DEVELOPMENT OF AN IONTOPHORESIS SYSTEM FOR TARGETED AND EFFECTIVE TRANSDERMAL DRUG DELIVERY

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CERTIFICATE

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ABSTRACT

The current deals with the Iontophoretic transdermal drug delivery system. Effective, non-invasive and targeted drug delivery is a critical challenge in modern medicine. It plays a vital role in optimizing therapeutic outcomes, minimizing side effects, and enhancing patient comfort and compliance. One promising approach to address this challenge is through the application of **iontophoresis**, a technique that utilizes mild electrical currents to drive the transport of charged drugs through biological barriers such as the skin, leading to non-invasive treatment for chronic epidermal diseases. Gelatin-sodium alginate hybrid hydrogel has been used as a drug carrier material. Three hydrogel films, i.e., Gelatin, Sodium Alginate and Gelatin-Sodium Alginate hybrid hydrogel have been synthesized and characterized through XRD, FTIR, SEM and EDX. In order to make this hydrogel conduvctive, so that it may respond to external stimuli, i.e., voltage, carbon nanotubes have been added in it. Mechanical properties of these hydrogels have been evaluated through the Tensile Test, and an approximately 7% increase in the strength of sodium alginate is seen after being blended with the gelatin, and by cross-linking between the chains of gelatin and sodium alginate using Calcium Chloride. Hybrid hydrogel has been coated over copper electrodes to be used as a cathode material in drug delivery systems. Moreover, the coating of hydrogel over copper electrode has been evaluated through Electrochemical Impedance Spectroscopy. Finally Drug Release Tests have been evaluated through UV-VIS spectroscopy. Drug release pattern indicated that drug release rate can be enhanced proportionally, upon application of DC voltage/current.

DEDICATION

With deep respect,

We dedicate this endeavor to our beloved parents, respected instructors, and lab engineers, whose leadership and unwavering support encouraged us during the entire project.

ACKNOWLEDGEMENTS

We are really grateful to Allah, the Most Gracious, for enabling us to do exceptionally well on our final year design project. We sincerely thank our supervisor, Dr. Usman Liaqat, for his unwavering support and direction, which were crucial to the project's successful completion. We could not have accomplished this difficult task to the best of our abilities without his commitment. We also thank our co-supervisor, Dr. Waheed Miran, whose insightful recommendations substantially advanced our comprehension and problem-solving abilities. We also appreciate the lab engineers, attendants, and research students for helping us become familiar with the equipment and processes that are necessary for our project. We would like to sincerely thank all of the Materials Engineering department's instructors and staff for their consistent support and direction during this journey.

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CHAPTER 1

INTRODUCTION

1.1 Background

Iontophoresis is a process in which an ionic drug is delivered inside the body through the skin, using a mild electric current [1]. Because of the non-invasive nature of this process, it is highly patient compliant as compared to other conventional drug delivery techniques e.g. surgery and oral medication etc. [2]. Iontophoresis being a targeted drug delivery system enhances the drug efficacy by passing the body metabolism. Moreover, it leads to reduced side effects a drug may have while circulating throughout the body, through blood. The exceptional barrier properties of the stratum corneum, which is the outermost layer of the skin, pose a significant challenge for delivering drugs through the skin [3]. Iontophoresis offers a solution for transdermal drug delivery by employing a mild electric current to transport charged molecules. In Figure 1, a positively charged drug is being attached to an anode and negatively charged buffer ions are being attached to the cathode. Upon oxidation of an anode through the battery, drug molecules are repelled and then pass through the skin. Simultaneously negatively charged buffer ions are repelled by the cathode and travel towards the anode by passing through the skin. During this process, the drug performs its action, and the excess drug is captured by the cathode through the electrostatic force of attraction. The remaining drug which is captured by the cathode can be delivered again into the body by reversing the terminals of the battery.

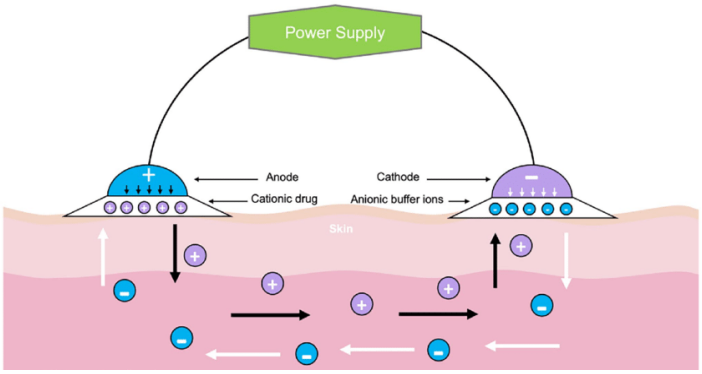


Figure 1: Schematic Diagram of Iontophoresis

This innovative delivery method is non-invasive and offers numerous advantages over traditional passive transdermal administration. These include on-demand drug release into the skin and improved control over dosage.

Hermann Munk is credited with one of the earliest instances of utilizing electricity for medication transfer, dating back to approximately 1879. Following a 20–25-minute exposure to an electrically charged solution of strychnine, Munk noted cramps in rabbits [1]. Nearly four decades later, Stéphane Leduc detailed techniques for employing electric current to deliver salicylic acid, aiming to relieve pain and accelerate wound healing.

1.2 Issues in conventional drug delivery systems

Conventional drug delivery systems, such as oral medication or surgical procedures, face several challenges. Oral medications often encounter barriers such as degradation in the gastrointestinal tract, variable absorption rates, and first-pass metabolism in the liver, which can reduce their effectiveness. Additionally, surgical procedures are invasive and carry risks such as infection, bleeding, and prolonged recovery times. Iontophoresis offers a non-invasive alternative that bypasses many of these limitations. By utilizing a low-intensity electric current to drive charged molecules across the skin, iontophoresis enables direct delivery of drugs into the bloodstream, avoiding degradation in the digestive system and bypassing first-pass metabolism. This approach allows for more precise control over drug dosage and can facilitate the delivery of larger molecules that may not be suitable for oral administration. Furthermore, iontophoresis typically results in rapid therapeutic effects. Psoriasis affects about 2-3% of the global population, amounting to over 125 million people worldwide. Iontophoresis can be used to deliver corticosteroids and other medications to manage psoriasis and other skin conditions, though the effectiveness varies depending on the individual and the severity of the condition.

1.3 Problem Statement

Currently, chronic diseases are the leading cause of adult mortality in nearly all nations, and projections suggest a further 17% increase over the next decade. Globally, approximately one-third of adults experience multiple chronic conditions. In the United States, six out of ten

adults have at least one chronic disease, while four out of ten have two or more. This alarming hike in chronic diseases requires a more effective and patient-friendly drug delivery system. Iontophoresis stands out as the most effective way to treat such chronic diseases by delivering medicine locally through the skin. For example, Psoriasis (A chronic skin condition characterized by the rapid buildup of skin cells, leading to thick, red, scaly patches) affects about 2-3% of the global population, amounting to over 125 million people worldwide. Iontophoresis can be used to deliver corticosteroids and other medications to manage psoriasis effectively. [Global Report on Psoriasis, WHO]. Similarly, Iontophoresis can deliver analgesics and anti-inflammatory drugs directly to the site of pain, making it a valuable tool in the management of localized pain conditions such as tendonitis and bursitis.

1.4 Working Principle

The working principle of our proposed Iontophoresis system can be understood with the help of a schematic diagram of a Galvanic cell in which CNTs + drug-containing hydrogel-coated electrode is used as an anode. Upon oxidation of an anode through the battery, the negatively charged Dexamethasone drug is released into the PBS solution through the electrostatic force of repulsion as well as hydrogel degradation.

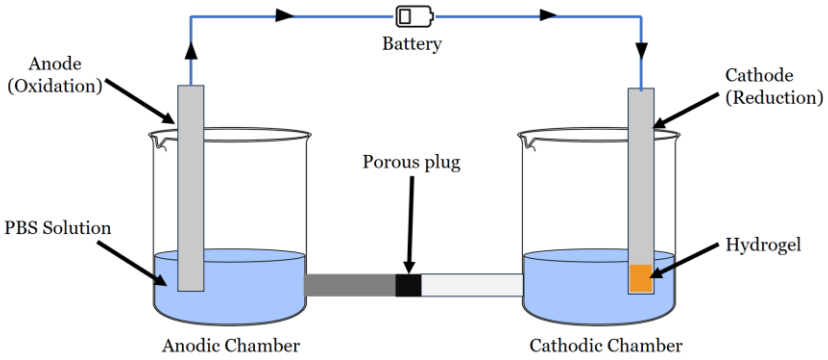


Figure 2: Working Principle of Iontophoresis

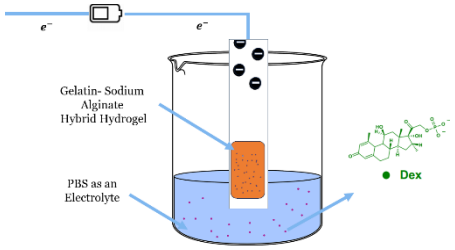


Figure 3: Drug Release in PBS solution

The amount of drug being released into PBS solution has been measured in a regular interval of time, through UV VIS spectroscopy.

1.5 Objectives

The objectives of our project are as follows.

1) Synthesize a conductive drug carrier material
2) Coat that drug carrier material (hydrogel) over a conductive electrode
3) Design an iontophoresis system on a lab scale.
4) To improve drug delivery efficiency by optimizing iontophoretic parameters such as voltage, current, and duration of application.

1.5.1 How to achieve these objectives?

The foremost task is to synthesize drug carrier material. We have prepared Gelatin-Sodium Alginate hybrid hydrogel for drug encapsulation.

This hydrogel is non-conductive. To make it conductive to let it respond to external power supply, Carbon nanotubes have been added in it [1]. Secondly, any charged drug needs to be encapsulated inside which will be released into the PBS solution upon application of power supply. Thus, we encapsulate Dex in hybrid hydrogel, an anti-inflammatory drug that carry negative charge.

The drug carrier material needs to be coated over an electrode. Copper electrode has been prepared through cold mounting and hybrid hydrogel has been coated copper electrode, through casting. Finally, the electro-stimulated release test will be carried out in an electrochemical cell in which Dex containing Gelatin-Sodium Alginate hybrid hydrogel coated over copper electrode will be used as a cathode material. Upon its reduction, this negatively charged drug will be released in a PBS solution and its concentration concerning time will be measured through UV-VIS spectroscopy [2].

CHAPTER 2

LITERATURE REVIEW

2.1 Drugs used for Iontophoresis process

Among the diverse range of medications used in iontophoretic therapy, local anesthetics, opioids, and steroids stand out for their ability to provide targeted pain relief and reduce inflammation, making them particularly beneficial in managing conditions such as arthritis and postoperative pain. Non-steroidal anti-inflammatory drugs (NSAIDs) are similarly effective in alleviating inflammation and pain, often used in sports injuries and chronic musculoskeletal disorders. Additionally, iontophoresis has proven valuable in the administration of antibacterial, antifungal, and antiviral drugs, offering a non-invasive alternative to oral or intravenous routes, particularly for localized infections and skin conditions. The technique's application extends to oncology, where anti-cancer drugs can be delivered directly to the tumor site, potentially enhancing therapeutic outcomes while minimizing systemic side effects. Fluorides, commonly used in dental care, also benefit from iontophoretic delivery, thereby improving the prevention and treatment of dental caries. We've chosen Dexamethasone sodium phosphate, an anti-inflammatory drug that carry negative charge for this project [4].

2.2 Drug Carrier Material

A literature review found out that researchers have used following drug carrier materials [5].

1. Poly (ethylene glycol) hydrogel
2. Silicon nanoneedles array
3. Poloxamer hydrogel
4. PEDOT functionalized gold electrode
5. AM-co SV hydrogel

We have exploited Hydrogels for the purpose of drug carrier material. Researchers have used different hydrogels for the purpose of Iontophoretic transdermal drug delivery [4]. In the very first research paper we've studied, Mg electrode has been used as an anode material

and viologen-based hybrid hydrogel (Acetalamide and Styrene bipyridine) as a drug reservoir as well as cathode material [4]. The delivery dosage was manipulated by optimizing parameters such as viologen content in a hydrogel and intensity and duration of electric current.

After further research, we found out that Gelatin-Sodium Alginate hydrogel is a potential candidate to be used for this purpose [5].

2.3 Making Hydrogel Conductive

Gelatin-Sodium Alginate hydrogel does not conduct any electricity. Following are the ways through which Gelatin-Sodium Alginate hydrogel can be made conductive so that it responds to external power supply.

(1) By incorporating nanoparticles like gold, silver, or copper can significantly increase the electrical conductivity of hydrogels [6].
(2) Ionically conductive hydrogels: Adding salts such as sodium chloride (NaCl) or lithium chloride (LiCl) can enhance the ionic conductivity of hydrogels [7]. The presence of ions facilitates the movement of electric charge through the gel.
(3) Using conductive crosslinkers during the hydrogel formation process can create a network that facilitates electron or ion transport. For example, crosslinking with boronic acid derivatives can enhance conductivity.
(4) Single-walled or multi-walled CNTs can be embedded in hydrogels, enhancing electrical conductivity and mechanical properties.

We added CNTs in Gelatin-Sodium Alginate hydrogel in order to make it conductive. Carbon nanotubes are composed of graphene sheets rolled into cylindrical shapes. They exhibit exceptional electrical conductivity due to the presence of π-electrons that can move freely along the length of the nanotube [7]. They provide continuous pathways for electron transport. This means that electrons can move through the CNT network within the hydrogel, facilitating electrical conductivity.

2.4 Enhancing Mechanical Strength

Gelatin Sodium-Alginate hydrogel lacks mechanical strength which pose it's limitations to use in various iontophoretic applications. This hydrogel does not have good mechanical properties. Through literature review, we found out that there are many physical and chemical cross-linking methods to increase the strength of this hydrogel.

2.4.1 Physical Cross linking

Gelatin is a protein which is composed of amino acids sodium alginate is a polysaccharide that contain carboxyl functional group. They can undergo ionic interactions in the presence of multivalent ions (e.g., calcium ions). Adding a divalent cation source, such as calcium chloride ($CaCl_2$), to the hydrogel precursor solution induces the formation of physical crosslinks between the polymer chains. This process is reversible and does not involve covalent bond formation, allowing for tunable gelation kinetics and mechanical properties. Amount of cross-linking agent to be added has a significant impact on mechanical properties of this hybrid hydrogel.

2.4.2 Photo cross linking

Photo crosslinking methods involve the use of photo initiators and light exposure to induce crosslinking reactions in hydrogel precursors containing photoreactive functional groups. Gelatin-sodium alginate hydrogels can be photo crosslinked using photo initiators such as riboflavin or photoreactive groups (e.g., methacrylate groups) incorporated into the polymer chains. Upon exposure to UV or visible light, these groups initiate crosslinking reactions, leading to the formation of covalent bonds and increased mechanical strength.

2.4.3 Chemical Cross linking

Chemical crosslinking involves the formation of covalent bonds between polymer chains using crosslinking agents. For Gelatin-Sodium Alginate hydrogel, common crosslinking agents include glutaraldehyde, genipin, and carbodiimides etc. These agents react with

functional groups present in gelatin (amino groups) and sodium alginate (carboxyl groups), forming stable covalent bonds that link the polymer chains together. Chemical crosslinking typically results in hydrogels with higher mechanical strength and stability compared to physically cross-linked hydrogels [5].

We physically cross linked our hydrogel by means of Ca^{+2} with $CaCl_2$ being used as a cross-linking agent.
Moreover, a specific reported ratio of Gelatin and Sodium Alginate (1:0.8) to be used for this purpose enhances the mechanical properties of this hybrid hydrogel significantly.

After defining the scope and objectives of our project, the next task was to synthesize Gelatin, Sodium Alginate and Gelatin-Sodium Alginate hybrid hydrogel. Protocols for the synthesis of these hydrogels have been found after a thorough literature review. Moreover, XRD, FTIR and SEM results of our prepared hydrogels have been matched with those in the literature to confirm the successful synthesis of our hydrogel.

2.5 Drug Release from Hydrogel Matrix

In order to monitor the amount of drug being released from a hydrogel matrix, there are many ways that we found through literature review. Let's jump into them one by one.

2.5.1 Ultraviolet-Visible (UV-Vis) Spectroscopy

UV-Vis spectroscopy is commonly used to monitor drug release from hydrogels by measuring the absorbance of the drug at specific wavelengths. As the drug diffuses out of the hydrogel matrix into the release medium, its concentration can be quantified by comparing the absorbance readings to a standard calibration curve.
Standard calibration curve is actually drawn to establish a relationship between absorbance of specific wavelength of light with the concentration of drug present inside the solution.

2.5.2 High-Performance Liquid Chromatography (HPLC)

In the context of drug release from hydrogels, samples from the release medium are injected into the HPLC system. The drug is separated from other components and detected, typically using UV detection.

2.5.3 Fluorescence Spectroscopy

Fluorescence spectroscopy make use of the fluorescent properties of certain drugs or incorporates fluorescent markers into the hydrogel matrix. As the drug is released, the change in fluorescence intensity or emission wavelength can be monitored. This technique is highly sensitive and can detect even low concentrations of the drug, providing detailed insights into the release dynamics.

Out of all these techniques, we opted for UV-Vis spectroscopy to measure the release profile of drug, with and without application of external power supply to the charged drug particles in a conductive Gelatin-Sodium Alginate hydrogel. A thorough literature review is done to understand about the calibration curve, and the calculations involved in it. Moreover, we did a literature review on how UV-Vis spectroscopy is employed to study release profile of drug from hydrogel matrix and how to calculate the concentration of drug being released into the solution over time, from hydrogel matrix, through a standard calibration curve.

CHAPTER 3

MATERIAL SYNTHESIS

3.1 Gelatin Hydrogel

The gelatin hydrogel was synthesized by dissolving gelatin powder in a solvent, agitating it and letting it dry at room temperature until we get a dried hydrogel film.

3.1.1 Material and Apparatus

The apparatus and materials used for synthesizing gelatin hydrogel are as follows.

- Gelatin powder.
- Deionized water.
- Petri Dish.
- Hot Plate.
- Spatula.
- Weighing Balance
- Magnetic Stirrer
- Aluminum Foil.
- 100 ml Beaker.
- Measuring Cylinder.

3.1.2 Procedure

1) 1g gelatin powder is added into 10 ml deionized water to get a 10% gelatin solution.
2) Heated the mixture in a water bath at 45°C for 50 minutes.
3) After that, the solution was poured into the petri dish.
4) Let it air dry at room temperature for 48 hours.
5) The gelatin hydrogel film was peeled after drying.

3.2 Sodium Alginate Hydrogel

Sodium alginate hydrogel was prepared by dissolving sodium alginate powder into DI water and agitating it. This time the hydrogel was dried in an oven and $CaCl_2$ was used for cross-linking between the chains.

3.2.1 Material and Apparatus

The apparatus and materials used for synthesizing sodium alginate hydrogel are as follows.

- Sodium Alginate powder
- Calcium Chloride
- Deionized water
- Measuring Cylinder
- 100 ml Beaker
- Petri Dish
- Hot Plate
- Spatula
- Weighing Balance
- Magnetic Stirrer
- Aluminum Foil
- Heating Oven

3.2.2 Procedure

1) 0.2 g sodium alginate powder was added to 10 ml DI water to prepare a 2% Na-Alg solution
2) Put it under agitation at room temperature for 4 h
3) Side by side we prepared 1.5% $CaCl_2$ solution to cross-link between the chains of sodium alginate
4) This $CaCl_2$ solution was prepared by adding 0.03g of $CaCl_2$ in 2ml DI water as the ratio of sodium alginate to CaCl2 was to be 10: 2
5) This $CaCl_2$ solution was added into it after 3 hours and 50 minutes of stirring of sodium alginate solution

6) After this cross-linking process was carried out, the solution was poured into the petri dish
7) Dried it in a drying oven for 48 hours
8) Sodium Alginate film was peeled off after drying to characterize the hydrogel sample

3.3 Gelatin-Sodium Alginate Hybrid Hydrogel

The Gelatin-Sodium Alginate hybrid hydrogel was prepared to get the optimized mechanical strength of the hydrogel. Gelatin and Sodium Alginate powder were mixed in a ratio of 1: 0.8 respectively, and further cross-linking between the chains of Gelatin and Sodium Alginate was carried out using Calcium Chloride.

3.3.1 Material and Apparatus

The apparatus and materials used for synthesizing gelatin hydrogel are as follows.

- Gelatin powder
- Sodium Alginate powder
- Calcium Chloride
- Deionized Water
- Measuring Cylinder
- 100 ml Beaker
- Petri Dish
- Hot Plate
- Spatula
- Weighing Balance
- Magnetic Stirrer
- Aluminum Foil
- Centrifuge
- Heating Oven

3.3.2 Procedure

1) 0.5g of sodium alginate was dissolved in 10 ml DI water.
2) The solution was heated to 50 ◦ C using a hot plate under agitation using a magnetic stirrer, for 1.5 hours.
3) After the complete dissolution of sodium alginate, 0.4g gelatin powder was added to the dissolved solution and mixed under agitation, at 50 ◦ C for 4 hours.
4) Then the degassing process was conducted in a centrifuge (3500 rpm, 15 min) to eliminate air bubbles.
5) After that, the hydrogel was cast into a petri dish.
6) A 2% solution of calcium chloride was prepared by adding 0.1 g $CaCl_2$ in 5 ml DI water. After mixing, the hydrogels were crosslinked by pouring the $CaCl_2$ solution over the hydrogel surface.
7) $CaCl_2$ solution was drained after 10 minutes.
8) Then it was left for air drying at room temperature for three days.
9) The film was prepared and then peeled off.

3.4 Conductive Drug containing Hybrid Hydrogel

To get an electro stimulated drug release, hydrogel needs to be conductive so that it responds to external applied voltage/current. In order to make it conductive, CNTs have been added to it. Moreover, Dexamethasone drug has been added in this hybrid hydrogel whose electro stimulated release is to be monitored with time.

3.4.1 Material and Apparatus

The apparatus and materials used for synthesizing gelatin hydrogel are as follows.

- Gelatin powder
- Sodium Alginate powder
- Carbon nanotubes
- Dexamethasone pallet
- Calcium Chloride
- Deionized water

- Measuring Cylinder
- 100 ml Beaker
- Petri Dish
- Hot Plate
- Spatula
- Weighing Balance
- Magnetic Stirrer
- Aluminum Foil
- Mortar and Pestle
- Centrifuge
- Heating Oven

3.4.2 Procedure

1) 0.5g of sodium alginate was dissolved in 10 ml DI water.
2) The solution was heated to 50 ◦ C using a hot plate under agitation using a magnetic stirrer, for 1.5 hours.
3) After the complete dissolution of sodium alginate, 0.4g gelatin powder was added to the dissolved solution and mixed under agitation, at 50 ◦ C for 4 hours.
4) 2000 µg of dexamethasone pallet is weighed and has been added in it.
5) Stir the solution for 2 hours to homogeneously mix the drug into the solution.
6) After that, 0.35 mg CNTs were added into the solution and placed over hot plate for 1.5 hours to stir it.
7) Then the degassing process was conducted in a centrifuge (3500 rpm, 15 min) to eliminate air bubbles.
8) After that, the hydrogel was cast into a petri dish.
9) A 2% solution of calcium chloride was prepared by adding 0.1 g $CaCl_2$ in 5 ml DI water. After mixing, the hydrogels were crosslinked by pouring the $CaCl_2$ solution over the hydrogel surface.
10) $CaCl_2$ solution was drained after 10 minutes.
11) Then it was left for air drying at room temperature for three days.
12) The film was prepared and then peeled off.

CHAPTER 4

HYDROGEL COATING OVER ELECTRODE

The hydrogel was coated over a copper electrode by preparing a Gelatin-Sodium Alginate hybrid hydrogel solution and simply casting it over that cold-mounted (epoxy) copper electrode.

4.1 Material and Apparatus

The material and Apparatus required for the process of cold mounting of the Copper electrode and coating hybrid hydrogel over it are as follows.

- Copper electrode
- Wire for connection
- Bottle cap
- Release wax
- Epoxy resin
- Hardener
- Bowl
- Weighing balance
- Vernier Caliper
- Grinding and polishing machine.
- Alumina paste

4.1.1 Procedure

1) Two copper electrodes were taken and had holes drilled into them so that connection could be done through the wire for their use as a working electrode in electrochemical testing.
2) Release wax was placed inside the bottle cap.
3) 7.6 g epoxy resin and 3.8 g hardener were mixed (2:1) and then placed in caps containing copper electrodes for the cold mounting of this electrode.
4) After that, they were left for two days for curing.

5) After cold mounting, the exposed surface of copper electrodes was cleaned through grinding and polishing of these electrodes.
6) The exposed surface area of copper electrodes was measured using a vernier caliper.

Exposed surface area of bare electrode = 58.08 mm^2

Exposed surface area of hydrogel-coated electrode = 61.05 mm^2

7) Gelatin-sodium alginate hybrid hydrogel was prepared side-by-side through the procedure discussed above. This time the hydrogel was cast onto the Copper electrode for coating purposes and left for drying at room temperature for three days.

Figure 4: Schematic illustration of cold mounting and hydrogel coating on copper electrode

4.2 Electrochemical Impedance Spectroscopy

EIS test was performed to test whether gelatin-sodium alginate hybrid hydrogel has been coated over copper electrode or not. This test was performed on an electrochemical workstation.

4.2.1 Material and Apparatus

The apparatus and materials used for performing the EIS experiment are as follows.

- NaCl
- Deionized water.
- Aluminum foil.
- Working electrode.
- The reference electrode (Platinum wire).
- Counter electrode.

4.2.2 Procedure

1) 5% NaCl solution has been prepared as an electrolyte by dissolving 1.966 g of NaCl in 56 ml Deionized water.
2) Both electrodes I.e., bare copper electrode and hydrogel-coated copper electrode were placed inside this electrolyte solution for 10 minutes in order to stabilize the open circuit potential.
3) Then OCP was run for 5 minutes.
4) After that, potentiostatic EIS was performed with 5V peak-to-peak AC voltage and exposed surface area of 58.08 mm^2 and 61.05 mm^2 for bare Cu electrode and hydrogel-coated Cu electrode, respectively.
5) Bode plot of EIS results were analyzed through GAMRY ECHEM analysis.

Figure 5: Hydrogel Coated Electrode dipped in Electrolyte (PBS)

4.3 Analysis of EIS results

EIS with an equivalent circuit model is used to gain a deeper insight into this electrochemical system. In the circuit, Ru is the bulk resistance; Rp is the polarization or charge transfer resistance; the constant phase element (alpha) is associated with the double-layer capacitance across the electrode/ electrolyte interface.

Solution resistance for bare and copper-coated electrodes came out to be 9.803 ohms and 8.388 ohm respectively, while Polarization/Charge transfer resistance is 4.226 X 10^3 ohm and 7.788 X 10^3 ohm. There is a significant increase in the charge transfer resistance of

hydrogel-coated electrodes. This means that there is a layer of non-conductive gelatin-sodium alginate hybrid hydrogel between the electrode/electrolyte interface that resists the charge travelling towards the electrode. This proves the coating of hybrid hydrogel over the Copper electrode.

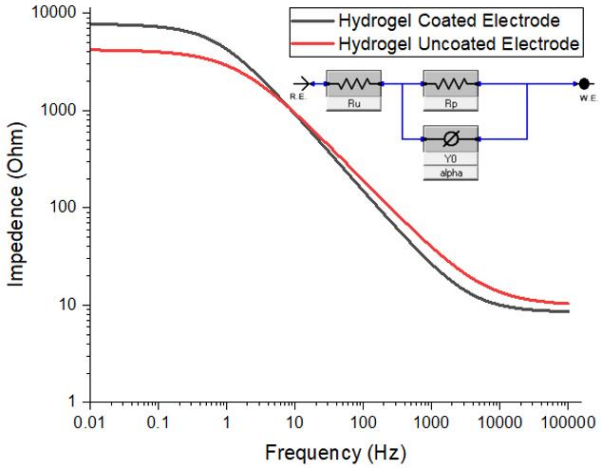


Figure 6: EIS of Bare Coated Electrode & Hydrogel Coated Electrode

CHAPTER 5

CHARACTERIZATIONS

Gelatin, Sodium Alginate and Gelatin-Sodium Alginate hybrid hydrogel samples were characterized using XRD, FTIR, and SEM. Mechanical properties were tested using a tensile test.

5.1 X-Ray Diffraction Analysis

Figure 3 shows the results of XRD analysis of prepared hydrogel films. Crystalline peaks of carbon tape can be seen in the XRD spectra of all three samples. This is because during analysis, a double-sided carbon tape was used to hold hydrogel films onto the sample stage. Pure gelatin hydrogel exhibited a broad diffraction peak near 2θ = 20°, indicating its short-range ordered structure. On the other hand, the Amorphous structure of sodium alginate and Gelatin-Sodium Alginate hybrid hydrogel is evident from their XRD spectra. Some crystalline peaks of calcite are seen in the sodium alginate and Gelatin-Sodium Alginate hybrid hydrogel spectra. These peaks correspond to the precipitation of $CaCO_3$ during the cross-linking process when $CaCl_2$ was added as a cross-linking agent during Synthesis of hydrogel. Similarly, in XRD spectra of drug and CNTs containing hydrogel, diffraction peak of Carbon nanotubes and Dexamethasone is evident at $2\theta = 24^0$ and $2\theta = 19^0$

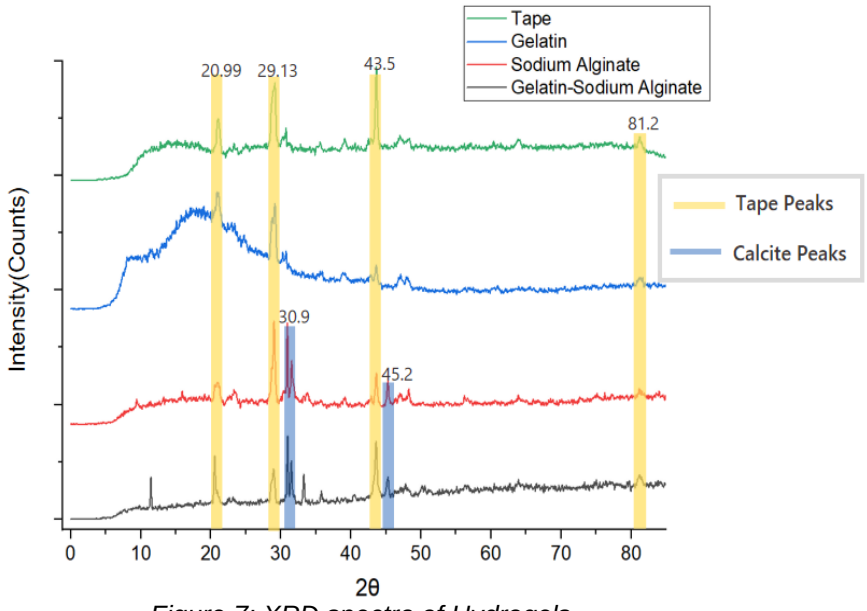


Figure 7: XRD spectra of Hydrogels

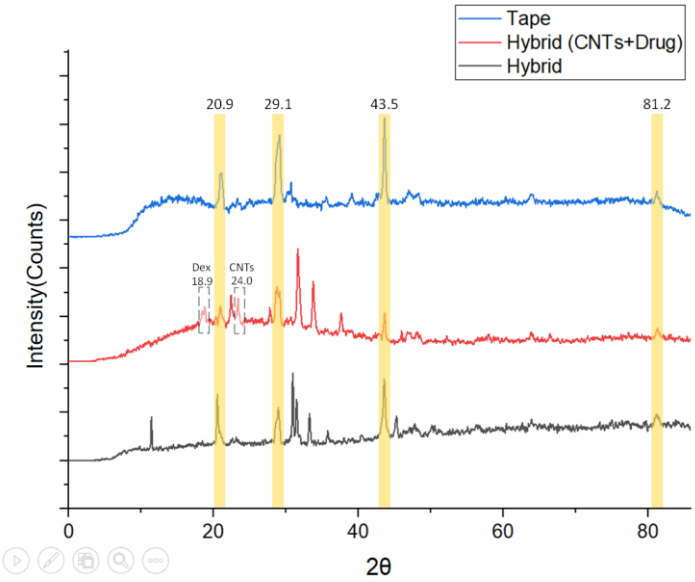


Figure 8: XRD spectra of Hydrogel containing CNTs and drug

5.2 FTIR analysis of Hydrogels

Furthermore, the hydrogel samples are characterized through FTIR. The peaks of absorption observed at 1600 and 1415 cm−1 in the infrared spectrum of sodium alginate in its pure form were linked to the asymmetric and symmetric stretching vibrations, respectively, of the −COO− group present in alginate. Similarly, the absorption peaks identified at 1633 and 1541 cm−1 in the infrared spectrum of pure gelatin were associated with the stretching vibrations of C=O and C−N (characteristic of the amide I band) and the bending vibrations of the −NH group (characteristic of the amide II band), respectively.

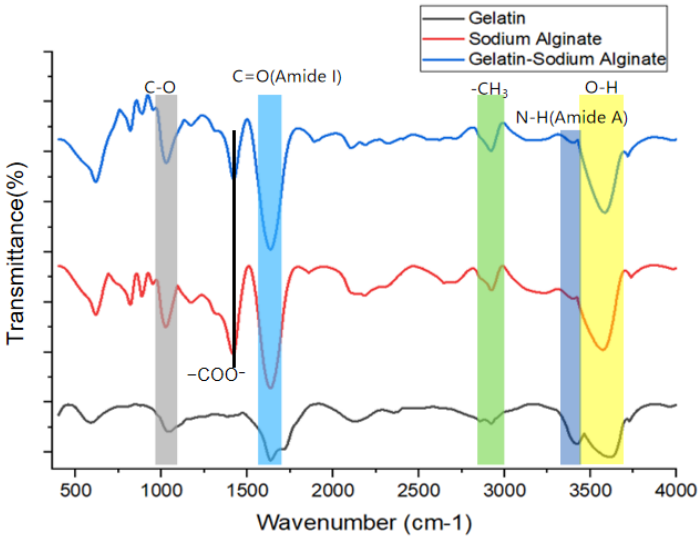
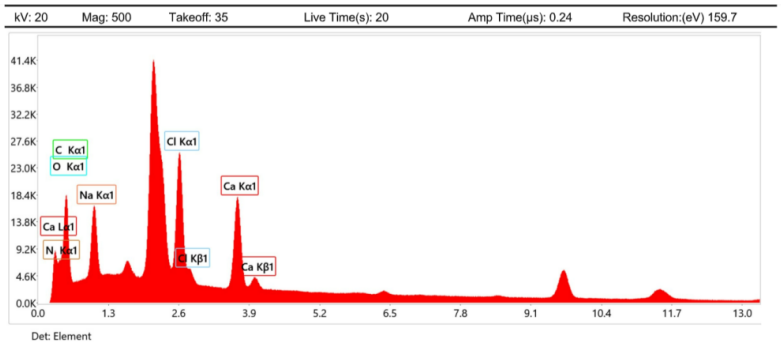


Figure 9: FTIR spectra of Hydrogels

5.3 Energy Dispersive Spectroscopy (EDS) Analysis

The elemental composition of Gelatin-Sodium Alginate hydrogel is found through EDS (Figure 5). EDS analysis shows the presence of Carbon, Nitrogen, Oxygen, Sodium, Chlorine, and Calcium in Gelatin-Sodium Alginate hydrogel. The presence of chlorine and calcium is due to the cross-linking of Gelatin-Sodium Alginate gel.



eZAF Quant Result - Analysis Uncertainty: 99.00 %

Element	Weight %	MDL	Atomic %	Error %
C	15.5	0.80	21.4	11.7
N	23.4	0.39	27.6	11.0
O	36.1	0.26	37.4	10.6
N a	8.9	0.10	6.4	8.8
C l	8.3	0.05	3.9	2.8
C a	7.8	0.06	3.2	2.3

Figure 10: EDX Analysis of Gelatin-Sodium Alginate Hydrogel

5.4 SEM Analysis of Hydrogel Samples

Figure 6-9 shows the secondary electron images of gelatin, sodium alginate and Gelatin-Sodium Alginate hybrid hydrogel, respectively. Dense hydrogel films have been prepared.

In the SEM Image of Gelatin-Sodium Alginate hybrid hydrogel, A polyelectrolyte complex is present. Gelatin, a protein-based polymer, and sodium alginate, a polysaccharide-based polymer, interact.

in a water-based solution. Both gelatin and sodium alginate contain charged functional groups (gelatin contains amino groups and sodium alginate contains carboxylate groups), making them polyelectrolytes. When a solution containing gelatin and sodium alginate is mixed, the oppositely charged groups on these polymers can interact through electrostatic attraction, leading to the formation of a polyelectrolyte complex which is seen in the secondary electron image. Moreover, the crystals which can be seen in the figure are the $CaCO_3$ crystals. During cross-linking, the local concentration of calcium ions can become high enough to exceed the solubility of calcium carbonate in the hydrogel. This can lead to the precipitation of $CaCO_3$ crystals.

Figure 15 illustrates the SEM image in which it can be seen that the drug particles are distributed inside Gelatin-Sodium Alginate matrix. The hydrogel film prepared this time has porosity in it as well, which can be seen from the image below.

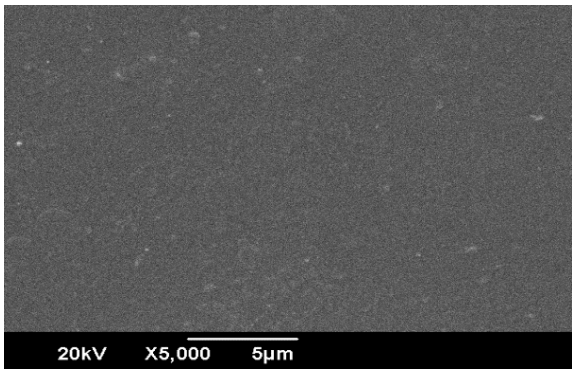


Figure 11: SEM image of Gelatin Hydrogel

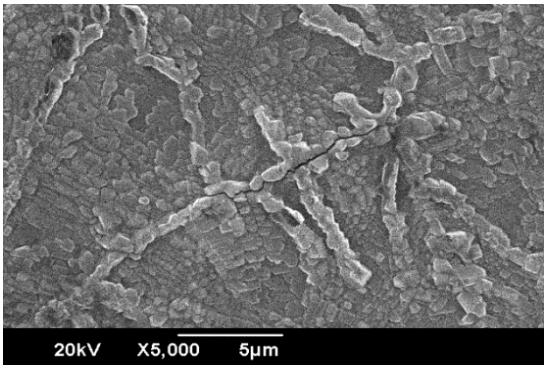


Figure 12: SEM image of Sodium Alginate Hydrogel

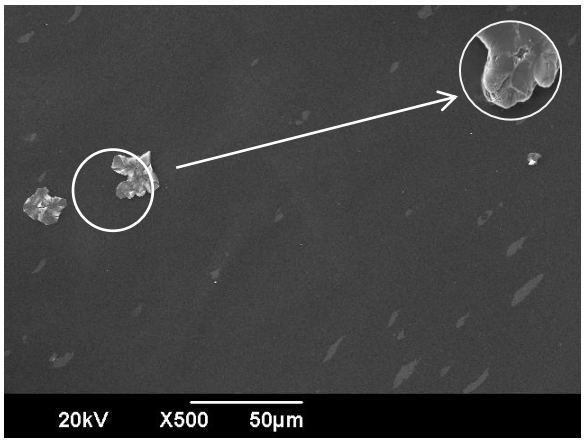


Figure 14: CaCO3 crystals in Gelatin- Sodium Alginate Hybrid Hydrogel

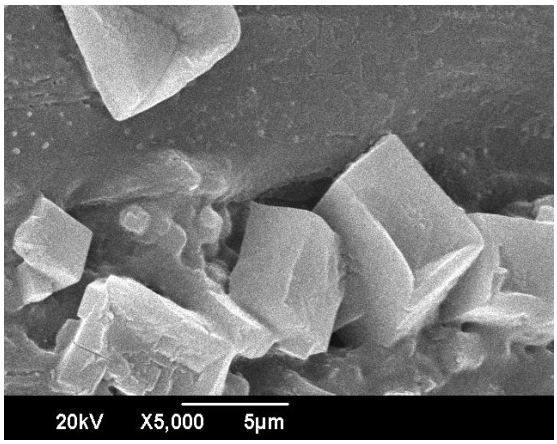


Figure 13: Polyelectrolyte complex in Gelatin-Sodium Alginate Hybrid Hydrogel

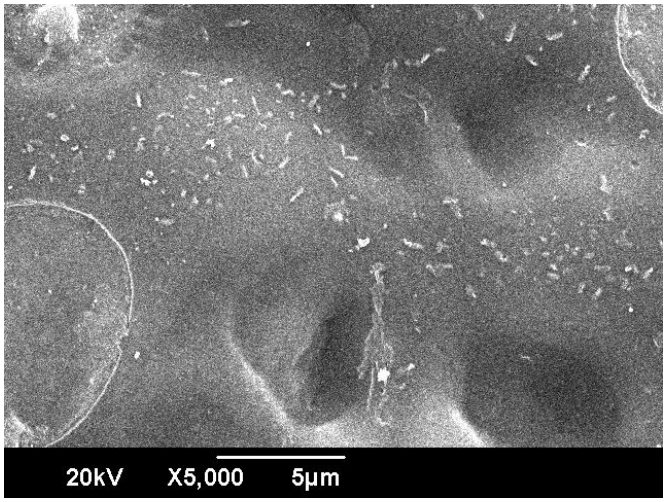


Figure 15: Drug loaded hybrid hydrogel

5.5 Tensile Test

Hydrogel needs to be stable enough to be loaded with the drug, applied to the skin, and worn during the iontophoresis process without disintegrating or losing its shape. Moreover, Hydrogel with enhanced mechanical properties can provide more controlled and sustained drug release profiles, allowing for better regulation of drug delivery rates during iontophoresis. This can improve the efficacy and safety of the treatment by preventing rapid or uneven drug release.

It can be seen in the figure that an 85% enhancement in mechanical strength is achieved by.

- The addition of Gelatin in sodium alginate in the ratio of 1: 0.8, respectively.

- Using calcium chloride as a cross-linking agent between the chains of gelatin and sodium alginate.

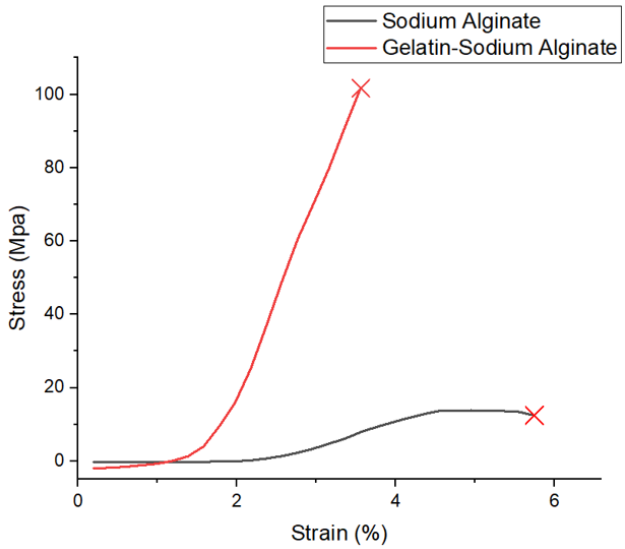


Figure 16: Tensile Test of Sodium Alginate and Gelatin-Sodium Alginate Hydrogel

CHAPTER 6

DRUG RELEASE TEST

Dexamethasone can be effectively monitored for release from hydrogels using UV-Vis spectroscopy. In this study, hydrogel loaded with dexamethasone is immersed in a suitable release medium, such as phosphate-buffered saline (PBS). At predetermined intervals, aliquots of the release medium are collected and analyzed using a UV-Vis spectrophotometer. Dexamethasone has a characteristic absorbance peak in the UV region which allows for its precise detection and quantification, typically around 240 nm, but this peaks shift occur if we do not use standard drug, i.e., drug in pallet form with other impurities inside. The absorbance of each sample at this specific wavelength, in our case 206 nm is measured and compared against a calibration curve generated from standard solutions of known dexamethasone concentrations. This allows for the determination of the drug concentration in each sample. By plotting these concentrations over time, we can construct a release profile, illustrating the cumulative amount of dexamethasone released from the hydrogel.

6.1 Calibration Curve

In a drug release test, a calibration curve is an essential tool used to determine the concentration of a drug in a solution by establishing a relationship between known drug concentrations (standard solutions) and their corresponding absorbance in UV-visible spectroscopy. This relationship is crucial for accurately quantifying the amount of drug released over time. To create this calibration curve, we prepared dexamethasone-containing phosphate-buffered saline (PBS) solutions with varying drug concentrations—specifically, 5 µg/ml, 15 µg/ml, and 25 µg/ml. By measuring the absorbance of these solutions across a wavelength range of 200-400 nm, we can plot absorbance against concentration to generate a standard curve. This curve enables the determination of unknown drug concentrations in similar solutions by comparing their absorbance values to the established curve. Thus drug release profile is established.

6.1.1 Preparation of standard solution

6.1.1.1 Material and Apparatus

In order to prepare standard drug solution for calibration curve, following material and apparatus is required.

1. Dexamethasone powder
2. PBS solvent
3. Beakers
4. Magnetic stirrers
5. Volumetric flask
6. Weighing balance
7. Pipette
8. Mortar and Pestle
9. Aluminum foil
10. Hot plate

6.1.1.2 Procedure for preparation of standard solution

Following procedure we have followed to prepare standard solutions of known concentration (5 µg/ml, 15 µg/ml and 25 µg/ ml)

(1) Crush a dexamethasone pallet using mortar and pestle

(2) Add 5 ml PBS solution in three different beakers

(3) Accurately weigh 5, 15 and 25 micro gram dexamethasone and transfer it in each beaker

(4) Put each beaker (drug containing PBS solution) over a hot plate with magnetic stirrer inside and stir it for 1 hour to homogeneously dissolve drug into PBS solution.

(5) Transfer 5 ml of each solution i.e., 5 µg/ml, 15 µg/ml and 25 µg/ ml in a separate cuvette in order to have a further UV VIS analysis of each standard solution

6.1.1.3 UV VIS Analysis for calibration curve

For UV-VIS analysis, one of the most crucial factors to consider is the wavelength range over which absorption must be studied. This range is important because it determines the specific

wavelengths at which the substance being analyzed absorbs light, thereby providing insights into its concentration in a solution. In our experiment, we focused on drawing the absorption spectra of a dexamethasone-containing PBS (phosphate-buffered saline) solution within the wavelength range of 200 to 400 nm. This range was selected to capture the relevant absorption characteristics of dexamethasone, which exhibit absorption maxima at 206 nm, as it is not in pure/standard form. This peak is significant as it represents the wavelength at which the highest absorption occurs, making it an ideal point for further quantitative analysis.

To quantify the concentration of dexamethasone in solution, we prepared a calibration curve by measuring the absorption at 206 nm for various concentrations of the drug. The calibration curve is essential for establishing a relationship between the drug concentration and its corresponding absorption, which can then be used to determine unknown concentrations in future samples.

6.1.1.4 Procedure for UV-Vis analysis to draw calibration curve

Initially, two cuvettes containing only PBS solution were placed in the spectrophotometer to perform a baseline measurement, commonly referred to as a sample run. This step is critical for ensuring that any subsequent measurements can be accurately attributed to the presence of dexamethasone rather than any background absorption from the PBS.

Following the baseline measurement, one of the PBS-containing cuvettes was replaced with a cuvette containing a solution of dexamethasone in PBS at various concentrations. Specifically, solutions with concentrations of 5, 15, and 25 micrograms per milliliter were sequentially placed in the spectrophotometer. For each concentration, we recorded the absorption spectra over the 200 to 400 nm wavelength range. By comparing the absorption data at these different concentrations, we constructed a detailed calibration curve. The intensity of absorption VS wavelength curve confirmed the absorption maxima at 206 nm, and the calibration curve demonstrated the linear relationship between absorption and concentration, which is fundamental for the accurate determination of drug concentrations with and without application of external voltage/power supply.

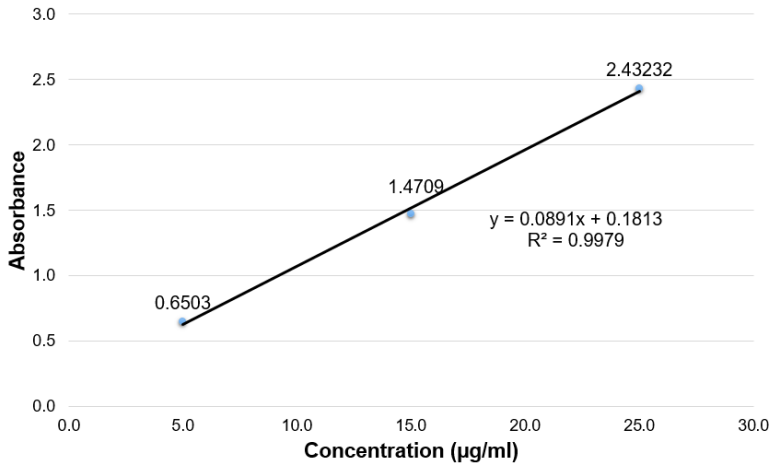


Figure 17: Calibration curve

From these points, we draw a best fit line so that we can linearly relate drug concentration with absorption which would further be used in the Drug release calculations, with and without power supply.

Absorbance = 0.0891 (concentration) + 0.1813 with **R^2 = 0.99**

6.2 Drug release with and without external voltage

After completing the calibration curve, the next crucial task is to evaluate the release profile of dexamethasone within a phosphate-buffered saline (PBS) solution, both with and without the application of direct current (DC) voltage. The experimental setup involves coating a conductive, drug-loaded hydrogel onto two electrodes. One of these electrodes is immersed in a PBS solution, while the other is connected to a series of three 1.5 V cells, generating a total of 4.5 V DC voltage.

The experiment is designed to assess how the application of an electric field influences the release of dexamethasone from the hydrogel. To achieve this, samples are collected from both the electrically stimulated PBS solution and the non-stimulated control at regular

intervals. These samples are then analyzed to determine their absorbance, which correlates with the concentration of dexamethasone released into the solution.

By plotting the absorbance against the concentration of the drug, we can visualize the release profile of dexamethasone under both conditions. The resulting graph, as illustrated in the figure 18, displays the Absorbance versus time spectra for both the electro-stimulated and non-electro-stimulated drug release profiles. This comparison highlights the impact of applying a DC voltage on the release kinetics of dexamethasone from the carbon nanotube (CNT)-containing gelatin-sodium alginate hydrogel.

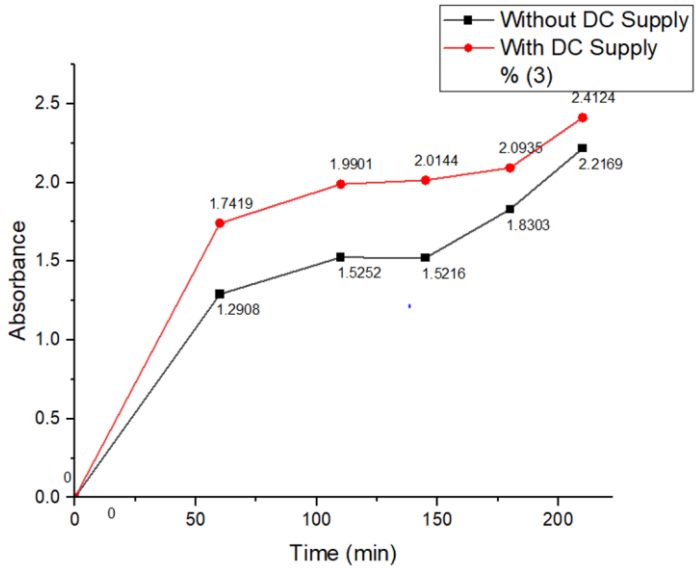


Figure 18: Drug release rate with and without DC Supply

Here it can seen clearly that upon application of mild DC voltage, drug release rate can be enhanced by making the hydrogel coated electrode, cathode so that negatively charged drug in it get repelled and release into the solution.

As **Absorbance = 0.0891 (concentration) + 0.1813**, So from this absorption spectra we can find the exact amount of drug that has been released into the PBS solution.

6.2.1 Calculations

6.2.1.1 Without voltage

Table 1: Drug Release Rate without DC Supply

Time (mins)	Absorbance	Concentration (µg)	Concentration (3 ml) (µg)	Cumulative (3ml) (µg)	Concentration (50 ml) (µg)	Cumulative (50 ml) (µg)	% age Drug Release
60	1.2908	12.45	37.35	37.35	622.5	622.5	31.25 %
110	1.5216	15.04	45.12	82.47	752	789.35	39.46 %
145	1.5252	15.08	45.24	127.71	754	836.47	41.82 %
180	1.8303	18.51	55.53	183.24	925.4	1053.21	52.66 %
210	2.2169	22.85	68.54	251.78	1142.5	1325.74	66.28 %

6.2.1.2 With 4.5 V DC voltage

Table 2: Drug release rate with 4.5 V DC Supply

Time (mins)	Absorbance	Concentration (µg)	Concentration (3 ml) (µg)	Cumulative (3ml) (µg)	Concentration (50 ml) (µg)	Cumulative (50 ml) (µg)	% age Drug Release
60	1.7419	17.52	52.56	52.56	876	876	43.8 %
110	1.9901	20.30	60.9	113.46	1015	1067.56	53.38 %
145	2.0144	20.57	61.71	175.17	1028.5	1141.96	57.1 %
180	2.0935	21.46	64.38	239.55	1073	1248.17	62.41 %
210	2.4124	25.04	75.15	314.7	1252	1290.55	74.53 %

To find %age drug released in PBS solution, following formula is used.

%age drug release = [Cumulative (50 ml) drug concentration) / Original (2000 µg) drug concentration] X 100 %

6.2.2 Drug Release VS Time Spectra

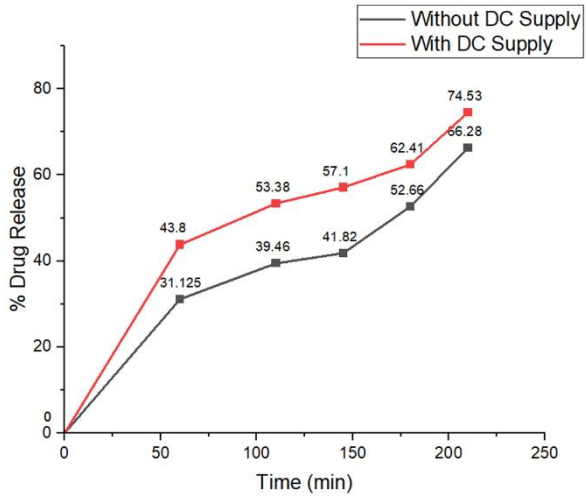


Figure 19:Drug Release Rate

To quantify the release of Dexamethasone from a hydrogel-coated electrode into a phosphate-buffered saline (PBS) solution, we can utilize the absorption spectra obtained at regular time intervals. This method allows us to track the concentration of the drug in the solution over time and assess the impact of an applied DC voltage on the release rate.

Beaker 1: The electrode was uniformly coated with a hydrogel containing Dexamethasone and immersed in PBS solution. At regular intervals, samples of the PBS solution were analyzed using a spectrophotometer, monitoring the absorption peak specific to Dexamethasone

Beaker 2: In this beaker, a mild DC voltage (1.5 V) was applied to the other drug containing hydrogel coated electrode to facilitate drug release

A calibration curve was prepared as explained above using a series of Dexamethasone standards with known concentrations. The absorbance of each standard was measured to create a linear relationship between absorbance and concentration, described by the following equation

$$A = \epsilon \cdot c \cdot l$$

where A is the absorbance, ϵ is the molar absorptivity coefficient, c is the concentration, and l is the path length. The absorbance of each PBS sample was measured, and the concentration of Dexamethasone was determined using the calibration curve. At each time point, the amount of drug released was calculated for both electro stimulated and non-electro stimulated process, by multiplying the concentration in the PBS solution by the volume of the solution, and the cumulative amount (calculated in table 1 and 2) of drug released over time was plotted.

6.3 What happened when even higher voltage is applied?

Following the observation of increased drug release rate with the application of DC voltage, our subsequent experiment was to determine whether the magnitude of the applied voltage directly influences the rate of drug release.

To conduct this study, we repeated the preparation of a conductive hydrogel solution containing the drug and cast it onto two cold-mounted copper electrodes. One electrode served as a control to monitor drug release without any external power supply, while the other electrode was connected to a 7V DC power supply. The absorption spectra obtained from the drug released into the PBS solution from both electrodes, with and without the external power supply, are illustrated in the figure. Notably, a significant enhancement in the release rate of the drug was observed upon the application of a 7V DC voltage. This huge difference confirms our hypothesis that the drug release rate can indeed be proportionally

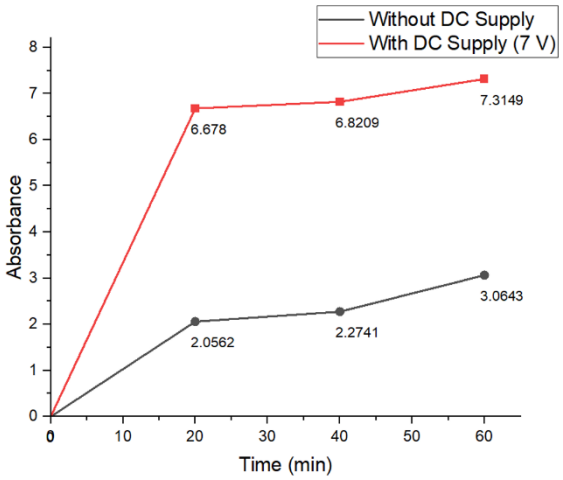


Figure 20: Drug Release Rate at Higher Voltage

enhanced by increasing the external applied voltage or current. dependency of drug release kinetics.

Moreover, the visual representation provided in the figure 21 illustrates drug release into the PBS solution, particularly the accelerated release phenomenon. Within just 30 minutes of the application of a 7V DC voltage, a noticeable amount of drug was released into the solution as compared to the case where no external power supply was connected.

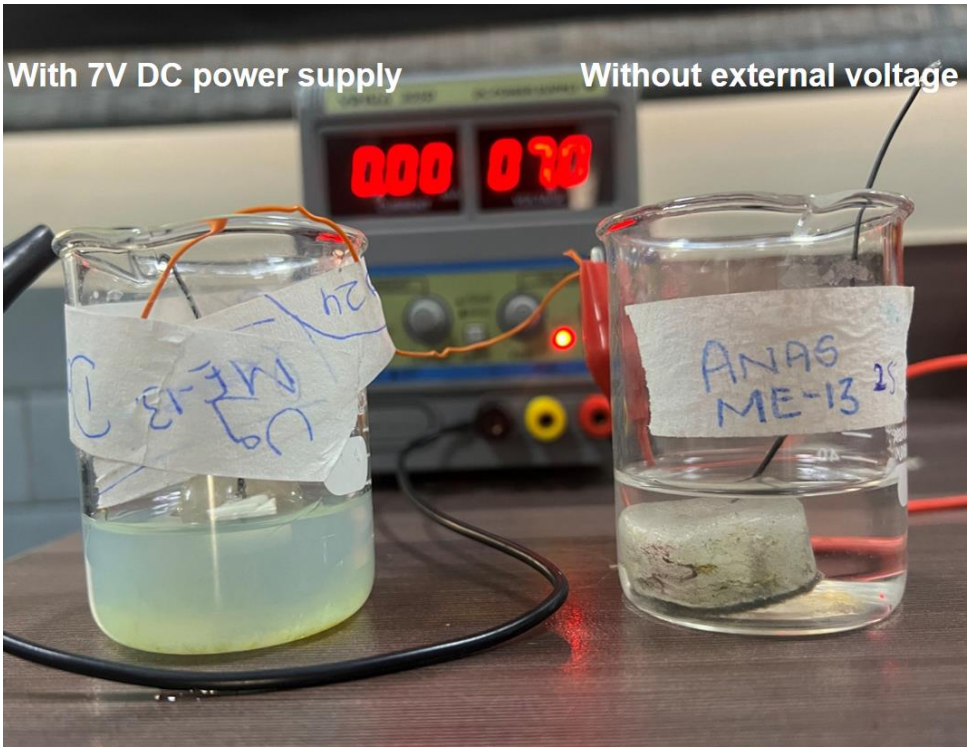


Figure 21: Visual Representation of Drug Release

CONCLUSION

We have synthesized CNTs containing drug (Dex) encapsulated Gelatin-Sodium Alginate hydrogel, characterized it using SEM, XRD, FTIR and EDX, coated it over copper electrode and finally did a drug release test through UV-Vis spectroscopy, with and without external applied voltage. We came to a conclusion that upon applying voltage, drug release rate can be enhanced proportionally.

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