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Release Study of Methylene Blue (As Drug Model) By Alginate-Gelatin Blend Macrospheres Prepared Via Crosslinking Method

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DECLARATION

I declare that this thesis entitled Release Study of Methylene Blue (As Drug Model) By Alginate-Gelatin Blend Macrospheres Prepared Via Crosslinking Method is the results of my own research except as cited in the references.

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ABSTRAK

Kajian ini memberi tumpuan kepada penyediaan makrosfera campuran alginat-gelatin untuk pengkapsulan dan pembebasan metilena biru sebagai ubat model. Makrosfera disediakan dengan menghubungkan silang natrium alginat dan polimer gelatin dengan kalsium klorida. Nisbah alginat-gelatin yang digunakan ialah 20:80, 35:65 dan 50:50. Manik yang terbentuk dicirikan oleh spektroskopi Fourier Transform Infrared (FTIR), dan spektrofotometri boleh dilihat UV. Kesan nisbah polimer, pautan silang kalsium klorida, saiz jarum dan pH pada pelepasan ubat telah disiasat. Analisis FTIR mengesahkan kehadiran kumpulan berfungsi tertentu yang menunjukkan hubungan silang antara polimer. Analisis makrosfera dan saiz zarah mendedahkan bulatan licin dengan diameter antara 6-10 mm berdasarkan jarum yang digunakan. Kajian UV-vis menunjukkan pelepasan biru metilena terkawal dan berterusan sehingga 60 minit daripada rumusan makrosfera. Corak pelepasan ubat yang lebih perlahan telah dicapai pada pH neutral 7 berbanding pH berasid 4 dan pH alkali 9. Hasilnya menunjukkan bahawa makrosfera campuran alginat-gelatin yang disesuaikan yang disediakan melalui pautan silang boleh berfungsi sebagai sistem yang cekap untuk pengkapsulan dan pelepasan terkawal ubat.

Kata Kunci: alginate, gelatin, kalsium klorida, pH, makrosfera,

ABSTRACT

This study focuses on the preparation of alginate-gelatin blend macrospheres for the encapsulation and release of methylene blue as a model drug. The macrospheres were prepared by crosslinking sodium alginate and gelatin polymers with calcium chloride. The alginate-gelatin ratios used were 20:80, 35:65 and 50:50. The formed beads were characterized by Fourier Transform Infrared (FTIR) spectroscopy, UV-visible spectrophotometry and optical microscopy. The effects of polymer ratio, crosslinker concentration, needle gauge size and release medium pH on the drug release profiles were investigated. FTIR analysis confirmed the presence of specific functional groups indicating crosslinking between the polymers. The microscopy and particle size analyses revealed round smooth beads with diameters ranging from 6-10 mm based on the needle gauge used. The UV-vis studies demonstrated controlled and sustained methylene blue release for up to 60 minutes from the macrosphere formulations. A slower drug release pattern was achieved at neutral pH 7 compared to acidic pH 4 and basic pH 9. The results indicate that tailored alginate-gelatin blend macrospheres prepared by crosslinking can serve as efficient systems for the encapsulation and controlled release of drugs.

Key words: alginate, gelatin, calcium chloride, pH, macrospheres

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
w/w	weight by weight
ml	milliliter
g	gram
G	gauge
mm	millimeter

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Alginate and gelatin microbeads can be chemically bonded with calcium chloride to encapsulate methylene blue, making them suitable for drug delivery purposes. Alginate and gelatin are commonly employed in drug delivery systems due to their inherent biocompatibility, biodegradability, and nontoxicity, making them favorable choices as natural polymers. Calcium chloride, when used as a crosslinking agent, can create a durable gel structure capable of enclosing methylene blue and other pharmaceutical substances. The resultant microbeads or hydrogel beads can be utilized for the purpose of controlled or prolonged discharge of medications (Long et al., 2023)

Sodium Alginate is an organic linear copolymer consisting of linked B-D mannnuronate and a-L-guluronate residues. Due to its distinctive properties, it is widely utilized in various applications. Sodium alginate possesses the notable characteristic of being able to dissolve in water. Consequently, it possesses the ability to readily dissolve in water, rendering it highly convenient for utilization in various applications (Indumathi Sathisaran & Balasubramanian, 2020).

In the agricultural field, sodium alginate is used for slow release of agrochemicals. Alginate gels can serve as excellent matrices for the controlled release of agrochemicals, such as fertilizers or pesticides, in the soil. Overall, the unique properties of sodium alginate make it a versatile and valuable material for various industries, including textile printing, food, biomedical, enzyme, agricultural, and environmental applications. Its ability to solubilize in water, form gels, and create films and fibers of calcium alginate contributes to its wide range of uses.

Gelatin it be dissolves in water, it must be cross-linked to be used as a carrier for application such as pharmaceutical, food application and cosmetics industries. Calcium chloride is the chemical crosslinking agent that is used the most often because it works so well to stabilize collagenous materials. In addition, alginate-gelatin crosslink with calcium chloride can be used as the encapsulating material, and their distribution can be tailored to the particular application by carefully adjusting the carrier's composition and size. These tactics may enable overcoming prior limits such as poor mechanical behaviours and high water vapour permeability. In order to combine the characteristics of materials formed of proteins and polysaccharides into a single polymer, alginate-gelatin polymer films are being created (Indumathi Sathisaran & Balasubramanian, 2020).

Methylene blue, a highly adaptable artificial colouring agent, serves a vital function when integrated into alginate-gelatin macrospheres. The intense blue hue acts as a valuable visual indicator, making it easier to track and observe macrospheres during experiments. Methylene blue functions as a prototype drug or indicator compound, enabling the investigation of controlled release mechanisms from the alginate-gelatin matrix. The dye's even distribution onto the polymer matrix guarantees consistent dispersion, facilitating meticulous analysis of release patterns and diffusion properties. In addition, methylene blue's biocompatibility not only serves as a dye but also plays a crucial role in evaluating the overall biocompatibility of the macrospheres. Essentially, methylene blue within alginate-gelatin macrospheres functions as a versatile tool, aiding in the visualisation, tracking, and comprehensive analysis of the macrospheres' behaviour in various applications.

Recently, blending polymers has been getting a lot of attention for research and improving the qualities of polymers. Cross-linking is a popular way to connect two chains of polymer with a chemical bond. It is used in several ways to improve the mechanical properties and performance of materials. Thus, calcium chloride is well-known as a crosslinking agent for compounds with hydroxyl groups, like polysaccharides. It has functional groups that are used in different ways, such as drug delivery, decreasing oxygen permeability, proton exchange film, evaporation systems, ultrafiltration films, and coatings. Alginate-gelatin mixes with cross-linked microstructures of

calcium chloride offer different ways to change pH-sensitive, drug carriers and biodegradability (Theeraphol Phromsopha & Yodthong Baimark, 2014).

1.2 Problem Statement

The production of encapsulation using natural polymer has been widely used in biomedicine, the food industry, and cosmetics and personal care products of production in the size of beads. alginate has certain properties that make it unsuitable that it becomes slightly unstable in the presence of a single-capacity cation, which means that it becomes less stable over time. This can lead to low stability and poor quality of the product. On another hand, the poor resistance of gelatin is hygroscopic, which means it has a propensity to absorb and retain moisture from its surroundings. This can lead to changes in the physical properties of gelatin in beads, including softening and increased susceptibility to microbial growth. Nevertheless, alginate-gelatin can combine to enhance their chemical properties for microsphere production. This mixture forms a stable gel matrix, improving drug encapsulation, stability, and bioavailability. The synergy also boosts microsphere mechanical strength, resistivity to degradation, and adaptability to different environments. Crosslinking agents like calcium chloride further enhance stability mechanical properties, and optical parameters. Moreover, the release studies on alginate-gelatin beads are vital for evaluating drug delivery performance. They provide essential insights into the efficiency and rate of drug release, guiding optimization for effective therapeutic outcomes of beads that into different pH, the bead's effect of pH and temperature that is responsive of natural polymers can be used to deliver the beads into specific site triggered by these conditions.

1.3 Objectives

1. To prepare alginate-gelatine beads using crosslinking method
2. To study the effect of parameter (ratio alginate-gelatine, and needle size) bead.
3. To analyse UV-vis research on methylene blue release at various pH levels for 24 hours

1.4 Scope of Study

In this study, the preparation of alginate-gelatin and calcium chloride using crosslinking method. Alginate and gelatin blend prepared as beads to encapsulate methylene blue as a model drug. After, alginate-gelatin crosslink with calcium chloride, release study of the beads were studied. Natural polymer as an alginate-gelatin used a different blend ratio and calcium chloride concentration permanently, to study the release of methylene blue an encapsulation.

1.5 Significances of Study

Alginate is a well-known natural polymer that has been utilized widely used in biomedicine and food industry for encapsulation in many years. Studies have demonstrated that release study made from alginate have high biocompatible, biodegradability and also non-toxic. The mechanical characteristics of alginate-gelatin is biocompatible with calcium chloride of beads were also discovered to be quite good for improve the performance of beads. It has potential to be drug delivery to be much safer and good for human to consume since its natural and also affordable. Therefore, encapsulation of alginate-gelatin with crosslink of calcium chloride is the most suitable alternatives to be more efficient in encapsulation in every application.

CHAPTER 2

LITERATURE REVIEW

2.1 Macrosphere

Macrosphere refers to the process by which a foreign substance or object is surrounded and isolated by a protective barrier or structure. This can occur naturally as a biological response to certain substances or materials introduced into the body, or it can be deliberately induced for therapeutic purposes. In the context of cell therapy, macrosphere provides increased retention time in the target tissue, improving its therapeutic efficacy. This concept has several applications in the medical field, including drug delivery systems, tissue engineering, and implantable devices.

Macrospheres, with their sphere-in-capsule structure, have the potential to function as a versatile multi-drug delivery system, enabling the achievement of combination therapy objectives. One can achieve this by enclosing several drugs within a single macrospheres, allowing for the simultaneous administration of multiple drugs. Methylene blue as drug delivery systems utilizing macrospheres have the capability to offer controlled release of drugs. This provides the body with the capacity to sustain an even drug concentration while simultaneously reducing the likelihood of undesirable effects. Alginate and gelatin are two examples of materials that can be used to achieve this objective. In addition, calcium chloride or other substances can be used to crosslink these materials.

2.2 Macrospheres Preparation Method

There are several methods to produce microsphere in medical field, for emulsification macrosphere techniques to produce of Alginate/Gelatin macrospheres for drug delivery involves several methods, including extrusion, ionic gelation, and coacervation. The extrusion method combines the encapsulating material, sodium alginate, with the substance for encapsulation. This mixture is forced through a nozzle into a solution, leading to the creation of stable macrospheres. Ionic gelation, achieved through external gelation, involves the gradual addition of a polysaccharide solution into a crosslinking solution containing divalent or multivalent ions. Coacervation, another method, utilizes the formation of a coacervate phase to encapsulate drugs, resulting in controlled-release macrospheres.

To enhance stability, crosslinking with calcium chloride is employed, linking polymer chains and preventing breakdown (Regina et al., 2023). In the production of Alginate/Gelatin macrospheres for drug delivery, the combination of extrusion, ionic gelation, coacervation, and crosslinking is crucial. These techniques allow the creation of stable and controlled-release macrospheres, enhancing the potential for effective drug delivery applications. The choice of methods and the control of crosslinking parameters play a significant role in determining the characteristics of the macrospheres, such as size, drug encapsulation efficiency, and release kinetics. Overall, the integrated approach involving these methods and crosslinking contributes to the development of advanced drug delivery systems with tailored properties for therapeutic applications (EREN BELGİN et al., 2023).

2.2.1 Extrusion

The Alginate/Gelatin macrospheres were produced using the extrusion method. This technique entailed amalgamating the substance intended for encapsulation with a solution of an encapsulating material, such as sodium alginate. Once the mixture was completely blended, it was subsequently forced through a nozzle into a solution that causes it to solidify, resulting in the creation of macrospheres. Alginate possesses a unique ionotropic-gelation effect that allows it to interact with divalent ions, leading to the creation of a stable hydrogel structure known as the "egg-box". (Regina et al., 2023)

2.2.2 Ionic Gelation

Ionic gelation is a method used in the production of macrospheres or microspheres for drug delivery. This technique enables the formation of a strongly interconnected structure by the interaction between a positively charged particle (or a negatively charged particle) and an ionic polymer compound. Ionotropic gelation can be achieved through three distinct methods: internal gelation, external gelation, and inverse gelation. The external method, also known as the controlled diffusion method, is currently the most commonly used technique for ionotropic gelation.

The ionic gelation technique entails the gradual addition of a solution containing polysaccharides into a solution that promotes crosslinking. This crosslinking solution usually contains ions that are either divalent or multivalent. The electrostatic reaction leads to the creation of interconnected microstructure particles. The selection of ionotropic medium, whether monovalent, divalent, or trivalent cations, and its concentration, affects various properties such as gelation rate, degree of derivatization, drug encapsulation efficiency, drug loading, and bead size (Piotr Gadziński et al., 2022).

2.2.3 Coacervation

Macrospheres are produced for drug delivery purposes using the coacervation process. The process involves the formation of a coacervate, which is a liquid phase that separates from a mixture of two immiscible liquids. After this procedure, the drug or active ingredient is introduced into the coacervate. Subsequently, the resulting mixture is either crosslinked or solidified to generate microspheres or macrospheres. This method has been used to produce drug delivery systems with controlled release properties, particularly for oral and colonic drug delivery systems, among others. The coacervation process enables the encapsulation of drugs and the attainment of controlled release kinetics. This technique is crucial for the development of macrospheres used in various drug delivery applications (EREN BELGİN et al., 2023).

2.2.4 Crosslink

To enhance the beads' stability, calcium chloride was used as a crosslinker. Crosslinking is a process that links the polymer chains together, making the microparticles more stable and less

likely to break down. The amount of crosslinker to alginate-gelatin in the mixture determined the typical size of the alginate-gelatin microparticles, crosslinking is a process that involves chemically bonding the gelatin molecules together to create a more stable structure. The importance of controlling the swelling and dissolution of macrospheres in drug delivery applications has been emphasized in research conducted by (Theeraphol Phromsopha & Yodthong Baimark, 2014).

2.3 Materials

There variety of materials that can used for macrosphere, this researcher (Long et al., 2023) used main point of materials is natural polymer where it uses a gelatin, and alginate where it used as beads to determined drug release. Alginate is a linear block copolymer composed of repeating monomeric units of α-1, 4-L-guluronate (G) and β-1, 4-D-mannuronate (M). Sodium alginate, a water-soluble salt of alginate, can be used to prepare alginate hydrogel fibers through the wet-spinning process. These fibers can be utilized in knitted fabrics or chopped for nonwoven materials. Alginate nonwoven materials have the ability to form hydrogels and have been applied for active agents due to their biocompatibility, biodegradability, and nontoxicity features. Alginate's gel-forming ability is based on the "egg-box model," where cross-linking with calcium ions creates a three-dimensional network structure. This gel formation is crucial in the preparation of silver nanoparticles (AgNPs)-loaded calcium alginate beads embedded in gelatin scaffolds, which have potential uses as wound dressings (Antezana et al., 2022).

Furthermore, materials that used in manufacturing food for most of the time, carbohydrates are used to encapsulate things that are used in food. Polysaccharides are long chains of sugar molecules that make up complex carbs. There are fatty acids and fatty alcohols, waxes, glycerides, and phospholipids among the lipid products that can be used in food. There are various materials used for encapsulation in food applications, but polysaccharides are the most widely used these materials must be food grade, biodegradable, and able to form a barrier between the internal phase and its surroundings.

As is well known, the materials used in encapsulation are crucial for producing capsules that dissolve readily and are safe for human consumption. The materials that were chosen have to through with the consultant. Materials that can be used for encapsulation in the medical field include biocompatible materials such as polymers, lipids, and alginate. Microencapsulation is a technique that can be used to trap the material of interest inside a mixture of these materials. Excipients such as polymers (typically polyesters such as poly(L-lactic-co-glycolic acid) and polycaprolactone), lipids (typically phospholipids, triglycerides, and natural waxes), or insoluble metal salts and oxides such as silica, calcium phosphate, and calcium carbonate are used to prepare such particles that are typically used to prepare such particles.

In addition, the purpose of this study is as drug carriers in the medical field. These nanoparticles are designed using various components such as synthetic polymers, metal ions, oils, and lipids.

2.3.1 Starch

Starch as a natural polymer for the experiment's primary material. Starch is a naturally occurring polymer that is commonly found in grains, potatoes, and corn. Starch is long chain where it made up of many repeating pieces of a single molecule and it consist of glucose. Starch is a polysaccharide of glucose made of two types of α-d-glucan chains, amylose and amylopectin it can occur of form amylose is a linear or straight-line polymer that scientists describe as amorphous or solid (Sherrell, 2022, January 19). Furthermore, it also has been widely used especially in drug delivery applications, for a delivery in molecules and targeting to specific sites in body. Disintegrants make it possible for tablets and pills to dissolve into smaller pieces so that the drug can be absorbed. Starches also take in water quickly, which lets pills break up in the right way (Drugscom,2022).

2.3.2 Gelatin

Gelatin is natural biopolymer that is biocompatible. Biodegradable, and safe for use in food and medical application, where is derived from collagen that can obtained through thermal

denaturation or physical and chemical degradation process. However, gelatin have a deficiency that poor in mechanical strength and water resistance. Other than that, if gelatin combine with another polymer, it can overcome the limited of gelatin, resulted the improve the product and the crosslink are the substances that help to strengthen the gelatin by create chemical bonds between the gelatin molecules making it suitable for various application. Gelatin and alginate can form a well-proportioned aqueous solution and hydrogel, making them suitable for use as a cell matrix in tissue engineering applications. Gelatin is known for its thermally reversible gelling properties, making it a useful gelling agent for encapsulation and tissue engineering. Gelatin has been extensively studied in combination with alginate to create gelatin/alginate hydrogel systems for tissue engineering. The gelatin/alginate hydrogel system offers advantages such as sound biocompatibility, ease of gelation, and the ability to control the pH for drug delivery applications.

2.3.3 Alginate

Sodium alginate is a type of organic linear copolymer that is composed of linked ß-D-mannuronate and α-L-guluronate residues. It is commonly used in various applications due to its unique properties. Alginate can form a gel-like structure when it is cross-linked with calcium ions. This process is known as the "egg-box model" because the calcium ions fit into the spaces between the sugar units, creating a structure that resembles an egg carton. The cross-linking with calcium ions gives alginate its unique gel-like properties. Alginate has a wide range of applications due to its ability to form gels and its biocompatibility. It can be used as matrices for encapsulating living cells, providing a protective environment for the cells to survive and function. Alginate is also used in wound dressings, where it can create a moist environment that promotes wound healing. The gel matrix provides stability and support to the loaded beads, allowing for controlled release and potential uses as wound dressings.

2.4 Release Study

In this research the beads need to be release in over a period of time. The materials within the beads form the core, while the shell coating functions as a wall. These micron-sized of beads are designed for release under specific conditions. The beads process takes advantage of self-

assembly, which means that the beads are formed by the spontaneous organization of the constituent materials.

Release study of encapsulation materials can be engineered to react to specific pH levels, allowing for the controlled release of medications in specific body regions. Numerous studies have concentrated on the development of pH-sensitive encapsulation systems for drug delivery. These investigations examine the release of encapsulated drugs at various pH levels, including the acidic pH of specific organs such as the stomach and intestines. Based on the pH conditions at the target site, the objective is to obtain optimal drug release and therapeutic efficacy.

2.4.1 pH on Release Study

The pH has a significant effect on the alginate-gelatin gelatinization consequently, the viscosity of food products. In addition to textural and sensory qualities, the pH may affect the solubility, extractability, and denaturation of the proteins in food products (Moon Ho Do et al., 2020). Moreover, pH influences gelation under certain conditions, a lower pH is required for gelation. With increasing protein concentration and heating time, acid-induced why protein gelation could result in harder gels, which was explained by a higher conversion rate of native protein. Gel rigidity diminished below pH 4.3 (Wang Wen-qiong et al., 2021)

CHAPTER 3

MATERIALS AND METHOD

3.1 Materials

Methylene blue (Co533) as a powder and Sodium Alginate powder was purchased from Aldrich, Gelatin powder from bovine skin (G93910), was used and Calcium Chloride (CaCl2) from Merck, as crosslink to form macrospheres.

3.2 Preparation of Microspheres

The preparation of alginate-gelatin in encapsulation consists of methylene blue in crosslinking with calcium chloride. A 25 ml emulsion was prepared by dissolving 0.5 g of gelatin and 4% sodium alginate in distilled water. To achieve a uniform mixture (referred to as solution 'A'), the emulsion was continuously agitated using a magnetic stirrer for a duration of 2 hours. To create the gelatin-alginate composite beads containing methylene blue, 50 ml of methylene blue was introduced into solution 'A' and thoroughly mixed. The resulting suspension was subsequently dispersed as droplets in a 0.2 M CaCl2 solution, left undisturbed for 2 hours, and subsequently rinsed with deionized water.

Table 1: Average particle size and alginate/gelatin blend microparticles in different pH

Sample Name	Ratio of Sodium Alginate (Al) : gelatin (Gl) g	Calcium Chloride(ml)	Size of needles (G)	Release pH
20Al/80Gl	20/80	100	18	4
			24	7
			30	9
35Al/65Gl	35/65	100	18	4
			24	7
			30	9
50Al/50Gl	50/50	100	18	4
			24	7
			30	9

3.3 Characterization of Optical

The characterization of alginate-gelatin were examined and carried out by using 2 main machines, Fourier Transform Infrared Analysis (FTIR) and Ultraviolet Spectroscopy (UV-Vis) characterized the structural change and measure the absorption spectra of target substances.

3.3.1 Fourier Transform Infrared (FTIR)

Fourier transform infrared (FTIR) spectroscopy is a technique used to study the molecular structure of a macrospheres. When infrared radiation is passed through a bead of alginate, gelatin, 20Al/80Gl, 35Al/65Gl, and 50Al/50Gl exhibited absorption in macrospheres, which it passes through. The sample of macrospheres was then pressed under a pressure of 10,000 to 15,000 pounds per square inch using a special disc. FT-IR spectra were recorded in disc method and scanned at the resolution of 4.0 cm−1 over the wave number region of 4000−400 cm−1. The resulting spectrum represents the macrospheres sample in absorption and transmission. The spectrum can be used to identify the types to varying sample macrospheres blend ratios.

3.3.2 Particle size analysis

The beads was characterized by using image J. In order to analyze the particle sizing of a, minimum of 150 beads was calculated to obtain d_{ave} size of the beads.

3.3.3 UV-Vis

UV-V is spectrophotometry used to track the release of the methylene blue and compare it to a standard curve for release solution. Cumulative methylene blue release was found by dividing the total amount of drug released at a given time by the amount of drug that was in the macrospheres sample at the beginning. The methylene blue using a UV-Vis spectrophotometer at a wavelength of 668 nm. The concentration of the methylene blue model drug in the medium was obtained by referring to a predetermined methylene blue concentration UV-Vis absorbance standard curve. It used a machine called a UV-Vis spectrophotometer to measure the amount of drug in the solution at a specific wavelength

CHAPTER 4

RESULT AND DISCUSION

4.0 FTIR analysis

Alginate gelatin beads were characterized by FTIR analysis where its characterization on the chemical and functional group from the sample 20Al/80Gl, 35Al/65Gl, and 50Al/50Gl where 20Al/80Gl is referring to 20% of the alginate(Al) and 80% of the gelatin (Gl). Therefore, FTIR spectra show in figure 4.1 illustrated that the sample were almost identical which is indicated no change in the functional group.

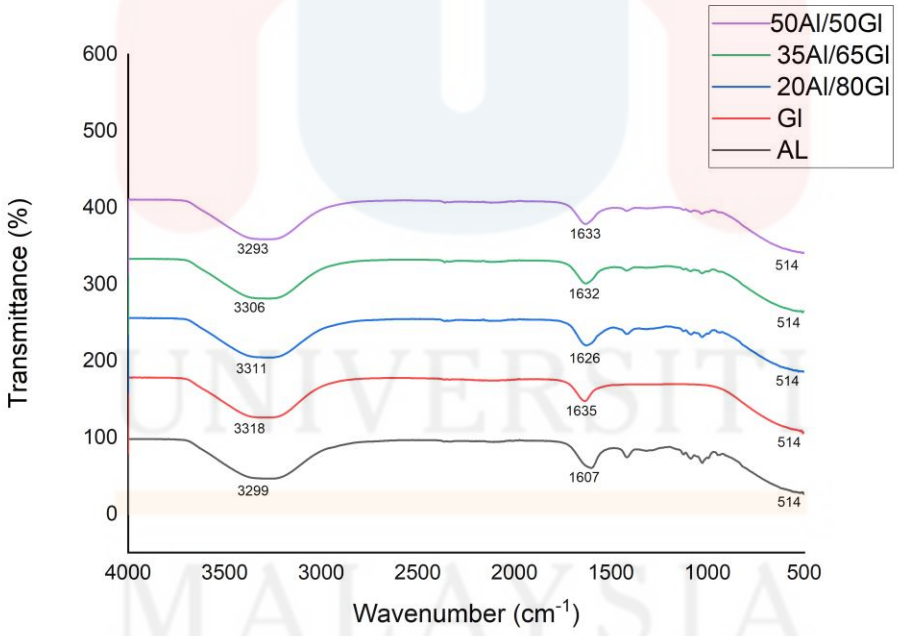


Figure 4.1 FTIR spectra of sample alginate, gelatin, 20Al/80Gl, 35Al/65Gl, and 50Al/50Gl

The provided FTIR peaks for alginate (Al) are represented by the colour black in figure 4.0. When the O-H stretching vibration is present, the peak that occurs at 3299 cm^{-1}, in the range 3000- 3500 cm^{-1} is typically associated with it. The presence of this peak is indicative of the presence of hydroxyl groups, which in turn indicates that alginate either contains a considerable amount of water or is in a state of being adequately hydrated. Due to the presence of the O-H stretching vibration peak, it can be deduced that alginate possesses hydrophilic properties, which is in line with its behavior as a material that forms hydrogels. Carboxylate groups COO- in alginate are responsible for the asymmetric stretching vibrations that are responsible for the peaks that take place at 1607 cm^{-1} in region 1000-2000cm^{-1}. Within the structure of alginate, the carboxylate groups are a component of the uronic acid units. Carboxylate functionalities are a distinguishing feature of alginate, and the presence of this peak is evidence that these functionalities are present. The presence of carboxylate groups and ether linkages, respectively, is confirmed by the peak at 514 cm^{-1}, which indicates that these essential structural components are present in alginate. The provided peaks, in general, are consistent with the characteristics that are anticipated to be present in an alginate FTIR spectrum. (Susiany Pereira Lopes et al., 2017)

Furthermore, the red line represents gelatin (Gl). The presence of N-H stretching vibrations in the amide A band is confirmed by the presence of amide groups within gelatin, as indicated by the peak at 3318 cm^{-1} in region of 3000-3500cm^{-1}, which is found in a sample of gelatin. Proteins are expected to have a peptide backbone structure, and this is consistent with that structure. Information regarding the conformation of the protein can be obtained from the peak that is located at 1635 cm^{-1}. This peak corresponds to the amide I band. Insights into the secondary structure of gelatin, such as the proportions of alpha-helices and beta-sheets, can be gained by analyzing the specific shape and position of this peak. The peak that is located at 514 cm^{-1} is most likely associated with bending vibrations. This peak provides information about the structural components of gelatin, which may be related to the C-O-C structure. On the whole, the peaks that have been provided are in agreement with what one would anticipate to find in a gelatin (Gl) FTIR spectrum (Dai et al., 2020).

Moreover, a sample 20Al/80Gl consisting of 20% alginate and 80% gelatin represents a line with a blue colour. The identification of gelatin in the mixture is confirmed by the peak observed at 3311 cm^{-1}, which corresponds to the N-H stretching vibration in the amide A bands.

This peak is present in the sample material with a composition of 20Al/80Gl. Due to the prominent peak, it is probable that the gelatin component is dominant in the mixture. This is due to the pronounced intensity of the gelatin content peak, which holds great significance. The existence of gelatin is additionally corroborated by the peak observed at 1626 cm^{-1}, which corresponds to the amide I band and signifies the stretching vibrations of the C=O bonds. This peak also yields information regarding the conformation of the protein. The unique attributes of this peak possess the capacity to offer valuable observations regarding the secondary arrangement of gelatin, particularly when examined within the framework of the mixture. The peak observed at 514 cm^{-1} may be attributed to the presence of alginate and gelatin, as it corresponds to a range of vibrations CONH2 group, indicating that the alginate is likely associated with gelatin. Overall, the observed peaks align with the expected properties of a blend of 20% alginate and 80% gelatin in the specified sample providing conclusive evidence of good molecular compatibility between the two polymers. (LOPES et al., 2017)

In addition, the sample of 35Al/65Gl indicated 35%alginate and 65%gelatin in line green can be utilized to analyze the relative intensity and position of these peaks, thereby facilitating comprehension of the interaction between the alginate-gelatin. The peak at 3306 cm^{-1} corresponds to the presence of that associated with the stretching vibration of the O-H (hydroxyl) bond. Hydroxyl groups are commonly present in polysaccharides like alginate and proteins like gelatin, and they play a significant role in determining the overall structure of these molecules. By examining the occurrence and intensity of this peak, one can gather data on the hydrogen bonding and water content of the sample. Meanwhile, the presence of the peak at 1632 cm^{-1} in the amide I band stretching vibrations indicates the occurrence of C=O stretching vibrations in proteins. Gelatin, being a protein derived from collagen, contains amide groups in its structure. The secondary structure of the proteins in the sample can be understood by analyzing the exact position and shape of this peak, which showed minimal changes after the alginate-gelatin mixture was formed. The peak at 514 cm^{-1} is likely associated with a distinct vibration in the polysaccharide component, which could be attributed to the stretching of the C-O-C glycosidic linkage in alginate. Additionally, it is plausible that the phenomenon is linked to specific vibrations within the gelatin component that are correlated with bending or stretching. A robust correlation exists between the skeletal vibrations of carbohydrates and proteins and the peaks that manifest in this particular region. Based on the observed peak shifting in the FTIR spectra, it concluded that the gelatin and

alginate formed weak hydrogen bonds. This conclusion was reached because the formation of strong hydrogen bonds leads to peak shifting due to changes in electron density at the hydrogen-bonded site (Dai et al., 2020).

In addition, the sample consisting of 50Al/50Gl, which indicate 50%alginate and 50%gelatin in the line purple reaches its maximum intensity at a wavenumber of 3293 cm^{-1}. The peak exhibits a robust correlation with the stretching vibration of the O-H (hydroxyl) bond. The presence of hydroxyl groups in both alginate and gelatin molecules in the sample may be the cause of this connection. By analyzing the intensity and position of the peak, it is feasible to gather information about the hydrogen bonding and water content in the sample interactions between 50Al/50Gl. The presence of the peak at 1633 cm^{-1} in the amide I band stretching vibrations suggests the occurrence of C=O stretching vibrations in proteins. Gelatin, being a protein, inherently contains amide groups in its structure. Obtaining the exact position and composition of this peak can provide a more comprehensive comprehension of the secondary structure of the proteins found in the sample. It may offer information about the gelatin component in this case. The peak at 514 cm^{-1} likely associated with a distinct vibration in the polysaccharide component, which could be attributed to the stretching of the C-O-C glycosidic linkage in alginate. Additionally, it is plausible that the phenomenon is linked to specific of bending or stretching within the gelatin component. A strong correlation exists between the carbohydrates and proteins in their skeletal structure and the occurrence of peaks within this specific region. The peaks can be analyzed to comprehend the interaction between the two components by considering the 50Al/50Gl ratio of the sample (Afzal et al., 2018).

4.2 Beads Characterization

The parameters used in alginate-gelatin such as diameter, ratio, and release methylene blue properties of the methylene blue-entrapped gelatine-alginate beads were determined in order to understand the physical properties of the beads in figure 4.1.

Table 4.1: Average particle size and alginate/gelatin blend microparticles

Sample Name	Ratio of Sodium Alginate (Al) : gelatin (Gl) g	Calcium Chloride(ml)	Size of needles (G)	Release pH	Size beads before release (mm)	Size beads after release (mm)	Weight beads before release (g)	Weight beads after release (g)
20Al/80Gl	20/80	100	18	4	9.63	8.33	2.33	1.14
			24	7	7.01	6.87	1.41	0.73
			30	9	6.15	-	1.55	-
35Al/65Gl	35/65	100	18	4	9.47	8.75	2.72	1.96
			24	7	7.03	6.89	1.71	1.51
			30	9	6.50	5.6	2.03	1.35
50Al/50Gl	50/50	100	18	4	9.05	7.52	2.64	1.22
			24	7	7.10	6.58	2.60	1.59
			30	9	6.87	5.57	1.85	1.432

4.3 Effect of Bead Size

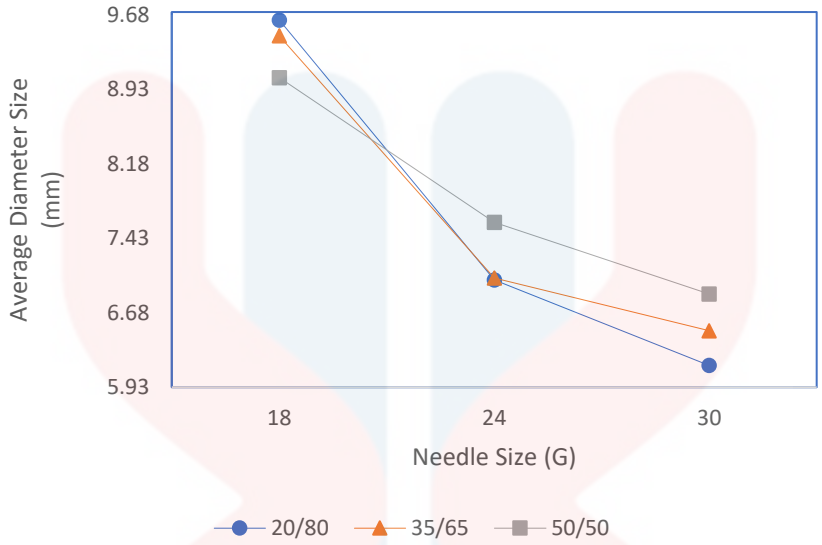
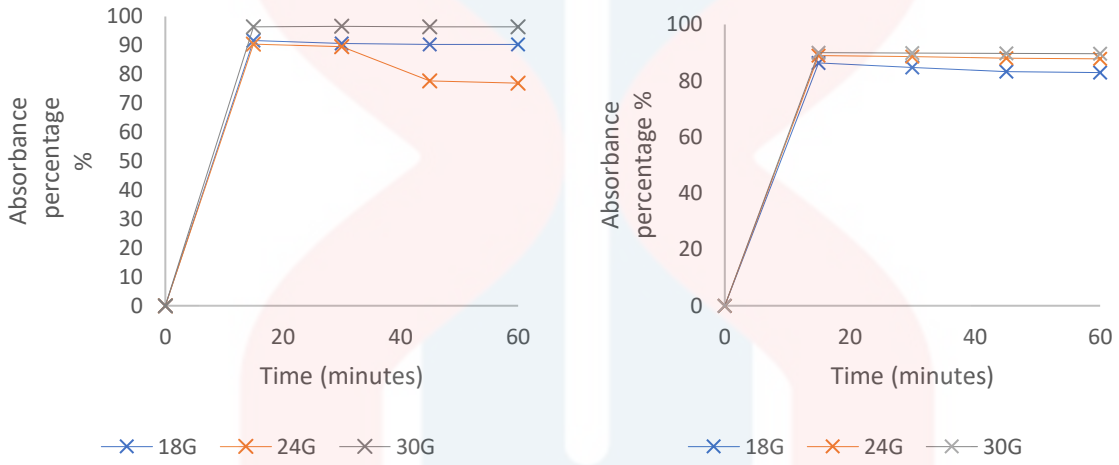


Figure 4.3 alginate-gelatin beads show the effect of needle size

Figure 4.3 demonstrates the effect of different needle sizes (18G, 24G, and 30G) on the average size of beads. It is shown that the needle size influences on the particle size of the beads. It is also shown that using an 18G, 24G, and 30G syringe, found that all forms of gel beads have the same external characteristics that are round and smooth and shiny surface. The use of an 18G needle resulted in the production of beads with a diameter of approximately 9.63 mm. In contrast, the use of a 24G needle resulted in beads with a diameter of approximately 7.01 mm, and a 30G needle generated beads with a diameter of approximately 6.15 mm. The presence of surface tension in the alginate-gelatin suspension is responsible for the decrease in bead size which be attributed to the usage of a smaller syringe size attract the drop, the drop will accumulate on the needle until its weight is balanced by the surface tension, at which point it will fall. (Hamed et al., 2017).

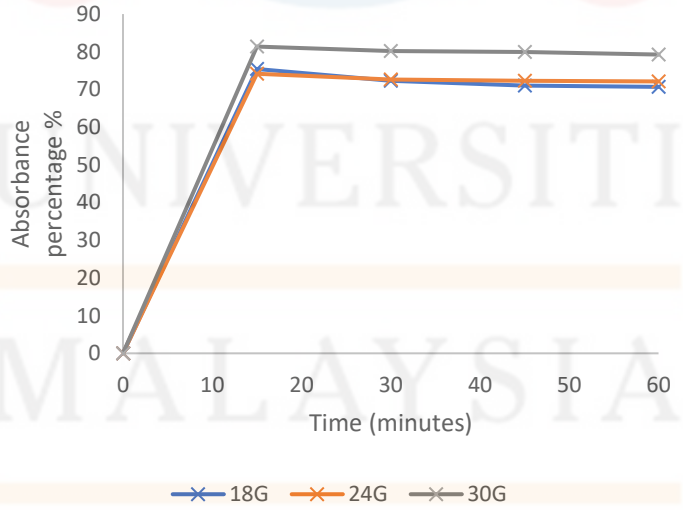
4.4 Release study

Data that present figure 4.4 shows the effect of release study on methylene blue with a different sample namely 20Al/80Gl, 35Al/65Gl, and 50Al/50Gl.



a)20Al/80Gl

b)35Al/65Gl



c)50Al/50Gl

Figure 4.4 release study of sample alginate-gelatin a)20Al/80Gl b)35Al/65Gl c)50Al/50Gl crosslinked with calcium chloride

Due to the hydrophilic qualities of both gelatin and alginate polymers, the medium can move more easily inside and outside of the particle. The information is derived from graph A, which illustrates that the liberation of the drug from the 20Al/80Gl sample necessitates the process of diffusion into the surrounding medium. To examine of methylene blue release shown in graph A, it measured the quantity of absorbance release in discharged during a 60-minute timeframe. Excel was utilized to generate a linear graph, enabling the calculation of the release from the 20Al/80Gl system (Figure 4.4). After a duration of 20 minutes, the discharge of methylene blue was observed to be uniform, and the spectrophotometric analysis indicated that the discharge was both regulated and consistent over the course of 60 minutes. Within the initial 30 minutes, it is evident that the needles with gauges 18G, 24G, and 30G have achieved a level exceeding 90%. After a duration of 60 minutes, the percentage of absorbance released was determined to be 96.45% for 30G needles, 90.39% for 18G needles, and 76.98% for 24G needles. This phenomenon occurs because the drugs release from the nano system necessitates the diffusion of the release medium into the nanoparticle and subsequent dissolution of the beads, which are subsequently released into the surrounding environment. In addition, gelatin and alginate polymers possess hydrophilic characteristics, facilitating the movement of the medium into and out of the nanoparticle (Eun Mi Lee et al., 2014).

Moreover, in the case of the (figure 4.4 B) sample, the 35Al/65Gl sample exhibits identical outcomes as the sample, specifically the discharge of methylene blue at needles 30G with an absorbance of 89.64% as measured by the UV-vis spectrometer. It was observed that needle 30G exhibited a slower release rate over a 60-minute time period. The increase in the ratio of sodium alginate had a significant effect on the modulation of drug release, as shown in Figure 4.4 B. Utilizing alginate with a high concentration of guluronic acid will enhance the gelling properties. Figure 4.4 B illustrates that despite increasing the ratio of sodium alginate, the drug administration was sustained for a significant duration. The drug release rate decelerated as the sodium to alginate ratio was raised. Increasing the polymer ratio resulted in a notable deceleration in the release of methylene blue. Nevertheless, there was minimal disparity observed in the release patterns (Kesavan et al., 2010).

Regarding the graph C sample of 50Al/50Gl, the release from the alginate-gelatin beads exhibited a sustained release pattern with a decreased initial release, which was the desired outcome. When compared to the 18G and 24G needles, the needle under consideration retains around 72% of the loaded protein. However, only the release of absorbance was observed after 60 minutes. In contrast, 72% of the loaded protein was released within the initial 30-minute period. The absorption of needles with a concentration of 30G exhibits a 70% release within the initial 30 minutes. Furthermore, the sample beads with a composition of 50Al/50Gl demonstrate a continuous release at 60 minutes, with an absorption rate of 79%. One can infer that the 30G needle demonstrates a slower release rate when compared to the 18G and 24G needle sizes.

Based on this observation, it can be concluded that the 30G needle size exhibits a more effective slow release within 60 minutes compared to the 18G and 24G needles for these three combinations. For the ratios Al20/Gl80, Al35/65Gl, and Al50/Gl50, it is recommended to use a needle size of 30G. This is because using a 30G needle size usually results in a 75% higher release of absorbance. This is in opposition to the 18G and 24G needle sizes. This phenomenon occurs due to the cohesive properties of gelatin and alginate, which are influenced by the concentration of the alginate solution used to create the beads. The increased ratio of gelatin beads can be attributed to the synergistic gelling effect of gelatin and alginate. As a consequence, the structure becomes more expanded and porous, facilitating the release of methylene blue from the beads. The 20Al/80Gl sample is considered a slow standard with a purity of 96.45%, and it is approaching a purity level of 100% (Indumathi Sathisaran & Balasubramanian, 2020).

4.5 Effect pH Release

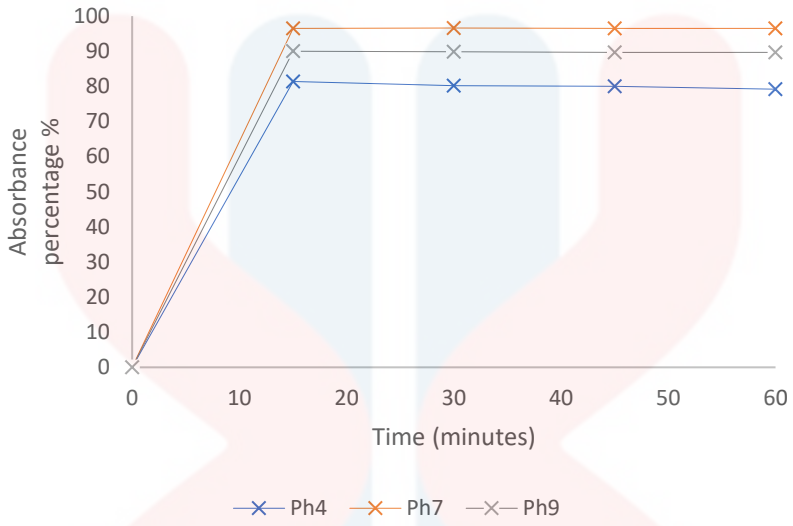


Figure 4.5 Release of pH for needle 30G

Conjugated delivery systems refer to a type of drug delivery system where methylene blue are attached or linked to a polymer. This allows for controlled of methylene blue release, meaning that the amount of release can be adjusted by changing the ratio of drug to polymer. The methylene blue is loaded onto the polymer by forming covalent bonds between the drug and the polymer. Reactive sites are specific locations on the polymer where the drug can form covalent bonds. The more reactive sites there are, the more drug can be loaded onto the polymer. By selecting specific covalent bonds between the drug and the polymer, the drug release can be controlled in response to certain stimuli. Stimuli-responsive drug release means that the drug is released from the polymer in a controlled manner when exposed to specific conditions or triggers. For example, hydrazone bonds are a type of covalent bond that show strong stability under a neutral pH environment but can undergo hydrolysis in a lower pH environment. This property allows for release in a lower pH environment, such as in the acidic conditions of certain tissues or cellular compartments.

The effect of pH variation on the release of beads, which are created through the interaction of sodium alginate and gelatin. The experiment involved using solutions containing sodium alginate and gelatin in to assess the impact of pH on the release of beads. The absorbance (%) represents the proportion of the beads in solution that release a specific pH value. The data was graphed to illustrate the correlation between pH and absorbance percentage (%). The figure displays the plot, which aids in displaying the trend. The absorbance (%) was measured at pH 4, pH 7, and pH 9. The optimal pH for the interaction between sodium alginate and gelatin in the release beads was found to be 7. The solution's pH is a critical factor in determining the degree of release

As it can see from figure 4.5 pH 7 show the result cumulative drug release of alginate-gelatin, were tested in phosphate buffer solution (PBS) pH 4, pH7, and pH 9 during 60 minutes, as shown in figure. 4.5. The cumulative release of beads alginate-gelatin pH 4, ph7, and pH9 from beads presented a constantly and steadily release during the first 30 minutes, which reached absorbance release at 80%. After 30 minutes, pH 4 only release at 79% and pH 9 absorbance release at 89% last but not least at pH 7 the drug release became more steady and slower, and the final release rate came up to 96%. Conversely, the initial release of alginate-gelatin during the first 30 minutes demonstrated an obvious burst release for the cumulative release above was 50%. It speculates that the sample was readily diffused from the aqueous network structures, which led to the burst release in the initial period. Compared with pH4 and pH9 the Ph7 exhibited a slower release that because, which is considered neutral, both gelatin and alginate are likely to be in a state conducive to gel formation. Gelatin may start to undergo conformational changes and gelation. Alginate, being stable in this pH range, can form a stable gel network in the presence of calcium ions. The combination of gelatin and alginate gelling effects might result in a slower

release of substances, as the gel matrix forms and hinders rapid diffusion. Furthermore, the macrosphere exhibits a greater water content. The amorphous region creates a void space that allows water to enter the alginate-gelatin beads, it allowing being released of methylene blue through diffusion into the surrounding environment. (Murat İnal et al., 2017). At pH 9, Alginate and gelatin separate in alkaline environments. This dissociation weakens the physical forces that bind gelatin and alginate polymers. These physical forces weaken over time. Thus, gelatin and alginate polymers lose adhesion, and deteriorating beads. Bead degradation is the disintegration of beads. Macrosphere degrade when gelatin and alginate polymers lose intermolecular forces. This degradation is more noticeable in alkaline pH. Degradation destroys macrosphere structure and expandability since pH becomes more basic, alginate-gelatin microsphere became shrink. (Indumathi Sathisaran & Balasubramanian, 2020)

Moreover, the result show in pH 4 and pH9 it indicated faster release that because lose its gel-like structure due to the protonation of amino acid residues. Alginate gelation might also be less effective in an acidic environment, leading to a less stable gel network. The weakened gel matrix and potential degradation of the gelatin structure can result in a faster release of substances due to increased permeability. In summary, the initial burst release is attributed to sample diffusion from aqueous networks. At pH 7, a slower release occurs due to the conducive neutral environment for gel formation, with gelatin and alginate creating a stable gel matrix. The macrosphere's greater water content and amorphous region enhance water entry, facilitating controlled release through diffusion. In contrast, at pH 4 and pH 9, a faster release is observed as gelatin loses its gel-like structure in acidic conditions, reducing alginate gelation effectiveness and destabilizing the matrix, leading to increased permeability. These findings provide insights into pH-dependent controlled release mechanisms. (Li et al., 2020).

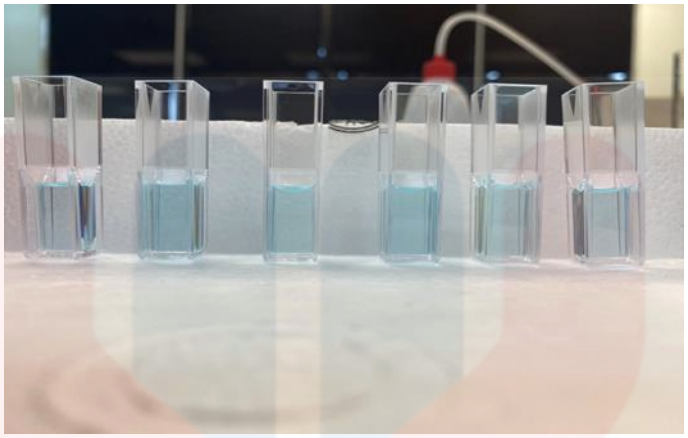


Figure 4.5: Release Methylene Blue

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Macrospheres of methylene blue as model drug, were successfully prepared from the materials used as alginate and gelatin that crosslinked with calcium chloride using an environmentally friendly and green chemistry approach that is by using the ionic liquid. The macrospheres was describe by analysis in diameter beads(Image J), FTIR, UV-vis, and it showed different patterns of drug delivery release in different ratio and pH.

For Image J in macrospheres, indicated the impact of needle size (18G, 24G, and 30G) on bead size in an alginate-gelatin suspension. The experiment revealed that needle size significantly influences the average beads diameter, with 18G resulting in approximately 9.63 mm, 24G in 7.01 mm, and 30G in 6.15 mm beads. Despite the variations, all beads exhibited consistent external characteristics round, smooth, and shiny surfaces. The observed decrease in bead size is attributed to surface tension in the suspension. In summary, needle size plays a crucial role in controlling bead dimensions during the production process.

Based on the FTIR spectroscopy, In conclusion, the FTIR analysis of alginate (Al) and gelatin (Gl) individually, as well as their blends in different ratios (20Al/80Gl, 35Al/65Gl, and 50Al/50Gl), reveals distinctive peaks indicative of their molecular characteristics. Alginate exhibits peaks associated with O-H stretching vibrations and carboxylate groups, consistent with its hydrophilic nature and unique structural features. Gelatin displays peaks corresponding to N-H stretching vibrations, providing insights into its peptide backbone and secondary protein structure.

The blends (20Al/80Gl, 35Al/65Gl, 50Al/50Gl) showcase peaks characteristic of both polymers, suggesting good molecular compatibility. The observed shifts and intensities in certain peaks indicate weak hydrogen bond formation between gelatin and alginate in the blends. Overall,

FTIR analysis provides valuable information about the molecular interactions and compatibility of alginate and gelatin in different ratios, crucial for understanding their potential.

UV-vis, in methylene blue release studies on alginate-gelatin bead formulations (20Al/80Gl, 35Al/65Gl,and 50Al/50Gl) reveal that the hydrophilic nature of both polymers facilitates effective diffusion of the drug into the surrounding medium. Needle sizes (18G, 24G, and 30G) significantly influence the release patterns. The 20Al/80Gl combination shows uniform and regulated release over 60 minutes, with 30G needles exhibiting the highest release (96.45%). The 35Al/65Gl sample demonstrates modulation in methylene blue release, with 30G needles showing a slower release rate. The 50Al/50Gl combination exhibits methylene blue release with 30G needles providing a slower release yet effective release. Overall, the study suggests that for these formulations, a 30G needle size is optimal for achieving controlled and methylene blue release over 60 minutes.

Furthermore, in delivery systems involving methylene blue linked to polymers enable controlled drug release, allowing adjustment of release amounts by varying the drug-to-polymer ratio. Covalent bonds formed between the drug and polymer, alginate gelatin where it offer controlled release in response to specific conditions. pH variation significantly influences the release of alginate-gelatin beads, with optimal release observed at pH 7. The study demonstrates that the pH-dependent release mechanism plays a crucial role, with a slower release at neutral pH due to the conducive environment for gel formation. Conversely, faster releases at acidic or alkaline pH result from destabilized gel-like structures, impacting the gelation effectiveness of alginate.

To sum up, it can be concluded that alginate-gelatin was the most successful treatment with drug release because it has biocompatible and biodegradable that suits to the real properties of the macrospheres. Hence, the regenerated materials of alginate-gelatin have been obtained in this study and the characterizations also have been analyzed by Image J, FTIR, and UV-vis. Hence, from all of the characterizations of combination those material can be correlate to the real properties and characteristics of alginate-gelatin. Thus, the purpose of this study was achieved.

For the recommendation for this study, to enhance the macrospeheres, further investigation into material modification is recommended in the results of this study. This action would be taken to enhance the characterization characteristics of the beads. It could potentially be employed to investigate various cross-linking agents or techniques for augmenting the alginate-gelatin

network's structure. Furthermore, it is feasible to fabricate beads whose expansion can be controlled in accordance with ratio and pH, respectively. This will facilitate our comprehension of the factors that impact the beads properties, as well as the techniques through which these properties can be altered to suit the needs of cosmetics, food supplement, medication delivery, and fertilizer.

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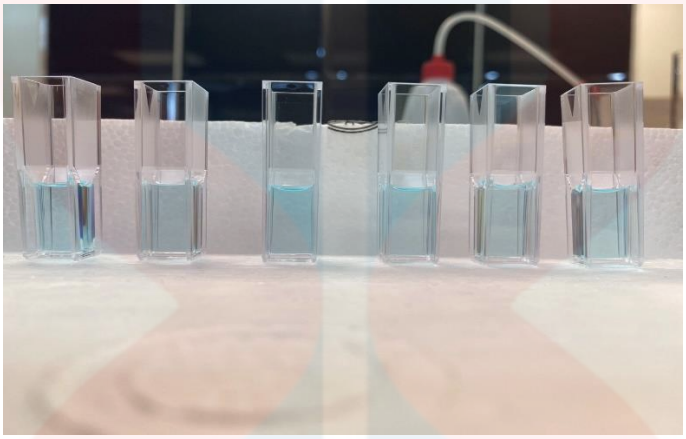
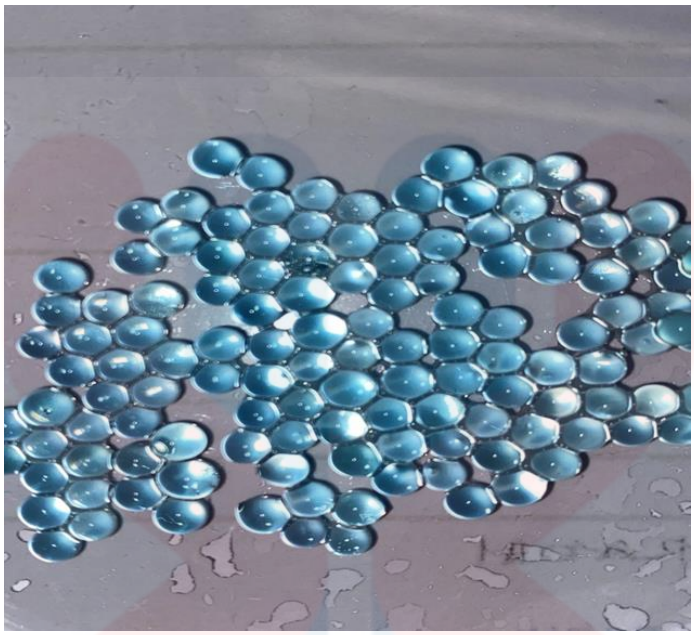
APPENDIX



A1: Release Methylene Blue

A2: pH paper for release study



A3: Macrosphere alginate-gelatin entrap methylene blue