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THEME

Characterization and encapsulation of bee venom with sodium alginate for pharmaceutical application

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DEDICATION

From the bottom of my heart, this work is dedicated to all those who are dear to me

TO MY DEAR MOTHER

No matter what I do or say, I can never thank you enough. Your affection embraces me, your kindness guides me, and your presence by my side has always been my source of strength to face various obstacles. I hope that your blessings are Always with me.

TO MY DEAR FATHER

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To My Dear Sisters and Brothers

for all the support that you give all of the time

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ملخص

في السنوات الأخيرة، اكتسبت الموارد الحيوية شعبيةً في مجال الأدوية نظرًا لانخفاض خطر آثارها الجانبية الخطيرة. تُركز أطروحتنا على سم النحل، المعروف بفوائده العلاجية، ولكنه محدودٌ بعدم استقراره وسرعة تحلله. لمعالجة هذه المشكلة، استخدمنا البوليمر الحيوي ألجينات الصوديوم لتغليف السم. عند إسقاطه في محلول كلوريد الكالسيوم، يُشكل خرزات واقية تُثبّت السم وتُمكّن من توصيله عن طريق الفم.

بعد تحسين ظروف التركيب والمعالجة، حصلنا على خرزات مُشكّلة جيدًا، وأجرينا توصيفًا ناجحًا باستخدام تقنيات تحويل فورييه للأشعة تحت الحمراء(FTIR) ، والقياس الطيفي بالأشعة فوق البنفسجية، وتقنية حيود الأشعة السينية .(XRD)وقد أكد اختبار الذوبان استمرار إطلاق السم. تُشير نتائجنا إلى فعالية هذه الطريقة في تثبيت سم النحل وتوصيله، مما يُوفر أساسًا متينًا للأبحاث المستقبلية.

الكلمات المفتاحية : سم النحل، التغليف، الخرز، توصيف البوليمر الحيوي

ABSTRACT

In recent years, bio-based resources have gained popularity in pharmaceuticals due to their lower risk of serious side effects. Our thesis focuses on bee venom, known for its therapeutic benefits but limited by its instability and rapid degradation. To address this, we used the biopolymer sodium alginate to encapsulate the venom. When dropped into a calcium chloride solution, it forms protective beads that stabilize the venom and enable oral delivery.

After optimizing the formulation and processing conditions, we achieved well-formed beads and conducted successful characterization using FTIR, UV spectrophotometry, and XRD. A dissolution test confirmed sustained venom release. Our findings suggest that this method is effective for stabilizing and delivering bee venom, offering a solid basis for future research.

Key words : bee venom. encapsulation. beads. Characterization Biopolymer

RESUME

Ces dernières années, les ressources biosourcées ont gagné en popularité dans l'industrie pharmaceutique en raison de leur faible risque d'effets secondaires graves. Notre thèse porte sur le venin d'abeille, reconnu pour ses bienfaits thérapeutiques, mais limité par son instabilité et sa dégradation rapide. Pour y remédier, nous avons utilisé l'alginate de sodium, un biopolymère, pour encapsuler le venin. Plongé dans une solution de chlorure de calcium, il forme des billes protectrices qui stabilisent le venin et permettent son administration orale.

Après avoir optimisé la formulation et les conditions de traitement, nous avons obtenu des billes bien formées et réalisé une caractérisation réussie par FTIR, spectrophotométrie UV et DRX. Un test de dissolution a confirmé la libération prolongée du venin. Nos résultats suggèrent que cette méthode est efficace pour stabiliser et administrer le venin d'abeille, offrant ainsi une base solide pour les recherches futures.

Mots clés : venin d'abeille. encapsulation. billes. Caractérisation. Biopolymère

SUMMARY

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LIST OF ABBREVIATIONS

A: Ampere

AC: Alternating Current

BV: Bee Venom

BPH: Benign Prostate Hyperplasia

CNS: Central Nervous System

FDA: Food and Drug Administration

FTIR: Fourier Transform Infrared Spectroscopy

HBV: Honey Bee Venom

HIV: Human Immunodeficiency Virus

h: Hour

Hz: Hertz

kDa: Kilodalton

MCD: Mast Cell Degranulating Peptide

mg: Milligram

min: Minute

mm: Millimeter

MS: Multiple Sclerosis

µL : Microliter

PNS: Peripheral Nervous System

pH: Hydrogen Potential

UV.vis: UV-Visible Spectrophotometry

V: Voltage

XDR: X-ray Diffraction

General

Introduction

General Introduction

In our days, scientists are trying to return to Mother Nature to develop new modern pharmaceutical drugs from natural sources as raw materials for new drugs on the market, because they have fewer side effects. There are several sources, such as plants, which have already provided many drugs. There are also biological sources, like using certain types of bacteria to produce insulin, and using venom from snakes, scorpions, and even bees. This is the foundation of our thesis.

Bees are economically beneficial insects whose existence dates back to the Cretaceous period during the Mesozoic era. Bees have provided several products to humans, such as honey, beeswax, pollen, royal jelly, and propolis. They also pollinate a wide variety of agricultural crops. Although bees are extremely beneficial to crops and humans, they do present a danger due to their ability to inflict painful and toxic stings. Fortunately, most honey bees are not aggressive towards humans and only attack when they feel threatened [1,2].

Honeybee venom is one type of the complex biological substances in nature. It is rich in components and extremely efficacious, therefore very attractive to the pharmaceutical industry which has attracted the attention of many companies. It has been used in bevies of apitherapy for treatment of diseases including arthritis and chronic pain and it is rich in bioactive compositions, such as melittin, apamin and phospholipase A2, which showed anti-inflammatory, antimicrobial and neuroprotective actions. Although the direct injection of bee venom has potential for therapy, it is hindered by its toxicity, instability, and allergenic properties.

Polymers materials are playing a very important role in making new devices and thus pave the way for substituting the old outdated materials. The 20th century witnessed an exploding activity in this area. This has resulted in the birth of new disciplines like molecular electronics, conducting polymers (including synthetic metals), organic semiconductors and plastic electronics. Polymers are gaining attention of scientific and technological community because of their wide range of applications in industrial, pharmaceutical, medical fields.

Polymers had appeared in their many natural forms like wood, cotton, cellulose, starch, etc. Which man began to use. Most of the synthetic polymers are of a relatively recent origin [3].

One of the problems with bee venom is that it has a protein and enzyme nature, which leads to instability and rapid degradation when it enters the human body, especially when it dissolves at the stomach level. That's why we need to protect it by using an encapsulation method.

Alginate is a natural polyacid, and has a unique property of gel formation in the presence of multiday lent cations, such as calcium ions in aqueous media, which takes place mainly at junctions in the G–G sequence rich chain region knows as the ''egg box junctions''. Therefore, alginate is used as an immobilization matrix for cells and enzymes as well as pharmaceutical and food adjuvants. When an aqueous solution of sodium alginate is added drop wise to an aqueous solution of calcium chloride, a spherical gel with regular shape and size is obtained. The spherical gel is termed an ''alginate bead'' [4].

Therefore, we see the need to optimize bee venom encapsulation with alginate, ensuring the formation of perfect spheres and conducting all necessary tests to confirm that the active ingredient can reach the site of release and be effective in its action.

CHAPTER 1:

STATE OF THE ART

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1.1. Bee Venom

1.1.1. Bee venom definition

Bee venom (BV) is a specialized secretion produced by the venom glands of honeybees, used mainly for defense against predators and as a warning signal to other bees. After stinging, volatile components of the venom are released into the air which act as a chemical warning signal to bees within range of the sting to avoid danger. Bee venom gland anatomy is located in the abdomen of the bee, between the venom-producing gland and a small duct that leads to the sting apparatus. [5,6].

The stinger consists of two barbed lancets designed to penetrate into the skin deep enough to inject about 50 to 140 micrograms of venom. Because the lancets are so barbed they may get stuck in vertebrates, the stinger (as well as the venom gland and injected muscle tissue) generally separate from the bee 's body and die within a few minutes thereafter.

Venom production rises for a few weeks after metamorphosis and begins to decrease during foraging activities (flying and collecting nectar). Bee venom has toxic effects on a number of systems in mammals, particularly the cardiovascular system and nervous system. It has comparable pharmacological activity to snake venom (the amount of venom released per sting is relatively low) [7].

BV causes hemolysis, inhibits blood coagulation, and increases vascular permeability, resulting in edema and, in very severe cases, internal hemorrhaging. In humans a typical response is localized inflammation (swelling, redness, pain) but particularly in areas of the body most sensitive to the sting such as the mouth/tongue/eyes. High doses of BV can cause death, either as a result of bronchospasm or paralysis of the respiratory center. In addition, BV stimulates the immune system and in the hypersensitive person can trigger severe allergic reactions such as anaphylaxis that can be life-threatening [8].

1.1.2. Harvesting bee venom

In fact, the most widely used and efficient technique for extracting bee venom by using electrical stimulation. This method was described first by Markova and Molnar in 1954, and then was refined later by a number of authors, most notably palmer (1961), Benton et al. (1963), Gunnison (1966), morse and Benton (1964a) and Nobre (1990). All the modern device for collecting bee venom is based on this principle.[9]

The chief advantage of electrical stimulation is that it triggers venom release without depriving the bee of its stinger, thus safeguarding the bee's life and allowing it to continue to serve its purpose normally inside the colony, as a consequence of which electrical stimulation is considered the safest ant most humane method for collecting bee venom.

Generally optimum yields of bee venom are achieved during the optimum time of the year (summer through fall) when bees are most active. Summer is best suited for collecting the honey bee venom, followed by autumn. The yield in spring is generally lower because of reduced activity of the bees.

As Bogdanov points out, nowadays the main components of modern bee venom collection devices consist of:

1. Power source :a battery of 12–15 volt and 2 ampere, or an alternating current (ac) supply of 25 v at 1200 hz with either a battery or direct connection to the electrical grid.
2. Impulse generator : a device that produces electrical impulses at a frequency of 50–1000 hz in durations of 2–3 seconds, with rest intervals of 3–6 seconds.
3. Electrical stimulator: a panel of uninsulated, evenly spaced wires (3–4 mm apart) that deliver the electric pulses that the bees will need to stimulate[9].

4. Collection surface - a glass plate under the wires that the bees lay their venom on and after it has dried it is scraped off of the glass and taken away for further processing.

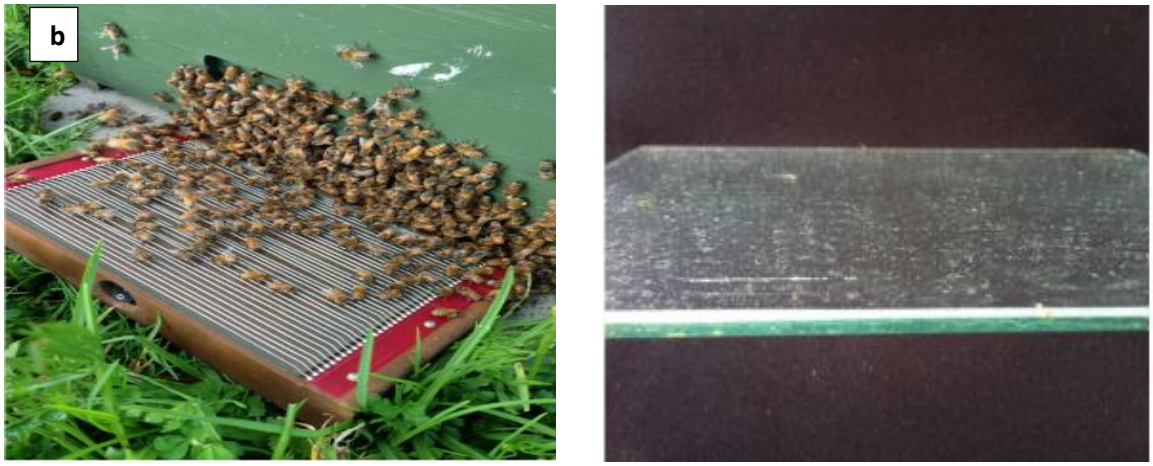


Figure 1.1. Bee venom collector Bee whisper 5.0 is based on the electric stimulation protocol In (a) it is shown how the equipment is put in front of the hive entrance. After completion of the collection cycle, the venom that has been deposited on the glass slide (b) can be scraped down. [9]

For the safe and effective collection of bee venom the electrical stimulator should be designed in such a way that the bees do not get trapped between the active conductors and the glass slide. Proper spacing and structural arrangement will prevent the physical harm to the bees when they are stimulated. Also, maintenance is very important. The active wires should be regularly cleaned at the end of each working day. Dry bee venom (non-conductive) can build up on the wires and cause serious degradation in the effectiveness of the device (up to 90% if not properly cleaned). It also induces a blockage in the current flow leading to poor stimulation. Effectiveness and safety of electrical stimulation is limited in large part by the characteristics of the electric pulses applied to the bees. These parameters involve maximum voltage, pulse width and current intensity. The control of these parameters should be considered to minimize possible effects on the bee; thus, they must be carefully controlled: in particular, the voltage must be within a safe limit throughout the device's operation cycle and pulse width should be relatively short so as to avoid physiological damage or stress to the bees. For best performance and reduced disturbance of the bees the device should be equipped with autonomous power supply via a battery. Therefore, no external wiring should be done which will cause irritation or alarm to the bees. Autonomous operation also increases portability and ease of use in the field[9].

1.1.3. Physical Properties, Chemistry, and Pharmacology of Bee Venom

HBV is a transparent liquid with a pungent, bitter taste. It has a specific gravity of about 1.13 and a pH between 4.5 and 5.5. On exposure to air, the venom dries and crystallizes quickly. In dried form, it usually appears pale yellow; however, commercially prepared venom is usually brown due to oxidation of some protein structures. Bee venom is highly soluble in water, but not in alcohol or ammonium sulfate. Due to the presence of volatile compounds, considerable care must be taken during collection to avoid their loss. Chemically, bee venom is a complex mixture of high- and low-molecular-weight components. It contains enzymes such as phospholipase A2, phospholipase B, acid phosphomonoesters, hyaluronidase, phosphatase, and Lys phospholipase. It also includes bioactive peptides (small proteins) such as melittin, apamin, adolapin, tertiapin, and secapin. Other components include phospholipids, biologically active amines such as histamine, dopamine, and noradrenaline, essential amino acids, sugars (glucose and fructose), pheromones, and minerals like calcium and magnesium. The most abundant and pharmacologically important compound in bee venom is melittin (a 26-amino acid peptide), which accounts for about 40–50% of its dry weight and is primarily responsible for its biological activity. Pharmacologically, bee venom has demonstrated a wide range of therapeutic activities, supported by both in vitro and in vivo studies:[10,11]

1. Anti-mutagenic
2. Anti-nociceptive
3. Radioprotective
4. Anti-hepatotoxic
5. Cytoprotective
6. Anti-oxidant
7. Anti-microbial,
8. Anti-viral, anti-inflammatory
9. Neuroprotective
10. Anti-arthritic
11. Anti-metastatic
12. Anti-tumor effect:[10,11].

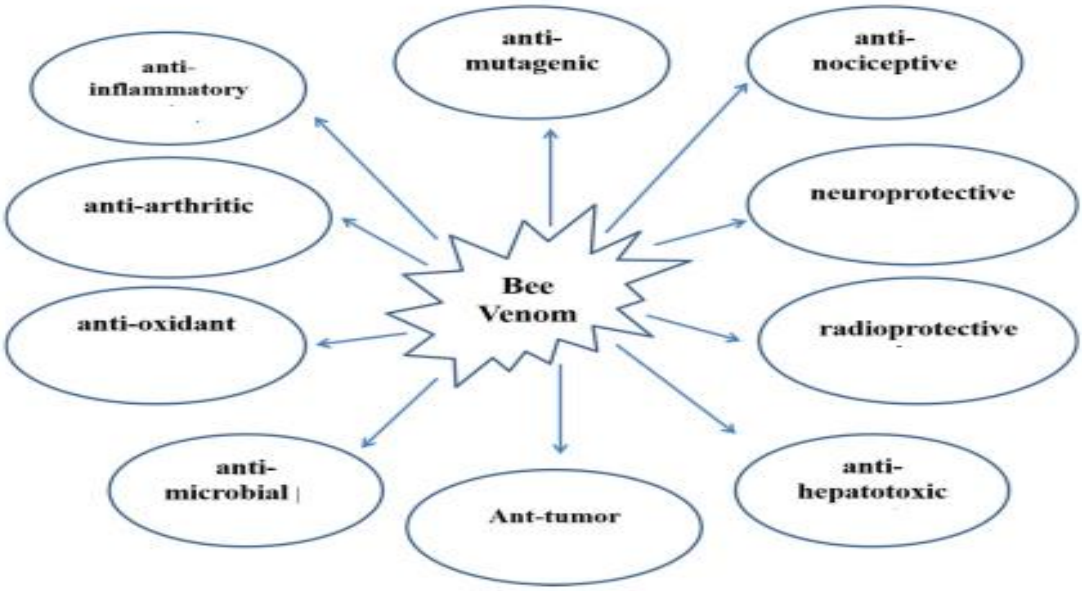


Figure 1.2. Pharmacological activities of bee venom [11]

1.1.4. Clinical Applications

Thinking about the extensive variety of biological properties of bee venom, it would not be surprising that its use could extend to therapeutic purposes for human diseases. Bee venom therapy, like many other complementary and alternative medicine approaches, has been used to treat various conditions. Before the use of injections by syringe, honeybee venom was usually delivered directly from honeybees through their stings. Even today, in some cases, bee venom is still applied using this traditional method. The live honeybee is gently held with tweezers or another tool by the practitioner, who then places the bee on the targeted area of the patient's body, at which point the honeybee reflexively stings[12].

It is worth mentioning here that while honeybees do not die after stinging other insects, they usually die when stinging a human. After stinging a human, the bee cannot pull the stinger back out. It leaves behind not only the stinger but also part of its digestive tract, as well as muscles and nerves. This extensive abdominal rupture is what kills the bee.

Depending on the condition being treated, the therapy schedule can vary. In this context, bee venom can be administered through different methods, including direct bee stings, bee venom injections, or bee venom acupuncture (also known as apitherapy).

Although administering bee venom via syringe is often preferred over direct stings, most studies and clinical practices use bee venom acupuncture because of the combined effects of the venom's bioactivity and the mechanical stimulation of acupuncture.

In clinical practice, bee venom injections into acupuncture points have been shown to be effective in treating Parkinson's disease, neuropathic pain, Alzheimer's disease, intervertebral disc disease, spinal cord injury, musculoskeletal pain, arthritis, multiple sclerosis, skin diseases, and cancer. The effectiveness of bee venom injections may be attributed to their anti-inflammatory, analgesic, and anti-apoptotic effects.

Regarding the therapeutic potential of bee venom, one human study found that bee venom acupuncture could be used as an adjunct therapy for Parkinson's disease when adults received treatment at ten acupuncture points twice a week for eight weeks[13].

According to a clinical study by Hauser et al., administering therapeutic bee venom to patients with multiple sclerosis (MS) was shown to reduce functional limitations associated with the disease. Over the course of the 12-month study, patients demonstrated significant improvements in balance, coordination, bladder and bowel control, upper and lower extremity strength, fatigue, endurance, spasticity, and numbness. Statistically significant progress was observed in walking, climbing stairs, driving, getting in and out of bed, using the toilet, bathing, and repositioning in bed.

In contrast, another clinical study by Hesselius et al. Found that treatment with bee venom did not reduce disease activity, disability, or fatigue, nor did it improve quality of life in patients with relapsing-remitting ms.

Liu et al. Reported that bee venom combined with other medications was more effective in treating rheumatoid arthritis than conventional drugs alone. They also observed that bee venom therapy could reduce the required dosage of western medicines and lower the relapse rate [11].

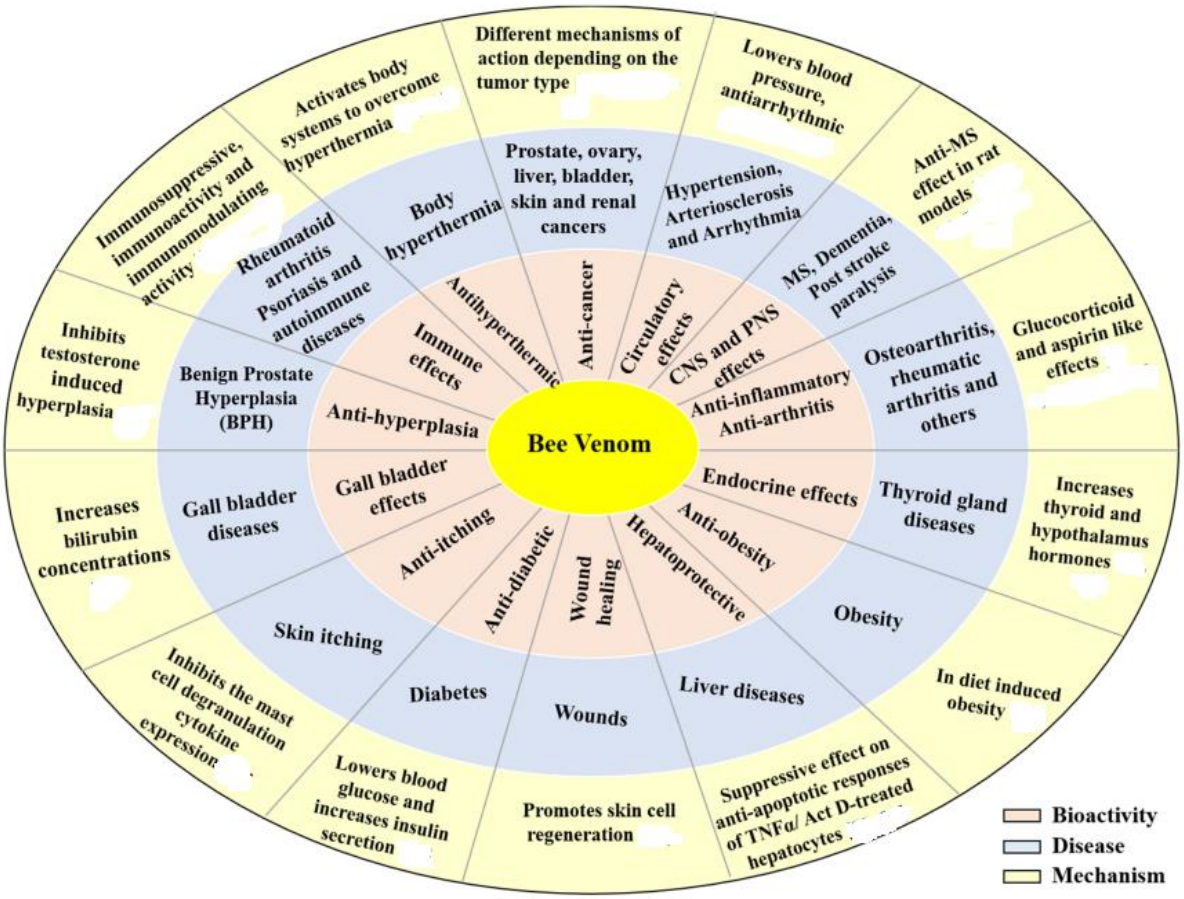


Figure 1.3. Therapeutic effects and mode of actions of bee venom. Abbreviations: CNS, central nervous system; PNS, peripheral nervous system; MS, multiple sclerosis; BPH, benign prostate hyperplasia; TNF-α, tumor necrosis factor-α; Act D-treated hepatocytes, actinomycin D-treated hepatocytes[11].

1.1.5. Composition of bee venom

It is composed mainly of water (80 %) and a mixture of peptides, enzymes, biologically active amines, amino acids, carbohydrates, volatile compounds, phospholipids, pheromones, and minerals such as Ca, Mg, and P (Table 1) The concentration of the components can be influenced by factors such as the method of collection, environment, time of year, species, and age of the bees [14-16].

Table 1.1. Percentage of the main components that make up bee venom [16]

Component	Group	% in dry matter HBV
Melittin	Peptide	50-60
Phospholipase A2	Enzyme	10-12
Complex ethers	Volatile compounds	4-8
P, Ca, Mg	Minerals	3-4
Glucose, fructose	Carbohydrates	2-4
Apamin	Peptide	1-3
Mast cell degranulating	Peptide	1-3
Hyaluronidase	Enzyme	1.5-2
Secapin	Peptide	1-2
Histamine	Biological amine	0.5-2
Dopamine	Biological amine	0.1-1
Adolapin	Peptide	0.1-0.8
Noradrenaline	Biological amine	0.1-0.5

1.1.5.1.Melittin

It is the compound with the most reported biological activities and with the highest concentration in the dry matter of hbv. It is a small and linear peptide, formed by 26 amino acids (figure 1.4); it is soluble in water, amphipathic, with a weight of 2,840 da. Melittin only induces mild allergic reactions, but it is the component that causes most of the pain associated with stinging due to its direct and indirect action on primary nociceptor cells it is classified as a lytic peptide due to its amphipathic nature, which allows it to bind to the surface of cell membranes, disturbing the integrity of phospholipid bilayers, creating pores that can cause lysis or necrosis of cells. The formation of pores is what allows this molecule to exhibit hemolytic, antimicrobial, antiviral, and antifungal activity; nevertheless, its nonspecific cellular lytic activity poses significant risks to healthy cells [16 - 18].

$$Gli - Ile - Gli - Ala - Val - Leu - Lis - Val - Leu - Tre - Tre - Gli - Leu - Pro - Ala - Leu - Ile - Ser - Trp - Ile - Lis - Arg - Lis - Arg - Gln - Gln - NH2$$

Figure 1.4: amino acid composition of melittin [16]

In studies carried out with cell cultures and animal models, it has been shown that this component has anticancer activity, part of this activity is due to the fact that it inhibits the angiogenesis process, which retards tumor growth; it also alters the cell membrane, causing

necrosis in the cell .it has also demonstrated in vitro antibacterial activity against borreli burgdorferi, the bacteria that cause lyme disease, and against different strains of staphylococcus aureus, including methicillin-resistant strains. In a study carried out with the human immunodeficiency virus (hiv), it was shown that nanoparticles prepared with a melittin solution form small pore-like attack complexes, which can injure or break the protective envelope of hiv-1, attacking a vital part of its structure.

In a study carried out with mice, the effect of this peptide was observed in injuries generated in the biceps femoris muscle of the animals; the mice that received the melittin treatment had less production of proinflammatory cytokines, an increase in the expression of biomarkers of muscle regeneration, and a better locomotor activity compared to the positive control, which received diclofenac; therefore, the authors suggest that melittin could serve as part of a treatment for muscle lesions [16].

1.1.5.2. Phospholipase a2

It is the main immunogenic and allergenic component present in hbv; it is an enzyme with a molecular weight of 19 kda, made up of 134 amino acids. It is the second component with the highest concentration in dry matter of hbv, and the second component in reported biological activities; it is also one of the main allergenic components of hbv, causing high allergic sensitivity. Phospholipases are enzymes that hydrolyze free and membrane associated phospholipids, converting them into fatty acids and other lipophilic substances, leading to tissue injury and cell death by lysis; it also lowers blood pressure and inhibits blood clotting.

This enzyme induces the synthesis of prostaglandins, which promotes inflammation. The injection of phospholipase a2 intraperitoneally and subcutaneously in mice has been shown to help prevent neurodegenerative diseases, such as Parkinson’s disease, because it has a neuroprotective effect and contributes to regulating pathological manifestations. It has been reported that this enzyme can cause lysis and prevent the proliferation of different cancer cell lines, such as human kidney carcinoma (a498), human breast carcinoma (t-47d), human prostate carcinoma (du145), and human bronchial epithelial cell line (beas-2b), in addition to stimulating monocyte-derived dendritic cells, cells with a fundamental role in the immune response. Depending on its concentration and exposure time, phospholipase has

demonstrated bactericidal (at 2 h) and bacteriostatic (at 12 h) activity against trypanosoma brucei, Enterobacter cloacae, Escherichia coli, and CitrobacterFreundii [19][16].

1.1.5.3. Apamin

It is the smallest neurotoxin in HBV; it is a peptide made up of 18 amino acids (figure 4), present only in HBV. It has neurotoxic action at the central and peripheral level, with nerve cytotoxic and nociceptive effects due to its ability to cross the blood-brain barrier and because it blocks potassium-dependent ca2+ channels. In addition, it inhibits neuromuscular transmission through the activation of m2 muscarinic inhibitory receptors in motor nerve endings, an effect that could improve the control of muscle excitability in patients with myotonic diseases, such as Parkinson's disease. In studies carried out on animal models, this peptide has been shown to protect dopaminergic neurons. Another study demonstrated its anti-inflammatory activity in gouty arthritis; its antioxidant, anti-apoptotic, and anti-inflammatory activity in acute kidney injuries has also been demonstrated. The results position apamin as a component of interest for research focused on the treatment of Parkinson's disease, gouty arthritis, and problems caused by acute kidney injury[16].

$Cis - Asn - Cis - Lis - Ala - Pro - Glu - Tre - Ala - Leu - Cis - Ala - Arg - Arg - Cis - Gln - Gln - His - NH2$

Figure 1.5. Amino acid composition of apamin [16]

1.1.5.4. Mast cell degranulating peptide (mcd peptide)

Also known as peptide 401, it is a peptide made up of 22 amino acids (figure 4). It possesses two antagonistic immune activities. In high amounts, it inhibits mast cell degranulation, inhibiting the release of histamine, acting as a powerful anti-inflammatory agent; however, at low concentrations, it has a powerful degranulating effect on mast cells, which causes the release of histamine, which plays an important role in the inflammatory to allergic processes; there is also release of autacoids, such as arachidonic acid derivatives, and serotonin. It is most responsible for the erythema that appears at the site of the sting. In the central nervous system, it acts as a neurotoxin with the ability to block potassium channels, and in the cardiovascular system, it acts as a hypotensive agent [16].

$Ile - Lis - Cis - Asn - Cis - Lis - Arg - His - Val - Ile - Lis - Pro - His - Ile - Cis - Arg - Lis - Ile - Cis - Gli - Lis - Asn–NH$

Figure 1.6. Amino acid composition of mast cell degranulating peptide [16]

1.1.5.5. Secapin

Peptide composed of 25 amino acids (figure1.7), which exhibits antibacterial, antifungal, antifibrinolytic, and anti-elastolysis biological activity. Its administration in mice causes a hyperalgesia and edematous response, producing inflammation and pain. [16]

$Tir - Ile - Ile - Asp - Val - Pro - Pro - Arg - Cis - Pro - Pro - Gli - Ser - Lis - Fen - Ile - Lis - Asn - Arg - Cis - Arg - Val - Ile - Val - Pro$

Figure 1.7. Amino acid composition of secapin [16]

1.1.5.6. Adolapin

Peptide made up of 103 amino acids; it is the only component that has been shown to possess antinociceptive effects, in addition to a strong anti-inflammatory, antipyretic, and inhibitory activity of phospholipase a2. Its properties are due to the fact that it inhibits the synthesis of prostaglandins by inhibiting cyclooxygenase[16].

1.1.5.7. Hyaluronidase

Hyaluronidases are enzymes widely distributed in nature, normally involved in pathological activities, such as the diffusion of toxins, inflammation, allergies, etc., and physiological activities, such as fertilization, wound healing, embryogenesis, and angiogenesis. The enzyme found in bee venom belongs to the ec group 3.2.1.35. It is the major allergen present in the venom of honeybees, wasps, hornets, and scorpions, because it stimulates the systemic anaphylactic response mediated by . It is an enzyme with a molecular weight ranging from 33 to 100 kda, made up of 349 amino acids, and is active at pH 4 to 6. It is considered a propagation factor because it hydrolyzes the hyaluronic acid of the interstitium, causes dilation and an increase in the permeability of blood vessels, increasing blood circulation, which facilitates the diffusion of the other components of HBV, causing the spread of inflammation and the entry of pathogens found at the site of the injury [16].

1.1.5.8. Biological amines

They are the main neurotransmitters present in HBV; they include histamine, dopamine, 5- hydroxy tryptamine, adrenaline, and noradrenaline. These components have inflammatory, vasoactive properties, in addition to being associated with pain. The histamine present in the HBV has the ability to increase capillary permeability, favoring the inflammatory response, promotes smooth and skeletal muscle contraction, and is the first mediator of the inflammatory cascade in anaphylactic shock. Catecholamines (noradrenaline and dopamine) increase cardiac output, which helps to improve the distribution of HBV [16].

1.1.5.9. Other components

The presence of carbohydrates, proteins, volatile compounds, amines, and hormones, among others, has been reported. Some authors consider the presence of carbohydrates in the HBV as contamination caused by pollen and nectar at the time of collection. The presence of the major royal jelly proteins PMJR8 and PMJR9 has been detected, and in addition to the fact that they have a nutritional function, their glycosylation has the potential to cause sensitization in patients hypersensitive to HBV. The presence of more than 20 volatile compounds has been identified, including isopentyl acetate and (Z)-11-eicosen-1-ol, pheromones that serve bees to warn other members of the colony of danger and stimulate stinging [16].

1.2. Microencapsulation

A microcapsule has drug located centrally within the particle, where it is encased within a unique polymeric membrane. The core can be solid, liquid, or gas, and the envelope is made of a continuous, porous or nonporous polymeric phase. A drug can be dispersed inside the polymeric envelope as solid particulates or dissolved in solution, emulsion, suspension, or com bination of both emulsion and suspension. In contrast, a microsphere has its drug dispersed throughout the particle; that is, the internal structure is a matrix of drug

and polymeric excipient (Figure1.8). Small molecular weight drugs, proteins, oligonucleotides, and genes can be encapsulated into microparticles to provide their sustained release at disease sites. A microcapsule is a reservoir-type system in which drug is located centrally within the particle, whereas a microsphere is a matrix-type system in which drug is dispersed throughout the particle. Microcapsules usually release their drug at a constant rate (zero-order release), whereas microspheres typically give a first-order release of drugs.

1.2.1. Fabrication of microencapsulation

Microencapsulation is a technique that involves the encapsulation of small particles or solution of drugs in a polymer film or coat. Different methods of microencapsulation result in either microcapsules or microspheres. The most common methods of preparing microparticles and nanoparticles are emulsion and interfacial polymerization, and coacervation. Microcapsule Microspheres [20].

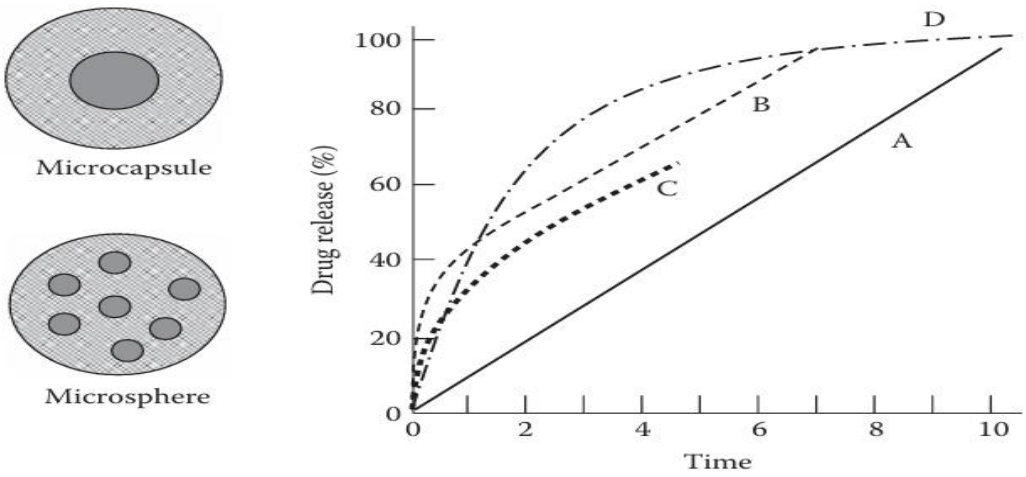


Figure 1.8. Schematic representation and drug-release profiles of microspheres and microcapsules: (a) microcapsules free of burst effect, (b) microcapsules with burst effect, (c) microspheres with square-root time release, and (d) micro spheres with first-order release [20].

1.2.1.1. Emulsification

The first step in almost any microencapsulation technique involves the for motion of an emulsion, usually of a polymeric solution inside a continuous phase. Similarly, to disperse nonsalable drugs inside polymeric solution, emulsions must be created. Thus, a thorough

understanding of emulsion formation and properties is extremely important. The emulsion formation determines the resulting particle size in the final process of encapsulation. An emulsion is achieved by applying mechanical force, which deforms the interface between the two phases to such an extent that droplets form. These droplets are typically large and are subsequently disrupted or broken into smaller ones. The ability to disrupt the larger droplets is a critical step in emulsification and in encapsulation, where an emulsion is prepared. The size of the oil-phase droplets obtained is determined by how rapidly the system is agitated when the oil phase is added to the aqueous phase; it also determines the size of the microparticles produced. However, protein and nucleic acid drugs are fairly labile and can be destroyed due to the application of mechanical shear, and thus, preventive measures should be taken to stabilize these drugs during emulsification process. A suitable surfactant is needed to produce a stable emulsion, a result achieved by lowering the surface tension. Devices commonly used for production of emulsions are the following:

- ultrasonicate
- homogenizer
- microfluidizer
- injection
- stirring
- many more

Albumin and some other water-soluble proteins can be used to prepare microspheres, involving the formation of a water-and-oil (w/o) emulsion and stabilization of the protein by cross-linking, using glutaraldehyde or heat denaturation. A mixture of petroleum ether and cottonseed oil (60:40) containing 0.5% v/v span® 80 can be used as a continuous phase (~100 ml). Serum albumin is dissolved in phosphate-buffered saline (pbs) con training 0.1% w/v sodium dodecyl sulfate (~1 ml). The albumin solution is added dropwise to the continuous phase, stirred with 2,500 rpm with a homogenizer. After 1 hour of mixing, glutaraldehyde solution (100 µl, 5%–12%) is added dropwise to the w/o emulsion, which is stirred for 1 hour at room temperature to allow cross-linking. Alternatively, microspheres can be stabilized by heat denaturation at 100°C–120°C. Following stabilization, microspheres are freed of oil by washing with petroleum ether (x3) and isopropanol (x2); they are then suspended in PBS and stored at 4°C until required. Biodegradation of albumin

microspheres and drug-release rate are dependent on the concentration of glutaraldehyde concentration or degree of heat denaturation. Apart from albumin, other proteins such as hyaluronidase and chitosan can also be used for preparation of microspheres by using cross-linkers [20].

1.2.1.2. Solvent evaporation

Solvent evaporation is the most popular method of preparation of mic particles. A core material and capsule wall material are dissolved in a water-immiscible, volatile organic solvent, and the resulting solution is emulsified in an aqueous solution. The solvent is allowed to evaporate, thereby producing solid microcapsules or microparticles. Another way is to form a double emulsion, where an aqueous core material solution is emulsified in a polymer-volatile organic solvent solution. The resulting emulsion is emulsified in water, giving a double emulsion. Evaporation of the volatile solvent yields a solid microcapsule with an aqueous core. Methylene chloride (CH2 Cl2) is a preferred solvent because of its volatility (boiling point, 41°C) and its capacity of dissolving broad range of polymers. Chloroform and ethyl acetate can also be used. A mixture of methylene chloride (a water immiscible solvent) and acetone (a water-miscible solvent) can also be used. The added drug may be completely dissolved in the polymer solution, or it may be completely insoluble and simply form a dispersion, suspension, or suspension–emulsion. In the latter case, the solid particles must be micronized, so that their mean diameter is much less than the desired mean micro sphere size. To aid emulsification, a surfactant is normally dissolved in the water phase before the oil-in-water emulsion is formed. A good example is partially hydrolyzed (88%) poly (vinyl alcohol) (PVA). After obtaining desired droplet size and emulsion stability, the system is stirred at constant rate, followed by solvent evaporation by using a rotary evaporator. Following solvent evaporation, the microparticles are separated from the suspending medium by filtration or centrifugation, washed, and dried. The maximum drying temperature must remain below the glass temperature of the polymer encapsulant or the microspheres fuse together. Although the solvent evaporation process is conceptually simple, the nature of the product can be affected by the following factors:

• Polymer molecular weight and concentration

• Polymer crystallization

• Type of drug and method of incorporation (solid, liquid, and suspension)

• Organic solvent used

• Type and concentration of surfactant used in the aqueous phase

• Ratio of organic phase to aqueous phase

• Rate of stirring

• Evaporation temperature

In general, semi crystalline polymers often give porous structures, with spherulites on the surface of the microspheres. Uniform, pore-free spheres are most readily obtained with amorphous polymers. Biodegradable polylactide (PLA) and its copolymer with glycolide (polylactide-co-glycoside [PLGA]) are commonly used for preparing micro particles, from which the drug can be released slowly over a period of a month or so. Microspheres can be used in a wide variety of dos age forms, including tablets, capsules, and suspensions. Table 14.1 lists some of the FDA-approved commercial products of microspheres. Lupron Depot from TAP Pharmaceuticals is an FDA-approved preparation of PLGA microspheres for sustained release of a small-peptide luteinizing hormone-releasing hormone (LHRH) agonist. More recently, PLGA microspheres of recombinant human growth hormone have been developed and marked successfully by Genentech, Inc., under the trade name of Notropis Depot. Polylactide-co-glycoside degrades into lactic and glycolic acids [20].

1.2.1.3. Interfacial (or in situ) polymerization

In interfacial polymerization, oil-soluble monomers and water-soluble monomers react at the water/oil interface of w/o or o/w dispersions, resulting in the formation of polymeric microcapsules. The process involves an initial emulsification step, in which an aqueous phase, containing a reactive monomer and a core material, is dispersed in a nonaqueous phase. This is followed by the addition of a second monomer to the continuous phase. Monomers in the two phases then diffuse and polymerize at the interface to form a thin film. The most widely used example of microcapsule preparation using this method is the interfacial polymerization of water-soluble alkyl diamines with oil-soluble acid dichloride's to form polyamides. Examples of other polymeric wall materials include polyurethanes, poly

sulfonamides, poly thiazides, and poly (phenyl esters). Interfacial polymerization of a monomer almost always produces microcapsules, whereas solvent evaporation may result in microspheres or microcapsules, depending on the amount of drug loading [20].

1.2.1.4. Complex coacervation

Complex coacervation uses the interaction of two oppositely charged poly electrolytes in water to form a polymer-rich coating solution called a com plex coacervate. This solution (or coacervate) engulfs the liquid or solid being encapsulated, thereby forming an embryo capsule. Cooling the system causes the coacervate (or coating solution) to gel via network formation. Gelatin and gum Arabic are primary components of most complex coacervation systems.

Coacervation uses the common phenomenon of polymer–polymer incompatibility to form microcapsules. The first step is to form a solution of gelatin in deionized water at 11 wt.% and 45°C–55°C. Once the gelatin and gum arabic solutions are prepared, the drug is emulsified or dispersed in the 45°C–55°C gelatin solution. Once the drug– gelatin emulsion or dispersion is formed, it is diluted by addition of a known volume of the 45°C–55°C deionized water and 11wt% gum arabic solution (45°C–55°C). The pH of the resulting mixture is adjusted to 3.8–4.4 by addition of acetic acid. After the pH is adjusted, the system is allowed to cool down to room temperature and then to below 10°C, and at this point, glutaraldehyde is slowly added to cross-link the polymer. The system is stirred gently throughout this cooling period. Alginates form gels on reaction with calcium salts: These gels consist of almost 99% of water and 1% or less of alginate. Cross-links are caused either by simple ionic bridging of two carboxyl groups on adjacent polymer chains via calcium ions or by chelation of single calcium ions by hydroxyl and carboxyl groups on each of the pair of polymer chains. Several types of viable cells (erythrocytes, sperm cells, hepatoma cells, and hepatocytes), tissues (pancreatic endocrine tissues and islets), and other labile biological substances are encapsulated within semi permeable alginate microspheres. The process involves suspending the living cells or tissues in sodium alginate solution, and the suspension is then extruded to produce microdroplets, which fall into a cacl2 solution and form gelled microbeads with the cells or tissues entrapped. These microbeads are next treated with polyline solution, which displaces the surface layer of calcium ions and forms a

permanent polysalt shell or membrane. Porous microspheres are formed by gelation of the following:

• Sodium alginate and chitosan

• Sodium alginate and CaCl2

• Sodium alginate and polyline [16].

1.2.1.5. Ionotropic Gelation Ionotropic gelation

Is a phenomenon exploiting the ability of polyelectrolyteto react with oppositely charged molecules (e.g., cations) and undergo the sol–gel transition, which results in the forming of structured physical materials (films, beads, hydrogels, nanoparticles). Recently, there has been a constant increase in interest in polysaccharide-based hydrogels for API encapsulation. A couple of techniques can be applied for hydrogel formation. Many of them are based on the preparation of spherical or oval beads composed of API and polymeric excipients. Crucial parameters determining the size and shape of droplets are the viscosity of the initial mixture, the surface tension, as well as the dynamic interactions between droplets and the matrix fluid (laminar or turbulent f low) as well as polymer concentration and molecular weight of the polymer [21].

1.2.1.6. Mechanisms of Ionotropic Gelation

In general, ionotropic gelation occurs between oppositely charged molecules. Positively charged polymer chains react with negatively charged divalent or multivalent ions. The electrostatic reaction leads to forming of the micro structured particles with interconnected nano-fibrillar networks. Such a structure can be achieved using three separate methods: internal, external, or inverse gelation [21].

In this thesis we aim to encapsulate bee venom as a drug by ionic gelation, using sodium alginate and calcium chloride. Standard alginate is an anionic polymer which has already been identified by clinical scientists from its natural origin and has a biochemical

activity which forms a hydrogel network when interacting with divalent calcium ions (Ca^{2+}) and forms calcium alginate beads. Encapsulating bee venom under conditions of environmental degradation (e. G. Enzymatic activity, degradation by oxidation and phchanging) yields-controlled release and improved stability. This material offers the potential for delivery of controlled bioavailability of biological active compound.

1.3. Polymers

Polymer is a natural or man-made material composed of very large molecules known as macromolecules, which are multiples of smaller chemical units known as monomers. Polymers are found in many biological organisms, including proteins, cellulose, and nucleic acids. Furthermore, they serve as the foundation for minerals such as diamond, quartz, and feldspar, and man-made materials such as concrete, glass, paper, plastics, and rubbers. Polymer refers to an undetermined number of monomer units. When the number of monomers is extremely high, the product is referred to as a high polymer. Polymers are not limited to monomers that have the same chemical composition, molecular weight, or structure [22].

Types of polymers based on their synthesis, there are of two type's natural and synthetic polymers.

1.3.1. Synthetic polymers

Synthetic polymers are produced by a variety of processes. Many simple hydrocarbons, such as ethylene and propylene, may be converted into polymers by adding monomers to the developing chain one after the other. Polyethylene is an additive polymer comprised of repeated ethylene monomers. It might include up to 10,000 monomers linked together in long coiled strands. Polyethylene is crystalline, transparent, and thermoplastic, which means that when heated, it softens. It is used to make coatings, packaging, molded components, and bottles and containers. Polypropylene, like polyethylene, is crystalline and thermoplastic, but it is tougher. Its molecules can be made up of 50,000 to 200,000

monomers. This compound is utilized in the textile sector as well as in the manufacture of molded products [22].

1.3.2. Natural polymers

They are of two type's organic and inorganic polymers. Organic polymers are important in living organisms because they provide basic structural components and participate in key life processes. Polymers, for example, make up the solid components of all plants. Among them are cellulose, lignin, and different resins. Cellulose is a polysaccharide, which is a polymer made up of sugar molecules. Lignin is made up of a complex three-dimensional network of polymers; wood resins are polymers of isoprene, a simple hydrocarbon. Rubber is another well-known isoprene polymer. Proteins, which are polymers of amino acids, and nucleic acids, which are polymers of nucleotides—complex molecules consisting of nitrogen containing bases, sugars, and phosphoric acid—are two other major natural polymers. In the cell, nucleic acids convey genetic information. Starches are natural polymers made of glucose that are essential sources of nutritional energy supplied by plants. Many inorganic polymers, such as diamond and graphite, can also be found in nature. Both are made of carbon. Carbon atoms in diamonds are bonded in a three-dimensional network, which gives the substance its strength. Carbon atoms join in planes that may glide across one another in graphite, which is employed as a lubricant and in pencil leads.[22].

1.4. Alginate

1.4.1. Sources

Commercial alginates are extracted from three species of brown algae. These include Laminaria hyperborean, Ascophyllum nodosum, and Macrocystispyrifera; in which alginate comprises up to 40% of the dry weight Alginate exists as a mixed salt of various cations found in the seawater such as Mg^{2+}, Sr^{2+}, Ba^{2+}, and Na^{+}. Bacterial alginates have also been isolated from Azotebactervinelandii and several Pseudomonas species [23].

1.4.2. Chemical structure

Alginate is a water-soluble linear polysaccharide extracted from brown seaweed and is composed of alternating blocks of 1–4 linked α-L-guluronic and β-D-mannuronic acid residues. Fig. 1.19 shows the structures of mannuronic and guluronic acid residues and the binding between these residues in alginate. Because of the particular shapes of the monomers and their modes of linkage interpolymer, the geometries of the G-block regions, M-block regions, and alternating regions are substantially different. Specifically, the G-blocks are buckled while the M-blocks have a shape referred to as an extended ribbon. If two G-block regions are aligned side by side, a diamond shaped hole results. This hole has dimensions that are ideal for the cooperative binding of calcium ions. The homo polymeric regions of β-D-mannuronic acid blocks and α-L-guluronic acid blocks are interspersed with regions of alternating structure (β-D-mannuronic acid–α-L-guluronic acid blocks)

The composition and extent of the sequences and the molecular weight determine the physical properties of the alginates. [23].

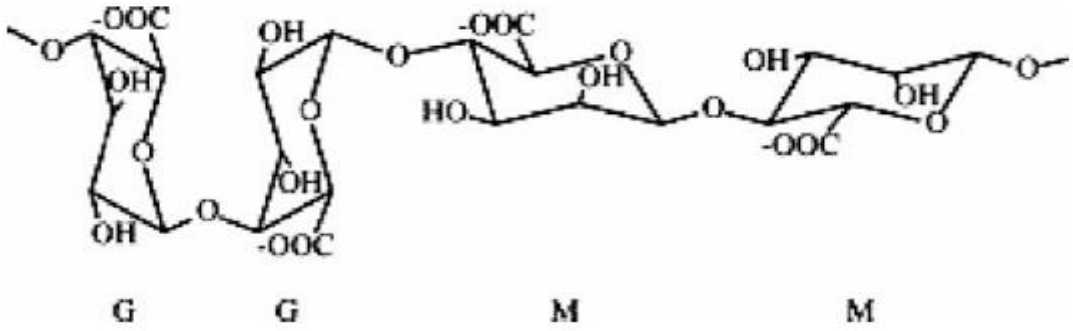


Figure 1.9. Chemical structure of alginate. Shown is a polymer chain of 2 guluronic acid (G) monomers and 2 mannuronic acid (M) monomers, with (1–4) linkages.[23]

1.4.3. Gel formation

The gelation of alginate can be carried out under an extremely mild environment and uses non-toxic reactants. The most important property of alginates is their ability to form gels by reaction with divalent cations such as Ca2+. Alginate beads can be prepared by extruding a solution of sodium alginate containing the desired protein, as droplets, in to a divalent cross-linking solution such as Ca2+, Sr2+, orba2+. Monovalent cations and Mg2+ ions do not induce gelation.

The gelation and cross-linking of the polymers are mainly achieved by the exchange of sodium ions from the guluronic acids with the divalent cations, and the stacking of these guluronic groups to form the characteristic egg-box structure showing Fig. 10. The divalent cations bind to the α-L-guluronic acid blocks in a highly cooperative manner and the size of the cooperative unit is more than 20 monomers. Each alginate chain dimerizes to form junctions with many other chains and as a result gel networks are formed. Thus, the calcium reactivity of algins is the result of calcium-induced dimeric association of the G-block regions. These gels which are similar to solids in retaining their shape and resisting stress, are 99–99.5% water with rest being alginate. Depending on the amount of calcium present in the system, these inter-chain associations can be either temporary or permanent. With low levels of calcium, temporary associations are obtained, giving rise to highly viscous, thixotropic solutions. At higher calcium levels, precipitation or gelation results from permanent associations of the chains. Numerous studies have shown that the chemical structure, molecular size as well as the gel forming kinetics and the cation has a significant impact on several of its functional properties including porosity, swelling behavior, stability, biodegradability, gel strength and the gel's immunological characteristics and biocompatibility[24].

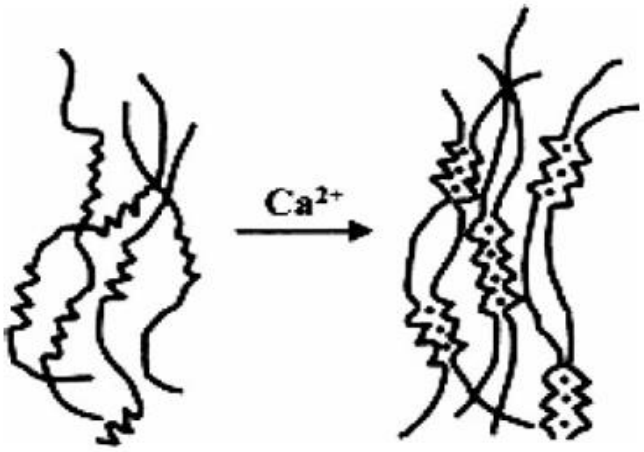


Figure 1. 10. Egg-box structure of an alginate gel formed by chelation of Ca2+ ions. [24]

CHAPTER 2

MATERIALS & METHODS

Chapter 2. Materials & Methods

2.1. Introduction

In our study, we aim to collect samples of Algerian bee venom from the beehives at ITELV (Institut Technique des Elevages) an institution specializing in animal husbandry. Since ITELV maintains beehives as part of its research activities, we will conduct the extraction and collection of bee venom on-site. This will ensure fresh and high-quality samples for our study. Furthermore, this bee venom will be incapsulated using alginate to insure that the bee venom is protected and kept stable.

2.2. Active ingredient and excipient

2.2.1. Bee venom

Bee venom is a biological substance that can be extracted from the hive of honey bees. It is secreted by the abdominal venom glands and is initially released as a liquid. Upon drying, it transforms into a solid powder, typically yellowish in color. Bee venom has a bitter taste and is odorless [11].

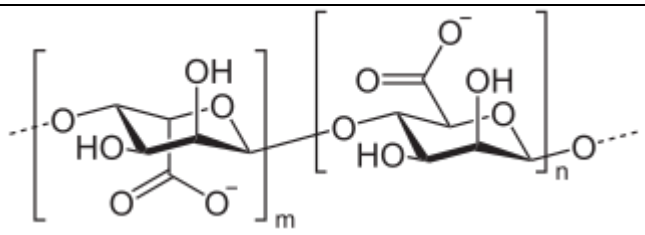
Table 2.1. Characterization of bee venom after Bhalotia, al[10]

Chemical formula	Mixed of peptide and enzyme
Chemical structure	It's complicated mixture of compound
Chemical name	bee venom
Molecular weight	Melittin peptide ~2,840 Phospholipase a_2 enzyme ~14,000 destro hyaluronidase enzyme ~38,000
Solubility	is highly soluble in water, but not in alcohol or ammonium sulfate
pH	4.5-5.5
Melting point:	It's complicated mixture of compound

2.2.2. Sodium Alginate

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder. [25].

Table 2.2. Characterization of sodium alginate [25]

chemical formula	(C6 H7 O6 Na)
chemical structure	
chemical name	sodium alginate
molecular weight	1. Low molecular weight sodium alginate: ~32,000 – 80,000 g/mol 2. Medium molecular weight: ~80,000 – 200,000 g/mol 3. High molecular weight: ~200,000 – 500,000+ g/mol
solubility	Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than30%.also,practically insoluble in o the organic solvent sand aqueous acidic solutions in which the his less than 3. Slowly soluble in water, forming a viscous colloidal solution.
pH	7.2
melting point:	instead of melting, sodium alginate undergoes thermal degradation when heated to high temperatures. **decomposition temperature**: begins around **200–250 °c**

2.2.3. Calcium Chloride Dihydrate

Calcium chloride occurs as a white or colorless crystalline powder, granules, or crystalline mass, and is hygroscopic (deliquescent)[21].

Table 2.3. Characterization of calcium chloride dehydrate after Gadziński, et al [21]

chemical formula	$CaCl_2 \cdot 2h_2o$
chemical structure	
chemical name	(calcium chloride dihydrate)
molecular weight	147.01 g/mol
solubility	In water is very high slightly soluble in ethanol
pH	7.2
melting point:	~176 °c (decomposes)

2.3. Bee venom collection

The bee venom is collected using the following most common and used method around the word:

2.3.1. Electric stimulation

This method is used all over the world, and the fact that it is common is because it's harmless and doesn't put the bees' lives in danger. The bees don't die, allowing us to preserve our hives while still collecting samples.

How it works is simple: a mild electrical current hits the bee, causing it to feel afraid and activate its defense mechanism, leading it to sting. This is when the venom is released and deposited into a glass or plastic plate. After we finish collecting the venom, we scrape the glass or plastic with a metal blade.

2.4. Materials used in the collection of bee venom

2.4.1. Bee venom collector device

The following treatments were collected bee venom from colonies by means bee venom collector devices. Characters of the bee venom device are (electric shock device)

-Input Voltage : 11.5-13.5vdc

-Timer-On : 0.5 - 2 Sec.

- Timer-Of: 3 -5 Sec.

- Collector Frames: 40cm X 50 cm

- Operation Mode: Semiautomatic

- Temperature: -5 C˚ to 40 C˚

- Humidity (Max) : 95% at 40 C˚

- Max Operating Time: 8 Hours [26].

Figure 2.1. Electroshock is the device is responsible for shock generation and pulses

2.4.2. Glass plate

In the context of a bee venom collector is a smooth, non-porous surface, typically made of glass, positioned beneath or within a collection frame or apparatus. It serves as the surface onto which bees deposit their venom after being stimulated, often by an electric shock device. The venom is released when bees sting the glass plate, leaving behind droplets that dry into a crystalline form for subsequent scraping and collection. The glass material is

chosen for its durability, ease of cleaning, and ability to preserve the venom's purity during collection.

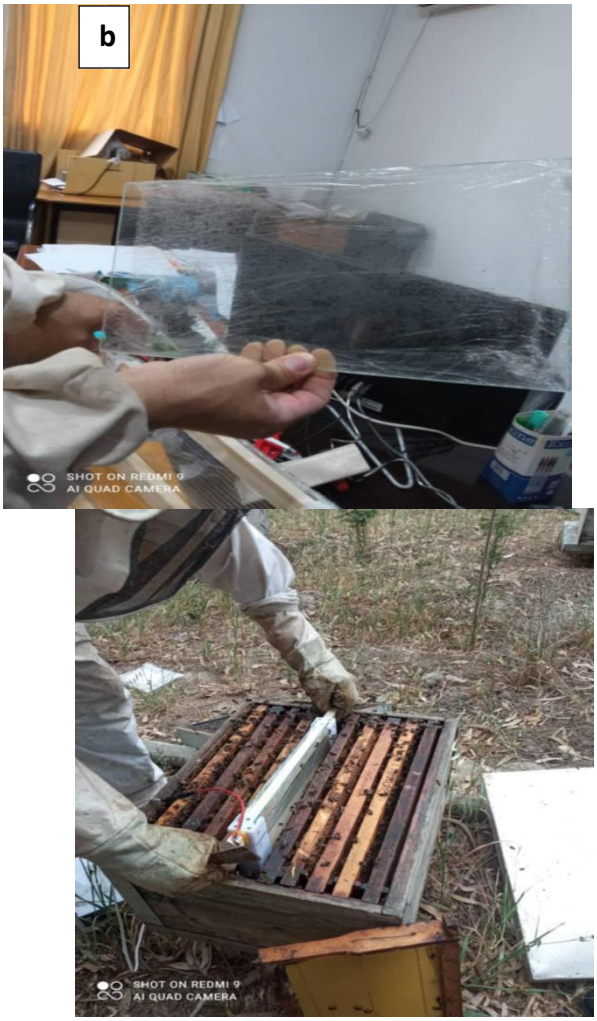


Figure2.2 (a) is the glass plate and (b) where we put it in the hive

2.4.3. Scraper

Which is a handheld tool often a flat, rigid blade or spatula made of metal or plastic designed to gently lift the crystallized venom residue from the glass without damaging the surface. The scraping process is a critical step in harvesting the venom, ensuring it is collected efficiently and in a pure form for further processing or use.

Figure 2.3 scrape collecting bee venom form the plate

2.4.4. Storage

Dried bee venom should always be kept in dark, opaque vials and refrigerated, or better yet, frozen. Dried bee venom should not be refrigerated for longer than a few weeks, but it can be frozen for several months. Both diluted and liquid venom can be kept in well-scaled, dark glass containers for comparable lengths of time.

The venom is stored in tightly sealed containers in a freezer at-20°c. It can be stored in this manner for at least five years, retaining its healing properties. Dates, locations of hives, and even weather conditions and other factors that may be of interest should be recorded. Some companies recommend the following: storage conditions for bee venom. Short-term storage: 3 weeks at 25-30°c; medium-term storage: 3 months at 4°c; long-term storage: up to 5 years at-20°c[27].

2.3. Encapsulation of bee venom

2.3.1. Tube pumps

lower pressure peristaltic pumps typically have dry casings and use rollers along with non-reinforced, extruded tubing. This class of pump is sometimes called a "tube pump" or "tubing pump". These pumps employ rollers to squeeze the tube. Except for the 360-degree eccentric pump design as described below, these pumps have a minimum of 2 rollers 180 degrees apart, and may have as many as 8, or even 12 rollers. Increasing the number of rollers increase the frequency of the pumped fluid at the outlet, thereby decreasing the amplitude of pulsing. The downside to increasing number of rollers it that it proportionately

increases number of squeezes, or occlusions, on the tubing for a given cumulative flow through that tube, thereby reducing the tubing life. [28].

2.3.2. material used in bee venom encapsulation

2.3.2.1. The dripping technique

Is a way in which a liquid is extruded thru an orifice, generally below the have an impact on of gravitational force alone, to shape discrete droplets. The length of those droplets is in most cases decided through the liquid`s viscosity and surface tension, and is usually independent of the orifice diameter. As flow rate increases, a essential flow rate can be reached, beyond which the method transitions from discrete droplet formation ("dripping") to a non-stop stream ("flow"). This essential go with the drift rate is influenced through surface tension, viscosity, and orifice dimensions [29].

The extrusion method is the most straightforward and popular method for preparing drug-filled microspheres by alginate ionic gelation involving simple diffusion and crosslinking reactions by ca2+ ions. The alginate solution is added dropwise to the crosslinking solution in the extrusion method [30].

2.3.2.2. Encapsulation of bee venom

The first step was to dissolve sodium alginate powder in distilled water. The solution was left to stand for at least two hours to ensure complete hydration. Separately, the active ingredient (bee venom) was dissolved in distilled water and stirred for 15 minutes. After preparation, the two solutions were combined and stirred together for at least 30 minutes to ensure complete homogenization.

Next, calcium chloride dihydrate was dissolved in distilled water to prepare the crosslinking solution. The solution was then set aside until use.

Then, a tube pump was used with green silicone tubing to formulate the beads. One end of the tubing was placed in the alginate–bee venom solution, and the other end was positioned above the calcium chloride solution, which was placed on a magnetic stirrer. The

pump was started, allowing the alginate–bee venom solution to pass through the tubing and form droplets that fell into the calcium chloride solution. Upon contact, the droplets instantly formed beads. Stirring was maintained throughout the process to prevent the beads from sticking together. After the pump finished we keep them strir for 30 min

2.3.2.3. Optimization of the protocol

At the beginning, we started with a fixed amount of alginate and gradually increased it step by step to determine the optimal mass required for proper bead formation. We then adjusted the flow rate of the pump, initially starting with a high flow and progressively decreasing it. We observed that a slower flow rate was necessary to obtain spherical-shaped beads. Additionally, we noticed that the stirring speed of the calcium chloride solution also affected bead morphology. Initially, we used a high stirring speed, which created a vortex in the solution and resulted in deformed beads. After several trials, we found that reducing the stirring speed helped achieve the spherical shape desired for our beads.

2.4. Characterization of bee venom

2.4.1. UV-VISIBLE spectrophotometer

Prepare the substance to be examined according to the instructions accompanying the reference spectrum/reference substance. Using the operating conditions that were used to obtain the reference spectrum, which will usually be the same as those for verifying the resolution performance, record the spectrum of the substance to be examined. The positions and the relative sizes of the bands in the spectrum of the substance to be examined and the reference spectrum are concordant in the 2 spectra [31].

Figure 2.4. UV-VISIBLE spectrophotometer

First, we dissolved our bee venom sample in distilled water. Then, we filled both cuvettes (reference and sample) with distilled water to establish the baseline. After performing the auto-zero calibration, we replaced one cuvette with the bee venom solution to begin the analysis. We selected a wavelength range between 200 and 400 nm. The spectrum showed that our bee venom sample had an absorption peak at around 284 nm in water.

2.4.2. Fourier transform infrared spectroscopy (FTIR)

For recording by attenuated total reflection attenuated total reflection (including multiple reflection) involves light being reflected internally by a transmitting medium, typically for a number of reflections. [31].

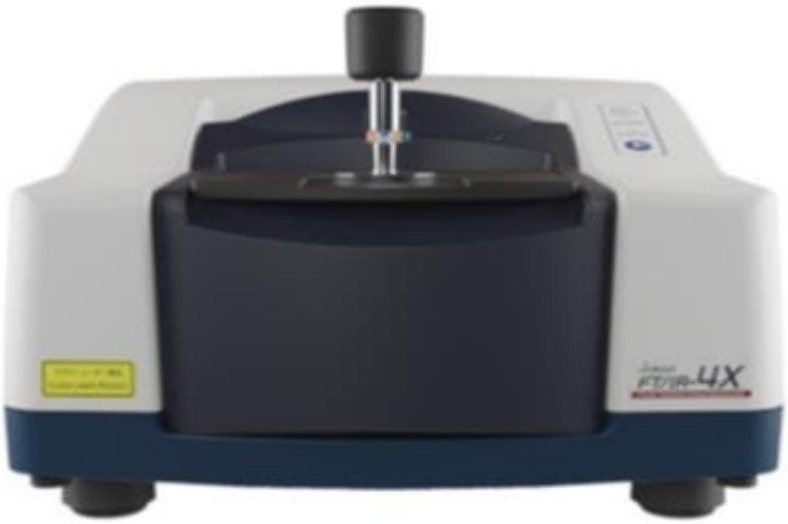


Figure 2.5. FTIR (Fourier transform infrared spectroscopy)

We took a sample of our bee venom and placed it inside the sample holder of the FTIR device. The instrument then analyzed it and produced a spectrum with peaks that help us identify various functional groups, such as double bonds and specific bonds like c=o, c–o, and others

2.4.3.X-ray diffraction (XDR)

The crystallinity and physical state of the solid was characterized by XRD of the sample as powder. The sample was lightly crushed and was distributed over a low-background sample holder. Measurements were obtained on a diffractometer with cu kα radiation (λ = 1.5418 å) at 40 kv and 40 ma. Xrd data were collected in the 2θ range of 5°–50° at a step size of 0.02° and a scan rate of 1°/min. From the obtained diffractogram peak positions and intensities were compared with those of patterns to the amorphous or crystalline phase.

Crystalline phases or amorphous nature of the samples were identified by matching the peak positions and intensities of the acquired diffractogram with the reference patterns. [32]

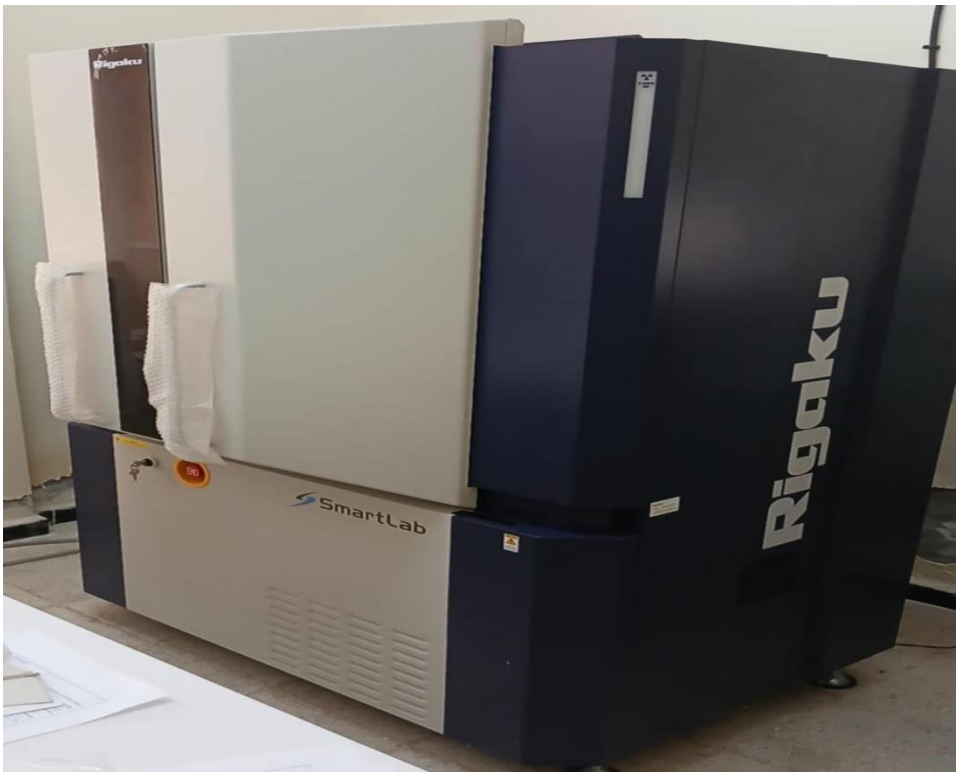


Figure 2.6. x-ray diffraction (XDR)

2.5. Test protocol for beads

2.5.1. Bead size measurement:

Article size and morphology of beads the diameter of beads was determined by screw gauge. For this purpose, 20 dried beads were randomly selected from each batch and the mean diameter was determined by screw gauge. The least count of screw gauge was 0.005 mm. Color and shape of dried beads of each batch was noted. Morphological examination of surface structure of beads was carried out by microscopy and microscopy 4 times more. [33].

2.5.2. Percent moisture loss%:

The microcapsules were evaluated for percentage moisture loss, which gives an idea about the hydrophilic nature. The weighed amount of microcapsules (w1) was initially kept in a desiccator containing silica gel at 37° for 24 h. The final weight (w2) was noted when

no further change in the weight of the sample was observed. Finally, the percent moisture content was determined by the following formula [34].

$$\text{Percent moisture loss \%} = (w1-w2/w1) \times 100 \ldots\ldots \text{(eq1)}$$

2.5.3. Yield %

individual weights of all the prepared beats of bee venom were measured and the percentage yield was calculated using the formula: [35]

$$\text{Yield \%} = \frac{\text{wight of the dry beads}}{\text{wight of the active ingrdit+exipant}} * 100 \ldots\ldots\ldots \text{(eq2)}$$

2.5.4. Swelling index:

Beads of bee venom, were soaked in 0.1n Hcl, 6.8 phosphate buffer and water. After 24 hrs., the beads were removed from their media's, excess water is removed and their weights are measured again. Swelling index was calculated using the formula: swelling index was calculated using the formula:

$$\text{Swelling Index \%} = \frac{\text{weight of wet beads} - \text{weight of dry beads}}{\text{weight of the dry beads}} * 100 \text{ [35] …eq (3)}$$

2.5.5. Drug entrapment efficiency:

The DEE (drug entrapment efficiency) was studied by taking the filtrate solution of the beads in a pH 6.8 phosphate buffer. The amount of bee venom loaded into the beads was determined by a UV spectrophotometer at 280 nm. The absorbance value for the drug in the filtrate gives the concentration of the drug in the filtrate. This amount of the drug in the filtrate solution was subtracted from the total amount of the drug added into the beads. This quantity was then divided by the total drug, and then multiplies with 100 to obtain the value of DEE.

All the experiments were carried out in triplicate

$$\text{Drug entrapment efficiency \%} = \frac{\text{drug recover on the beads}}{\text{beads recovery}} * 100 [35] \ldots\ldots \text{eq4}$$

2.5.6. Dissolution test

2.5.6.1. Procedure

Place the stated volume of the dissolution medium (± 1 per cent) in each vessel of the apparatus. Assemble the apparatus, equilibrate the dissolution medium to 37 ± 0.5 °c, and remove the thermometer. Place 1 dosage unit in each of the reciprocating cylinders, taking care to exclude air bubbles from the surface of each dosage unit, and immediately operate the apparatus as specified. During the upward and downward stroke, the reciprocating cylinder moves through a total distance of 9.9-10.1 cm. Within the time interval specified, or at each of the times stated, raise the reciprocating cylinders and withdraw a portion of the medium from a zone midway between the surface of the dissolution medium and the bottom of each vessel. Perform the analysis as directed. If necessary, repeat the test with additional dosage units.

Replace the aliquot withdrawn for analysis with equal volumes of fresh dissolution medium at 37 °c or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered with the evaporation cap for the duration of the test and verify the temperature of the medium at suitable times. Dissolution medium. Proceed as described for conventional-release dosage forms under apparatus 1 and 2. Time. Proceed as described for conventional-release dosage forms under apparatus 1 and 2 [31].

2.5.6.2. Calibration curve

The calibration of a method involves comparison of the value or values of a particular parameter measured by the system under strictly defined conditions with pre-set standard values. Examples include calibration of the wavelength and absorbance scales of a UV/visible spectrophotometer.

Most analytical methods are based on processes where the method produces a response that is linear and that increases or decreases linearly with analyte con centration. The equation of a straight line takes the form: $y = ax + b$.

Where a is the intercept of the straight line with the y axis and b is the slope of the line. Taking a simple example, a three-point calibration curve is constructed through readings of absorbance against procaine concentration [36].

2.5.6.3. Preparation of the solutions of the calibration curves of bee venom has ability in the two solutions:

A stock solution of bee venom (25 mg in 25 ml phosphate buffer) was prepared to obtain a concentration of 1 mg/ml. A series of dilutions (1.2.4.6.8 ml) were made by transferring aliquots of the stock solution into 10 ml volumetric flasks and diluting with the same buffer. The absorbance of each dilution was measured at 280 nm using a uv-vis spectrophotometer. A calibration curve was constructed by plotting absorbance against concentration and we do the same steps with 1.2 pH for the gastric environments.

2.5.6.4.Preparation of physiological solution:

The preparation of physiological solution was carried out according to the European pharmacopoeia, 6th edition, published on july 16, 2008.

a)Simulated stomach fluid

pH 1.2: in a suitable Erlenmeyer flask, dissolve 5.8 g of Nacl with stirring in 500 ml of distilled water and add 860 ml of Hcl (0.2m), then fill with distilled water to 2 liters and then adjust the pH. [37].

b) simulated intestinal fluid

pH 6.8: in an Erlenmeyer flask, dissolve 26.248 g of dibasic sodium phosphate in 2 liters of distilled water while stirring, then add 14.088g of monobasic sodium phosphate until completely dissolved, then adjust the ph. [37].

2.5.6.5.Dissolution principle:

The dissolution test was conducted to evaluate the release of the active ingredient from the prepared beads in simulated gastric (pH 1.2) and intestinal (pH 6.8) fluids. A known amount of beads containing a quantified dose of bee venom was introduced into two separate dissolution vessels—one filled with simulated gastric fluid (pH 1.2) and the other with simulated intestinal fluid (pH 6.8).

The dissolution medium (900 ml) in each vessel was maintained at a temperature of 37 ± 0.5°c, simulating human body conditions. The test was performed using a sup type ii (paddle) apparatus at a rotation speed of 100 rpm.

At predetermined time intervals, 5 ml samples were withdrawn from each vessel using a syringe. At 20 min .40 min 1h 2h 4h 6h and 8h to maintain the volume constant, each withdrawn sample was immediately replaced with 5 ml of fresh pre-warmed dissolution medium.

The collected samples were analyzed using a UV-visible spectrophotometer at 280 nm, and the concentration of bee venom released was determined using the calibration curve previously established.

CHAPTER 3:

RESULTS & DISCUSSIONS

CHAPTER 3 : RESULTS AND DISCUSSIONS

3.1. Introduction

In this chapter, we present all the work that has been carried out since the beginning of the project. We share our results and discuss the outcomes of each stage. Our goal is to provide a clear and structured summary of our findings so that future researchers can build on this work and improve it even further.

3.2. Harvesting of bee venom

After we choose a day with temperature of 28° and low rate of humidity and our hive was prepared for harvesting operation and using electrical stimulation and scraping the glass plat we harvest about 300 mg of bee venom powder.

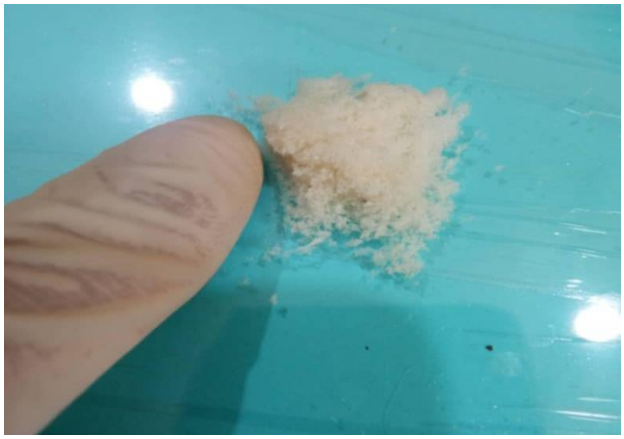


Figure 3.1. Amount of the harvested bee venom

3.3. Optimization of encapsulation bee venom

We started from the original protocol and modified one parameter at a time until we obtained the optimal bead shape for the encapsulation of bee venom, we gone show them in the next table

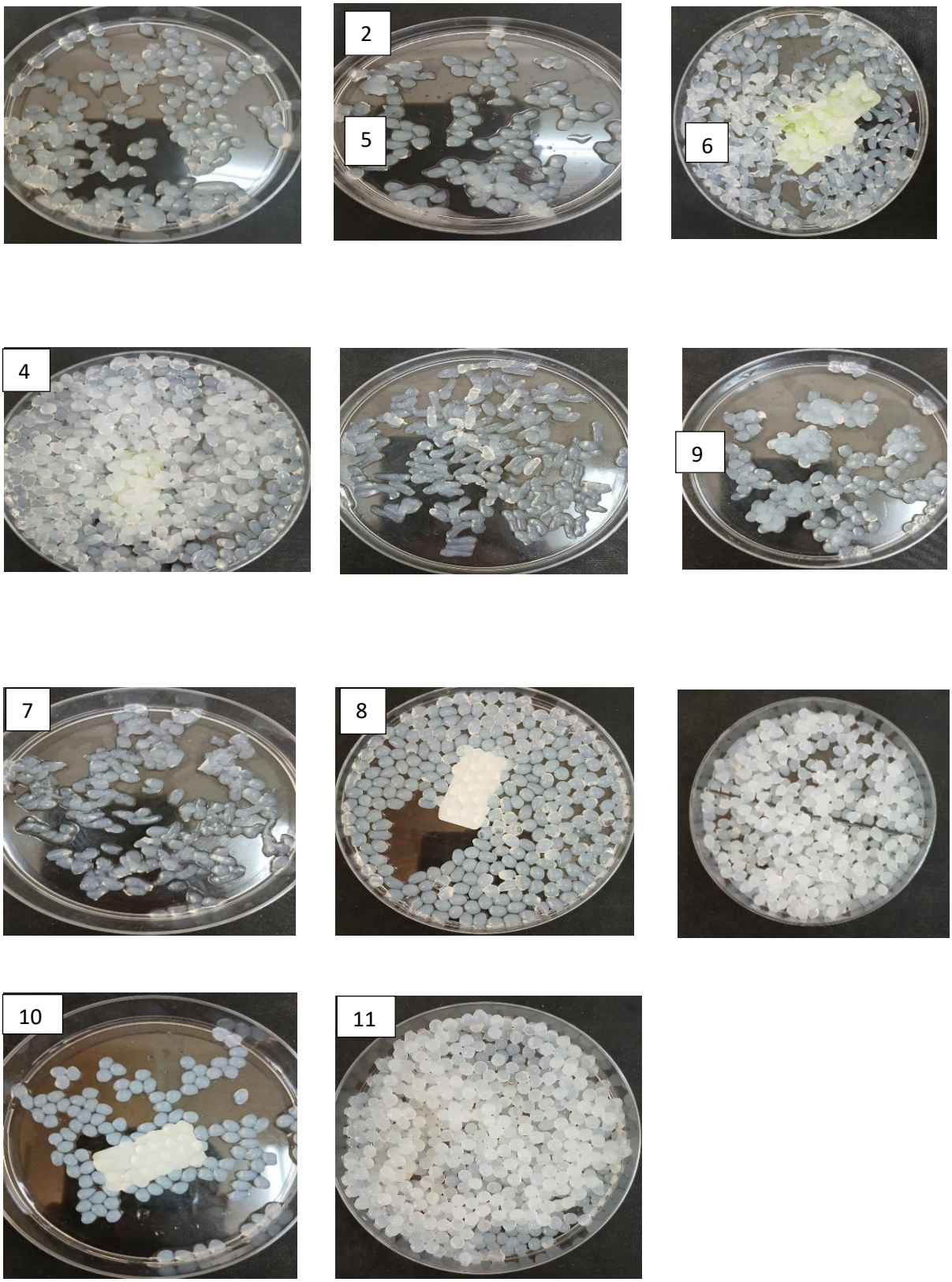


Figure 3.2. Global outcome of the achieved work

Table 3.1. Optimization of encapsulation bee venom

N°	**Asseys**	**Results**
1	We use 1g of alginate in 50ml of distilled water 15mg of bee venom in 50 ml distilled water and flow rate of 100 ml/min The stirring speed was set to 65 rpm	Although the encapsulation of bee venom was successful, the produced beads exhibited poor shape definition and lacked mechanical stability.
2	We use the same test condition just we decreased the flow rate to 95 ml/min	We encountered the same problem as in Trial No. 1, and the beads stuck together.
3	At this time, we increase the amount of alginate to 1.6g and kept the author test condition same as N°2.	The beads did not stick together, but they consistently had an irregular shape.
4	We always increase the amount of alginate this time we went to 2g and kept the test condition same.	The mechanical strength of the beads improved; however, the issue of irregular shape persisted.
5	Increased the amount of alginate 2.6 and save the pervious test condition.	It turned out that the more alginate we used, the stronger the beads became; however, the problem of irregular shape still persists, preventing us from achieving perfect beads.
6	We kept the same test conditions but reduced the flow rate to 95 mL/min and 3g of alginate	As before, the beads were shapeless and stuck together.
7	This time drop the flow to 80 mL/min and kept every think else same.	This time the bead didn't stick together but still shapeless but not like the pervious time. It gets less shapeless.

8	This time drop the flow to 60 mL/min and kept every think else same.	The beads have developed egg shape is better than shapeless from that we had but still not the perfect shape for our beads.
9	We didn't just drop the flow rate to 50mL/min but also, we drop the stirring speed was set to 25 rpm. .	We still obtained egg-shaped beads, but there was no sticking together.
10	At this time, we drop the flow rate intel 3.3 mL/min and kept everything the same.	At this stage, we obtained a mixture of egg-shaped and spherical beads, which is what we aimed for; however, the egg-shaped beads predominated over the spherical ones.
11	We hold all the test condition at the same we just drop the flow rate to 1 ml/min and also the stirring speed set to 25 rpm.	At this stage, we obtained spherical-shaped beads that were also stronger than the initial ones.

After a series of modifications to the test conditions and formulation parameters, we gradually improved the bead quality. Initially, the beads were fragile, irregular in shape, and tended to stick together. By adjusting factors such as alginate concentration, stirring speed, and flow rate, we were able to enhance the mechanical strength of the beads. While early trials resulted in predominantly egg-shaped and aggregated beads, later trials produced a mixture of egg-shaped and spherical beads. Eventually, we obtained strong, well-formed spherical beads—precisely the result we were aiming for. This confirms that the progressive changes in the test conditions had a positive impact on both the structural integrity and morphology of the encapsulated beads.

3.4. Characterization of Bee Venom

3.4.1. UV-Visible Spectrophotometer

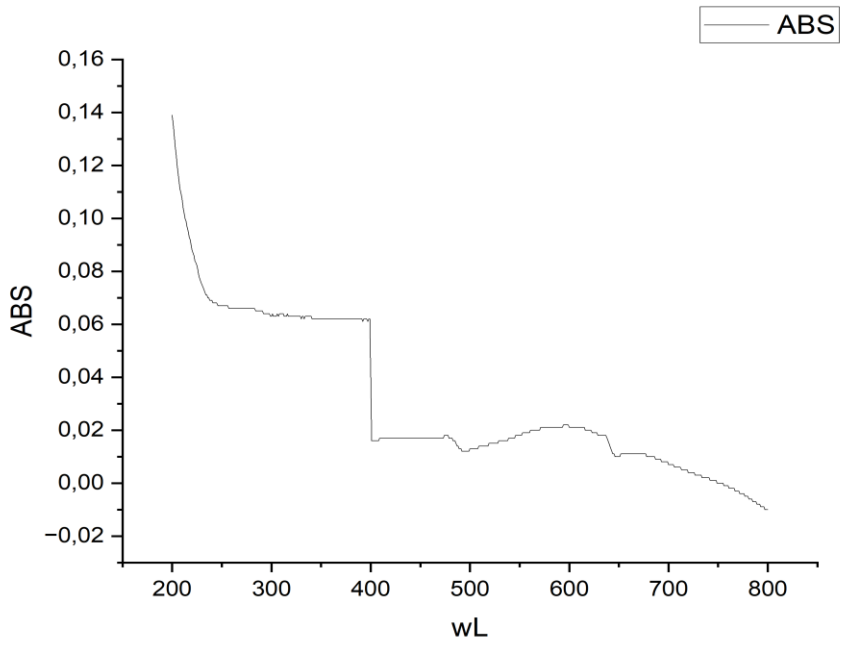


Figure 3.3. UV absorption curve of bee venom

After we dissolve our bee venom powder in the suitable amount of distilled water and set the measured in uv between 200-400 nm we found our peak in the 284 nm not far from the reference that is saying: «the components of the honey bee venom. The optical density of the fractions was measured on a Hitachi-557 spectrophotometer at an absorption wavelength at λ=280nm.” [38]

The reason of the difference between them and me possibly from the impure in the bee venom or the environment of bees because changing the environment or food of bees that has effect on bee venom

3.4.2. Fourier transform infrared spectroscopy (FTIR)

Following FTIR analysis of our sample, the resulting spectrum displayed several characteristic peaks. The next step involves identifying the functional groups associated with these peaks to better understand the molecular composition of the bee venom

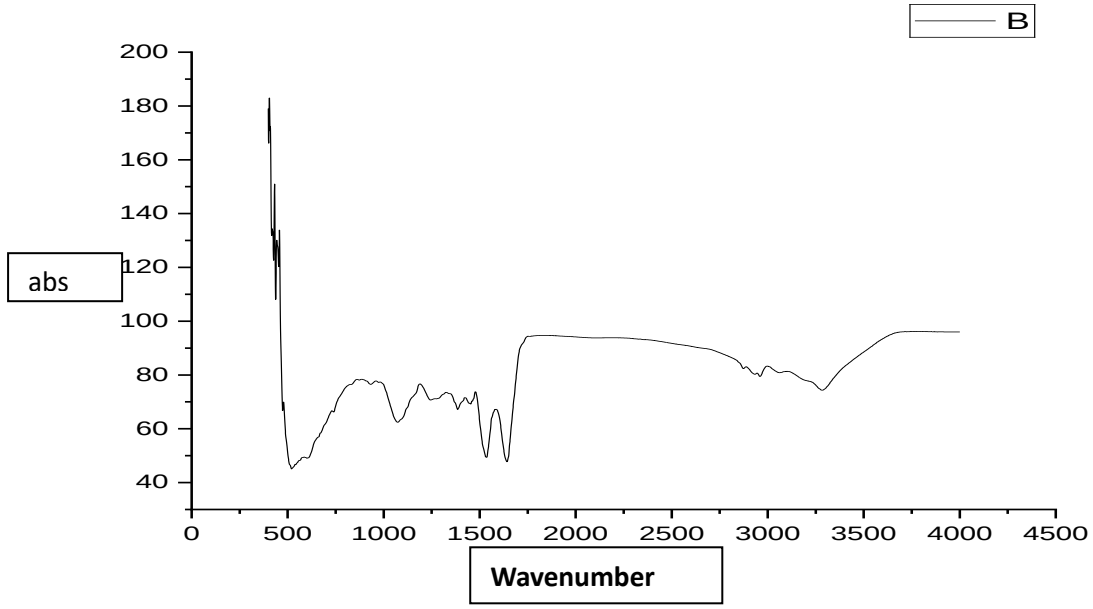


Figure 3.4. Fourier transform infrared spectroscopy (FTIR) of bee venom

The FTIR spectrum of bee venom reveals the presence of several characteristic functional groups, mainly associated with its proteinaceous and peptide content. A broad absorption band around 3265 cm^{-1} indicates O–H and N–H stretching vibrations, typical of hydroxyl and amine groups found in amino acids and peptides such as melittin. The peaks between 2873 and 3002 cm^{-1} correspond to aliphatic C–H stretching, suggesting the presence of $–CH_2–$ and $–CH_3$ groups, commonly found in the side chains of amino acids. A strong absorption near 1650 cm^{-1} represents the amide I band, primarily due to C=O stretching of peptide bonds, while the peak around 1540 cm^{-1} corresponds to the amide II band, which involves N–H bending and C–N stretching—both confirming the presence of proteins. Additional weak bands between 2100 and 2360 cm^{-1} may be attributed to minor components or environmental contaminants, such as C≡C or C≡N stretching vibrations. Peaks in the region from 1450 to 1300 cm^{-1} are related to C–N stretching and N–H bending, characteristic of amide III bands. The fingerprint region (1000–600 cm^{-1}) is complex and shows several peaks, including one at 1073 cm^{-1}, which may indicate C–O or P–O stretching, suggesting the presence of carbohydrates or phosphate groups, which are common in biologically active molecules. A sharp peak around 614 cm^{-1} might result from out-of-plane bending of aromatic rings, possibly related to specific amino acid residues like tyrosine or phenylalanine. Overall, the spectrum confirms the presence of peptides, proteins, and other organic functional groups consistent with the known composition of bee venom.

Reference of bee venom (FTIR)

A medium-intensity absorption band from 3500 to 3100 cm^{-1} (max at 3288 cm^{-1}) corresponds to N–H stretching vibrations (amide A) of peptide/protein structures, with a weaker signal at 3060 cm^{-1} (amide B), possibly an amide II overtone. Absorptions at 2960 and 2928 cm^{-1} are due to CH_2 asymmetric stretching, while signals at 2873 and 2855 cm^{-1} relate to CH_2 symmetric stretching.

In the fingerprint region (1700–700 cm^{-1}), key bands appear at 1645 cm^{-1} (amide I, C=O stretching) and 1537 cm^{-1} (amide II, N–H bending and C–N stretching), consistent with bee venom spectra reported by Park et al. Peaks at 1454 and 1386 cm^{-1} arise from protein side-chain COO^- groups. The amide III band (1290–1240 cm^{-1}) involves C–N stretching, N–H bending, and C–O stretching vibrations. Absorption at 1088 cm^{-1} is attributed to C–H in-plane deformation of aromatic structures, while a weak signal at 805 cm^{-1} relates to symmetric CNC stretching of proteins. Amide IV and V bands are found at 660 and 730 cm^-[39]

3.4.3. Powder X-ray Diffraction (PXRD)

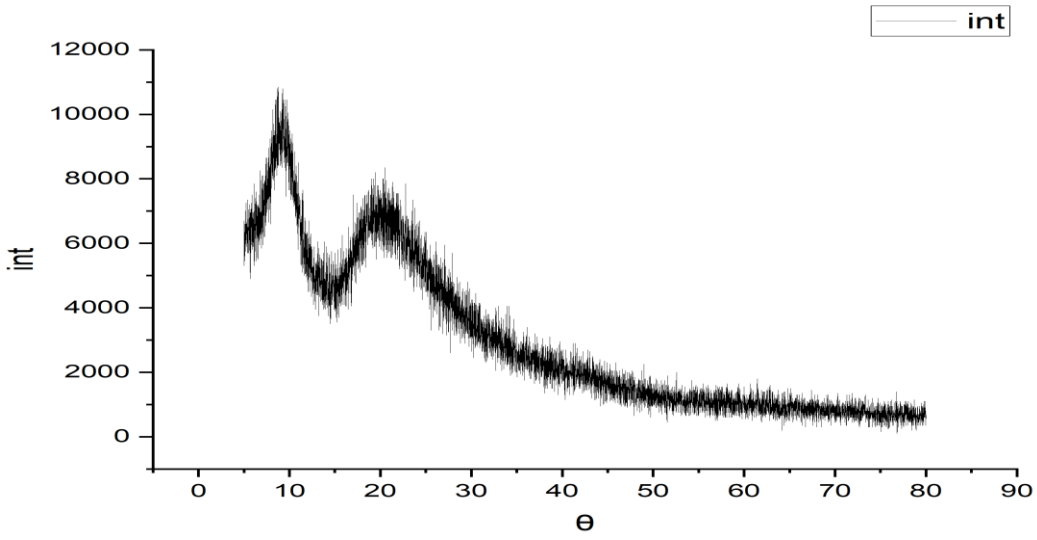


Figure 3.5. result of XRD for bee venom powder

The pattern indicates a broad peak around 10-20° with a maximum intensity near 10,000, suggesting the presence of amorphous or semi-crystalline structures in bee venom. The intensity decreases gradually beyond 20°, with smaller fluctuations, which could indicate minor crystalline components or noise. This is consistent with the complex molecular composition of bee venom, which includes proteins, peptides, and other biomolecules.

Reference of bee venom (XDR)

According to XRD analysis, BV showed an amorphos structure peaks at 2θ=9.9° and 21.3°. DSC thermogram of BV showed an endothermic peak at approximately 73.1°C, which is associated with the melting point of the specimen itself, and endothermic peak at 186°C which could be attributed to thermal degradation of the BV sample. On the other hand, on the DSC curve of the cooling segment, no phase transformations were observed, which confirms the decomposition of the sample.[40]

This reference may not always apply, because bee venom can vary depending on location, diet, and environmental conditions.

3.5. Test protocol for beads

3.5.1. Bead size measurement

Table 3.2. Diameter for 20 beads

3.91mm	3.59mm	3.89mm	3.61mm	3.65mm
3.47mm	3.77mm	3.97mm	3.41mm	3.92mm
3.18mm	3.96mm	3.71mm	3.66mm	3.87mm
4mm	3.39mm	3.82mm	3.61mm	3.77mm

The average diameter for the beads is 3.72 mm

3.5.1.1. Macroscopic appearance

This is our beads in wet and dry from

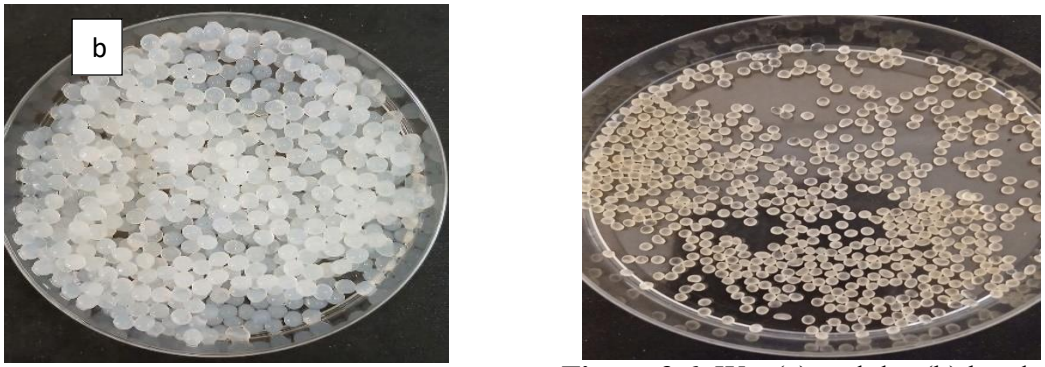


Figure 3.6. Wet (a) and dry (b) beads

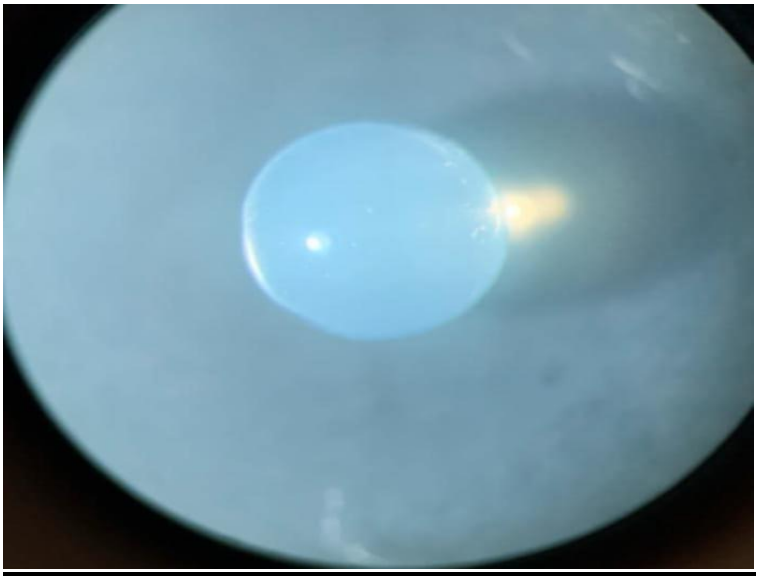


Figure 3.7. Macroscopic image of the formulated bead

As seen in figure 3.7, the beads exhibit a perfect spherical shape. The uniform color and appearance suggest that they possess strong physical characteristics and are not easily breakable. And there is no air bubble inside the bead.

3.5.1.2. Microscopic appearance

In this time, we used microscopic that are attached to a computer so we can see more details we zoom in 4 time more than last one and we have two results in all of the head and the second we cut in half so we can see more details.

A) Full beads

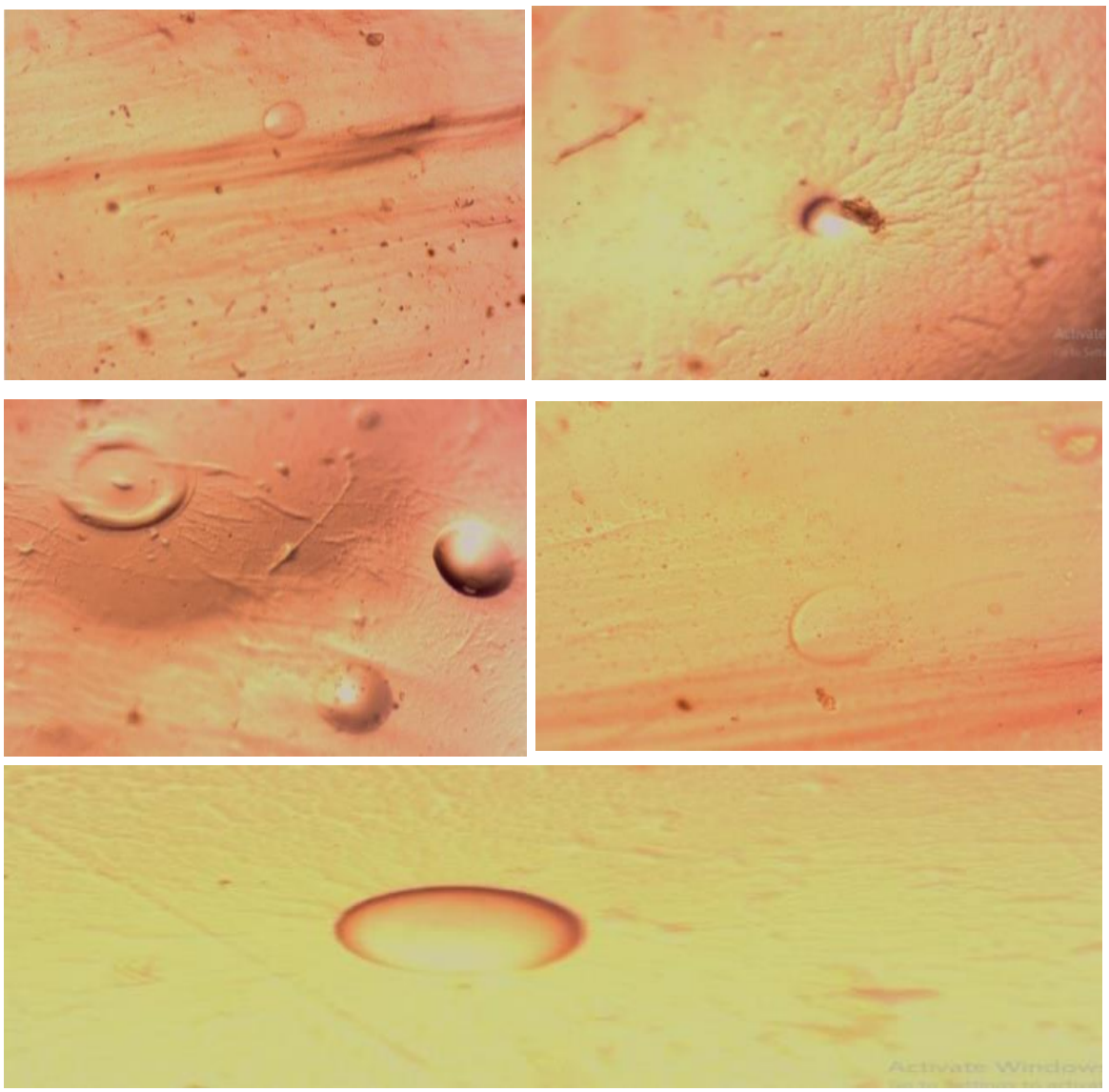


Figure3.8. Microscopic image of the full bead

This image reveals the internal matrix encapsulating the bee venom within the spherical beads. Additionally, the presence of certain impurities and aggregates is also observed and should not be overlooked".

B) Bead cut in half

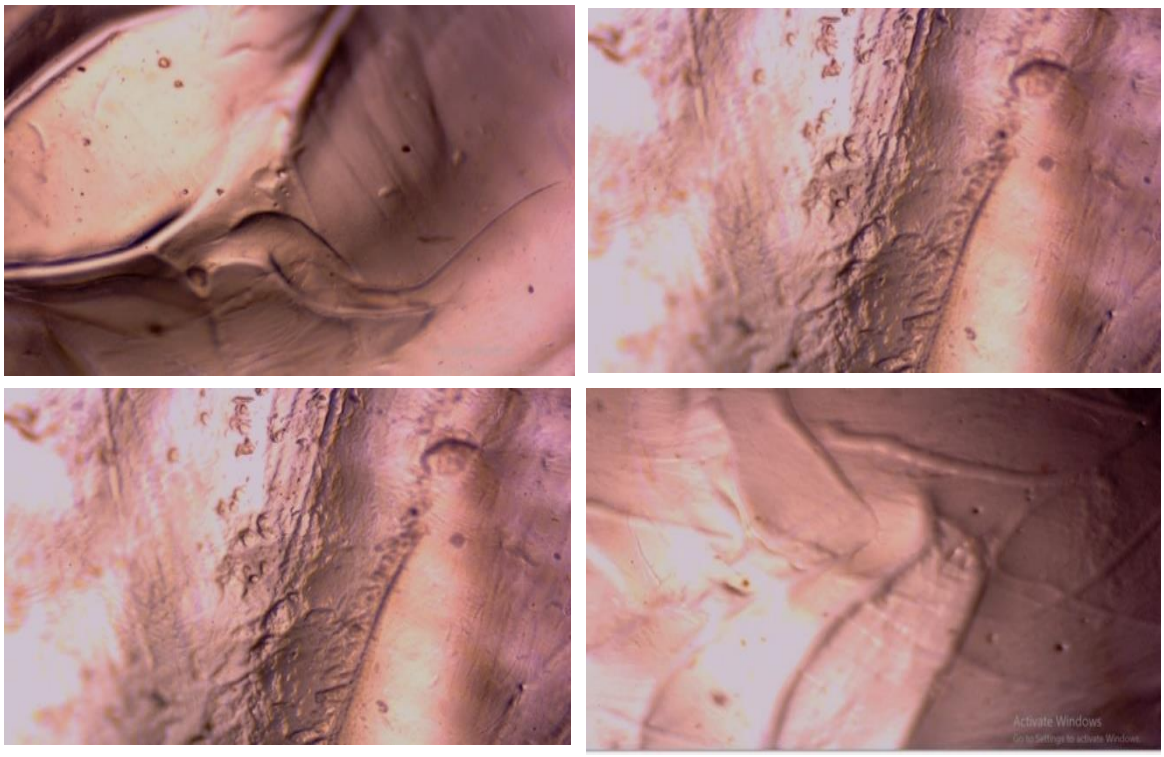


Figure 3.9. Microscopic image of the bead cut in half

From this angle, we can observe some holes on the surface of the bead where we cit it, which confirm the presence of an internal matrix. These openings also indicate that a significant amount of matrix deeper inside the bead.

3.5.2. Percent moisture loss

From eq (1), our Percent moisture loss equals 90%. This indicates that our beads are capable to lose moisture without losing shape and that active ingredient is in solid form which its more stable.

3.5.3. Yield

From eq (2) we get the Yield equals 315%

In this test, it is necessary to eliminate all the water inside the beads. Normally, an oven at 105 °C is used for drying, but this temperature causes degradation and dispersion of the bee venom. We tried using a desiccator with silica gel, but it does not appear to be effective

enough in removing all residual moisture from inside the beads. But from this we know that's our materials active ingredient + excipient)

3.5.4. Swelling Index

From eq (3) we have this result

A) In phosphate buffer

We don't have result because all of the bead has dissolved completely

Table 3.3. Result of swelling index

	Hcl	distilled water
Swelling index %	10.71%	13.52%

B) In Hcl

That mean our beads don't swell in Hcl so is not going to dissolved and maintain the shape also our bee venom is inside beads

C) In water

That mean our beads don't swell in water so is not going to dissolved and maintain the shape also our bee venom is inside beads

3.4.5. Drug Entrapment Efficiency

From eq (4) we get this result = 68%

That means your bead encapsulate 68% of our active ingredient (bee venom) which indicate your result is our beads have high capability of Entrapment Efficiency for bee venom.

3.4.6. Dissolution test

3.4.6.1. Calibration curves

a) This for 6.8 pH

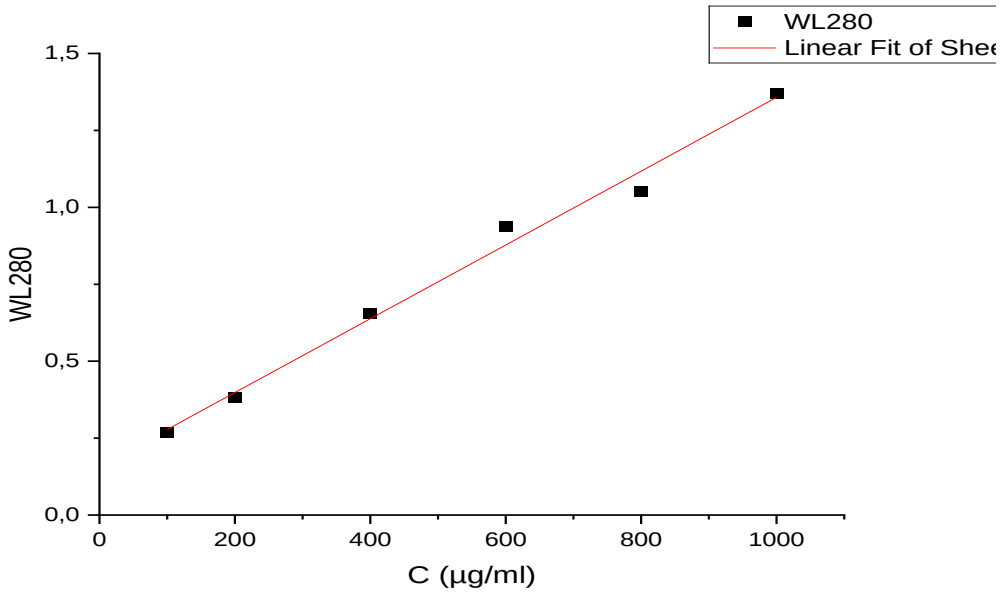


Figure 3.10. Calibration curve for 6.8 pH

b) This for 1.2 pH

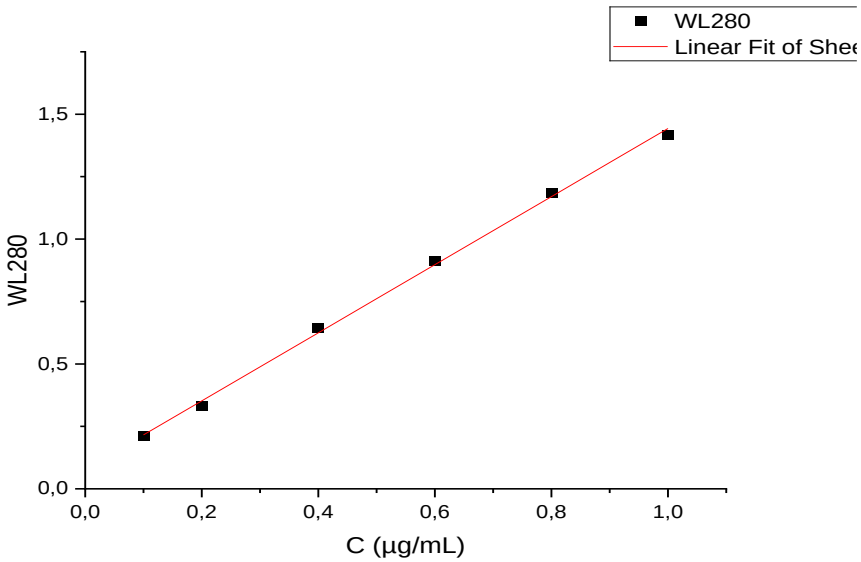


Figure3.11. Calibration curve for 1.2 pH

From the calibration curves of the different media illustrated in Figures 14.15 we obtained respectively for the acidic media pH 1.2 and the neutral media pH 6.8 the following equations: **y = 0.0012x +0.1582**, **y = 1.364x + 0.0794** and the values of the concentrations of the samples collected at different time intervals and analyzed by UV-Visible were calculated using these equations from their absorbances.

The dissolution percentage of bee venom from polymeric microcapsules was calculated using the following equation:

$$\textbf{Pourcentage dissolution (\%)} = \frac{C}{C_0} * \textbf{100 (eq 5)}$$

Knowing that:

C0: is the initial concentration of the sample

- **Calculations of dissolution pourcentages:**

The biopharmaceutical results of the two trials are presented in the form of dissolution profiles expressing the dissolved pourcentage of active ingredient as a function of time.

The two tables and two figures represent these results, respectively:

Table 3.4. dissolution pourcentages for pH= 6.8

Time	**C (µg/ml)**
20 min	Undetermined
40 min	Undetermined
1h	Undetermined
2h	Undetermined
4h	743,885996
6h	Undetermined
8h	1191,317

Table 3.5. dissolution pourcentages for pH=1.2

Time	C (µg/ml)
20 min	52,0361991
40 min	119,909502
1h	1364,25339
2h	244,343891
4h	Undetermined
6h	Undetermined
8h	1183,25792

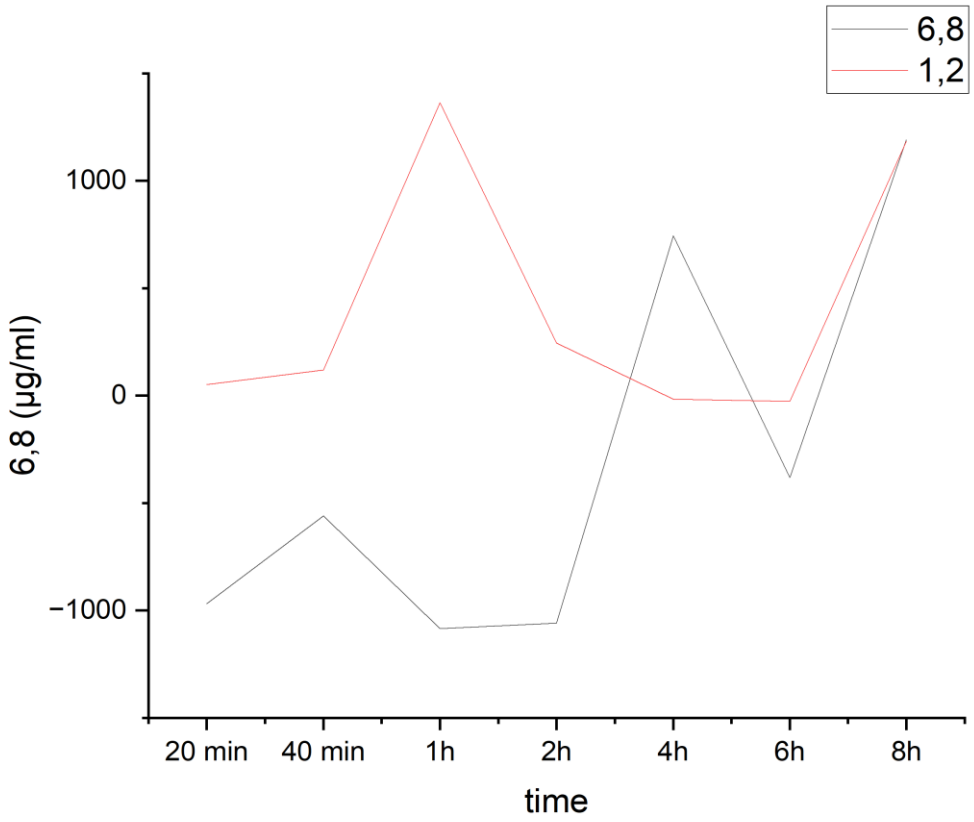


Figure 3.12. Release profile of bee venom beads

After we obtained the results from our dissolution test, they appeared to be inaccurate or misleading. This could have occurred for several reasons. We suspect possible degradation of the bee venom or interference during measurement with the UV-Vis spectrophotometer. Another possible explanation could be the aggregation of bee venom within the beads, which may have affected its release and solubility during the test.

And for the lack of time, we didn't get to optimize dissolution test, and try to obtain a better result.

General Conclusion

General Conclusion

At the end of our journey we wish gone get step forward in the unknown of bee venom encapsulation because of the benefits and a lot of therapeutic action its gone be step in the pharmaceutical industry

We began by harvesting bee venom from the hive using electrical stimulation. This method has been used for a long time and is considered the safest way to collect bee venom without killing the bees or putting their lives in danger. It also allowed us to obtain a sufficient amount of venom to complete our study.

After obtaining our samples, the first step was to encapsulate the bee venom using sodium alginate, a biopolymer capable of encapsulating the venom inside beads. This was done using a tube pump and the dripping method, where the solution was dropped into a calcium chloride solution to form the beads.

After that, we began performing the necessary tests, starting with bead size measurement. The beads had an average diameter of approximately 4 mm and exhibited a nearly perfect spherical shape. We conducted a macroscopic examination to observe the general shape of the beads, and also used a light microscope at 4× magnification to visualize the internal matrix structure, where the bee venom was encapsulated.

We also performed a moisture content test, which showed a result of approximately 90%, indicating that the majority of the bead composition was water. Following this, we conducted the encapsulation yield test; however, the results were not optimal due to certain issues, which are discussed in detail in the Results and Discussion section.

Next, we carried out the swelling index test, which revealed that the beads were stable in hydrochloric acid (hcl) and distilled water, showing no significant swelling or degradation.

However, in a pH6.8 buffer solution, the beads completely dissolved, indicating their potential for pH-dependent release behavior.

The Drug Entrapment Efficiency (DEE) test resulted in an outcome that was neither very high nor very low, with an entrapment efficiency of approximately 68%. This indicates a moderate level of bee venom encapsulated within the microspheres, representing an average efficiency for this type of formulation. And for the dissolution test our result we didn't came as we hoped and that was step back for our study and for those who came after me there is same point need to focus on:

- Try to obtained more quantity of bee venom for more characterization.
- Try to improve the yield test results.
- Optimization of dissolution test for better results.

In conclusion, I sincerely hope that someone will come forward in the future to continue and build upon my work. This research holds a great deal of potential and carries many promising ideas that could contribute meaningfully to the pharmaceutical field. With further development and deeper investigation, I believe this study could open new doors for innovative drug delivery systems and natural therapeutic approaches. It would be incredibly rewarding to see this work serve as a stepping stone for future advancements and real-world applications.

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