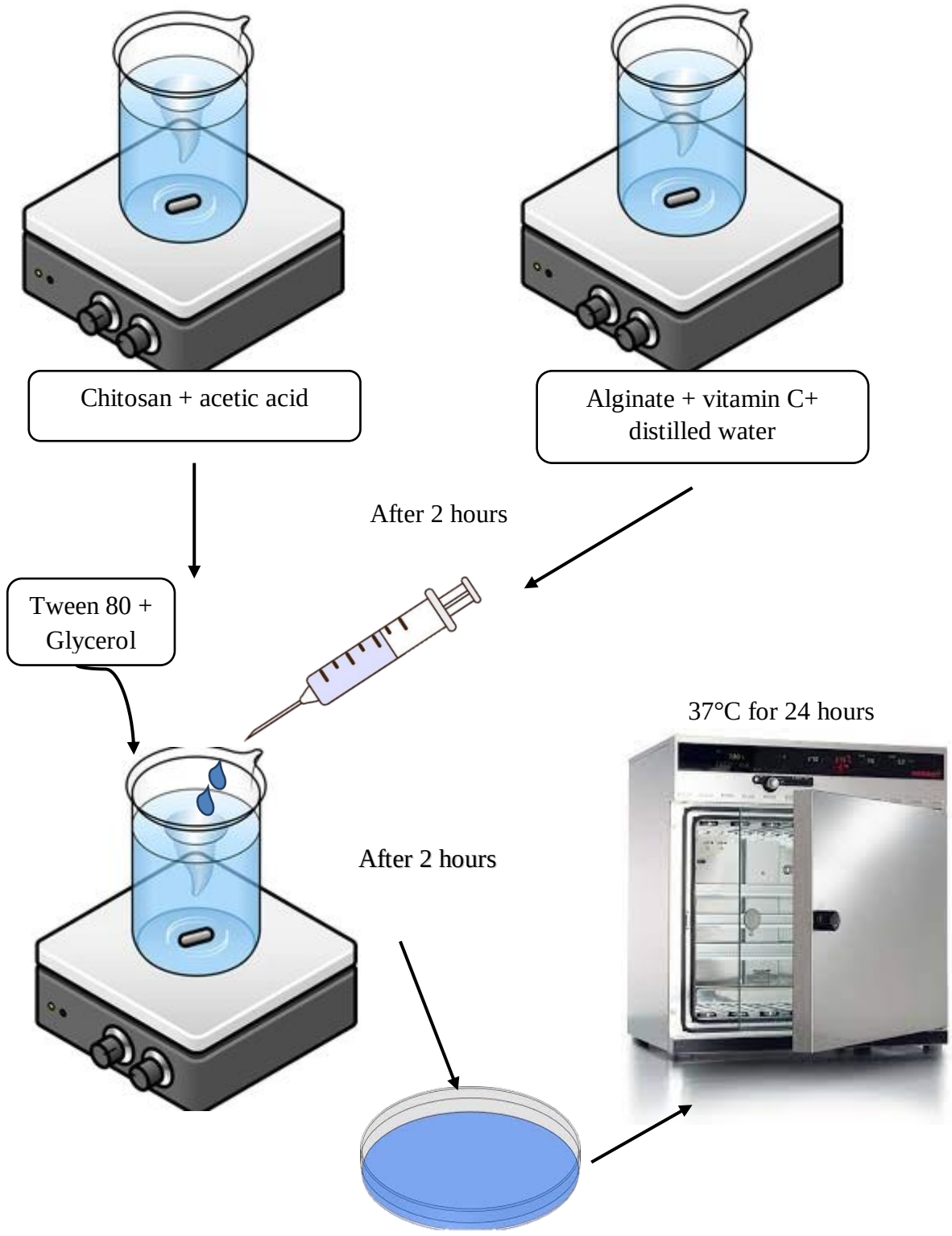


Figure IV.3: different steps to preparing a topical patches based on chitosan and sodium-alginate.

IV.1.4.Physic-chemical characteristics of the membranes

A number of assessment and evolution tests should be performed in order to descript and characterize transdermal patches such as dissolution, in vitro drug release. These tests are mentioned below and they comply with the European Medicines Agency Guidelines on the quality of transdermal patches made by the Committee for Medicinal Products for Human Use **(75)** Moreover, other types of physical, chemical and biological tests, evaluation and assessments explained below should be performed such as interaction of materials, patch thickness, weight uniformity, moisture content and moisture uptake or weight gain.

IV.1.4.1.Physical appearance:

Transdermal films of vitamin C were evaluated by visual inspection in terms of color, transparency, degradability, flexibility, and homogeneity. **(76)**

IV.1.4.2.membranes Thickness test

Studying the thickness of membranes aims to maintain the uniformity in transdermal formulations. It is determined by measuring the thickness of patch at three places using an optical microscope. **(7)**

IV.1.4.3.Folding endurance

The folding endurance of the patches was determined by repeatedly folding a patch at the same place until it broke or was folded up to 250 times without breaking**. (77)**

IV.1.4.4.Uniformity of membranes Weight

It is calculated by weighing ten different patches individually, and then the average weight and standard deviation were calculated. The acceptance criteria are that none of the weight should show a big deviation from mean weight. **(7)**

$$\xi = \mathbf{wf} - \overline{X} \qquad \textbf{EQ IV.1}$$

With:

- ξ**:** standard deviation (g)
- **wf**:membranes weight (g)
- $\overline{\mathbf{x}}$**:** the average in weight (g)

IV.2.Spectrophotometric characteristics of membranes

IV.2.1.Study of interactions between the drug and polymers

IV.2.1.1.Fourier Transform Infrared Spectroscopy (FTIR):

Most often, the infrared analysis of organic substances, and particularly polymers, gives rise to the obtaining of spectra or characteristic peaks of all the types of bonds existing in the substances which composed the membranes under study. It is for this purpose that a spectroscopy analysis Fourier Transform Infra-Red (IRTF) was affected out to better studying and interpreting the characteristics functions of the synthesised membranes. The analysis is carried out using a spectrometer which transmits infrared radiation to the sample and measures the wavelengths for which the material absorbs with the aim to study the chemical interaction between the alginate and chitosan, FTIR test was carried out by attenuated total reflection (ATR) method using a Thermo Nicolet 6700 Fourier transform infrared spectrometer connected to a PC with OMNIC software analysis in the National Institute of Crime and Criminology (INCC) in BOUCHAOUI- Algeria, all spectra were recorded between 400 and 4000 cm-1 over 64 scans with a resolutions of 0.8 cm^{-1}.

IV.2.2.Materials and reagents

- FTIR spectrometer,
- Pastille presser,
- A mortar,
- Chitosan, sodium-alginate, ascorbic acid, tween 80, glycerol, acetic acid,
- KBr.

IV.2.3. Experimental procedure

In general, we put 97% of weight from the KBr and 3% of weight from the powder that we want to analyse it.

In our experience we have weight 0,003 g of (chitosan powder, alginate powder ascorbic acid powder) successively and we complete weighting until 1g with the KBr powder, then we have grinding our mixture using the mortar. We put it in the pastille presser to formulate a fin pastille, after this we put the final pastille into an infrared apparatus until we obtained the desired pics figure IV.4.

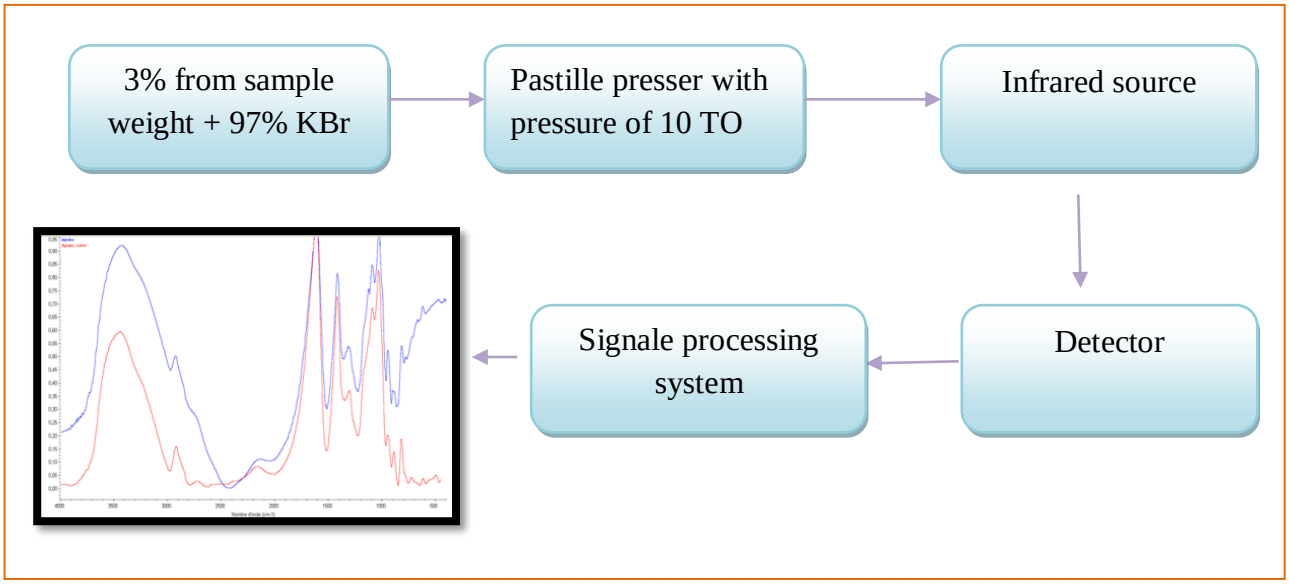


Figure IV.4: representative schema for the procedure of FTIR analyses.

IV.2.4.characterization by Surface microstructure and morphology (scanning electron microscopy « SEM »)

Morphological characterization is performed using a scanning electron microscope (SEM). The principle of this technique is based on electron-matter interactions and a fine beam of electrons sweeps the surface of the sample to be analyzed. Secondary and backscattered electrons are detected; each point of impact will correspond to an electrical signal ad the intensity of this signal will depend on both the nature of the sample and its topography at the point in question. It is therefore possible by scanning the surface of the sample with an electron beam, to obtain a map of the analyzed zone.

A scanning electron microscope is essentially composed of an electron gun, an electron column (electron lenses and scanning coils) whose function is to produce a fine electron probe on the sample, a stage move the sample in the 3 directions (x, y and z) and finally, sensors make it possible to detect and analyze the radiation emitted by the sample. The whole is subject to a vacuum. The images resulting from the SEM analysis were taken on a FEI model Quanta 600 device (INCC-GN), which operates under a high vacuum and under a high voltage of 15 KV.

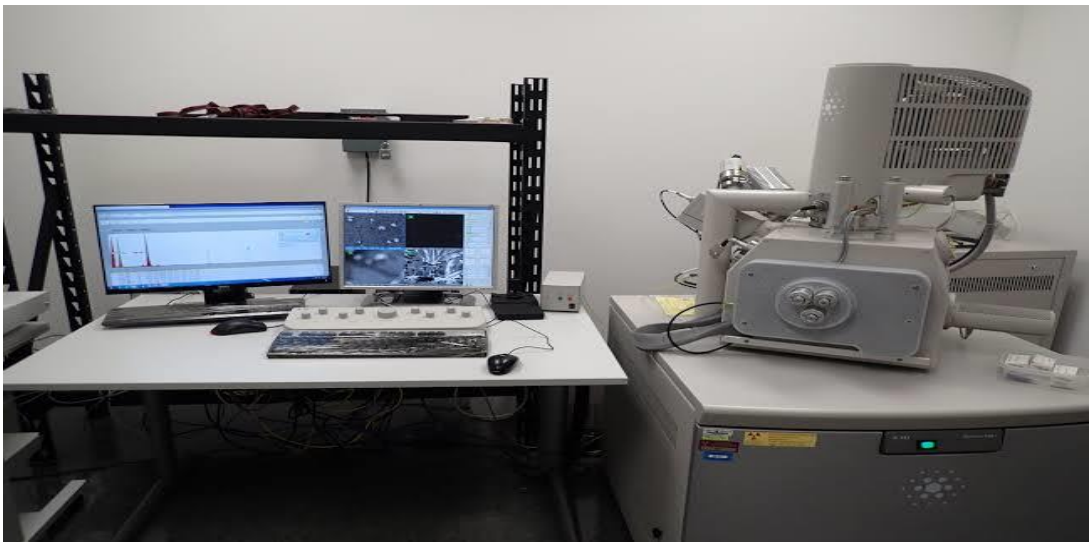


Figure IV.5: SEM model Quanta 600 device (INCC-GN).

IV.3.Release of vitamin C

IV.3.1.Ultraviolet and visible absorption spectrophotometer

After preparing and preserving our membranes, we applied diffusion and liberation test of the active ingredient which is the vitamin C contained in the pores of the membranes, this test was done by a UV/Visible spectrophotometric method using “Shimadzu Europe-UV mini-1240.

The principle of the ultraviolet and visible absorption spectrophotometer is based on the absorption of radiation by molecules in the range from 190 to 800 nm, which corresponds to the ultraviolet (190-400 nm) and the visible (400-800 nm). Uv-visible spectroscopy provides qualitative access to information about the nature of the bonds present within the sample, but also to quantitatively determine the concentration of absorbing species in this spectral range. **(78)**

Before the release of vitamin C passed through the skin, it was necessary to determine the wavelength of vitamin C. we have obtained the spectrum of vitamin c and the wavelength is λ max = 366 nm and the absorbance of vitamin C (DO= 3,259 nm).

IV.3.2.Materials used

- ✓ Beaker,
- ✓ Magnetic stirrer,
- ✓ Volumetric flask,
- ✓ Watch glass,
- ✓ Balance,
- ✓ Spatula,
- ✓ pH meter,
- ✓ Digital scale,
- ✓ UV-Visible spectrophotometer device.

IV.3.3.Preparation of a Phosphate Buffer Saline pH=7.4

The used products are:

- ✓ Distilled water,
- ✓ K_2HPO_4,
- ✓ KH_2PO_4.

In a volumetric flask we fill 800ml of H_2O after we weigh 9,343g of K_2HPO_4 and 6,309g of KH_2PO_4 and put it in the volumetric flask and complete with distilled water up to 1000ml, stir the solution well and measure the pH (pH= 7,04), as shown in the figure IV.6 **(55).** Using pH meter we measured the solution's pH and we adjust solution to final desired pH using KH_2PO_4 or K_2HPO_4.

Figure IV.6: measure the pH of Phosphate Buffer Saline solution.

IV.3.4.The experimental protocol:

First we cut an area of 3*3 cm^2 for each membrane and weigh it (w0), then we emerged the pieces of the membranes in the beakers with a temperature of 30-37 C° and put the beakers on the stirrers (50 rpm) and every 20 min the sample is taken and the release results are determined by UV-Visible spectrophotometer, as shown in the figure IV.7.

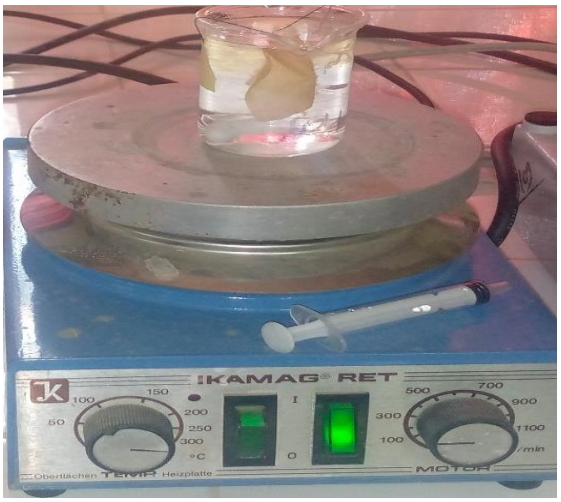


Figure IV.7: peace of membrane during the release in the Phosphate Buffer Saline.

The wavelength of vitamin C is fixed at 366nm. We then followed the variation of vitamin C release as a function of time. Each result was confirmed by two tests.

After this, vitamin C release study, we removed the membranes from the beakers for drying for 12 hours in the oven to weight the final weight (Wf) of the membrane and also we measured the final surface.

IV.3.5.Characteristics of the membranes since release

IV.3.5.1.Calculation of the encapsulation

The encapsulation was estimated for the different systems from the LOADING relationship, expressed as a fuction of the mass of the encapsulated active ingredient and weight of the dry membrane.

$$T = \frac{\text{mass of encapsulated active principle}}{\text{dry membrane weight}}\% \quad \text{EQ IV.3}$$

IV.3.5.2.Porosity

The porosity P of our membranes was calculated from the determination of the quantity of water absorbed by the membranes. **(79)**

$$P = \frac{wi - wf}{wi} * 100 \quad \textbf{EQ IV. 4}$$

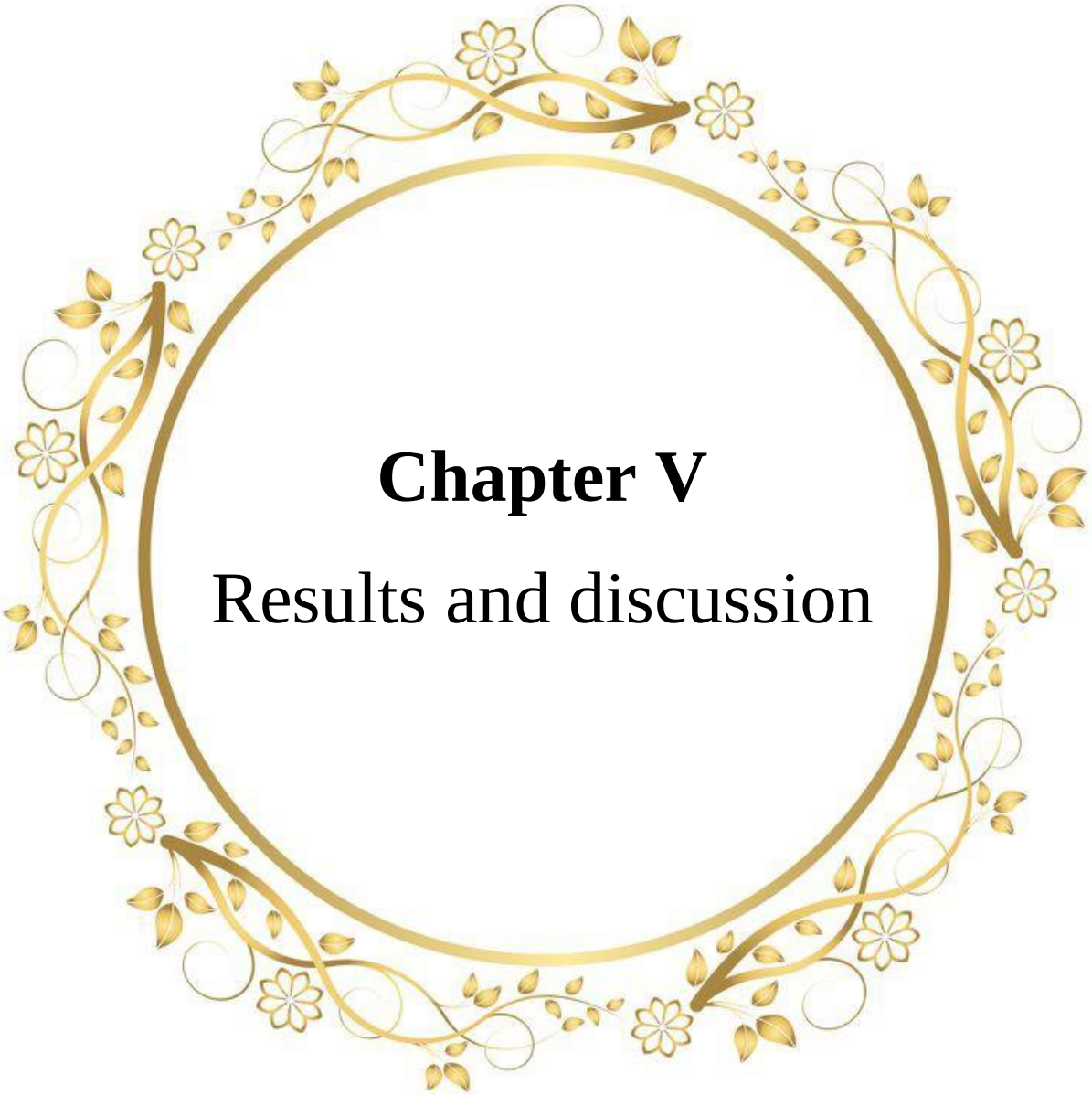
With:

Wf: final weight of the dried membrane.

WI: initial weight of the membrane.

IV.4.Conclusion

This chapter was intended for the detailed description of the equipment, methods and techniques of qualitative and quantitative analysis used in this work for the encapsulation of vitamin C in different concentrations of membranes based on (chitosan-alginate) besides the characterization of the prepared membranes, the study of the release of the active molecule in an pH=7.4 « phosphate buffer saline ».



Chapter V

Results and discussion

V. Introduction

As part of this study, we obtained membranes of different concentrations based on Chitosan-Alginate. The objective of this study is to know the diffusion of vitamin C through the skin. The choice of polymers was made taking into account the following characters: their biodegradability, their availability and the low cost of encapsulation.

In this chapter we have explained the different characterizations of the membranes before and after the release of vitamin C by physic-chemical analysis techniques such as: FTIR, UV-visible, and SEM. And the second step is to follow the release of vitamin C from its different concentrations Alg/SC in a Phosphate Buffer Saline Solution of pH=7.4

V.1.Characteristics of the membranes

V.1.1.Physical appearance

Color, transparency, rigidity, and the flexibility of membranes are the most important characters to check the physical state of membranes as it shown in table V.1.

Table V.1: color and transparency of different membranes.

	Colors and transparency		**Colors and transparency**
F1 100% Chitosan		F2 90% Chi; 10% Alg	
F3 80% Chi; 20% Alg		F4 70% Chi; 30% Alg	

F5 60% Chi; 40% Alg		F6 50%Chi; 50% Alg	
F7 40%Chi; 60% Alg		F8 30% Chi; 70% Alg	
F9 20% Chi; 80% Alg		F10: 10% Chi; 90% Alg	
F11: 100% Alg			

The obtained membranes have deferent degree of flexibility and rigidity as shown in figure V.1 even though we added an equal quantity of adhesive (tween80 and glycerol).

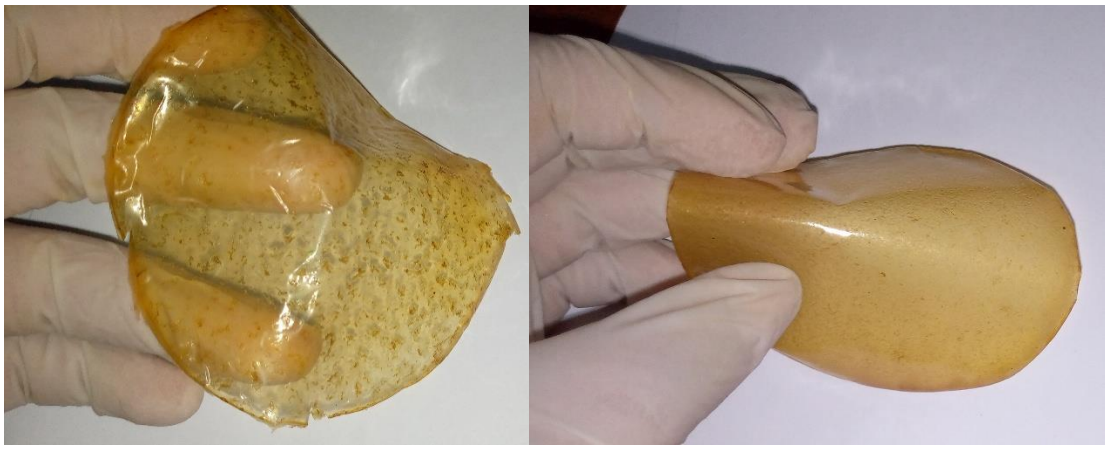


Figure V.1: flexibility between two membranes.

➢ Discussion:

According to the results we obtained, the color of the de deferent formulation of membranes becomes darker by the increase of the concentration of the sodium-alginate in the composition of the membranes, also they become less transparent. For example, the membrane that has composition of 90% Chitosan and 10% alginate was the most transparent membrane.

The good agitation of the chitosan and alginate solutions besides chitosan/alginate mixture results a smooth mixture without coagulation and undissolved particles, so a flat membrane with a smooth surface.

The rigidity of our membranes was due to the adhesives added to the mixture (Tween80, Glycerol) which gives it a flexibility to have applied them at anywhere.

All the polymers used for the formulation of transdermal patches showed good film forming properties. The patches formed were thin, flexible, smooth and transparent. The method used.

V.1.2.Membranes Thickness results

In this test we have measured the thickness of membranes at a maximum of 5 points, but we have chosen 2 parts from our membranes; a smooth and granulated part; the average between the thicknesses they have already measured corresponds to the thickness of the membrane. Four membranes were chosen as an example: F2, F6, F7 and F9 and the thickness are shown in table V.2.

Table V.2: results of the thickness between the smooth part and granulated part of membranes.

Membranes	Thicknesses (mm)		Average of thickness (mm)
	Smooth part	Granulated part	
F2	0.12	0.32	0.44
F6	0.13	0.17	0.30
	0.07	0.16	0.23
F7	0.11	0.18	0.29
F9	0.02	0.10	0.12

The higher the percentage of alginate (80%) for F9, the thickness is small (0.12 mm) between the smooth part and the granule part. And we explain the appearance of the granules the difference in thickness can influence directly on the drug delivering mechanismaccording to Fick's first law; as thickness increases, the release of vitamin C decreases, therefore a membrane that contained a high ration from alginate solution resulting the evaporation of the water in which we dissolved the alginate so the thickness will decrease, on the other hand membranes containing a large quantities of chitosan shows a high thickness as in formulation F2.

V.1.3.Folding endurance

The membranes that we have prepared are rigid, it doesn't break when bend them this is due to the adhesives that we have added during the preparation (Tween 80, Glycerol) which are plasticizers. Except when the percentages of alginate are higher than chitosan, the membranes are not very rigid so it breaks easily.

V.1.4.weight uniformity results of membrane

To verify that the prepared membranes are uniform to the weight, we have measured the weight of 10 individual membranes and the average between them, then we determined the standard deviation as it expressed in the following.

$$\bar{x} = 0.665 \text{ (g)}.$$

The results of weight variation tests of the membranes are shown in the table V.3.

Table V.3: results of weight variation tests of the membranes.

	WF (g)	ξ(g)
F2	0,729	0,064
F3	0,740	0,075
F4	0,738	0,073
F5	0,781	0,116
F6	0,509	-0,156
F7	0,519	-0,146
F8	0,802	0,137
F9	0,590	-0,065

➢ **Discussion**

Weights of the membranes ranged from 0,729 ± 0,064 (g) to 0,802±0,137 (g) for the membranes F (2, 3, 4, 5,) and from 0,509 ± 0,156 (g) to 0,590 ± 0,065 (g) for the membranes F (6, 7,8, 9), even though we made the same measurement for the adhesives but the final weight of the membranes are different, this can be explained by difference in the percentage of the polymers in the formulation; more the chitosan amount was significant in the membrane, more the weight will moderately increase comparing with those containing a high rations of sodium-alginate.So there is not a strict uniformity for all the membranes. And this is due to the difference in Chitosan-Alginate concentrations.

V.2.Characterization by infrared spectroscopy

V.2.1.Study of interactions between the drug and polymers

In order to see the completion of the encapsulation of the vitamin C under consideration in this study, IR spectroscopy characterisation was carried out. The analysis of the different ingredients included in the composition of our membranes (chitosan, sodium-alginate, vitamin C) besides the mixtures (Ch/Alg, Ch/Alg/Vit C) and the mixture with the solvents

(Ch/Alg/VitC/Tw80/Gly), using an FTIR from THERMO NICKOLET brand, at 37°C, gave the spectrums showing in figure V.2, V.3, V.4, V.5, V.6.

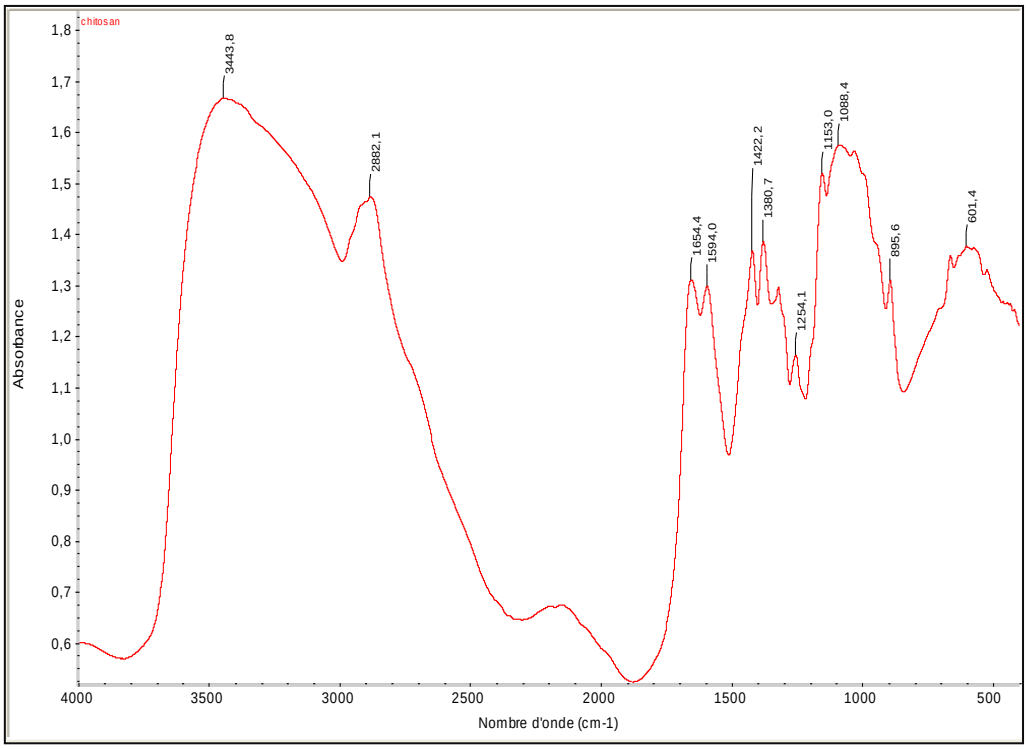


Figure V.2: FTIR spectrum of sample chitosan.

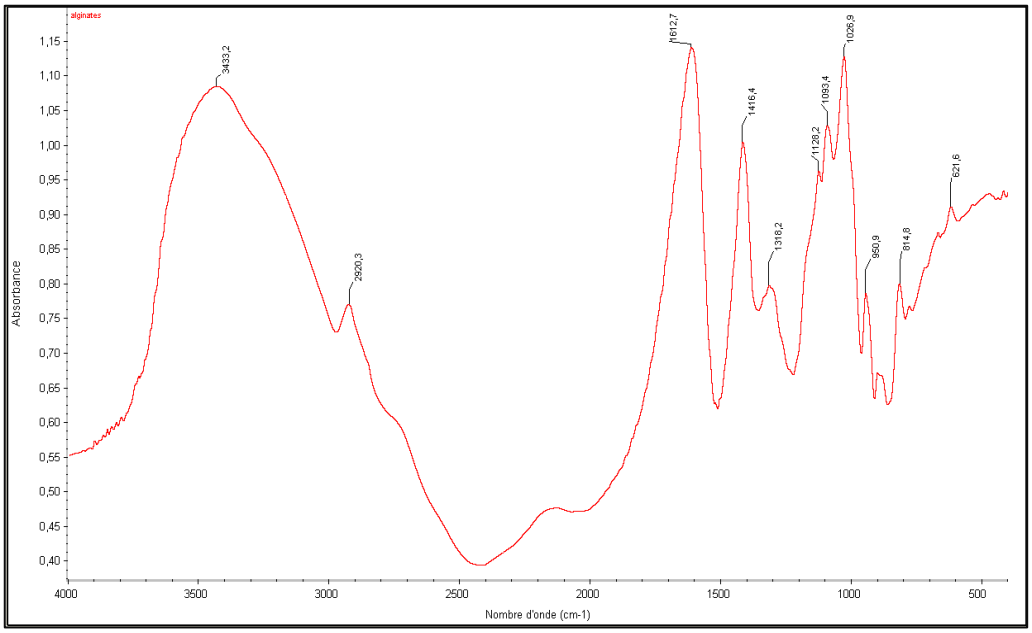


Figure V.3: FTIR spectrum of sodium-alginate sample.

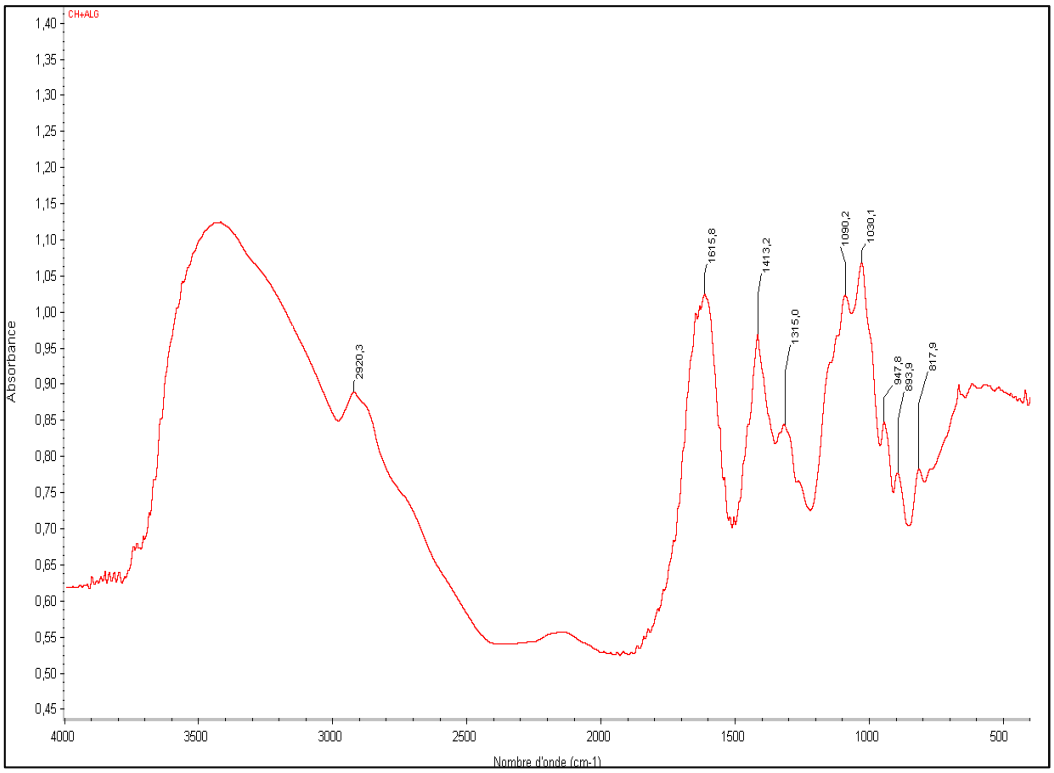


Figure V.4: FTIR spectrum of (Ch/Alg) mixture.

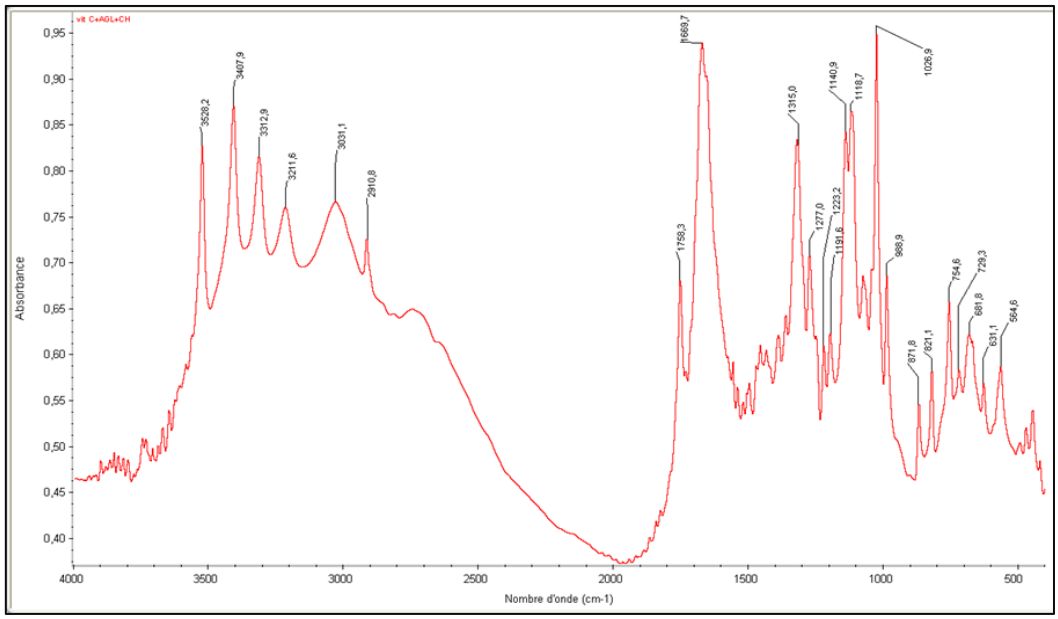


Figure V.5: FTIR spectrum of (Ch/Alg/VitC) mixture.

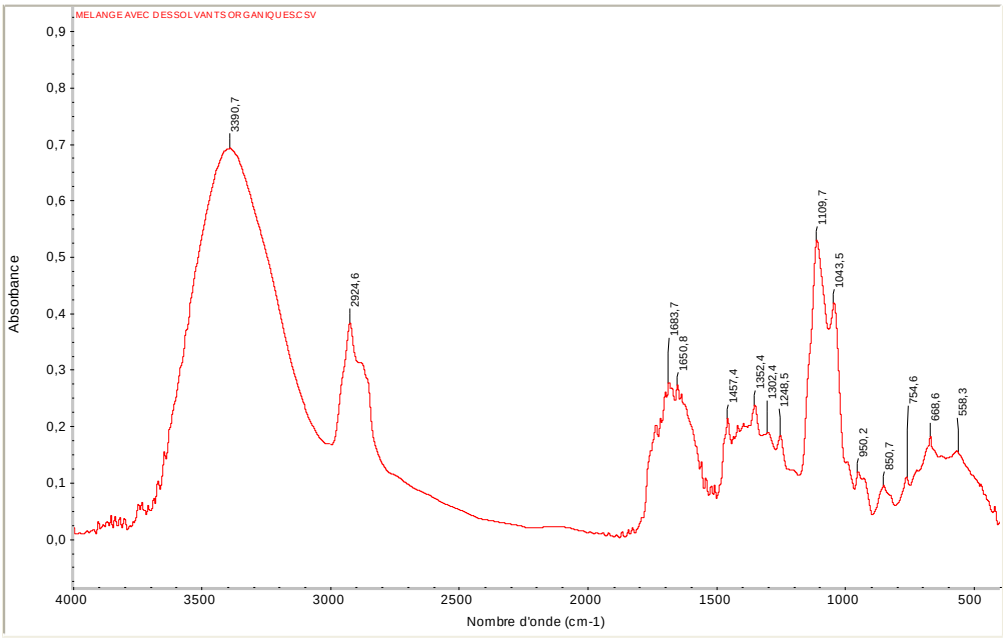


Figure V.6: FTIR spectrum of (Ch/Alg/VitC/Tw 80/Gly) mixture.

As an example we illustrate the following spectra in table V.4 which allows us to detect the characteristics bands.

Table V.4: allocation of the different peaks of SC, Alg, and vitamin C detected by FTIR.

Liaisons chimiques	**Nombre d'onde cm^{-1}**					
	Vitamine C	Chitosan	alginate	Ch/Alg	Ch/Alg/VitC	Ch/Alg/VitC/tw80/Gly/AA
O-H	3528,2	3443,8	3433,2	3440,7	3407,9	3390,7
$(C-H)_{CH2}$	2910,8	2883,1	2920,3	2920,2	2910,8	2924,6
C=O	1758,3	1654,4	1612,7	1615,8	1758,3	1683,7
C=C	1669,7	1594,0	-	-	1669,7	1650,8
$(C-H)_{CH3}$	1454,2	1554,8	1416,4	1413,2	1454,2	1457,4
$(C-O)_{OH}$	1315,0	1380,2	1318,2	1315,0	1315,0	1352,4
$(C-O)_{COOH}$	1233,2	1254,1	1245,9	-	1233,2	1248,5
C-OH	1118;7	1088,4	1093,4	1090,2	1118,6	1109,7
$(N-H)_{amine}$	-	3441,8	-	3440,2	3407,2	3390,7
Aromatics cycles	988,9-821,1	895,6-801,4	950,0-814,8	947,8-817,9	871,8-821,1	950,2-850,7

The FT-IR spectra of chitosan (Ch), alginate (Alg) and their mixtures (Ch/Alg) are shown in figures V (2; 3; 4; 5; 6). The (Ch) spectrum shows a wide and intense band centred at about 3443.8 cm^{-1}, which corresponds to the stretching vibrations due to the bonds of O-H and N-H. the peaks observed at 2883,1 cm^{-1} are due to the symmetrical or asymmetrical CH2 stretching vibrations of the pyranosic ring, characteristic chitosan absorption bands are generally observed between 1654,4 cm^{-1} and 1594,0 cm^{-1} corresponding to the stretching of C-O (amide I) and the bending of N-H (amide II). The absorption bands at 1380,2 cm^{-1} and 1088,4 cm^{-1} are associated to the skeletal vibrations involving C-O stretching, which are characteristic of the structure of the polysaccharides. **(55)**

Alginate absorption bands closed to 1612.7 cm^{-1} and 1416.6 cm^{-1} are associated with asymmetrical and symmetrical stretching vibrations of the carboxylate anions respectively, as other have observed. The alginate spectrum also shows a strong and wide band at 3433,8 cm^{-1} linked to the stretching of the O-H bonds and a weak band of aliphatic C-H stretching at 2920,3 cm^{-1} due to its polysaccharide structure, bands of about 1245,9 cm^{-1} (C-O stretching) and 1093,4 cm^{-1} (C-O-C stretching) have also been observed.

In the FTIR spectra of the mixed polysaccharides of the opposite charges we observed changes in the placement and disappearance of certain bands and the appearance of new peaks compared to the chitosan and alginate peaks. The Ch/Alg mixture has a narrowest and more intense band at about 3440,9 cm^{-1} which is caused by the formation of new hydrogen bonds between O-H and $N-H_2$ groups of chitosan and the C=O and O-H groups of alginate. The bands attributes related to the movement of the sodium carboxylate group were invisible after complication this disappearance is due to the lower tenure in excess of alginate and chitosan.

The possible shift towards the shorter wavelengths and the widening of the vibration bands of the functional groups implies could explain the presence of suspected interactions to develop a shift which can be observed from the peaks of 1615.8 cm^{-1}.

Encapsulation of vitamin C in the simple chitosan and alginate membrane was indicated by appearance of its characteristics peaks at 1118.7 cm^{-1}, 1315.0 cm^{-1} and 1758.3 cm^{-1} and 1669.7 cm-1 due to C-O ester linkage, bending vibration of CH2 and stretching vibration of ester carbonyl and C=C groups respectively, stretching vibration of CH3 and O-H was observed at 3031 cm^{-1} and 3221.6 cm^{-1} to 3528,2 cm-1 respectively. Peaks that appeared at 1380,2 cm^{-1} and 3031 cm^{-1} are due to the bending vibration of CH3 and stretching vibration

of NH group of chitosan and thus 1318,2 cm^{-1} of the alginate. The presence of these characteristics peaks showed the encapsulation of vitamin C in the membranes.

V.2.2.Morphological Characterization by scanning electron microscopy « SEM »:

All the membranes that have analysed by SEM have a fairly rough surface with numerous undulations on its external surface, as shown in figure V.7 which increases the surface area. This is a beneficial quality because a higher surface area allows better mass transfer.

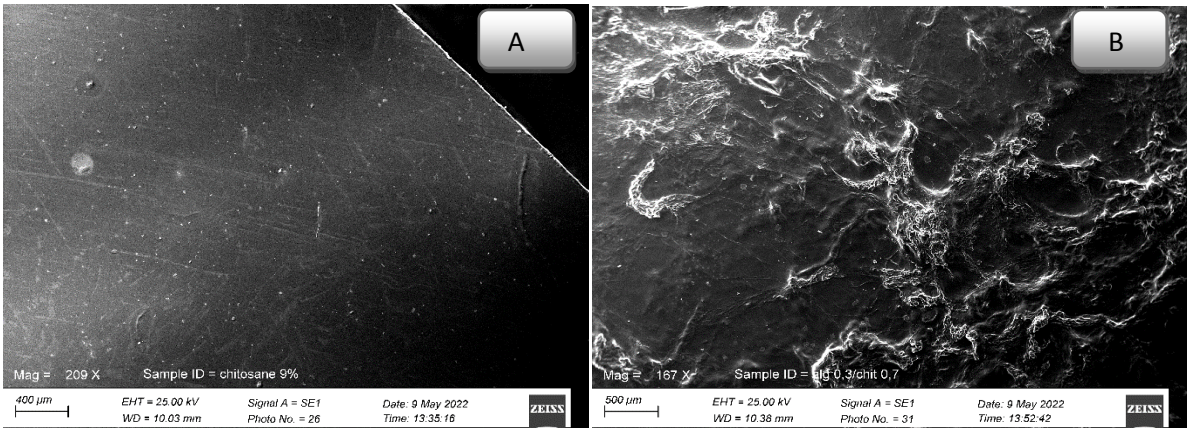


Figure V.7: surface of membrane shown by SEM (A) smooth surface, (B) rough and undulated surface.

According to the works of FABIAN **(80),** shows that the encapsulation by sodium alginate are characterized by a smooth and straited surface. These streaks due to big holes which allow the release of the active ingredient and degradation of the membrane (Fig V.8).

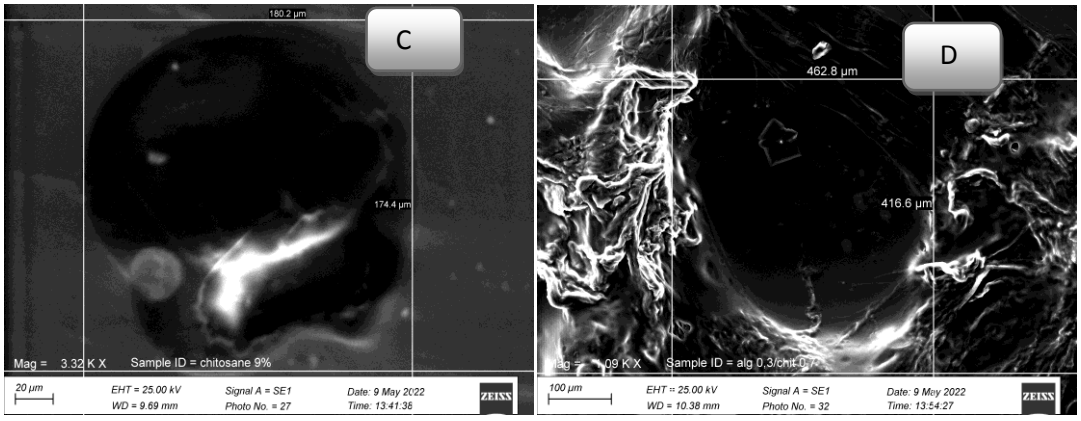


Figure V.8: cristographic defects.

V.3.Release test results

V.3.1.Calibration curve of vitamin C as a function of concentration

This curve is established using ascorbic acid as a reference and the results are expressed as the concentration of ascorbic acid, by (g/ml).

The calibration curve is established with a correlation coefficient.R^2= 0.9857 (Figure V.9).

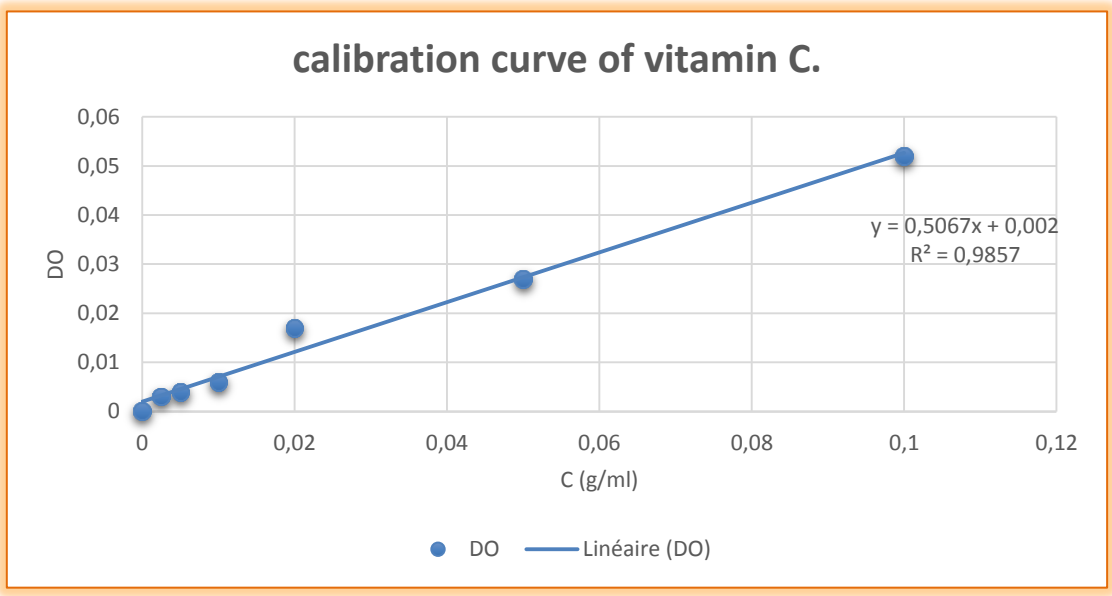


Figure V.9: calibration curve of vitamin C.

This calibration curve is a straight line; from it we can determine the concentrations of vitamin C release (table V.5).

V.3.2.Absorbance and concentrations since release:

The determined absorbance shown in table V.5 is converted to concentration using the following equation “y= 0.506X+0.002" from the calibrating curve of the vitamin C.

Table V.5: vitamin C absorbance values since release.

Percentages concentration SC/Alg (%)		**90% SC 10% Alg**	**80% SC 20% Alg**	**70% SC 30% Alg**	**60% SC 40% Alg**	**50% SC 50% Alg**	**40% SC 60% Alg**
Do (nm)	**T0**	0	0	0	0	0	0
	T1	0,274	0,218	0,248	0,072	0,115	0,399
	T2	0,278	0,248	0,250	0,209	0,243	
	T3	0,281	0,250	0,278	0,218	0,260	
	T4	0,305	0,263	0,305	0,314	0,273	
	T5	0,309	0,323	0,309			
	T6	0,315	0,327	0,332			
	T7	0,317	0,377	0,340			
	T8	0,332	0,386	0,356			
	T9	0,336	0,399	0,359			
	T10	0,349	0,405	0,365			
	T11	0,350	0,412	0,367			
	T12	0,352	0,415	0,388			
	T13	0,368	0,447	0,405			

- **Remark:**

According to these results of the concentrations and the absorbance we have noticed that at each time the concentration released of the vitamin C increases, and for the membranes (F5-F6-F7) are degraded after a few minutes. And the same for the membranes (F8-F9-F10) which degraded just after the first contact of the surface of the membrane with a phosphate buffer saline solution.

After converting the absorbance to the concentrations we have obtained the following cumulative drug released curves shown in figure V.10.

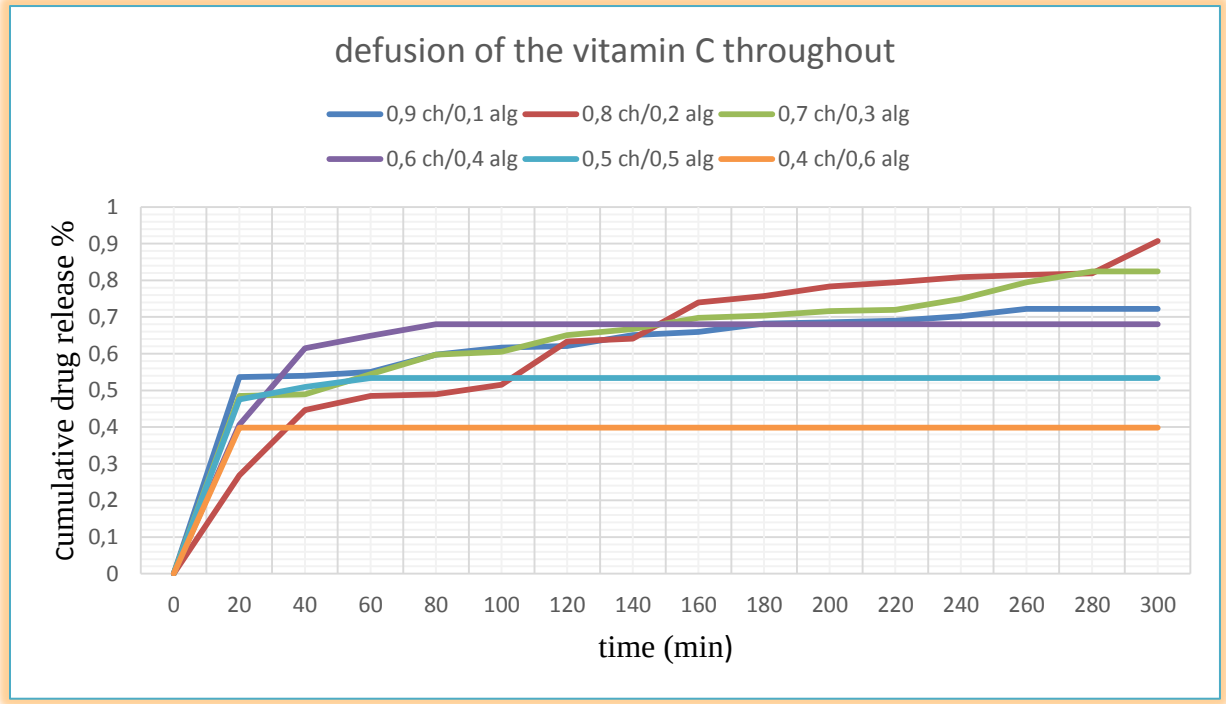


Figure V.10: cumulative drug release (%).

V.3.3.Characterization of membranes during release

Before calculating the encapsulation and porosity test, the results of the weight of the membranes before and after release, are indicated in the table V.6.We have calculated the encapsulation and porosity percentages just for membranes that are not degraded in a Phosphate Buffer Saline solution.

Table V.6: characterization of the membranes before and after release.

Membranes	F2	F3	F4	F5	F6	F7	F8	F9	F10
Initial weight Wi (g)	0,1118	0,0644	0,0484	0,0913	0,115	0,151	0,105	0,136	0,204
Final weight Wf (g)	0,1040	0,0573	0,0396	0,0629	_	_	_	_	_

According to this table we note for each the final weight for the different percentages of the membranes is reduced after releasing and drying compared to the initial parameters, after the diffusion of vitamin C.

V.3.4.Percentage of encapsulation:

Table V.7: percentage of the encapsulated vitamin C.

	Mass of encapsulated PA (g)	**Dry membrane weight (g)**	**T (%)**
F2	0.02	0.1040	19.23
F3		0.0573	34.90
F4		0.0396	50.50
F5		0.0629	31.79

From the results showed in the table V.7 we noticed that in the membrane F2 the percentage of chitosan is higher when compared to the alginate so the amount of alginate reacts with an equivalent amount of chitosan by producing pores where vitamin C is going to be encapsulated. Then there will be less binding between the two polymers and less cavity when alginate is introduced in small fractions. This leads to a low level of vitamin C encapsulation.

Unlike the F4 membrane which has a high encapsulation percentage where chitosan and alginate are introducing in almost the same fractions. Then more bonds between polymers, and more cavities that were formed.

V.3.3.2.Variation of the porosity of membranes in pH=7.4

Table V.8: membranes porosity (%) results.

Mass concentration (g/ml)	Wi (g)	Wf (g)	Porosity (%)
90% SC/10% Alg	0,1118	0,1040	6.97
80% SC/20% Alg	0,0644	0,0573	11.02
70% SC/30% Alg	0,0484	0,0396	18.18
60% SC/40% Alg	0,0913	0,0629	31.10

We have noticed through the results of the porosity that more than percentages are almost similar in the composition of our membranes, more than there would be encapsulation of active ingredients (vitamin C).

According to the curves obtained, we noticed that after 5 (min) of the release of vitamin C, the concentrations of vitamin C in the membranes increase.

- For the membrane F2 the concentration is raised up to c= 50% and after 5 (min) of release, the continuous concentration has increased throughout the duration of release and at t=240 (min) the concentration remains constant.
- And for the membrane F10, the concentration has evolved to c=40% and after 10 (min) of release, the membrane is degraded in a phosphate buffer saline so that their release concentration is constant throughout the duration of release. And the same case for the membrane F6 at t= 80 (min) the membrane is degraded.
- And for the membranes (F3-F4-F5) the diffusion of the vitamin C in this case is higher,
- And finally for the membranes (F7-F8-F9) since the first second of contact with a phosphate buffer saline, it degraded.

➢ General discussion of the release of vitamin C

We have noticed from the graphs (figure V.10) that the release of vitamin C is a uniform release before 10 min, then that there are disturbances during the duration (20-80 min) assuming that it is the error of the device then the release in this interval is not uniform that is

to say there is a difference in the diameters of the pores, the chains are flexible (not rigid) an inflation of the por. Thus we explain the constant release of vitamin C that there is no swelling in the pores.

Then the results that we got, we noticed that the variation of chitosan/alginate concentrations they influence the diffusion of vitamin C. then we saw that the release in a phosphate buffer saline (pH=7.4) is a sustained release such as the membranes (F5-F6).

And for the membranes (F7-F8-F9-F10) they are non-rigid membranes, because they are degradable in a phosphate buffer saline and this is due to the concentration of alginate higher than the concentration of chitosan; Because alginates dissolve quickly in aqueous medium, while chitosan does not dissolves in water, which gives the rigidity for the membrane (such as the membrane F2, the concentration of vitamin C released is very raised). Thus the release of vitamin C in this case is a rapid release and all the concentration diffuse in the first seconds and then they are constant.

Therefore these membranes which are degraded from the first time of release, according to **(81),** shown that the encapsulation of the active principle by sodium-alginate is unstable and degrades rapidly in basic or acid medium.

Then for the equal concentrations (SC/Alg), the release of vitamin C in this case is a prolonged and constant release after a few seconds because the polymers they are favoring the creation of bonds between them, therefore reduction of pores and release sufficient. That is, as the molecular weight between the two polymers increases, the movement decreases.

And from him the ideal case of release of vitamin C at pH=7.4 (pH of the skin) is the case when a greater concentration of the active substance is released in the first seconds.

And the relationship between the curves obtained and the calculations of vitamin C encapsulations, we have noticed that despite we have the same amount of vitamin C in all the membranes, but each membrane encapsulates a different amount of vitamin C to the other membrane. And each the concentration of chitosan more than the concentration of Alginate the encapsulation of vitamin C is large and release high. In this case can be said that the membranes they are favored increase of the pores.

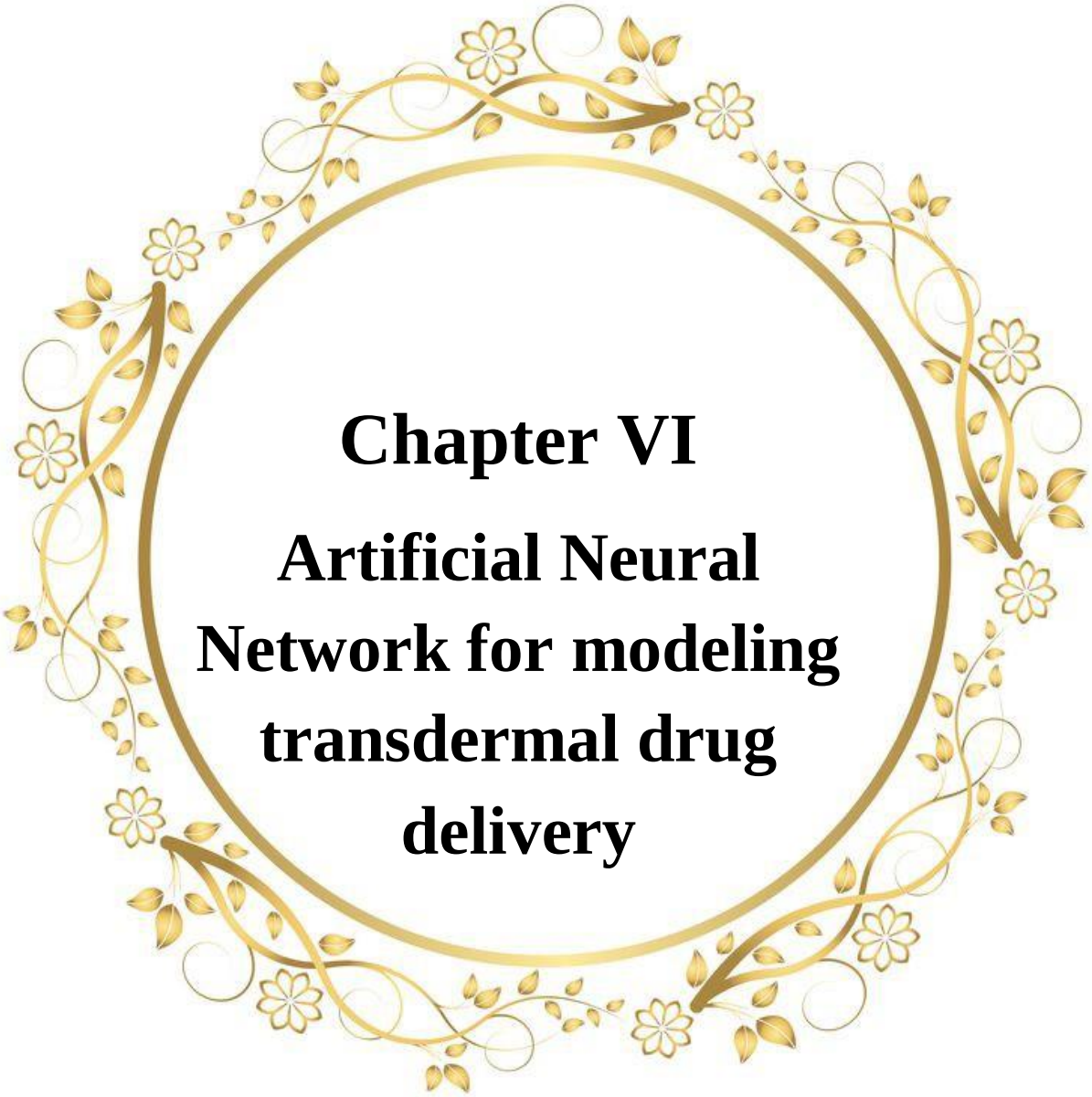
According to the results we can explain that there is a release by inflation (the pores in the initial state are small and when we put the membranes in a phosphate buffer saline, the

water will enter the membranes so the pores will swell which allows the release of vitamin C and there are the release by degradation.

Finally to conclude, the results obtained in this study must be improved by adding cross linkers which are generally aldehydes such as glutaraldehyde, but the latter is a toxic and harmful product for the skin so it must be avoided. If not by other proposal, we propose that it is necessary to add a sufficient percentage of vitamin C in the preparation of the membranes for better results. And the results which confirm this remark is the calculation of the encapsulation (T=19.23%) is an insufficient result (the membrane does not encapsulate a lot of vitamin C). And according to the graphs if possible leave the patches on the skin for a maximum of 40 minutes, this is the excellent and sufficient time for the diffusion of vitamin C in this period is (70%). Otherwise we did the release for 6 hours in this study just to see how the release reacted for a long time.

V.4.Conclusion

Based on the results obtained in this study, it can be concluded that the variations in the percentages of chitosan and alginate included in the composition of our membranes influence on the mechanical and physiochemical properties such as weight, flexibility, thickness. This can also effect on the delivering of the vitamin C which is encapsulated by the biopolymers (Chi/Alg), in addition we have noticed that some membranes have a great power of release of the active ingredient, thanks to the bonds cried between the two polymers which have been demonstrated by the FTIR technique.



Chapter VI

Artificial Neural Network for modeling transdermal drug delivery

VI. Introduction

A neural network is a massively parallel distributed processor made up of simple processing units; which are nodes that perform some usually nonlinear mathematical functions. This type of non-algorithmic calculation is characterized by a system that resembles the structure of a human brain. One of the great advantages of these models is their ability to learn (store experimental knowledge), generalize (make knowledge available) or extract rules automatically from complex data.

The technique of artificial neural network (ANN) has been used in many scientific fields such as: in solar energy, solubility of solid drugs. An artificial neural network (ANN) is used to develop predictive models to estimate the molecular diffusion coefficient of various gases at multiple pressures over a wide temperature range.

From the experimental data (vitamin C release) we made a data base and develop for an artificial neural network model. In this contribution we seek to propose an optimization methodology to achieve the best MLP (Multi-Layer Perceptron) network; based on almost every aspect of ANN modeling such as activation function, training data, training algorithms, pre and post-processing, hidden layer count and network size.

The main objective of this study is to develop an accurate model to predict a drug diffused on the skin using the ANN approach.

VI.1.Approach to artificial neural networks

Despite the growing power of computers and the implementation of theoretical approaches; several problems are posed within the framework of this research.

By approaching this definition of an ANN, reproduced from one can then imagine the vast fields of potential application of ANN **(82),** conceive the interest which is reserved for them during the last two decades, translated by the enormous number of publications, and finally understand why they have become a robust tool for scientific computing in several science and engineering disciplines.

Due to the availability and richness of the bibliography dealing with the foundations and applications of ANN, it is unnecessary to present in detail all the theoretical notions of ANN.

Therefore, we examine in this chapter the major factors effecting the successful application of ANN.

ANN is an adaptive nonlinear statistical data modeling technique consisting of interconnected artificial neurons processing data in parallel. The multi-layer perceptron (MLP) type was used in this figure VI.1, and the supervised learning process was used for the adjustment of the network parameters.

VI.2.Normalization technique

Data normalization refers to the analysis and transformation of input and output variables to minimize noise, highlight important laws, detect trends, and flatten the distribution of the variable to help the network of neurons in the learning of relevant patterns. In general, normalization is performed to confirm that all variables used in model inputs have equal importance during training; therefore, the normalization of the data must be understood from the upper limit of the activation function.

Finally, the outputs of the neural network are de-normalized before being presented. Input and output data were normalized in the range of (y_{min}= -1, y_{max}= 1) using a **mapminmax** algorithm given by equation EQ VI.1. This performs a normalization of the maximum and minimum value of each row.

$$y = \text{map}\min\max(x) = \frac{(y_{max} - y_{min})(x - x_{min})}{(x_{max} - x_{min})} + y_{min} \quad \textbf{EQ VI.1}$$

VI.3.Transfer function

The main difference between the various types of networks is in the type of activation function used by the hidden neurons. In MLP, a type commonly used by hidden neurons has a tangent sigmoid function shown in equation EQ VI.2; and that, by the output neurons a purlin function shown in EQ VI.3.

$$f(w) = \frac{e^{w} - e^{-w}}{e^{w} + e^{-w}} \quad \textbf{EQ VI.2.}$$

$$f(z) = z \quad \textbf{EQ VI.3.}$$

Where **w** is the weighted sum of input, and **z** is the input to the output layer.

After examining a considerable number of differently structured neural networks, the adequate ANNs selected in this study have a single hidden layer with 8 neurons and output layer with one neuron. The hidden layer has a tansing transfer function. The output layer has a purlin transfer function. The typical structure of ANN is shown in figure VI.1**.**

The mathematical formula that relates the inputs to the output of the optimized neural network (ANN) is given in EQVI.4.

$$DD(\%) = \sum_{n=1}^{n}\left[w_{ij(1.n)} \left(\frac{2}{1+\exp\left(-2\left(\left(\sum_{m=1}^{m} w_{i(n.m)} In_{(m)}\right)+b_{i(n)}\right)\right)} - 1 \right)\right] + b_j$$

EQVI.4.

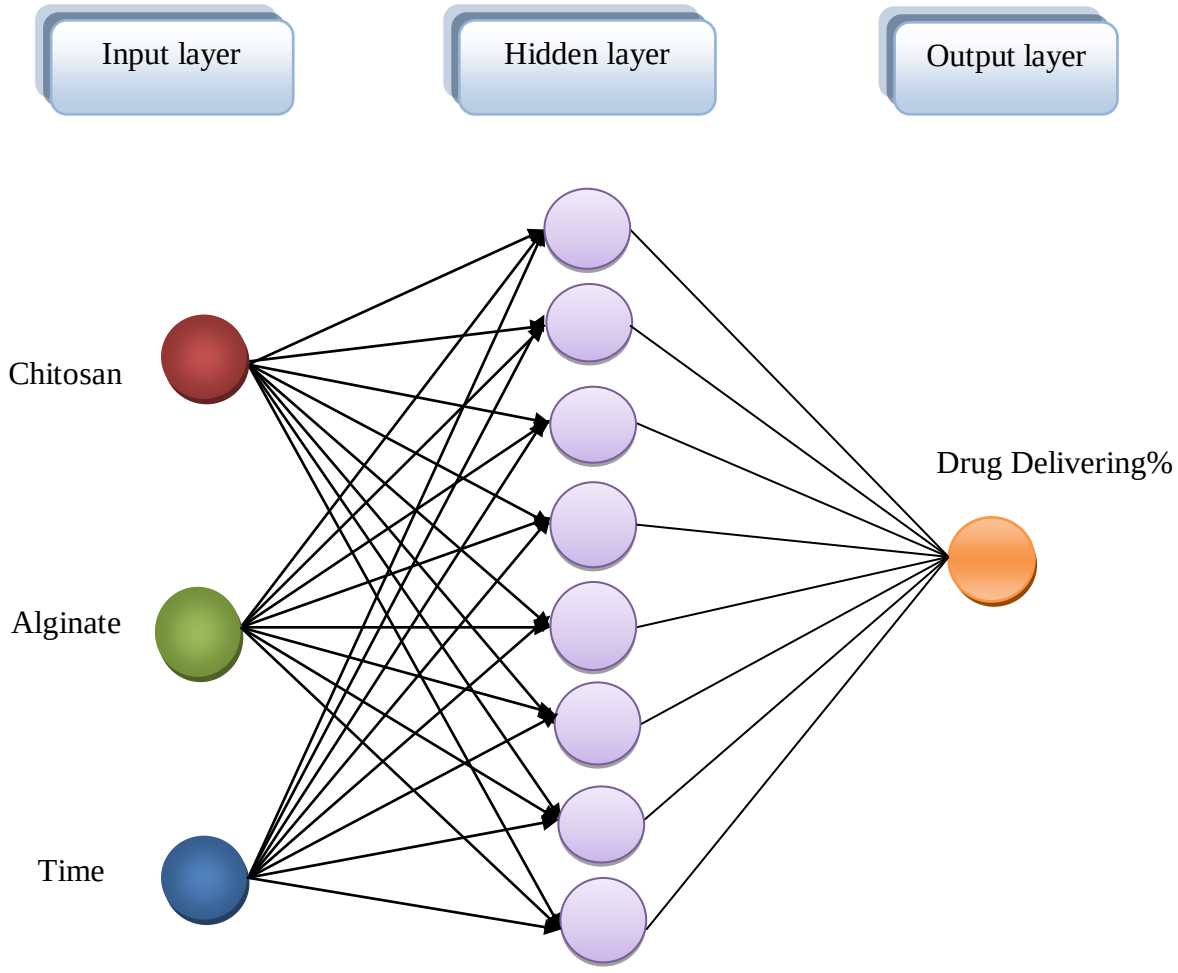


Figure VI.1: structure of the ANN to predict the delivering percentage of vitamin C.

VI.4.Modeling procedure

VI.4.1.Database collection

The database was formed from the experimental result based on the phenomenon of vitamin C release in a phosphate buffer saline solution. This experimental database was formed by the students *Amrouche Yassemine*, *Rahmoune Fatima Zahra* and *Dr Hammoudi Mounir*. We have transformed the dimensional experimental inputs into a dimensionless form. This database was transformed into dimensionless numbers including the different chitosan alginate percentages of concentration values and time.

In this study, a methodology was given to select the ANN model to predict the diffusion of vitamin C on the skin. The objective of this study was to select the best model. The methodology begins with extensive research to select a model with minimum complexity and optimal performance. The number of observed data used in ANN is 320, which are divided into three sections: the training set (256 data), the test set (32 data) and validation set (32 data).

The training, test and validation subsets of ANN were obtained by selecting 80% from the dataset as the training, 10% from the dataset as the test and 10% from the dataset as the sub-validation sets. A summary of the range of different variables is presented in the table VI.1.

Table VI.1: range of dimensionless variables

Settings	Units	Min	Max
Chitosan	%	10	90
Alginate	%	10	90
Times	Min	1,27246162	79,8794096
Concentration of release of vit C (%)	%	0,44390554	69,7183099

The difference between observed and predicted values was filtered through the system and used to adjust connections between layers, improving performance. Root mean squared

error (REQM) is the main criterion for evaluating the performance of ANN, which is defined as follows:

$$RMSE = \left[\frac{1}{n} \sum_{i=1}^{n} \left(y_i - y_i^t \right)^2 \right]^{\frac{1}{2}} \quad \textbf{EQ VI.5.}$$

The statistical quality of ANN for the three sets of training, testing and validation was then assessed using the coefficient of determination (R^2), the absolute relative error (ARE), as it indicated in EQ VI.6, EQ VI.7 and EQ VI.8

$$R^2 = 1 - \frac{\sum_{i=1}^{n} \left(y_i - y_i^t \right)^2}{\sum_{i=1}^{n} \left(y_i - y_0 \right)^2} \quad \textbf{EQ VI.6}$$

With:

$$y_0 = \frac{1}{n} \sum_{i=1}^{n} \left(y_i - y_i^t \right) \quad \textbf{EQ VI.7}$$

$$ERA_i(\%) = \left[\frac{\left| |y_i| - |y_i^t| \right|}{|y_i|} \right] \times 100 \quad \textbf{EQ VI.8}$$

VI.5.Modeling with neural networks

The objective of this modeling is to build a model to predict the diffusion of the vitamin C (drug) into the skin. ANN was chosen as the main modeling tool to perform the task.

The different percentages of chitosan, alginate and time were input arguments for the network (independent variable), while the adhesives were target argument (dependent variable). The structure of the database used for the model is presented in the table VI.2 and figure VI.2.

Table 6.2 : structure of ANN.

Type of network	Number of neurons in the hidden layer	Transfer function of hidden layer neurons	Transfer function of hidden layer neurons	Learning algorithm	Number of iterations or cycles
MLP 1 couche	8 neurons	Tangential sigmoid	Identity function	Levenberg-Marquardt	516

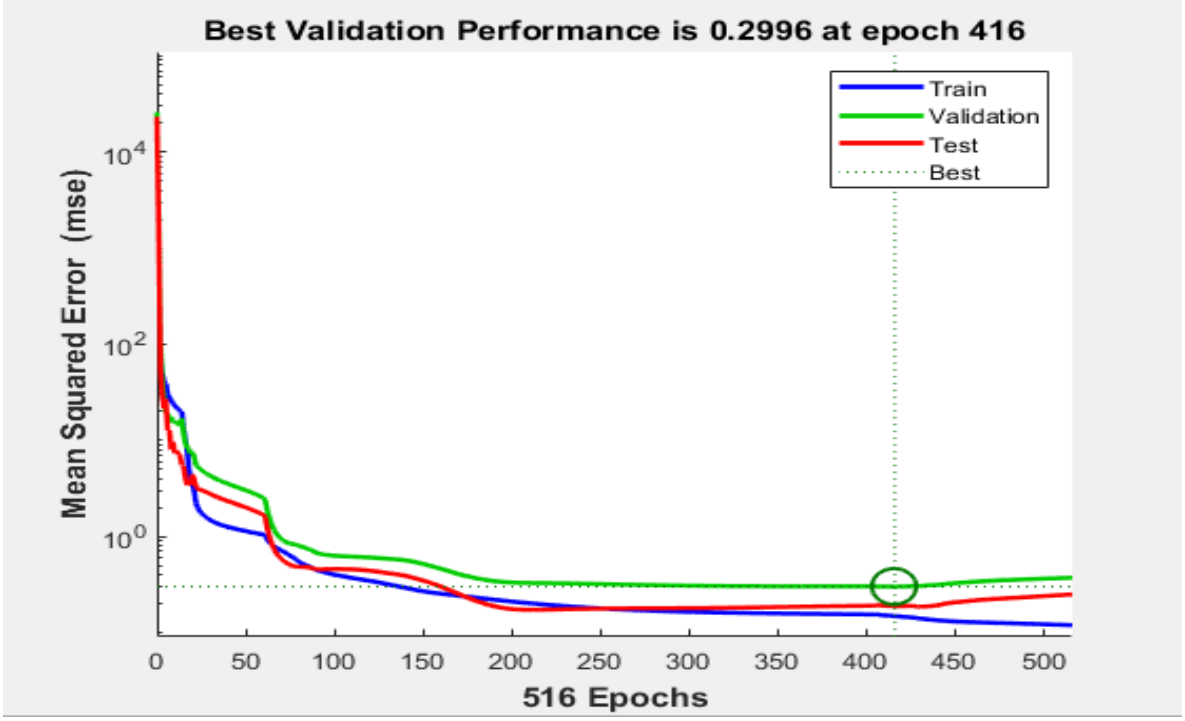


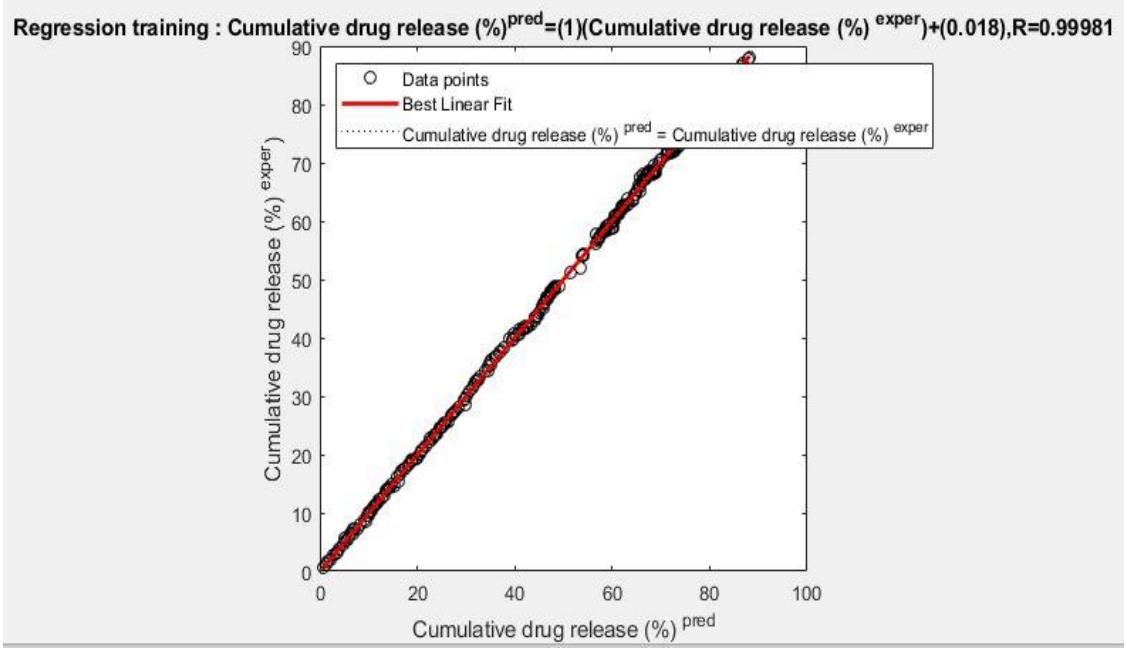
Figure VI.2: Validation with the TRAINLM functional diagram.

In this part we have listed all the important steps that led to the solution of the optimization of neural networks. The following parameters were considered and optimized when developing the best network for the prediction of vitamin C release: selection of input and output data, possible transfer function, learning mode, stopping criteria, algorithm training, normalization technique, number of hidden layers, number of hidden nodes and performance evaluation measures. The results are summarized in the table VI.3.

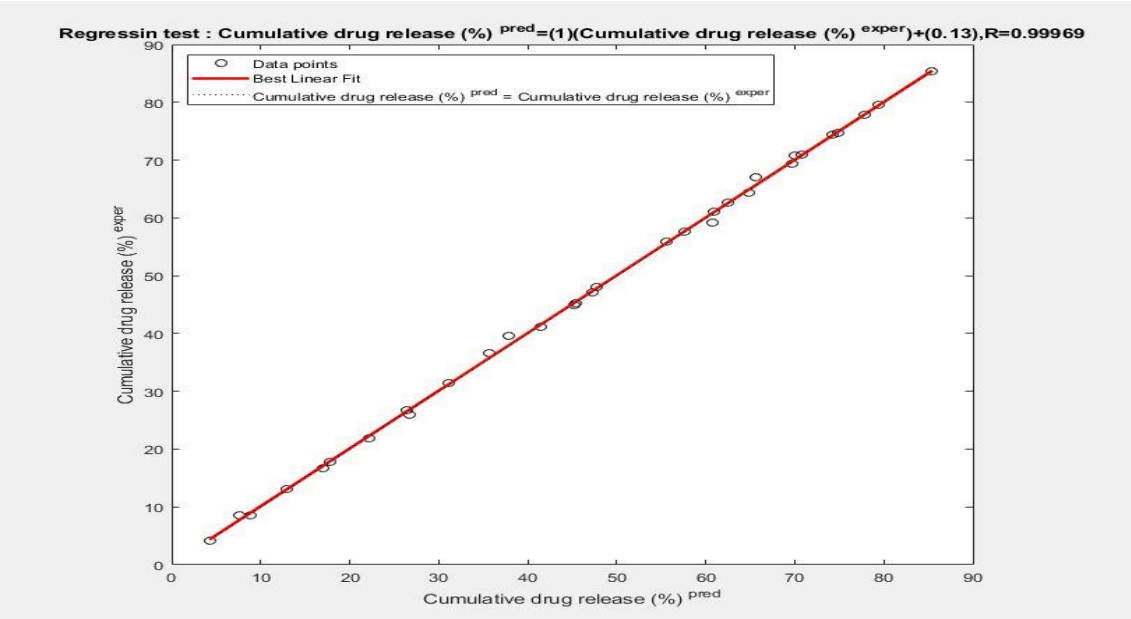
Table VI.3: the performance statistical of an ANN model.

	ARE_{min} (%)	ARE_{max} (%)	RMSE	R
Learning	$9.8820*10^{-4}$	72.5001	0.4718	0.99981
Test	0.00126	12.7266	0.5925	0.99969
Validation	0.02481	2.4488	0.5954	0.99971
Global	$9.8820*10^{-4}$	72.5001	0.4940	0.99979

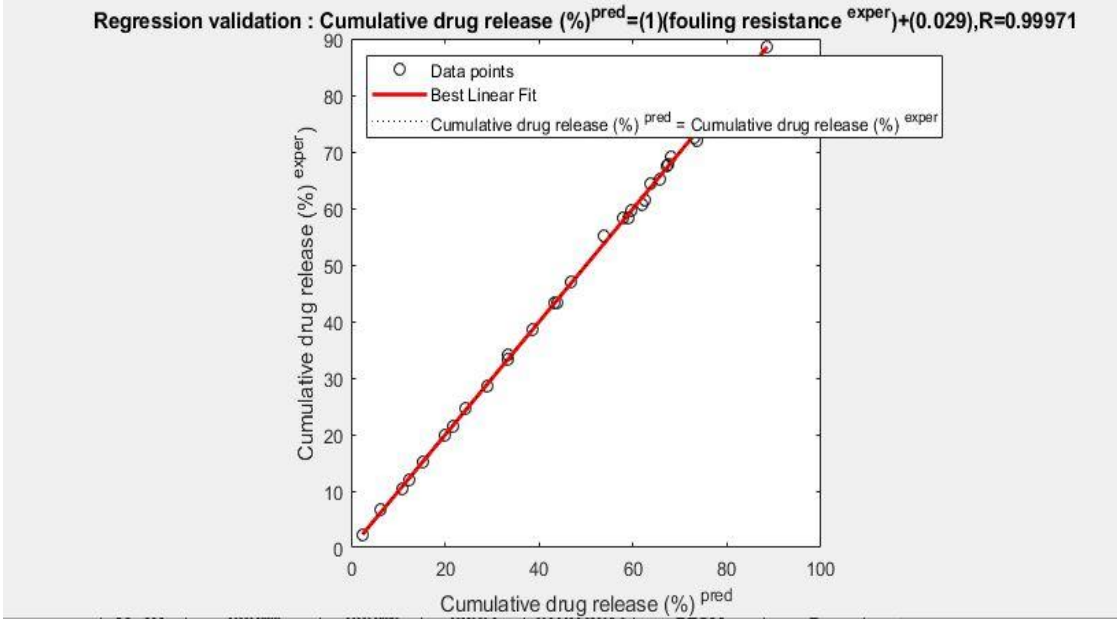
The performance of a trained network can be measured to some extent by the errors on the training, test, validation, and total sets, but it is often useful to study the response of the network in more detail. An option which is to perform a regression analysis which is a measure of how well the output succeeds in explaining variations in the target. The values of R obtained for the estimated output are 99.981%, 99.969%, 99.971% and 99.979%, as shown in the figure VI.3. The linear fit of the output-target relationship is near the 1÷1 line (output= target), which is a good sign of accurate network training.



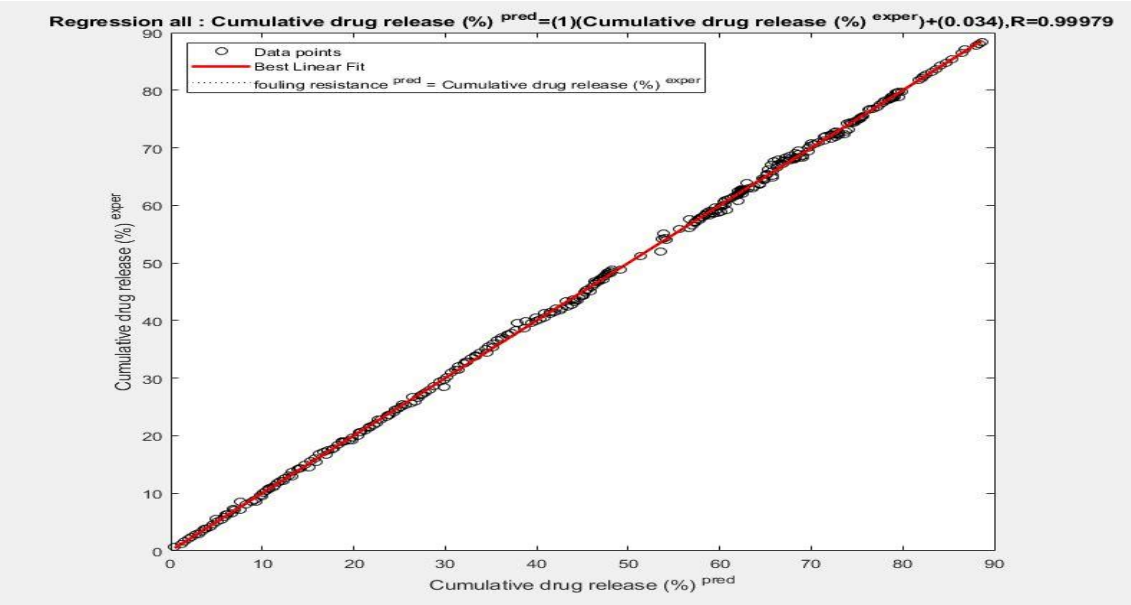
(A)


Regressin test : Cumulative drug release (%) pred=(1)(Cumulative drug release (%) exper)+(0.13),R=0.99969
Data points
Best Linear Fit
Cumulative drug release (%) pred = Cumulative drug release (%) exper
Cumulative drug release (%) exper
Cumulative drug release (%) pred
0
10
20
30
40
50
60
70
80
90

(B)


Regression validation : Cumulative drug release (%)pred=(1)(fouling resistance exper)+(0.029),R=0.99971
Data points
Best Linear Fit
Cumulative drug release (%) pred = Cumulative drug release (%) exper
Cumulative drug release (%) exper
Cumulative drug release (%) pred
0
20
40
60
80
100

(C)



(D)

Figure VI.3: plot between experimental cumulative drug release and ANN prediction for (A) the learning data set, (B) test data set, (C) the validation data set and the global data set (D).

VI.6.Results and discussion

VI.6.1.Neural network

Selecting an appropriate ANN learning algorithm has always been a difficult task. Its importance is also as network architecture and geometry.

We have conducted extensive experimental tests to determine the best learning algorithm in total; eleven (11) different learning algorithms for MLP network algorithms available in MATLAB were used.

A single hidden layer was adopted with a variation in the number of neurons from 1 to 20 for each algorithm tested. For each new configuration of the network, eight testes were carried out because the responses of the networks are not stable, which means that the performance of the same network varies from one training session to another.

A detailed comparison of the performance of MLP neural networks driven by the different algorithms mentioned above is represented in table VI.4, in this study ,the Levenberg-Marquardt algorithm was considered the learning algorithm as it could have an absolute error Maximum relative (ARE_{max}) smaller than other learning algorithms.

Table VI.4: comparison of 11 back propagation algorithms with 20 neurons in the hidden layer.

Algorithm of back propagation	Function	Absolute relative error(ARE_{max})%	Epoch	Coefficient of determination R^2
Levenberg-Marquardt	Trainlm	2.441	1000	0.99973
Regularization of Bayésienne	Trainbr	3.288	1000	0.99979
Conjugated gradient Powell-Beale	Traincgb	6.537	1000	0.99941
Conjugated gradient Fletcher-Reeves	Traincgf	7.718	1000	0.99864
Conjugated gradient Polar-Ribiere	Traincgp	18.405	1000	0.99451
Conjugated gradient at scale	Trainscg	11.789	1000	0.99917
Descent gradient with adaptive learning rate back propagation	Traingda	335.251	1000	0.96193
Back propagation with variable learning rate	Traingdx	193.530	1000	0.9795
Cross-sectional back propagation in one step	Trainoss	22.545	1000	0.99403
Resilient back propagation	Trainrp	58.820	1000	0.99197
Quasi-Newton back propagation BFGS	Trainbfg	5.916	1000	0.99957

However, the performance of the RNA model was statistically by R^2 and ARE_{max} according to EQ VI.6 and EQ VI.8, respectively, which was calculated using experimental values and network predictions, therefore, ARE_{max} was used as an error function that measures network performance. The maximum (ARE_{max} and R^2) network was selected as the best ANN model.

To obtain the optimal number of neurons in the hidden layer, a topology series was used, using the Levenberg-Marquardt conjugated gradient algorithm, but at the scale such that the number of neurons varies from 1 to 20 figure VI.4. To maximize ARE_{max} by looking for a set of weights to produce outputs equal or close to target values.

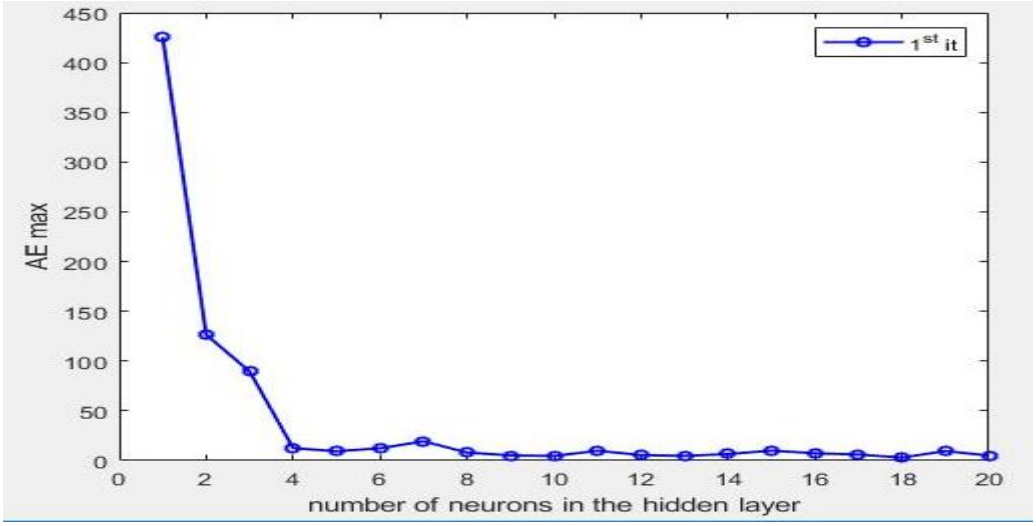


Figure VI.4: effect of the number of neurons in the hidden layer on the ARE_{max} of the neural network.

Prediction of ANN versus experimental results for the learning test and validation data sets are plotted in figure VI.5 for cumulative drug release. The performance of the selected network (3.8.1) is described in figure VI.5.

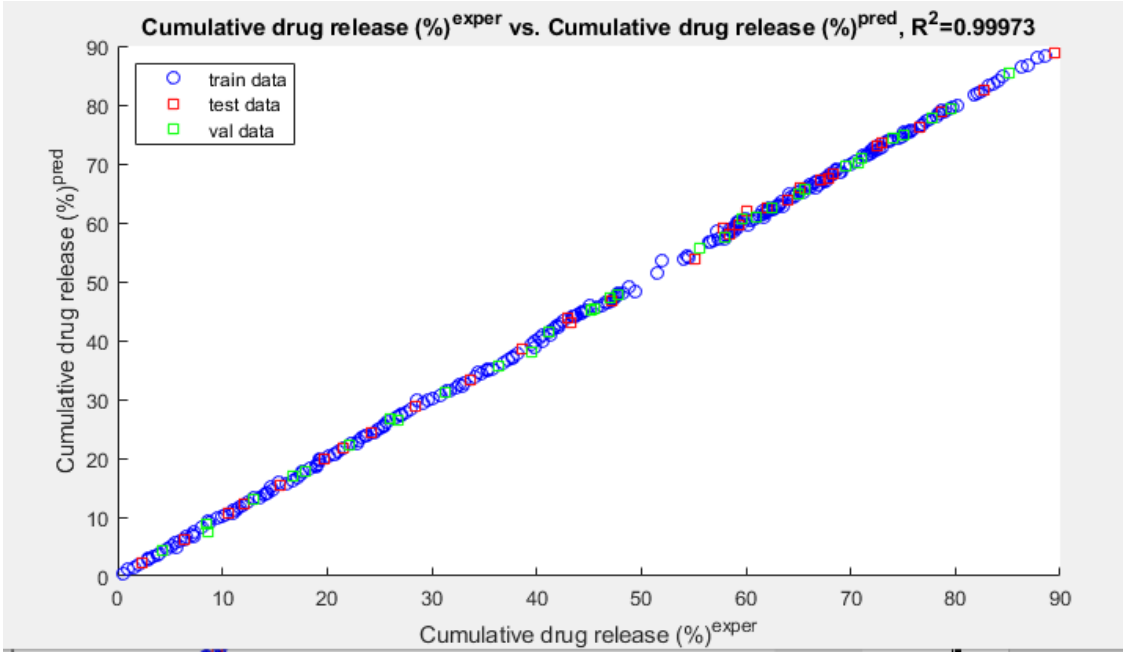


Figure VI.5: plot between experimental data and prediction of delivering drug in ANN.

VI.7.Analysis of sensitivity

In order to study the effects of the selected input parameters on the expected outputs, a sensitivity study is performed, where the model chosen to study 3 inputs, 1 output and 8 neurons in its hidden layer, we use the max-min method as a normalization technique and Levenberg-Marquardt as a learning algorithm. When the network has been formed and optimized, the weight matrix will be generated in table VI.5.

In order to evaluate the relative importance of the input variables, the weight matrix was used in EQ VI.9 proposed by D.G. Garson.

$$I_J = \frac{\sum_{m=1}^{m=N_h} \left(\left(\left| w_{j_m}^{i_n} \right| \Big/ \sum_{k=1}^{N_i} \left| w_{k_m}^{i_h} \right| \right) \times \left| w_{m_n}^{h_0} \right| \right)}{\sum_{k=1}^{k=N_i} \left\{ \sum_{m=1}^{m=N_h} \left(\left(\left| w_{j_m}^{i_n} \right| \Big/ \sum_{k=1}^{N_i} \left| w_{k_m}^{i_h} \right| \right) \times \left| w_{m_n}^{h_0} \right| \right) \right\}} \quad \textbf{EQ VI.11.}$$

Where $\mathbf{I_J}$ represents the relative importance of the input variable over the output variable, $\mathbf{N_i}$ and $\mathbf{N_h}$ are the set of input and hidden neurons respectively, **W** is the connection weights, the exponents " **i**, **h** and **o**" refer to hidden input and output layers, respectively, and "**k**, **m**, **n**" refer to input, hidden and output neurons, respectively. Note that the numerator of EQ VI.9 describes the sum of the products of absolute weights. However, the denominator in EQ VI.9 represents the sum of all the weights feeding the hidden unit, taking the absolute values. A summary of the results is shown in figure VI.6 where it was found that all input variables have a strong effect on the estimation of the drug delivery.

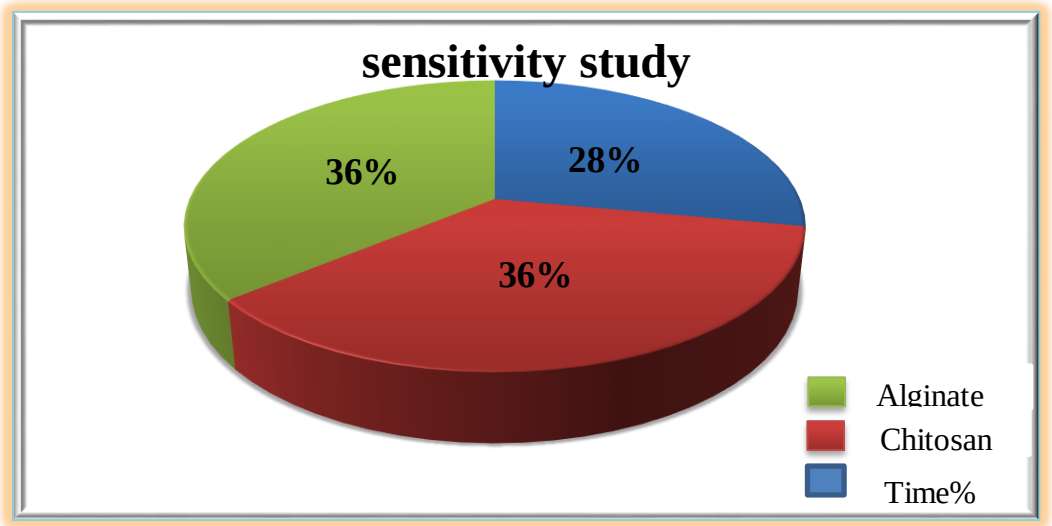


Figure VI.6: relative importance of the input variables on the calculated delivering percentage.

VI.8.Graphic interface for dimensional drug delivering (DD %)

Our model based on the nonlinear mathematical formula of the optimized neural network (ANN) and is presented in the EQ VI.10, linking inputs to outputs.

$$DD(\%) = \sum_{n=1}^{n}\left[w_{ij(1.n)} \left(\frac{2}{1+\exp\left(-2\left(\left(\sum_{m=1}^{m} w_{i(n.m)} In_{(m)}\right)+b_{i(n)}\right)\right)} - 1 \right)\right] + b_j \qquad \textbf{EQ VI.10}$$

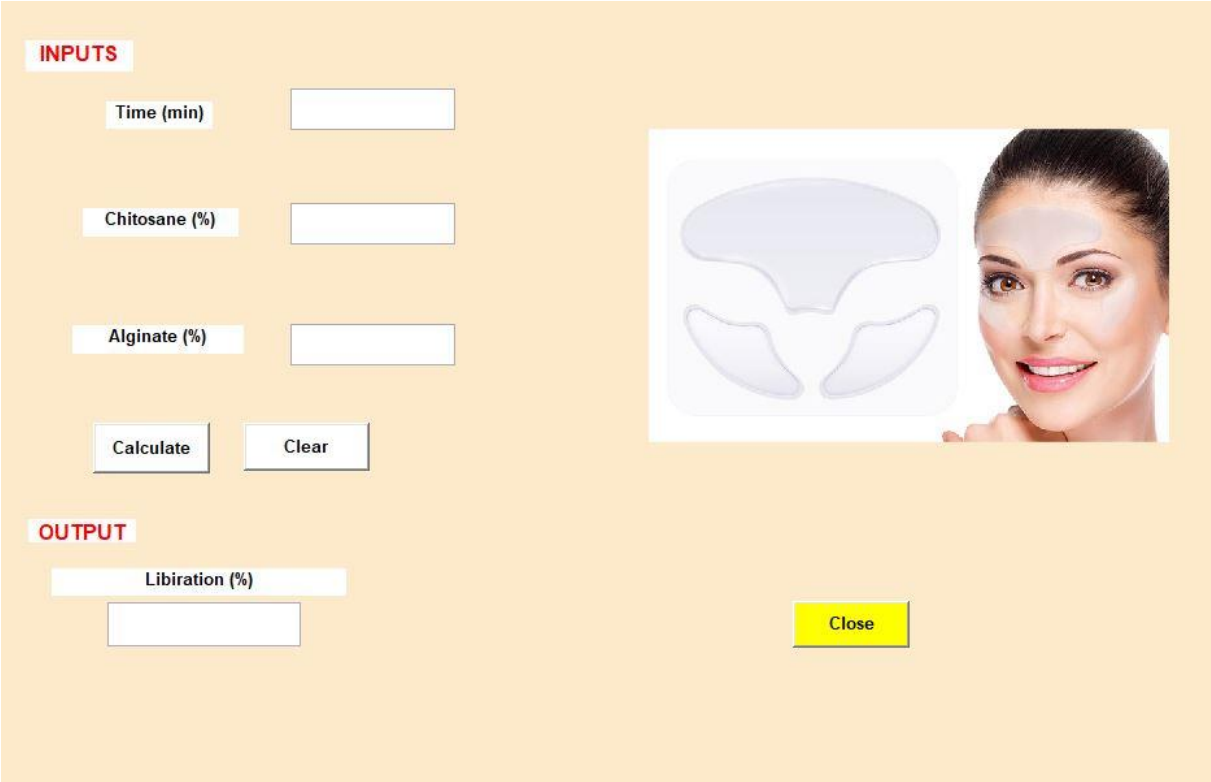


Figure VI.7: graphic interface from MATLAB of the drug delivering from the transdermal patches.

After optimizing the neural network model, a computer program was developed in MATLAB (figure VI.7) this allows the user to have all the inputs needed to run the model to calculate the percentage of liberating the active ingredient from the patches. The interface has been designed to offer more flexibility in the use of ANN model for quick and easy drug delivering calculation.

VI.9.Conclusion

In this chapter, we have used a methodology for selecting the ANN model to predict the percentage of delivering the active ingredient which is encapsulated by polymers in our membranes. The purpose of this study was to select the best model. The methodology starts with extensive research in order to select a model with minimal complexity and optimizing performances.

The ANN modeling method has many advantages, such as speed, adaptability, generalization and simplicity, which make it an interesting choice for the modeling of complex systems (drug delivering).

During the learning process, we used several configurations of neural networks. We started with the use of 11 training algorithms, observing the effect of each of them on the performance of the networks, so we chose the first three of them (trainlm, trainbr, and trainbfg) and we tried to improve their performance using a normalization technique. Among the three networks obtained, we selected the best prediction network, which was also tested with a hidden layer and several neurons. It has been found that a hidden layer with 8 neurons can provide better prediction.

Our model was developed by a direct-acting MLP back propagation architecture with the Levenberg- Marquardt formation algorithm. In fact, the results confirmed that MLP neural networks are sufficiently competent to predict the delivering of the active ingredient through the membranes based on chitosan and alginate which are considers as inputs.

We observed also that the network with a layers of (3, 8, 1) gives a correlation coefficient closer to 0.99973 with maximal absolute relative error (REA_{max}= 2,441%) for the whole database which means that the neural network was well formed, the ANN is an adequate interpolation tool for the non-linear behavior of RE for all the above mentioned operating conditions, in which an excellent prediction was obtained. Based on sensitivity analysis, we found that all input variables (chitosan, alginate, time) had a significant effect on the estimation of delivering the active ingredient which is the vitamin C included in our membrane.

General conclusion

General conclusion

As part of this thesis, we have adopt the method of drug encapsulation (vitamin C) based on a mixture of polymers (Alginate/Chitosan) to prepare membranes to be used as transdermal patches.

According to the obtained results during release we selected the values of the percentage of drug deliver and we formed a database which was processed by the MATLAB software in order to build a mathematical model which will always be reliable in this case, then the realization of an electronic interface which can be a reference to the future studies to facilitate the task of practice.

First of all, we prepared membranes based on two polymers (Chitosan/Alginate) at different percentages, as well as the addition of adhesives (glycerol, tween 80) results flexibility and rigidity of the membranes. We have chosen these two polymers, because they showed a good combination between them due to the interactions characterized by electrostatic which create some cavities in order to encapsulate the active ingredient (vitamin C). This technique makes it possible to protect the pharmacokinetics of any active ingredient and the purpose of this study is to control the release rate of vitamin C in a phosphate buffer saline.

Then we studied the release by UV-Visible spectrophotometer, the results that we obtained showed the best percentage of delivering about 70% for F5 (60% Sc/40% Alg). Also we noticed some perturbation during the release such F7, F8, F9 which was degraded at the first times of release. Thus the membranes were characterized by FTIR technique in order to identifying the bonds created between alginate and chitosan. As a result we noticed that there are some bonds created between the two polymers, on the other hand the absence of interaction of the bonds between the mixtures (Alg/Sc/Vit C) leads to predict that there is an encapsulation of vitamin C and there was no interactions between the active ingredient and the composition of our patches, and this goal we want to achieve.

And for the morphological study characterized by the SEM microscopic, we conclude from the pictures that we obtained that there are a rough surface with many undulations on its external surface, which increased the release surface. This is a beneficial quality as it allows better transfer of vitamin C across the membrane.

On the other hand, we have developed our work by using a methodology for selecting the ANN model to predict the percentage of delivering the vitamin C, the aim of this methodology was to select a model with minimal complexity and optimizing performances. , We have used several configurations of neural networks during the learning process. We started with the using 11 training algorithms, then observing the effect of each of them on the performance of the networks, and we have chosen the first three of them (trainlm, trainbr, and trainbfg) and we improve their performance using a normalization technique (-1; +1) to make a scan of all the points, then we selected the best prediction network, which was also tested with a hidden layer and several neurons. It has been conclude that a hidden layer with 8 neurons can provide better prediction.

Our model was developed by the direct action of multilayer perceptron MLP with the Levenberg- Marquardt formation algorithm, and we concluded from the obtained results that MLP neural networks are sufficiently able to predict the delivering of the active ingredient.

Also we have concluded that a network with one hidden layer and architecture of (3 inputs, 8 neurons, 1 output) gives a correlation coefficient closer to 0.99973 with maximal absolute relative error (REA_{max}= 2,441%) for our database which means that the neural network was well formed. And we have confirmed efficacy of all the inputs (Chi, Alg, Time) in our system, by the sensitivity test, which means that all the absence of one of the inputs can influence directly on our results.

And finally we have prepared an interface; the latter was prepared by a Hash tags technique in order to test our database and the neural network, this aimed to facilitate the work and the practice of future studies.

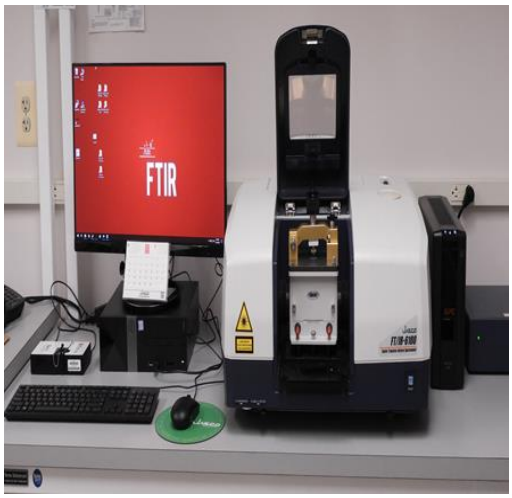
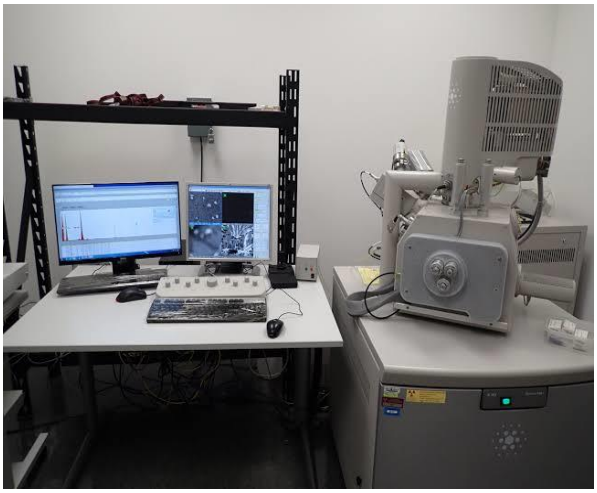
Our future perspectives about transdermal patches was to maximize the percentage of encapsulating the active ingredient by using a crosslink adhesives but without undesired effect on the skin, also we aspire to use other branches of the artificial intelligence such as Deep Learning in order to well improving the final results and to eliminate definitively all the problems and errors that remain in the experimental part of any research.

List of tables

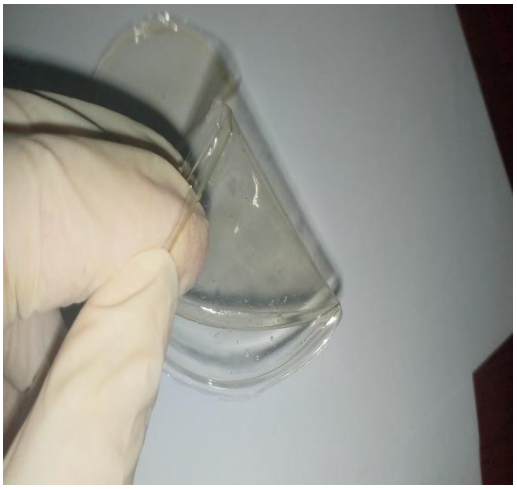
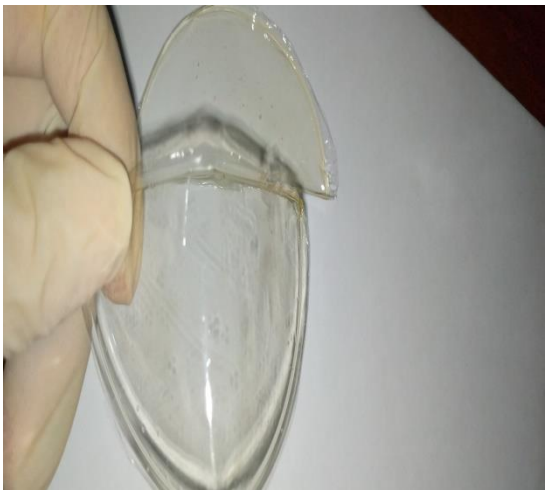
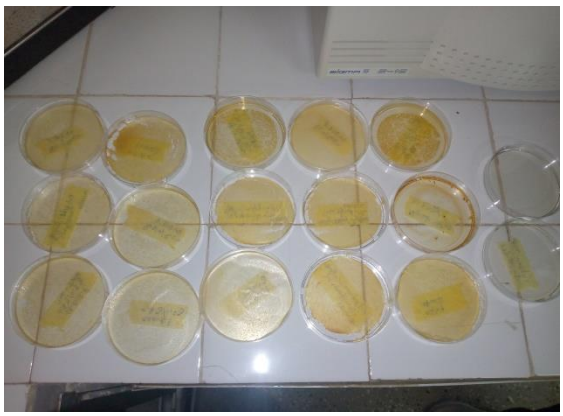
List of figures

Equipment

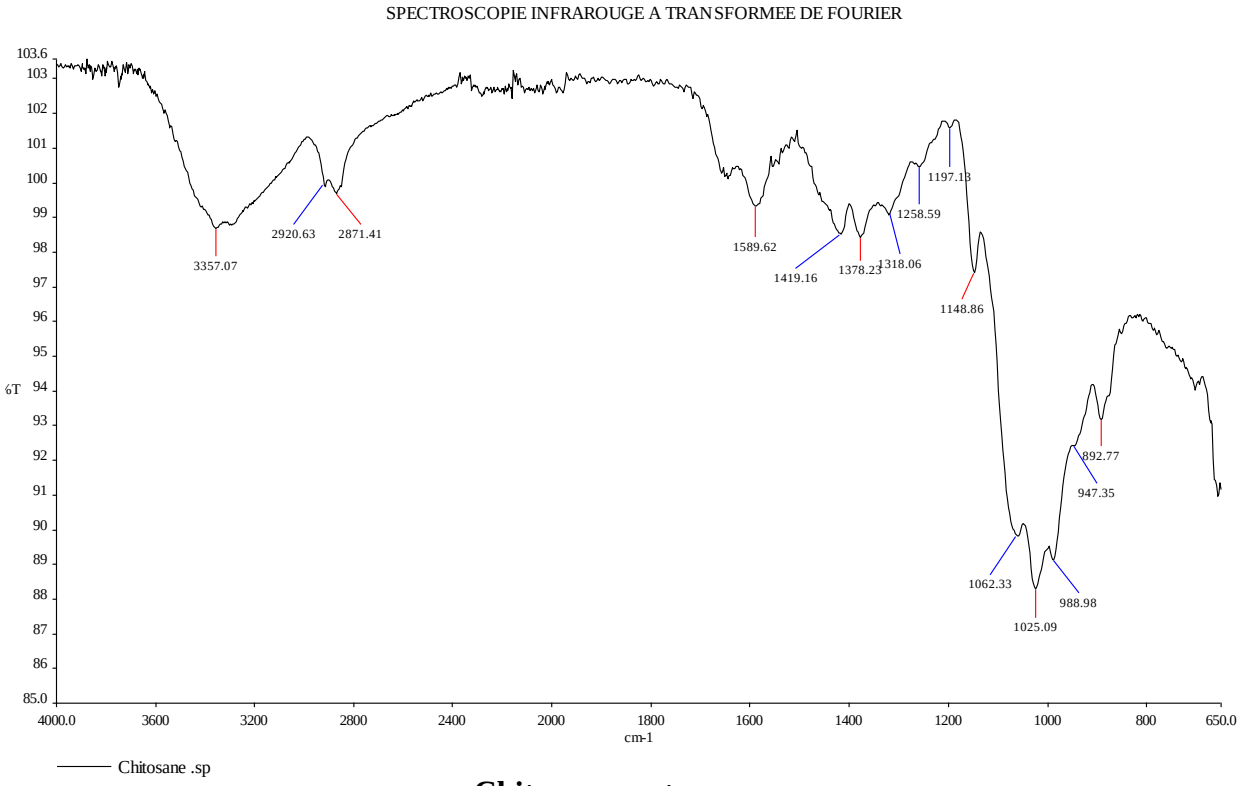
Oven : Universelles Memmert (+300°C)	Digital scale: Scaltec SBC31 (0,01g – 220g)
	
Magnetic stirrer: Agimatic-N (60-1600 rpm)	UV/Vis spectrophotometer: Shimadzu 1240 ONEDP
	

FTIR spectrophotometer : INCC/GN	MEB : SEM Quanta 600 (INCC/GN)
	

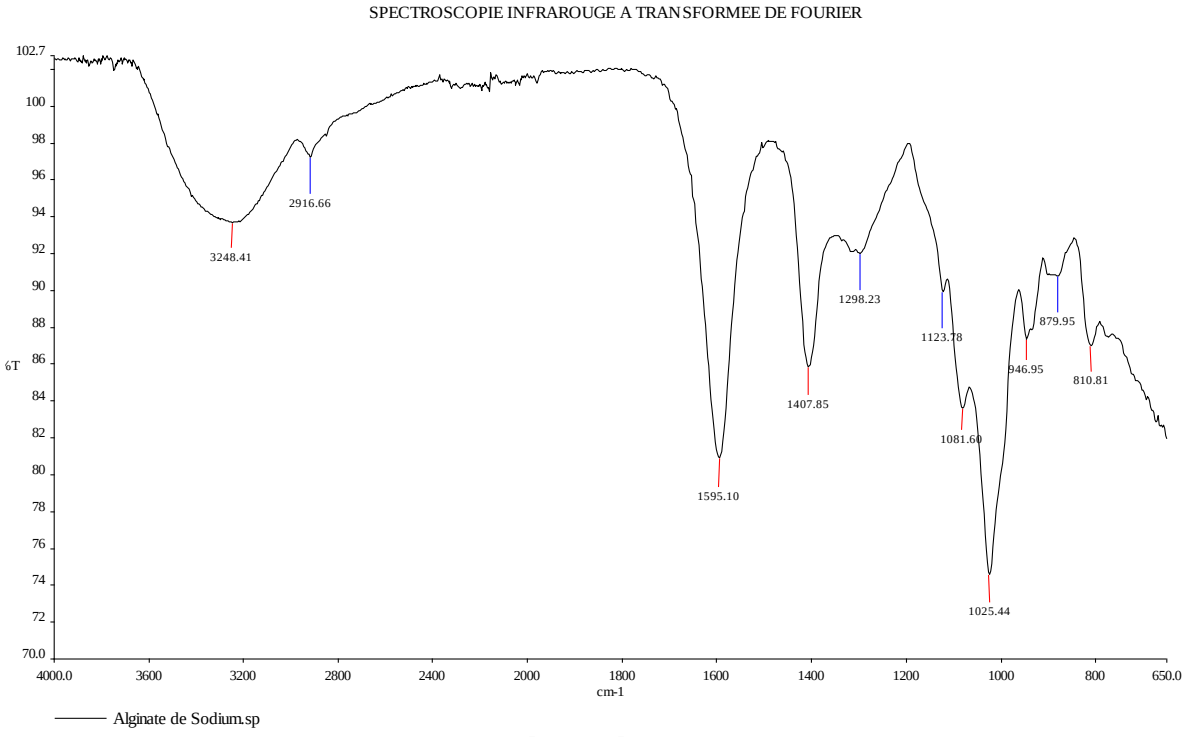
Preparations

Alginate membrane	Chitosan membrane
	
Membranes with different concentrations	Membranes degraded during release
	

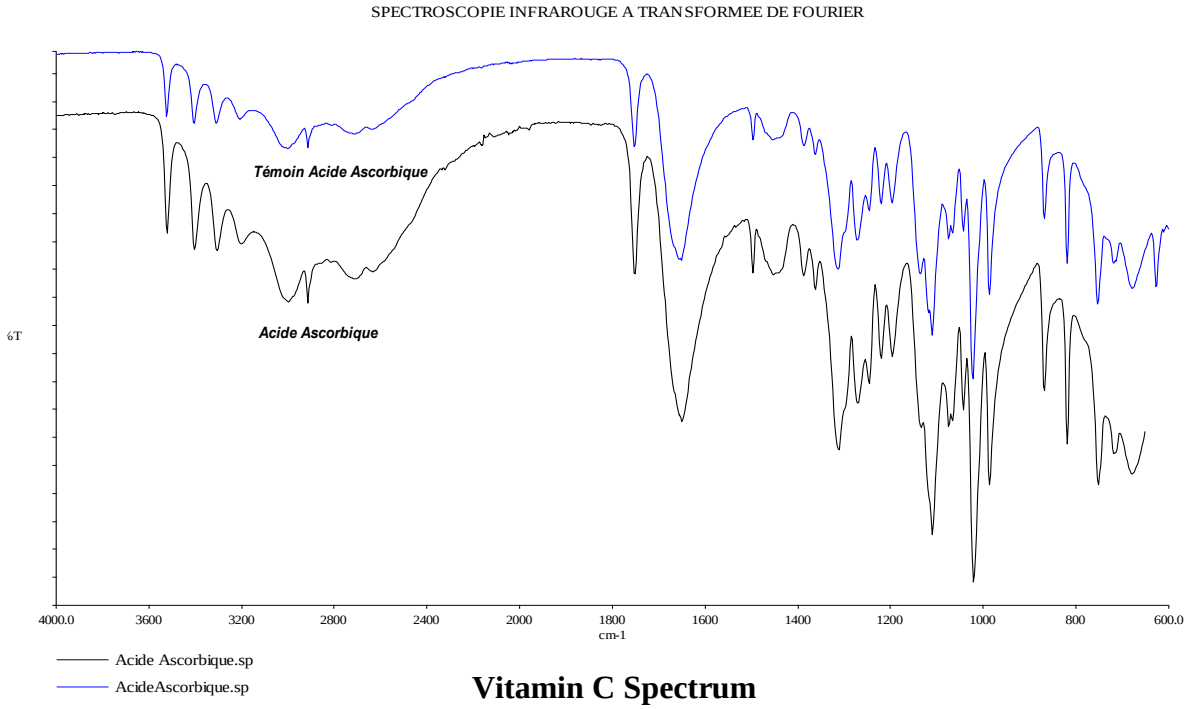
FTIR spectrum of components of patches



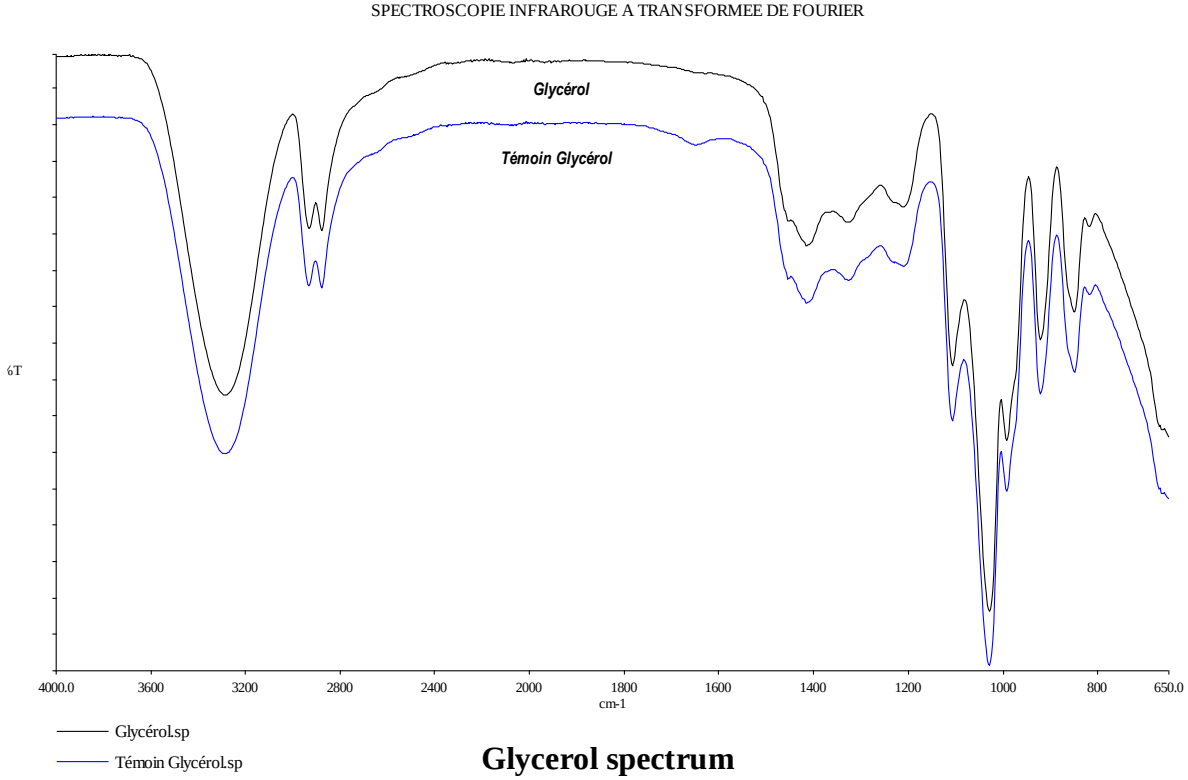
Chitosan spectrum



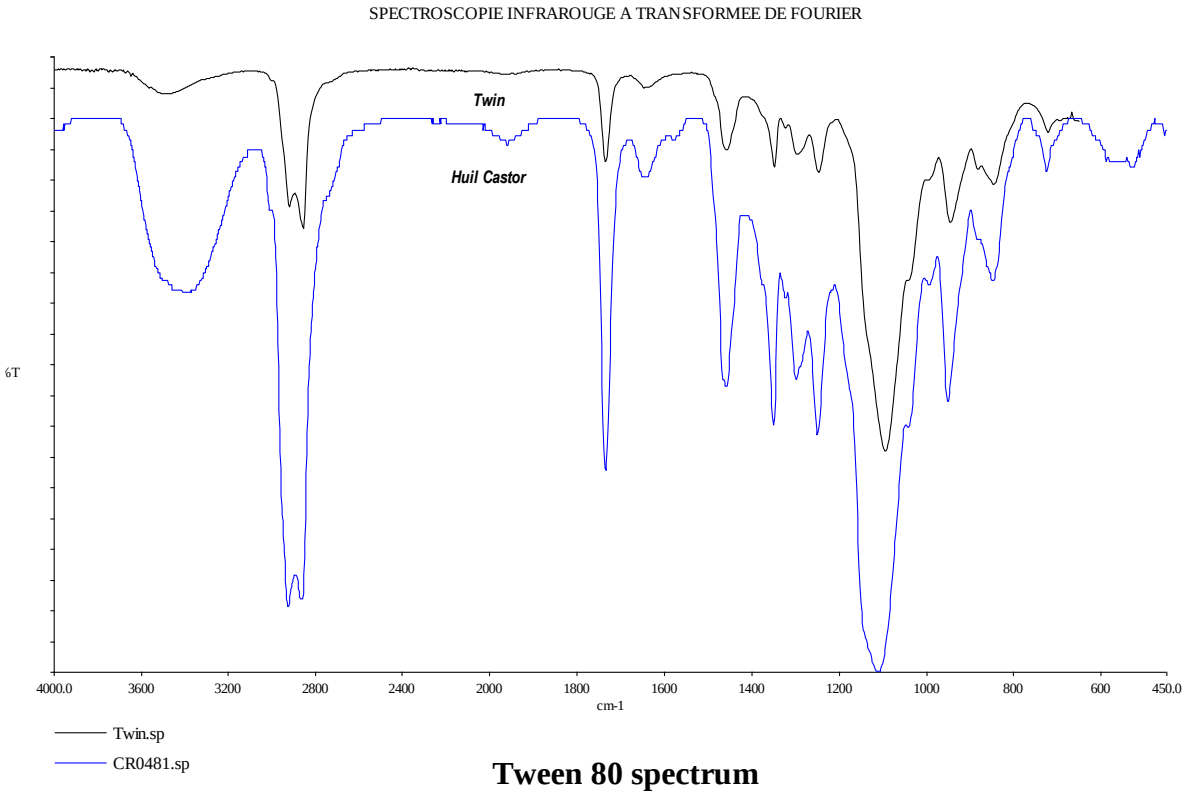
Sodium-alginate Spectrum



Vitamin C Spectrum



Glycerol spectrum



Tween 80 spectrum

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