An Appraisal of Wound Condition Mediated Drug Release by Comparing Two Hydrogel Systems

MASTER'S THESIS (Fall 2017-2019)

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AN APPRAISAL OF WOUND CONDITION MEDIATED DRUG RELEASE BY COMPARING TWO HYDROGEL SYSTEMS

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A thesis submitted in partial fulfillment of the requirements for the degree of MS Biomedical Science

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Titled: An Appraisal of Wound Condition Mediated Drug Release by Comparing Two Hydrogel Systems be accepted in partial fulfilment of the requirements for the award of MS Biomedical Sciences degree. Grade (___)

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Certified that the final copy of MS/M. Phil Thesis written by Ms. Rabail Gul, Registration No. (00000206344), of School of Mechanical And Manufacturing Engineering (SMME) has been vetted by the undersigned, found complete in all respects as per NUST Statutes/ Regulations, is free of plagiarism, errors and mistakes and is accepted as partial fulfillment for award of MS/MPhil Degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis.

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I certify that this research work titled "*An Appraisal of Wound Condition Mediated Drug Release by Comparing Two Hydrogel Systems"* is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources it has been properly acknowledged/referred.

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I am grateful to **Allah**, the Almighty for his guidance and enlightenment through his beloved **Prophet** (Peace be Upon Him). I would like to pay my special gratitude to my beloved family who encouraged and supported me throughout this degree, be it hostel or academic issues.

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Dedicated to my exceptional parents and loving husband whose tremendous support and cooperation led me to this wonderful accomplishment.

Abstract

Topical, sustained drug delivery according to the physiological need at the wound site enhances the healing rate of chronic wounds. Natural (Chitosan-Gelatin) and semi-synthetic (2-Hydroxyethyl Methacrylate-Gelatin) types of hydrogel complexes were prepared and tested to form flexible wound dressings. They smartly respond to the pH changes in wound by releasing drug accordingly, aiding in fast healing and successful wound management. The morphological and mechanical properties of hydrogels were determined using various characterization techniques, that comply with the in-vitro experimentation, which includes drug release pharmacokinetics, degradation profiles and anti-microbial study. Further, in-vivo studies were performed on mice models to test the efficacy of wound dressings.

Keywords: Hydrogels, Chitosan, Hydroxyethyl Methacrylate (HEMA), Gelatin, Wound Healing,

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List of Abbreviations

HEMA: 2-Hydroxyethyl methacrylate

TEGDA: Tetra (ethylene glycol) diacrylate

APS: Ammonium persulfate

$Na_2S_2O_5$: Sodium metabisulfite

PBS: Phosphate Buffer Saline

NaCl: Sodium Chloride

EWC: Equilibrium Water Capacity

CG: Chitosan-Gelatin

HG: HEMA-Gelatin

MR*SA*: Methicillin-resistant *Staphylococcus aureus*

IRB: Institutional Review Board

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Chapter 1 Introduction

Cutaneous wound healing is a widely discussed topic in scientific world [1]. Unhealed chronic wounds that progress in elderly people or patients with metabolic disorders e.g. diabetes, cause serious problems for not only the individual, but also society. With the passage of time, wound management has become an important part of medical care [2], [3]. Its standards are now defined better than ever, especially the characteristics of an ideal wound dressing. An ideal wound dressing can maintain moist environment, cleanse debris from the wound, manage exudate, promote granulation and epithelization, minimize discomfort and pain, promote angiogenesis, allow gaseous exchange, maintain appropriate tissue temperature, provide protection against bacterial infection and is sterile, non-toxic and non-allergic, non-adherent and easy to remove [4].

Among which moist wound healing is the most important one, in which an optimally moist environment is maintained in order to promote faster healing. The cutaneous wounds are more prone to bacterial colonization if kept uncovered and untreated [5], [6], so from simple gauze dressing to lint and paraffin impregnated dressings [7], new bandages are continuously being developed to immediately cover the wound in case of a cut, a burn, a fall or a bad knock [8].

Hydrogel is a well-known wound dressing candidate that has the high moisture content, it is highly absorbent as it absorbs all the exudate from the wound but still keeps it moist enough for its noninflammatory and painless recovery [9]. Hydrogels also allow gaseous exchange that helps promote granulation and epithelization [10]. There are a number of natural and synthetic hydrogel based wound dressings available in the market, for acute or chronic wounds.

The use of topical antimicrobial agents can help prevent the progression from colonization to infection without any risk of drug resistance [11]. Hydrogels containing the antimicrobial drugs, can be used for a range of wounds that are leaking little or no fluid, and are painful or necrotic wounds etc [12]. So, we need a wound dressing that also delivers drug to prevent any microbial infection [13]. But the question is that how to control the drug delivery at the wound site.

Hydrogels are a type of stimuli-responsive materials, they respond to a wide range of stimuli, such as light, temperature, pH, solvent composition, chemical species, and electrical field etc. Among these the wound pH influences indirectly and directly all biochemical reactions taking

place in the process of healing thus it's an important parameter for therapeutic interventions in wound-care [14], [15].

Normally pH of skin is acidic i.e. 4-6, which is disturbed in wounds and becomes more alkaline thus facilitating bacterial colonization. Restoring the natural acidic environment on the skin helps to improve tissue oxygenation, reduce inflammatory proteases, and stunt the growth rate of pathogens in the wound biofilm. Hence, a pH-responsive sustained release drug delivery system could be beneficial for effective treatment of wound [16], [17] i.e. pH sensitive hydrogels.

Gelatin is one of the most common and abundantly used pH sensitive polymer that has various applications in medical and pharmaceutical domain, due to its biocompatibility and biodegradability. Gelatin hydrogels are especially used in drug delivery and tissue engineering owing to their low-toxicity and advantageous anti-microbial and cell adhesion properties [18]. To further increase their mechanical strength and improved properties they are paired up with other polymers, natural or synthetic [19]. Chitosan is a natural and pH sensitive polymer that has been combined with gelatin [20] as well as other polymers in several studies for wound healing applications [21], [22], [23], [24], [25] and its pair with Gelatin particularly has proved to be remarkable natural composite for drug delivery and wound healing.

Alternatively, Gelatin can also be paired with synthetic, pH-sensitive polymer, such as Hydroxyethyl methacrylate (HEMA) for superior wound healing properties. Their blend has been considered for scaffold formation in tissue engineering [26], [27] due to their commendable biocompatibility and degradability [28].

In this research, two pH sensitive hydrogel composites have been prepared, one is composed of natural components- Chitosan and Gelatin and the other is semi-synthetic including HEMA, a synthetic polymer and Gelatin, a natural polymer. These both composites are compared to select the most suitable candidate for drug release at the wound site. The novel aspect is that the drug release is according to the wound condition and pH of its individual phase of healing. It allows the quick healing of the wound, without the risk of any infection or drug toxicity that is often seen in other drug containing wound dressings, due to initial burst release of drug.

This research work has been presented in three parts. The first part consists of designing and fabrication of a flexible, drug containing hydrogel based wound dressing; of both natural and synthetic origins. Their viable structure is proved by their morphological characterization by Scanning Electron Microscopy and flexibility is tested in the grips of a Universal Testing Machine. In second part, their comparative drug release analysis is explained, in which drug release profile of bacitracin zinc from both composites is described at wound's pH range (4-8). The effective drug release is verified by its anti-bacterial activity in disc diffusion method. Material characterization, by FTIR spectral analysis of both composites with and without drug justifies the structure of hydrogels during drug release. The last part includes in-vivo testing of the developed wound dressings on mice. The objective of this part is to verify the effective wound closure of cutaneous wounds in a duration of 5 days.

Chapter 2 Methodology

2.1. Hydrogel fabrication using solvent casting method

2.1.1. Chitosan-Gelatin Hydrogel

2% Chitosan solution was formed by dissolving pre-weighed amount of Chitosan (Santa Cruz Biotechnology) in 1% acetic acid (MERCK, Germany) solution at 25°C till homogenized. 7% Gelatin solution was prepared by dissolving pre-weighed amount of Gelatin (Daejung Chemical Co., Korea) in distilled water at 37°C till homogenized. Prepared Chitosan and Gelatin solutions were mixed in various ratios, at 25°C and the total volume of mixture was kept 20ml. 0.25% Glutaraldehyde (Sigma-Aldrich) solution was added in the mixture as a cross-linker. The composite mixture was then poured in a glass petri dish and cured at room temperature. 1:2, 1:3 and 1:4 gel ratios of Chitosan-Gelatin were finalized on the basis of fabrication feasibility & handling [29].

2.1.2. HEMA-Gelatin Hydrogel

30% HEMA solution was formed by dissolving pre-weighed amount of HEMA (Alfa Aesar, USA) in distilled water at 25°C for 2 min. 10% Gelatin solution was prepared by dissolving preweighed amount of Gelatin (Daejung Chemical Co., Korea) in distilled water at 37°C till homogenized. Prepared HEMA and Gelatin solutions were mixed at 25°C for 10 min, in various ratios but the total volume was kept 20ml. Next, HEMA crosslinker: TEGDA, HEMA crosslinking initiators: APS & $Na_2S_2O_5$ and Gelatin crosslinker: Glutaraldehyde (Sigma-Aldrich) were added in the mixture and further stirred for a few minutes. The composite mixture was then poured in a glass petri dish and cured at room temperature. 1:3, 1:4 and 1:5 gel ratios of HEMA-Gelatin were finalized on the basis of fabrication feasibility & handling.

2.2. Comparison of the physical characteristics

2.2.1. Uniformity

Both Chitosan-Gelatin (CG) and HEMA-Gelatin (HG) films were characterized in terms of weight and thickness uniformity [20]. Three specimens of size 1.0 cm × 1.0 cm of all films were weighed on electronic balance and their mean weight was calculated. The thickness of films was

measured by an electronic Vernier caliper at three different positions of the film with 0.001 mm of accuracy. The results were expressed as a mean of the measurements ± standard deviation (SD).

2.2.2. Swelling Kinetics/Equilibrium Water Capacity (EWC)

The swelling kinetics of the film in distilled water were measured [20]. The pre-weight dried film samples were immersed in distilled water at room temperature to reach the equilibrium state. At fixed time intervals, weight was measured after slightly removing the surface water by filter paper.

The Equilibrium water capacity was calculated by the following equation [30]:

$$\textbf{EWC (\%)} = \textbf{(Ws–Wd)/Ws x100}$$

where **Wd** was the weight of dried sample, and **Ws** was the weight of swollen samples, at different time intervals.

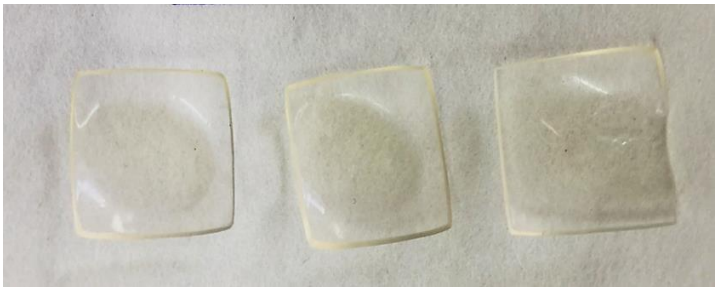


Figure 1. Swollen samples of Chitosan-Gelatin hydrogel film during EWC study in triplicates.

Figure 2. Swollen samples of HEMA-Gelatin hydrogel film during EWC study in triplicates.

One composite ratio of each hydrogel was chosen on the basis of their equilibrium water capacity & highest time of degradation, and further testing was carried out.

2.3. In-Vitro Studies

2.3.1. Drug Incorporation

The drug used in this study was Bacitracin zinc. It's a non-allergic antibiotic that helps prevent bacterial infections caused by minor cuts, abrasions or burns. Permeation method was used to load drug in hydrogel. It is the easiest drug loading method in which fully formed hydrogel is placed into medium saturated with the drug. The other methods of drug loading in hydrogel are described below.

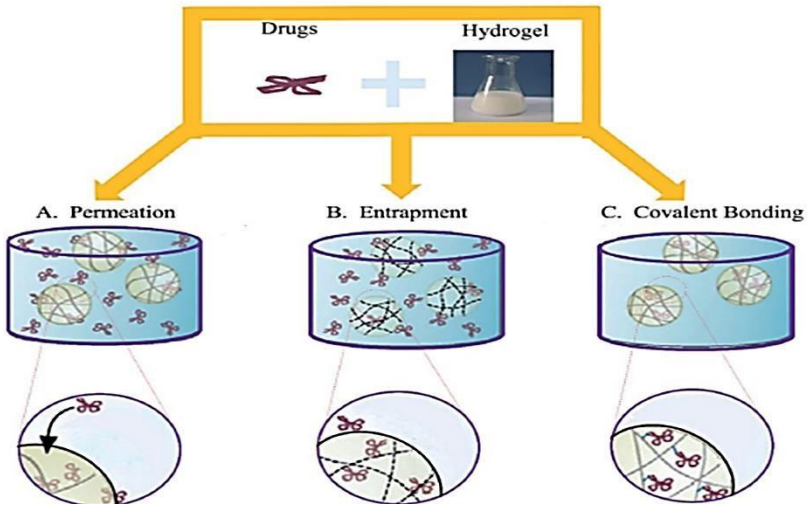


Figure 3. Methods of Drug loading in hydrogels.

	Permeation	Entrapment	Covalent bonding
Loadable drugs	Small molecules	Small molecules, peptides, proteins, micro/nanospheres	Small molecules, peptides, proteins
Network formations	Physical, covalently cross-linked, and IPN gels	Physical and covalently cross-linked gels	Physical and covalently cross-linked gels
In situ gelation possible	NO	YES	YES
Degree of burst release	High	Moderate	None
Smart delivery mechanisms	pH-Sensitive swelling, polymer dissolution and degradation	pH-Sensitive swelling, polymer dissolution and degradation	Enzyme-sensitive release, polymer dissolution and degradation
Release durations	Hours to days	Days and weeks	Days to months
Comments	High loading efficiencies for hydrophilic drugs, low chance of drug deactivation	Suitable for loading hydrophilic and hydrophobic drugs, moderate chance of drug deactivation, chance of toxic material leaching	Best suited for hydrophilic drugs, possible drug deactivation during polymer bonding

Figure 4. Comparison between methods of drug loading in hydrogels.

Hydrogel samples (1x1cm) of chosen gel ratios were placed in a filtered drug saturated solution for 60 minutes, keeping in view the maximum time taken by samples to reach the EWC. After 60 minutes, the samples were air dried. Gel drying and consequent changes in weight were monitored carefully. The concentration of drug per sample is given below (Table 1). The appearance of the gel samples after drug loading and drying is as follows. **(Figure 5)**

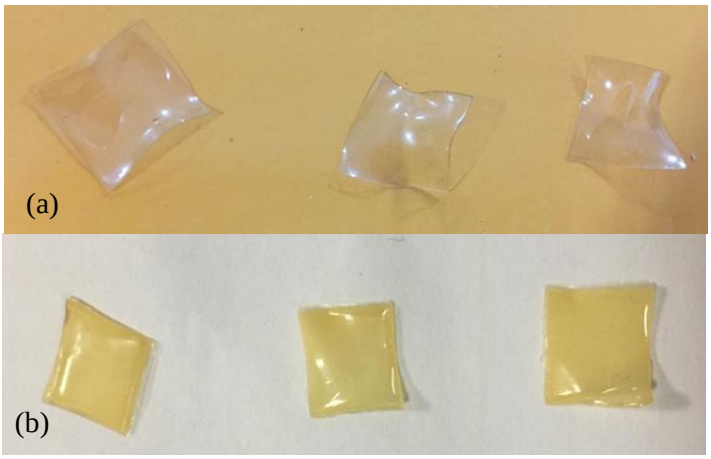


Figure 5. Drug loaded and dried samples of hydrogels. (a) Chitosan-Gelatin, (b) HEMA-Gelatin.

Table 1. Concentration of drug (mg) per sample weight (mg).

Samples	Sample Weight (mg)	Bacitracin Zinc (mg)
CG Hydrogel (1x1 cm)	20	0.5
HG Hydrogel (1x1 cm)	60	0.51

It shows that 0.025mg drug was incorporated in each mg of CG sample and 0.0085mg drug was absorbed by each mg of HG sample. The presence of drug was further justified by Fourier transform infrared spectroscopy.

2.3.2. Drug Release Study

In this study, the drug incorporated in the hydrogel was subjected to release under certain conditions and its amount was calculated after certain time intervals. It indicates the amount of drug to be released at the wound site with the passage of time. The study was done within wound's pH range (4-8).

Three 1x1 cm samples of both drug containing hydrogel films were immersed in 10 mL Phosphate Buffer Saline (PBS) solution at pH 4, 6, 7.4, 8 and 10. At various time points an aliquot (3mL) of eluted drug medium was removed for quantification; this volume (3mL) was replaced with fresh buffer to prevent sink conditions. The study was carried out for 48 hours and the amount of drug in the aliquot was then measured spectrophotometrically at 470 nm. The average of triplicate samples at each pH was calculated.

Following are the mechanisms of drug release from hydrogels **(Figure 6)** [31]. In this study, all three mechanisms were observed at different times.

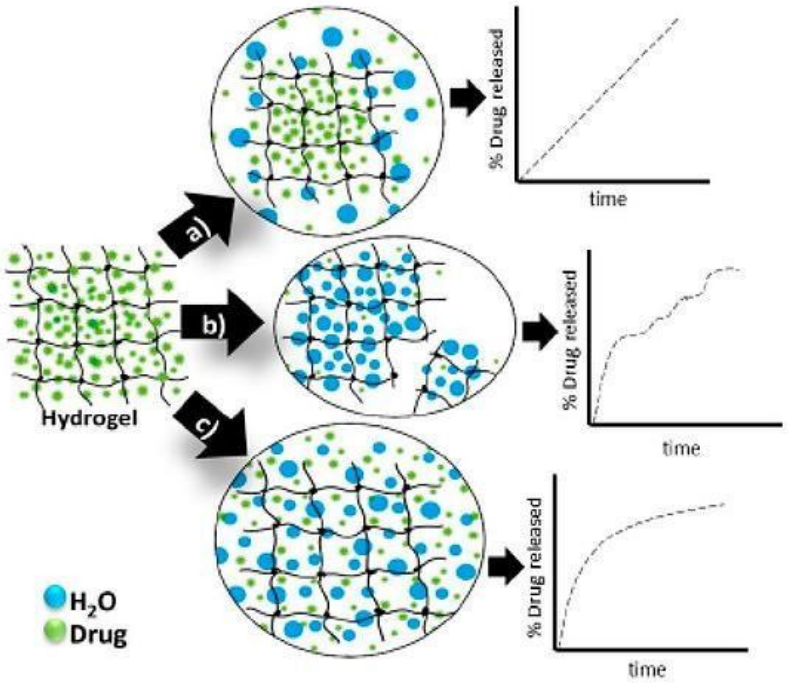


Figure 6. Hydrogels drug release mechanisms, (a) drug diffusion, (b) degradation of the polymeric matrix and (c) swelling.

2.3.3. Degradation Study

Purpose of this study was to find out the changes in hydrogel's integrity when placed under specific conditions in a specified amount of time, and to know when one change the hydrogel dressing before its degradation time, and also to check and compare both hydrogels as to which has longer time of degradation.

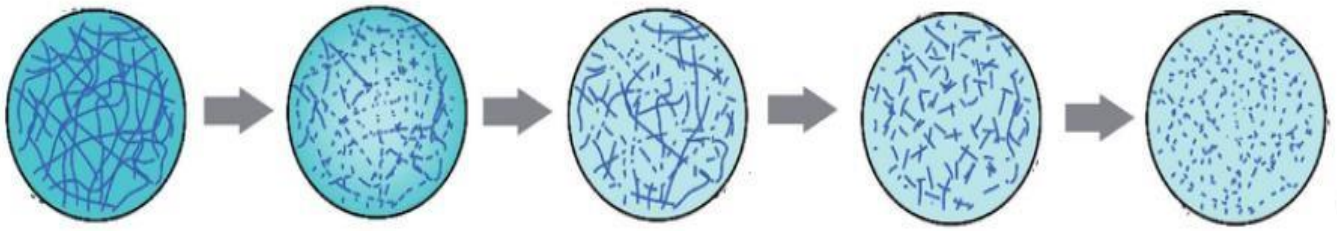


Figure 7. Degradation of Hydrogels

Three 1x1 cm samples of both hydrogel films were immersed in 10 mL PBS at pH 4, 6, 7.4, 8 and 10, each. At various time points the PBS was replaced and weight of the sample was measured using electronic measuring balance. The change in weight of the samples was observed and measured for 96 hours and average of triplicate samples at each pH was reported. The gel samples showed different results at different pH. Their appearance at each pH is shown below. **(Figures 8 and 9)**

Figure 8. Appearance of CG samples at different pH.

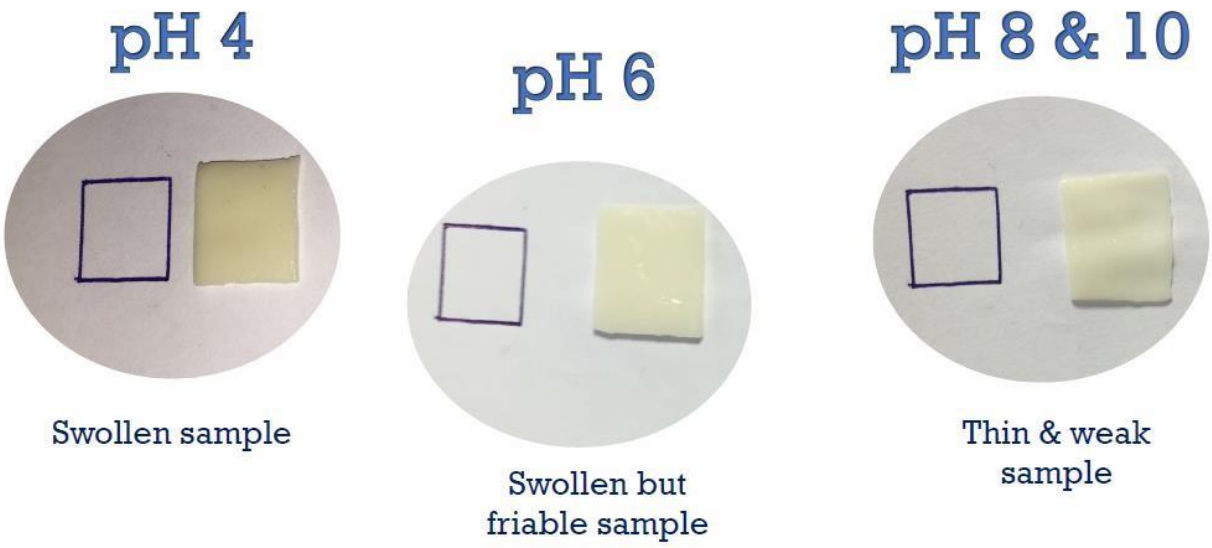


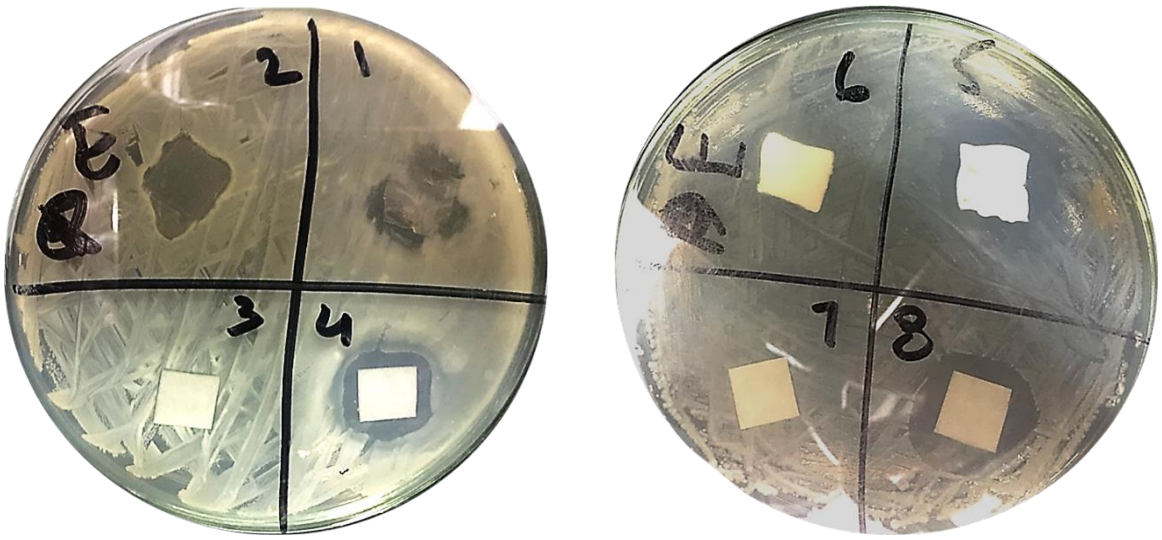
Figure 9. Appearance of HG samples at different pH.

2.3.4. Anti-Bacterial Study

The drug released from CG and HG films were tested for antibacterial activity, whether it's potent enough to prevent infection at the wound site. This was done by the disc diffusion method using *Escherichia coli*, *Pseudomonas aeruginosa* and Methicillin-resistant *Staphylococcus aureus* (MR*SA*) as test organisms kept in nutrient media. Nutrient agar media was used to prepare agar plate by pouring into sterilized petri dishes and solidified. Sterilized Agar media was prepared by dissolving 28 g of Nutrient Agar (Oxoid) in 1 L of distilled water and the solution was autoclaved at 121°C for 2 hrs. Luria Bertani (LB) broth for bacterial cultivation was prepared by mixing 10 g of tryptone (BioWorld, USA), 5 g of yeast extract (MERCK, Germany), 10 g of NaCl (MERCK, Germany) and 1 L of distilled water followed by autoclaving at 121°C for 2 hrs.

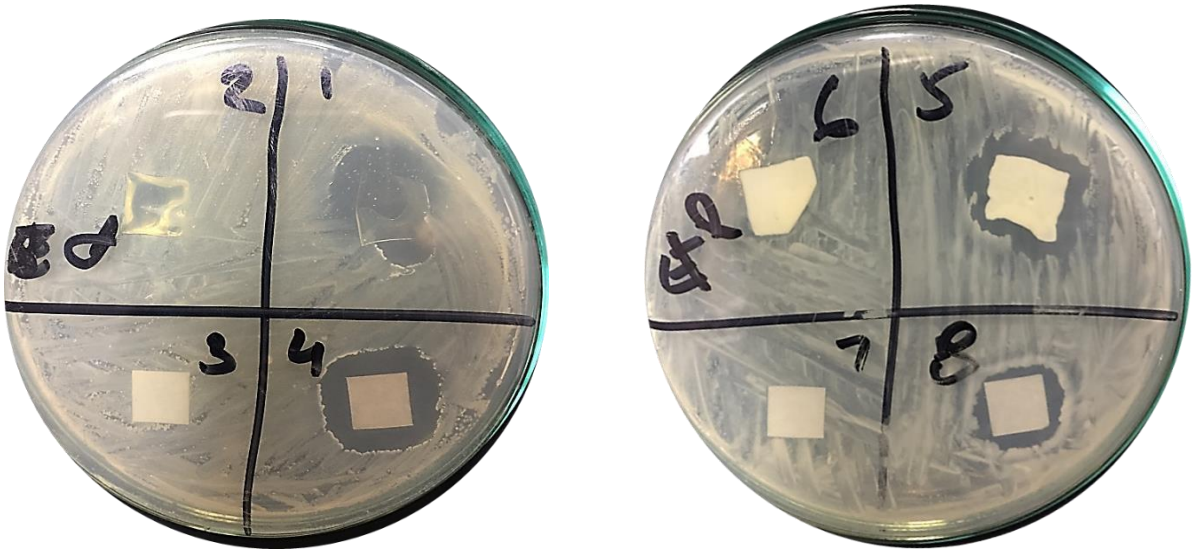
The prepared culture plates were inoculated with the microbes' culture and marked. Gel samples, with and without drug along with control were placed on the plates and incubated for 24 h at 37±0.5°C. Filter paper of same size as gel sample, soaked with distilled water was used as negative control and filter paper soaked with drug solution was used as a positive control. By using a caliper, the diameters of inhibition zones surrounding the samples were observed. Each experiment was carried out in triplicate.

Petri plate of each bacteria, for both hydrogels depicting inhibition zones are shown below.



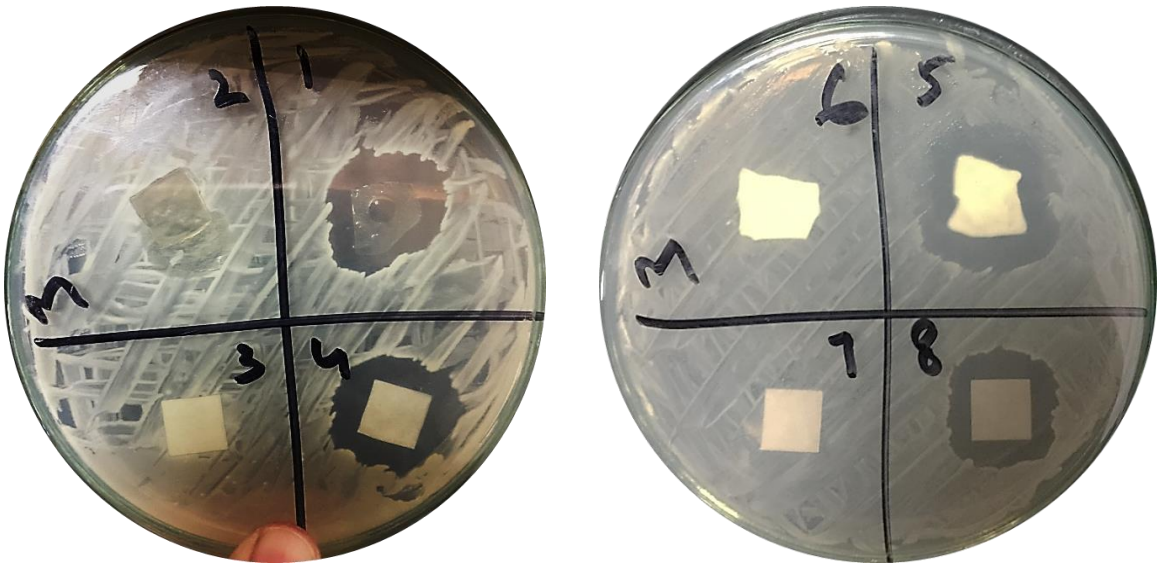
(a) (b)

Figure 10. Culture plates of *E. coli* containing hydrogel samples of (a) CG and (b) HG. 1,5: drug loaded gel samples, 2,6: gel samples without drug, 3,7: negative control, 4,8: positive control.



(a) (b)

Figure 11. Culture plates of *P. aeruginosa* containing hydrogel samples of (a) CG and (b) HG. 1,5: drug loaded gel samples, 2,6: gel samples without drug, 3,7: negative control, 4,8: positive control.



(a) (b)

Figure 12. Culture plates of MR*SA* containing hydrogel samples of (a) CG and (b) HG. 1,5: drug loaded gel samples, 2,6: gel samples without drug, 3,7: negative control, 4,8: positive control.

2.4. Material Characterization

2.4.1. Scanning Electron Microscopy (SEM)

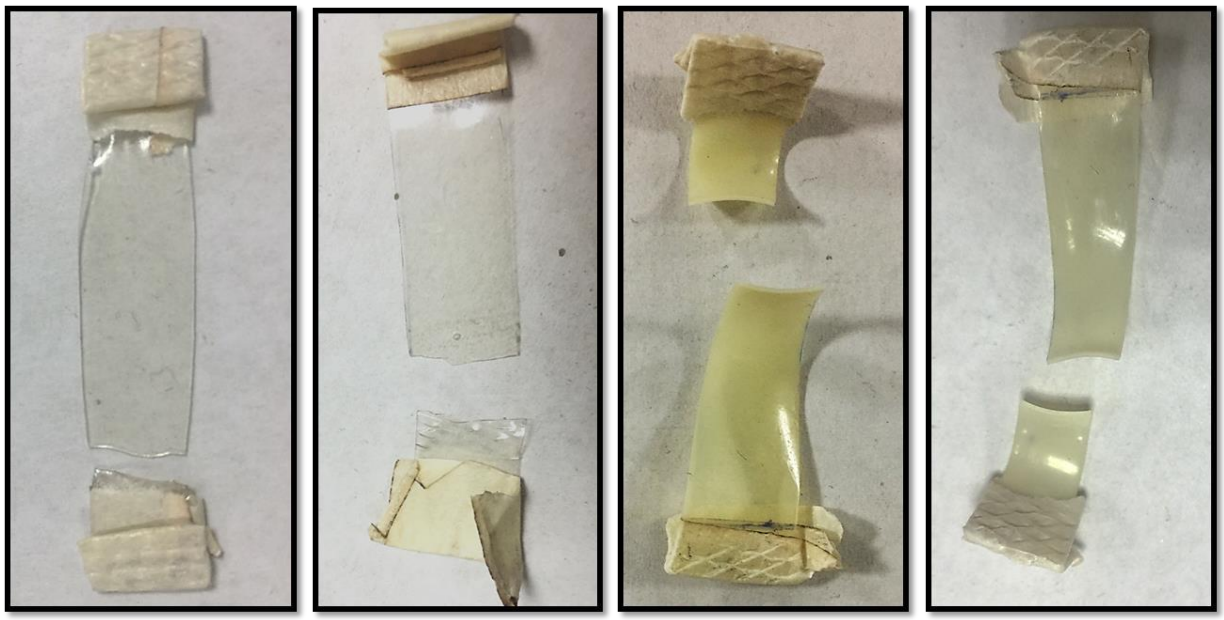
The surface topography and morphology of hydrogel inter-polymeric network were investigated by characterization of both Chitosan-Gelatin and HEMA-Gelatin surface and cross-section areas, using Scanning Electron Microscopy (SEM). The samples were placed on the standard specimen mounting stubs and were coated with a thin layer (20 nm) of gold by sputter coater unit JFC-1500 before analysis, to make the surface conductive.

2.4.2. FTIR Spectral Analysis

The FTIR spectral analysis was used to evaluate the chemical configuration of both Chitosan-Gelatin and HEMA-Gelatin films, with and without drug, to assess the possible interactions between the compounds in prepared films. The FTIR was carried out in ATR mode and the hydrogels were chemically finger-printed at the wavelength range between 4000–800 cm^{-1}. The transmission spectra were recorded and interpreted to identify the bond stretching in functional groups [32]. Essential FTIR software was used to analyze the FTIR spectra of the hydrogels.

2.4.3. Tensile Testing

Samples (1cmx5cm) of both Chitosan-Gelatin and HEMA-Gelatin were placed in the grips of a Universal Testing Machine at a specified grip separation and pulled until failure. Standard method ASTM D638 (Standard Test Method for Tensile Properties of Plastics) [33] was followed for this test, and 10mm/min speed was used in order to check the ultimate tensile strength of the hydrogel films in both wet and dry forms. Other factors such as, maximum stress, strain, force, time and displacement, calculated at entire areas were also calculated.



(a) (b) (c) (d)

Figure 13. Hydrogel samples after mechanical tensile testing, (a) CG dry, (b) CG wet, (c) HG dry, (d) HG wet.

2.5. In- Vivo Study

After acquisition of satisfactory results from in-vitro studies and material characterization, the two types of wound dressings were further tested on mice, to check their efficacy and to compare them for procurement of final conclusion, as to which dressing is better and does it really provide good results on a living subject [34].

2.5.1. Approval from Ethical committee

Institutional Review Board (IRB) ethical approval was needed to conduct the animal study. To attain the approval, all the required documentation was done, and the objective and necessity of this study was explained clearly to the officials. The biocompatibility of all the required materials was justified individually and it was made sure that the minimum number of animals to be used for this study. The approval letter is shown in Figure 14.

2.5.2. Animal Groups

After the acquisition of Institutional review board (IRB) letter, 12 Male BALB/c mice were taken and divided into four groups of three mice each.

- Negative Control group
- Positive Control group
- Experimental group (Chitosan-Gelatin)
- Experimental group (HEMA-Gelatin)

The mice of all group were kept in standard conditions i.e. 21°C temperature, 12hr light-dark cycle, and free access to food and water [35].

2.5.3. Wound formation

Hair from the dorsal side of mice were removed using a depilatory cream. Local anesthesia of Ketamine and Xylazine cocktail (1:7) was prepared and administered in intraperitoneal cavity, according to the weight of mice (0.1mL/20g) [36]. After confirming the unconsciousness of mice, their bare skin at dorsal side were wiped using alcohol swab and abrasion wound of 1x1cm was created on each mouse, using sterile surgical blade.

ASAB

ATTA-UR-RAHMAN SCHOOL OF APPLIED BIOSCIENCES
NATIONAL UNIVERSITY OF SCIENCES & TECHNOLOGY

Ref: No: IRB-138

Date: 06 May, 2019

IRB APPROVAL LETTER

Project Title: Fabrication and evaluation of pH sensitive Hydrogel films for Sustained drug delivery to the wound.

Name of Principal Investigator I: Dr. Murtaza Najabat Ali

Name of Principal Investigator II: -

Duration of Project: 21 Days

Field and Subfield of Project: Biomedical Science

Name of the Department: School of Mechanical and manufacturing Engineering, SMME, NUST

The aforesaid project has been reviewed by Institutional Review Board (IRB) Committee, ASAB, keeping in view the following selection criteria:

- Qualification, Expertise and Scientific Caliber of the Principal Investigators
- Proposed Goals of the Study
- Subject Selection
- Selection Criteria of Subjects
- Informed Consent Process
- Potential Problems
- Research Design and Methods
- Potential Benefits of the Study
- Risks of the Study
- Management of Risks
- Assessment of Risk
- Confidentiality
- Conflict of Interest

The committee thus **APPROVES** the project on **"Fabrication and evaluation of pH sensitive Hydrogel films for Sustained drug delivery to the wound"** on the scales and criterion set by IRB.

Dr. Attya Bhatti
Head of IRB, ASAB, NUST

Dr. Muhammad Tahir
Member IRB, ASAB, NUST

Dr. Najam us Sahar Sadaf Zaidi
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Figure 14. Institutional Review Board (IRB) Approval Letter.

2.5.4. Wound monitoring and treatment

The wounds of negative control group were left untreated and uncovered and the wounds in positive control group were covered with a commercially available ointment, Polyfax. The wounds of both experimental groups were shielded with their respective wound dressings, first drug loaded hydrogel and then covered with surgical tape. The pH and diameter of the wounds were measured daily for five days. Their pictures were also taken daily, to monitor the wound condition. The wound dressings in both experimental groups were replaced daily, after wiping the wound with alcohol swabs and applying fresh ointment on the wounds of positive control group. The results of all the groups were compiled and compared at the end of the experiment.

Figure 15. Pictorial description of in-vivo study.

Chapter 3 Results and Discussion

3.1. Comparison of Physical characteristics

Table 2. Average thickness and weight of Chitosan-Gelatin hydrogel film formulations.

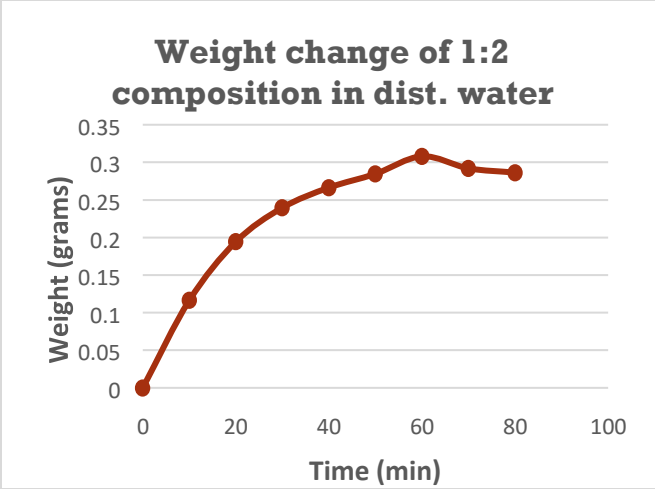
Hydrogel film	Film Ingredients		Characterization Parameters	
	Chitosan (ml)	Gelatin (ml)	Thickness (mm)	Weight (g)
1:2 (1x1 cm)	6.67	13.33	0.16 ±0.01	0.019 ±0.015
1:3 (1x1 cm)	5	15	0.17 ±0.01	0.018 ±0.013
1:4 (1x1 cm)	4	16	0.18 ±0.01	0.018 ±0.015

Table 3. Average thickness and weight of HEMA-Gelatin hydrogel film formulations.

Hydrogel film	Film Ingredients		Characterization Parameters	
	HEMA (ml)	Gelatin (ml)	Thickness (mm)	Weight (g)
1:3 (1x1 cm)	5	15	0.62 ±0.01	0.048 ±0.015
1:4 (1x1 cm)	4	16	0.59 ±0.01	0.047 ±0.013
1:5 (1x1 cm)	3.33	16.67	0.60 ±0.01	0.049 ±0.013

Equilibrium Water Capacity Study Results (Chitosan-Gelatin)

(a)



Equilibrium Water Capacity

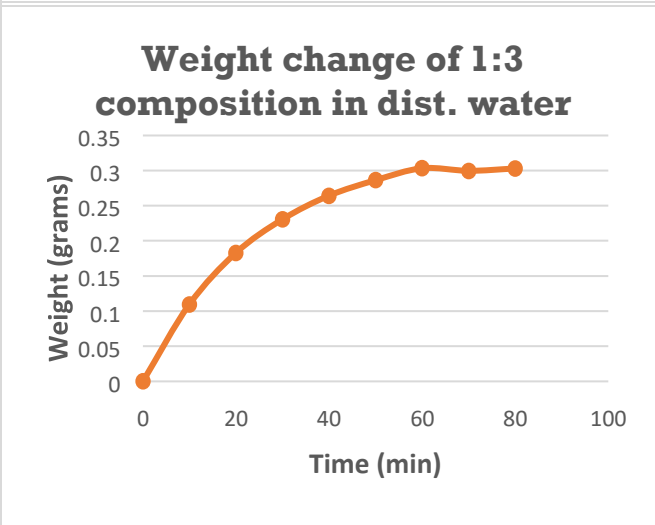
$$EWC\ (\%) = \frac{Ws - Wd}{Ws} \times 100$$

$$= \frac{0.30 - 0.015}{0.30} \times 100$$

$$= 95\ \%$$

Time	0	10	20	30	40	50	60	70	80
STD	0	0.0060	0.0066	0.0170	0.0158	0.0144	0.0079	0.0147	0.0077

(b)



Equilibrium Water Capacity

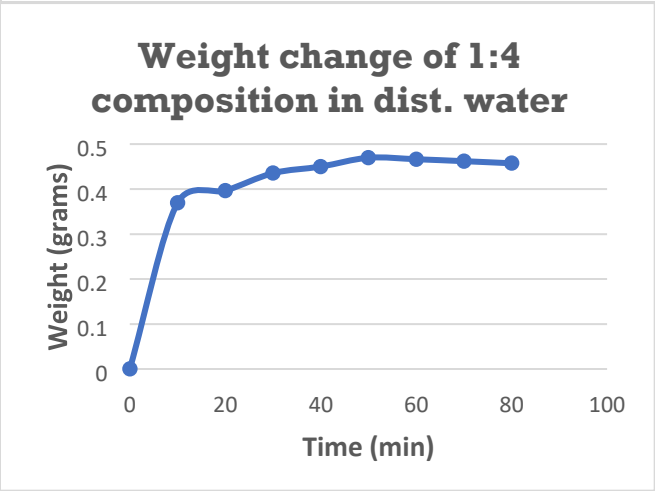
$$EWC\ (\%) = \frac{Ws - Wd}{Ws} \times 100$$

$$= \frac{0.30 - 0.017}{0.30} \times 100$$

$$= 94\ \%$$

Time	0	10	20	30	40	50	60	70	80
STD	0	0.0035	0.0165	0.0124	0.0150	0.0153	0.0323	0.0293	0.0228

(c)



Equilibrium Water Capacity

$$EWC\ (\%) = \frac{Ws - Wd}{Ws} \times 100$$

$$= \frac{0.31 - 0.016}{0.31} \times 100$$

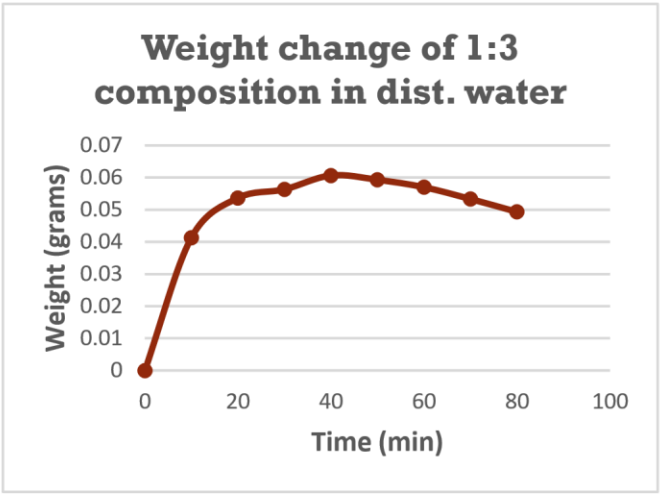
$$= 94\ \%$$

Time	0	10	20	30	40	50	60	70	80
STD	0	0.4251	0.4239	0.4442	04364	0.4454	0.4249	0.4278	0.4215

Figure 16. EWC results of CG at different ratios, (a) 1:2, (b) 1:3 and (c) 1:4.

Equilibrium Water Capacity Study Results (HEMA-Gelatin)

(a)



Equilibrium Water Capacity

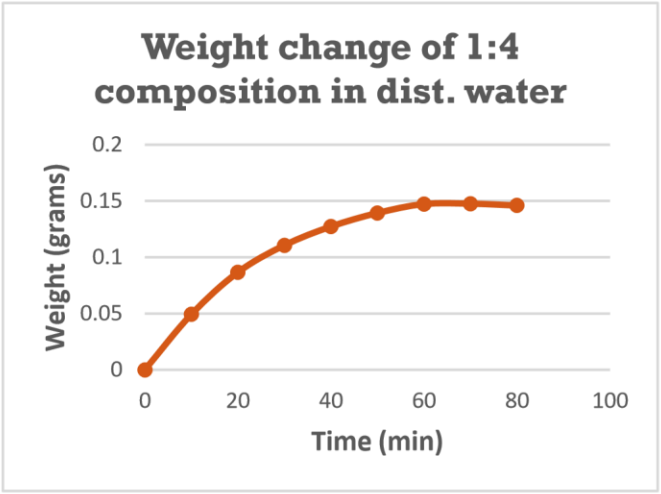
$$EWC\ (\%) = \frac{Ws - Wd}{Ws} \times 100$$

$$= \frac{0.060 - 0.022}{0.060} \times 100$$

$$= 63\ \%$$

Time	0	10	20	30	40	50	60	70	80
STD	0	0.0045	0.0068	0.0089	0.0110	0.0115	0.0115	0.0124	0.0124

(b)



Equilibrium Water Capacity

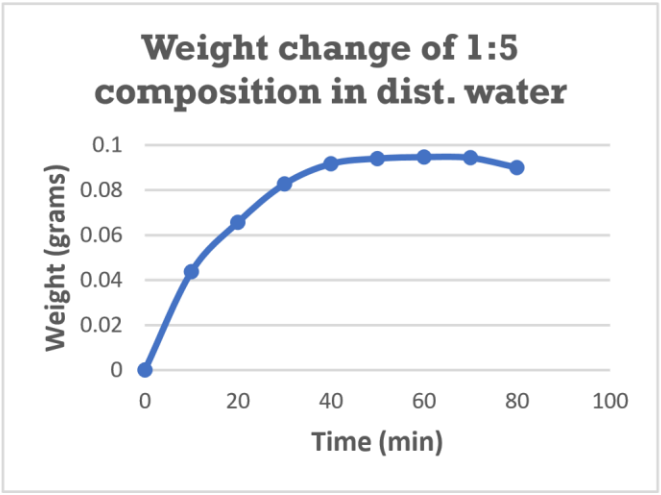
$$EWC\ (\%) = \frac{Ws - Wd}{Ws} \times 100$$

$$= \frac{0.147 - 0.046}{0.147} \times 100$$

$$= 69\ \%$$

Time	0	10	20	30	40	50	60	70	80
STD	0	0.0046	0.0051	0.0056	0.0040	0.0041	0.0035	0.0032	0.0026

(c)



Equilibrium Water Capacity

$$EWC\ (\%) = \frac{Ws - Wd}{Ws} \times 100$$

$$= \frac{0.094 - 0.033}{0.094} \times 100$$

$$= 65\ \%$$

Time	0	10	20	30	40	50	60	70	80
STD	0	0.0020	0.0058	0.0087	0.0135	0.0140	0.0161	0.0162	0.0153

Figure 17. EWC results of HG at different ratios, (a) 1:3, (b) 1:4 and (c) 1:5.

Table 2 and 3 depict the various measurements of CG and HG hydrogels respectively, in different ratios. The average values show that HG films are a little thicker and heavier than CG films, may be due to the greater number of ingredients used in their fabrication. Other than this observation, both films have uniform thickness and weight throughout and can make a suitable wound dressing which will conform to the skin surface and is breathable as their thickness is less than human skin thickness.

Figures 16 and 17 represent Equilibrium Water Capacity results of both Chitosan-Gelatin and HEMA-Gelatin hydrogels in three ratios each. Each part of figure has further three parts, including a graph that depicts the weight change of hydrogel samples at each interval when placed in distilled water for 80 minutes. The Standard deviation results are also stated in the table and EWC percentage has also been calculated through the formula. On comparison of the results, it can be seen that CG films have more swelling rate and water capacity than HG films, even though they are thinner than HG films. They allow more water uptake than HG films and can absorb more exudate from the wound site.

From the derived results, one ratio from each hydrogel was picked out for further studies, based on two factors; their maximum water capacity and highest time of degradation (i.e. time where the weight of gel started decreasing). The ratios that qualified for drug loading and in-vitro studies included, 1:3 of CG and 1:4 of HG.

3.2. Comparison of Drug Release Kinetics

The drug release study of both hydrogels was conducted in triplicates and the PBS solution at different pH values was used as drug eluting medium. The pH range was selected on the basis of wound pH that changes throughout its different stages of healing. The behavior of hydrogels at pH other than skin and pH was also monitored, to exclude the risks of its function in extreme conditions. The following graphs **(Figure 18)** demonstrate the average amount of drug released at pH 4, 6, 7.4, 8 and 10 from both hydrogels over the timespan of 48 hours.

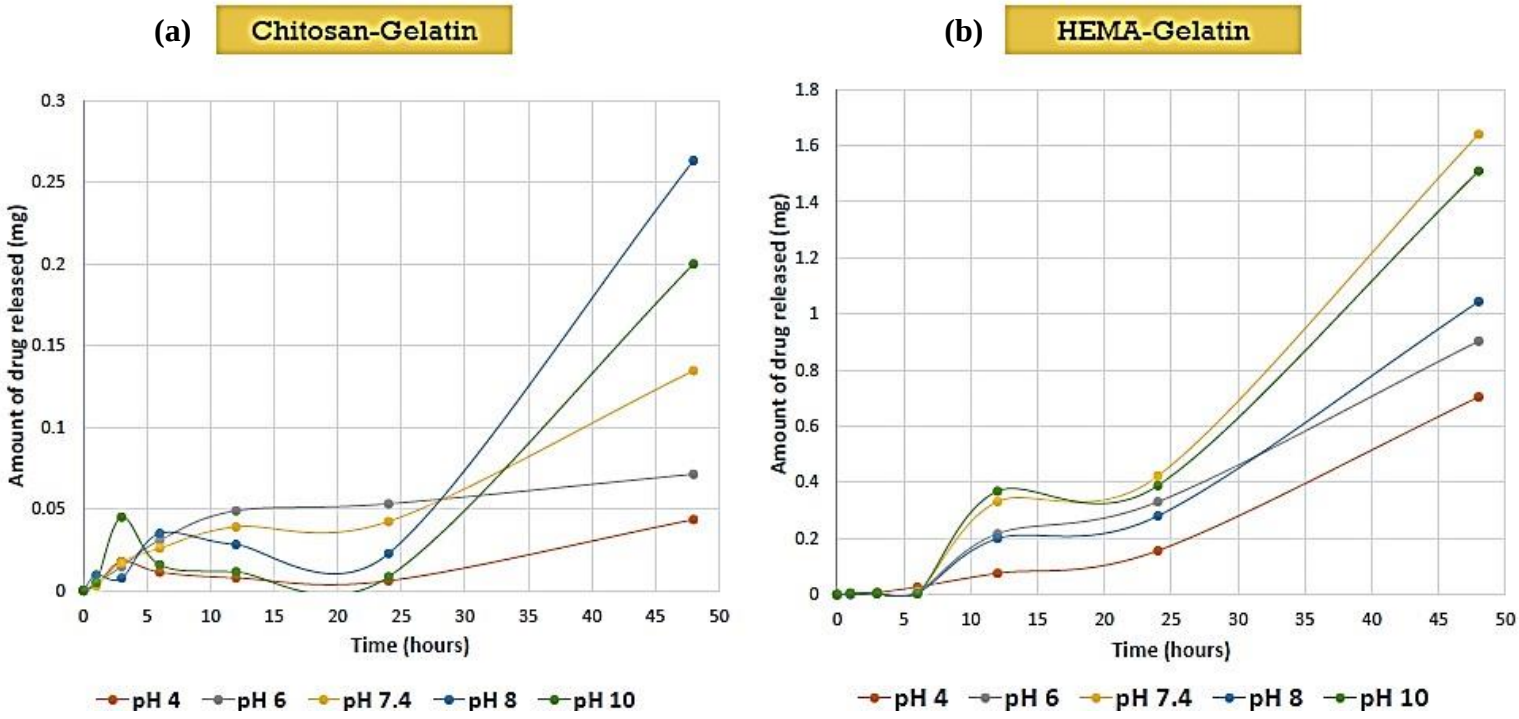


Figure 18. Average drug release of hydrogels. (a) Chitosan-Gelatin, (b) HEMA-Gelatin

The above graphs show three mechanisms of drug release over time, swelling at start when solution enters the hydrogel structure, then the embedded drug starts diffusing out of hydrogel into the solution. In both hydrogels, the drug release at lower (acidic) pH values is less than at the higher (alkaline) pH values, which is favorable, as more drug is needed in the initial stages of wound healing when the pH is alkaline and is susceptible to infection. Then with the passage of time and progression of wound healing, the pH starts decreasing towards the normal acidic range of skin i.e. about 5.5. The drug is not much needed at acidic pH which is what observed in the drug release. As for comparison between Chitosan-Gelatin and HEMA-Gelatin composites, the former shows more compliant release as in the case of HG, the drug release is also higher near the neutral pH i.e. 7.4 which is not necessary. Moreover, in HG, the drug is not released in first five hours, when the wound is more vulnerable and there is high susceptibility of infection.

Whereas in CG, the drug release is higher within initial hours at higher pH and gradually decreases later, uniformly. But one little shortcoming is he behavior of CG at pH 6, the drug release is higher between 10-15 hours than at all other pH values.

3.3. Comparison of Degradation

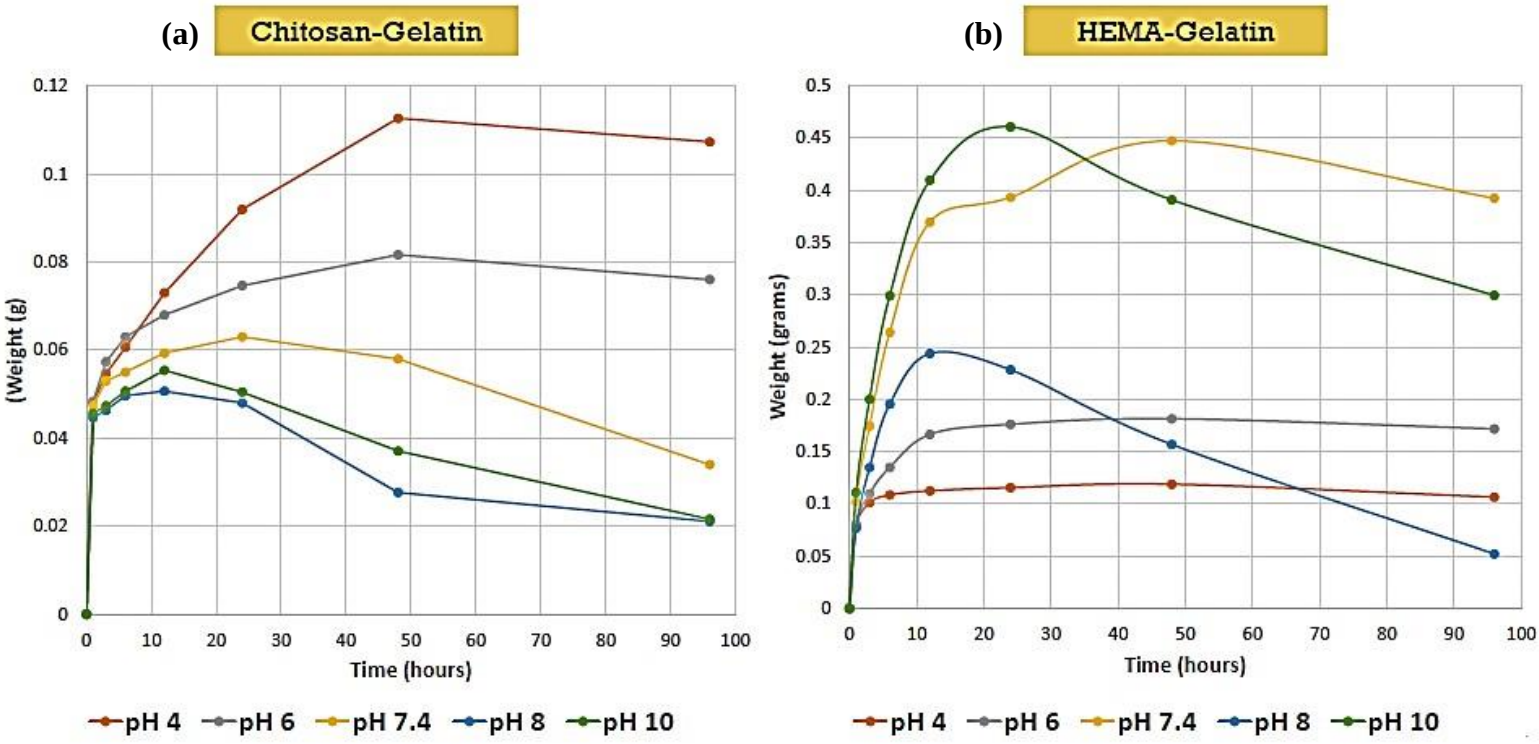


Figure 19. Average degradation of hydrogels. (a) Chitosan-Gelatin, (b) HEMA-Gelatin

Degradation of both hydrogels was also monitored in PBS at different pH over 96 hours. In case of CG, the degradation was comparatively uniform. Its gel samples degraded rapidly at alkaline pH; within 12 hours, but at acidic pH they kept swelling up to 48 hours. The response of gel at pH 7.4 was kind of neutral, in between acidic and alkaline pH; it degraded after 24 hours.

The hydrogel samples of HG on the other hand showed stable behavior at acidic pH. They neither swell much nor degrade rapidly. They survived till 48 hours after keeping almost constant weight for a long time. As for the gels at pH 8 and 10, the weight kept increasing for 12 and 24 hours respectively and then dropped quickly. The behavior of samples at pH 7.4 was also a blend of acidic and alkaline pH, they absorbed plenty of solution like samples at alkaline pH, but also remained constant like the samples at acidic pH after their degradation at 48 hours.

Overall, the samples of both hydrogels at alkaline pH became shrunk and small whereas remained swollen in acidic pH till their degradation. They were swollen but friable and weak at neutral pH.

3.4. Anti-Bacterial Study Results

Anti-bacterial study was also done in triplicates, the average diameter of zones of inhibition formed near drug loaded samples and positive control groups of both hydrogels, for three bacteria; *E. coli*, *P. aeruginosa* and MR*SA* are shown in **Figures 20**, **21** and **22** respectively.

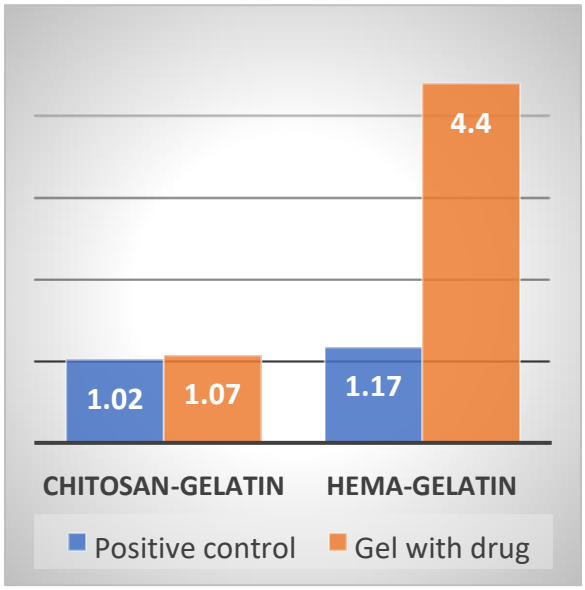


Figure 20. Average diameter of inhibition zones formed by bacitracin zinc in *E. coli* culture.

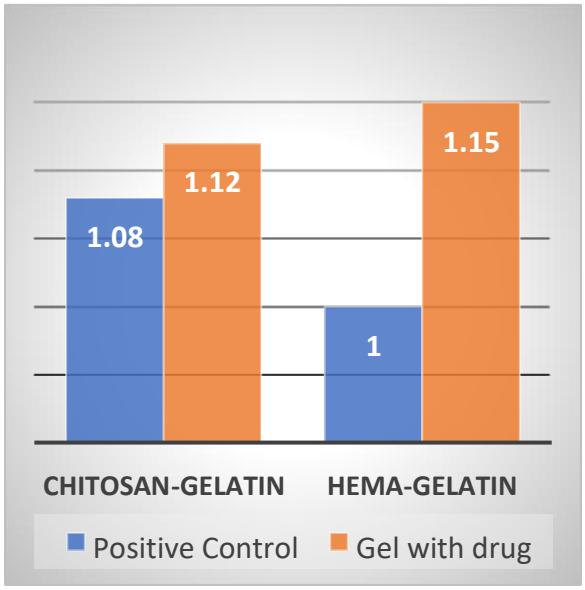


Figure 21. Average diameter of inhibition zones formed by bacitracin zinc in *P. aeruginosa* culture.

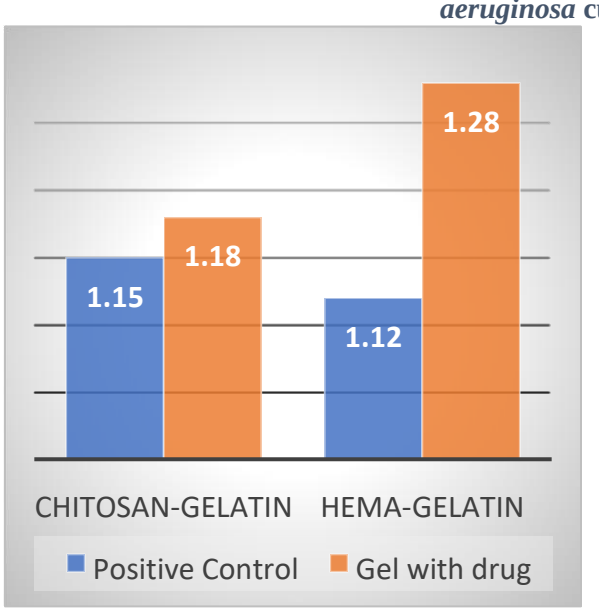


Figure 22. Average diameter of inhibition zones formed by bacitracin zinc in MR*SA* culture.

The results clearly display that both CG and HG have better antibacterial activity than positive controls in all three bacterial cultures when the amount of drug was kept equal in them. Next, between the two hydrogels, HG show comparatively better results than CG.

3.5. SEM Results

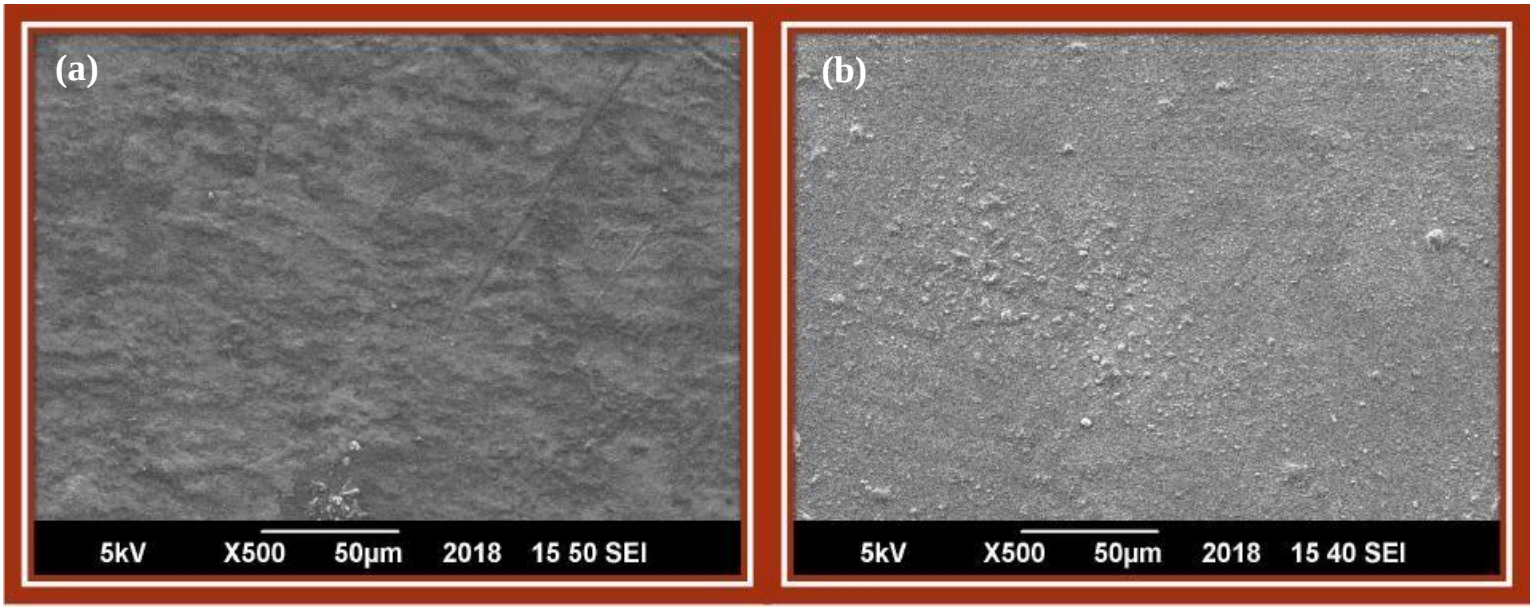


Figure 23. SEM images showing surface morphology of hydrogels. (a) Chitosan-Gelatin, (b) HEMA-Gelatin

Surface analysis of both hydrogels show homogenous, compact and continuous structures **(Figure 23)**. The CG surface is much smoother than HG surface, which is because of the little particles of the initiators used for the formation of HG hydrogel [37].

The cross-section areas of both hydrogels were also analyzed through Scanning Electron Microscopy **(Figure 24)**. Generally, both the cross-sections have homogeneity, but again CG's cross-section is shown to have more even structure than HG's, which has shreds of dried particles inside it as well.

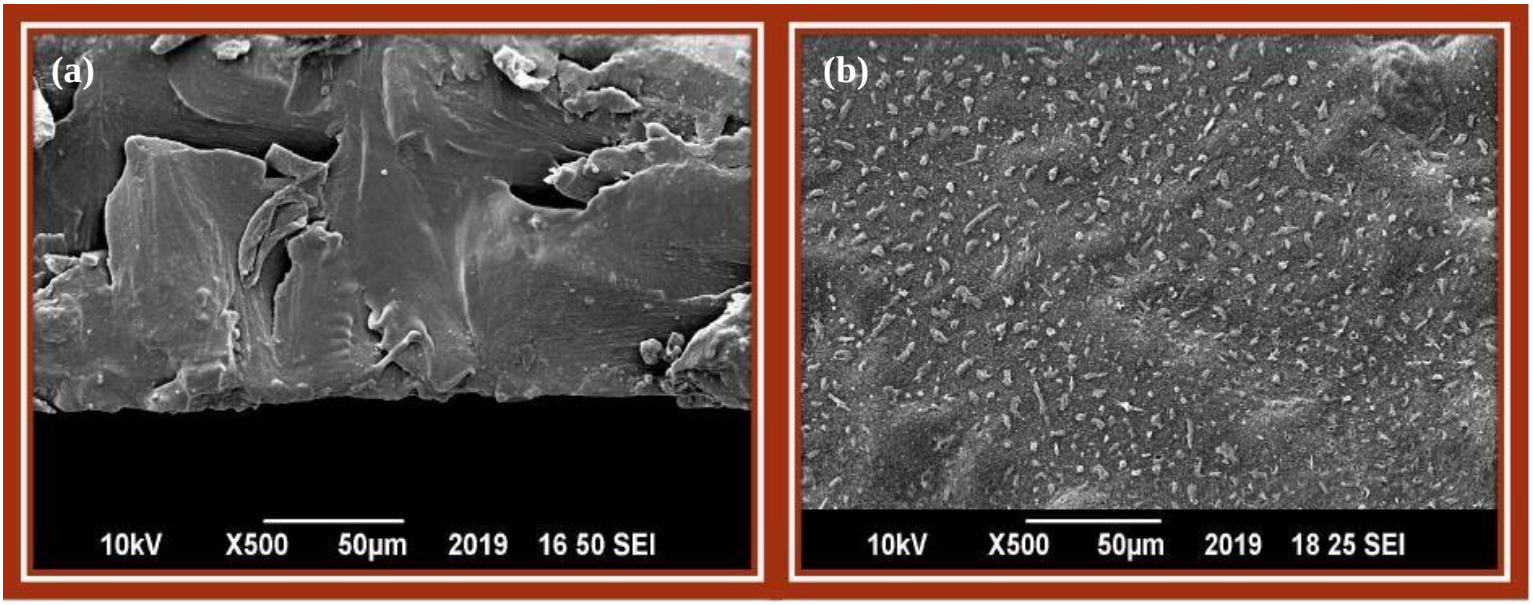


Figure 24. SEM images showing cross-sections of hydrogels. (a) Chitosan-Gelatin, (b) HEMA-Gelatin

3.6. FTIR Results

FTIR result of CG hydrogel without drug **(Figure 25)** depict the Amide I and Amide II (Chitosan & Gelatin), Amide III (Chitosan) functional groups, N-H group bending vibration and imine bond (C-N) indicating the formation of covalent linkage between Gelatin and Chitosan through the Glutaraldehyde cross linker moiety. The only difference in the FTIR result of CG hydrogel with drug **(Figure 26)** is the increase in intensity of band at 1696 cm^{-1} shows Bacitracin zinc binding to the gel matrix.

In the FTIR result of HG hydrogel without drug, **(Figure 27)** stretching vibration for the OH group can be identified at 3600 cm^{-1}. A peak at 1014 cm^{-1} may be attributed to the C-O-C group and the addition of Gelatin in the composite hydrogel may be identified by the 1762 cm^{-1} peak. Increase in the intensity of band at 1600-1700 cm^{-1} shows Bacitracin zinc binding to the gel matrix in the FTIR result of HG hydrogel with drug **(Figure 28)**.

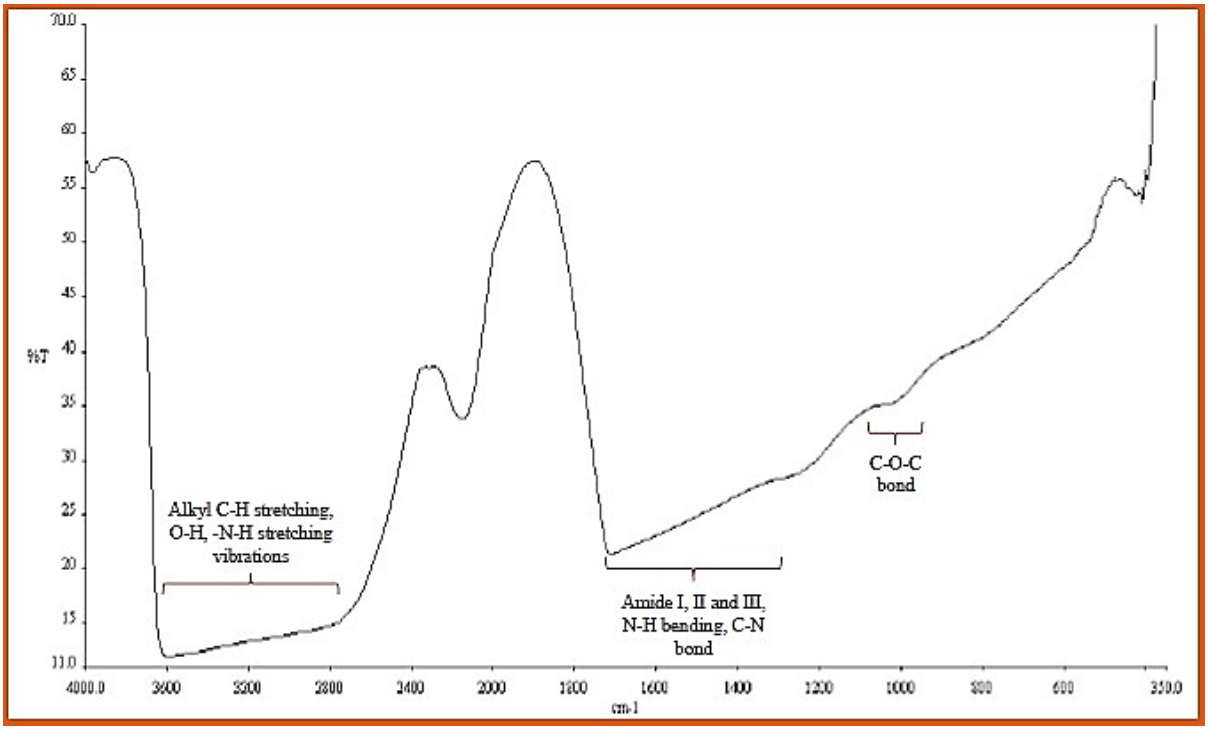


Figure 25. FTIR Spectrum of CG hydrogel without drug.

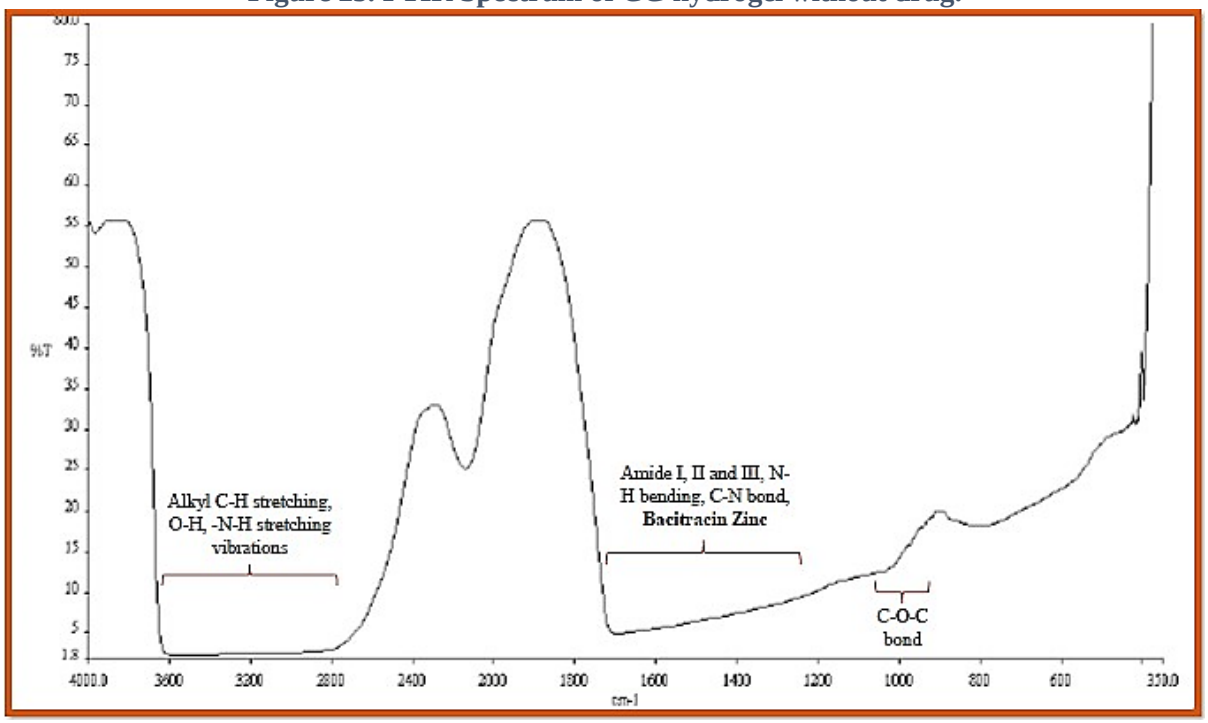


Figure 26. FTIR Spectrum of CG hydrogel with drug.

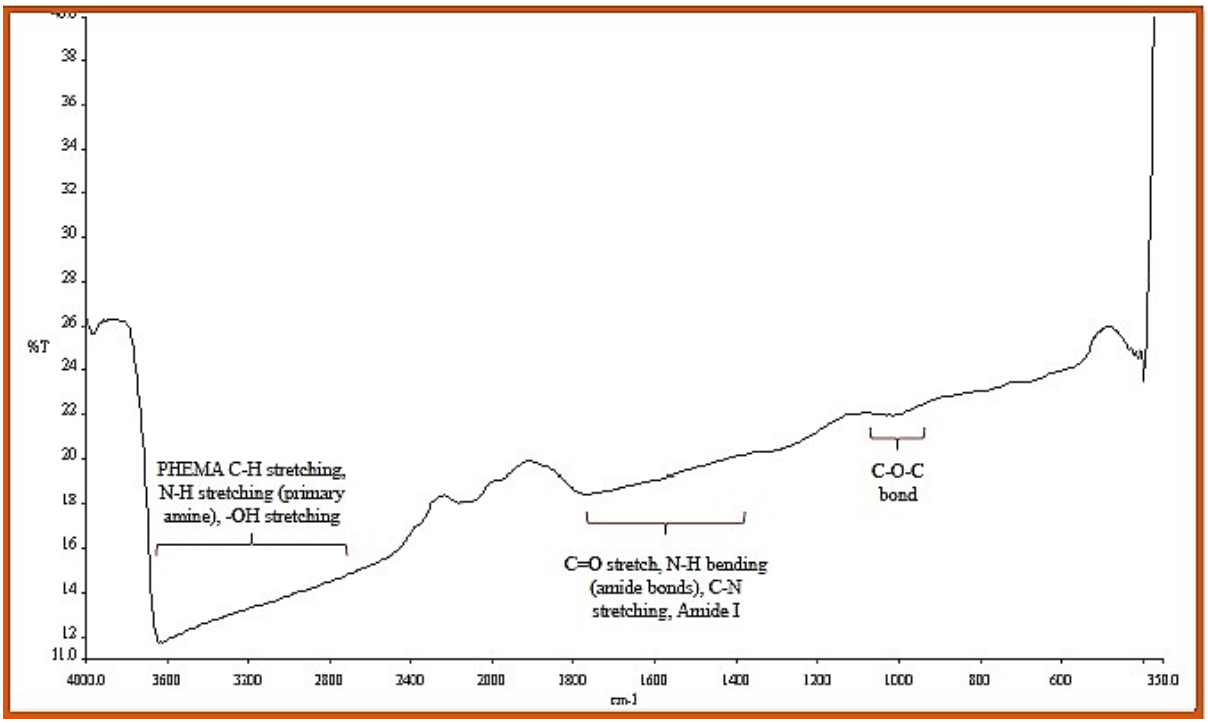


Figure 27. FTIR Spectrum of HG hydrogel without drug.

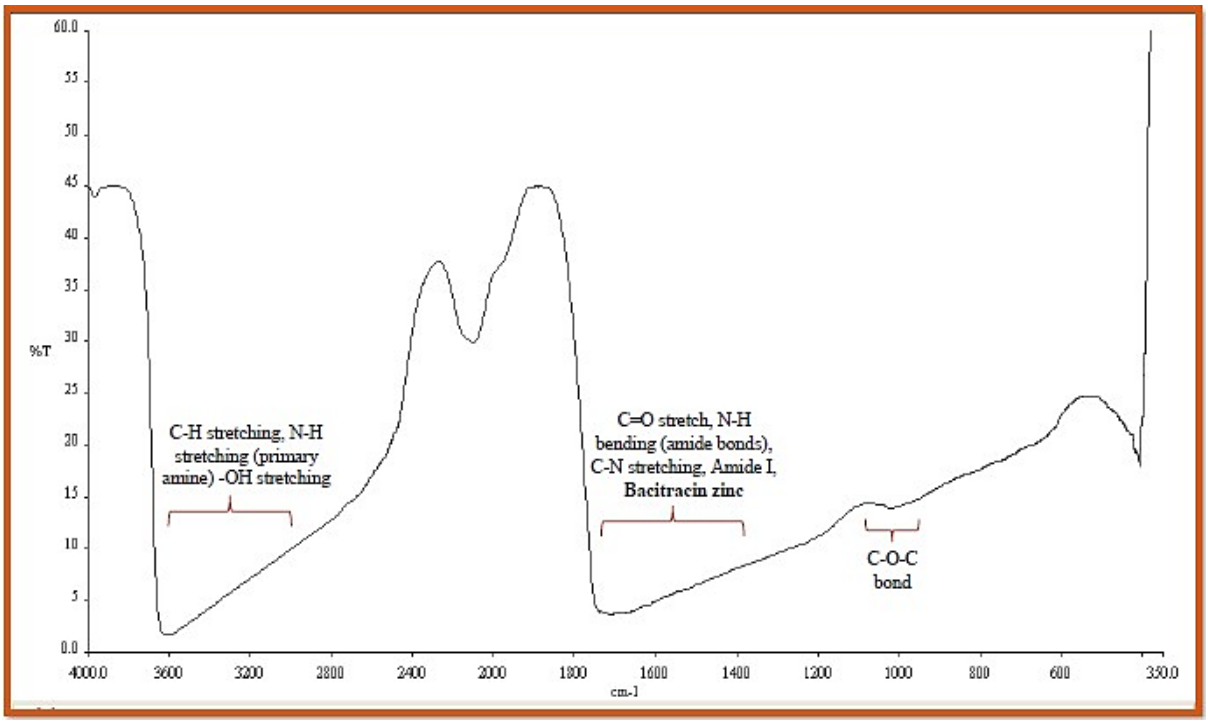


Figure 28. FTIR Spectrum of HG hydrogel with drug.

3.7. Tensile Testing Results

The difference in the ultimate tensile strength of both hydrogels in dry and wet forms are depicted in **Figure 29**. It is the measure of the maximum stress that a material can endure. The results show that CG dry has the maximum Ultimate tensile strength of 38.21, and in wet form it's much decreased to 0.0321. Whereas, the ultimate tensile strength of dry HG is much less than dry CG, being 1.619 and even lesser in wet form which is 0.0179.

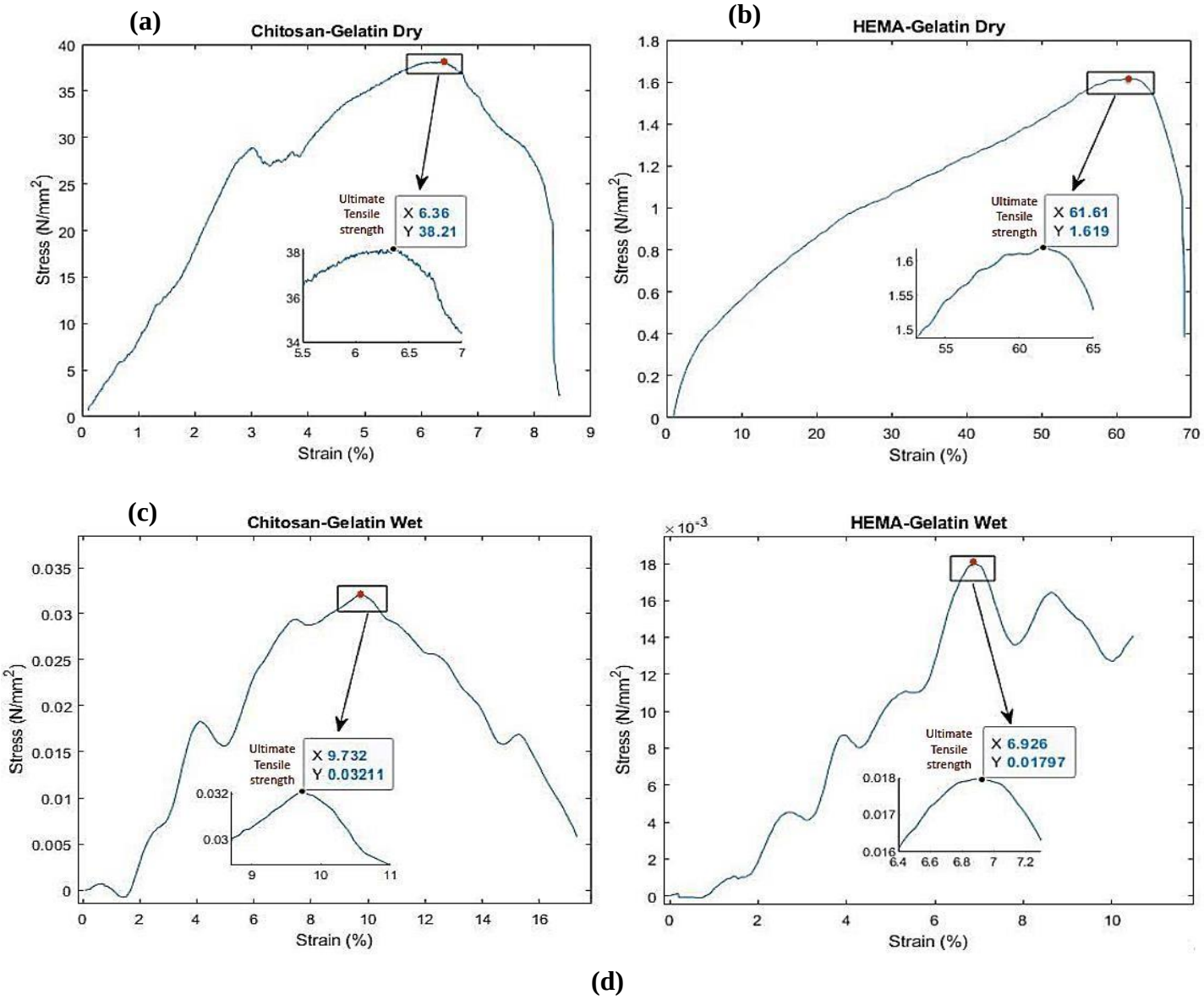


Figure 29. Mechanical tensile testing results of hydrogel samples in wet and dry forms. (a) CG dry, (b) HG dry, (c) CG wet, (d) HG wet.

3.8. In-Vivo Study Results

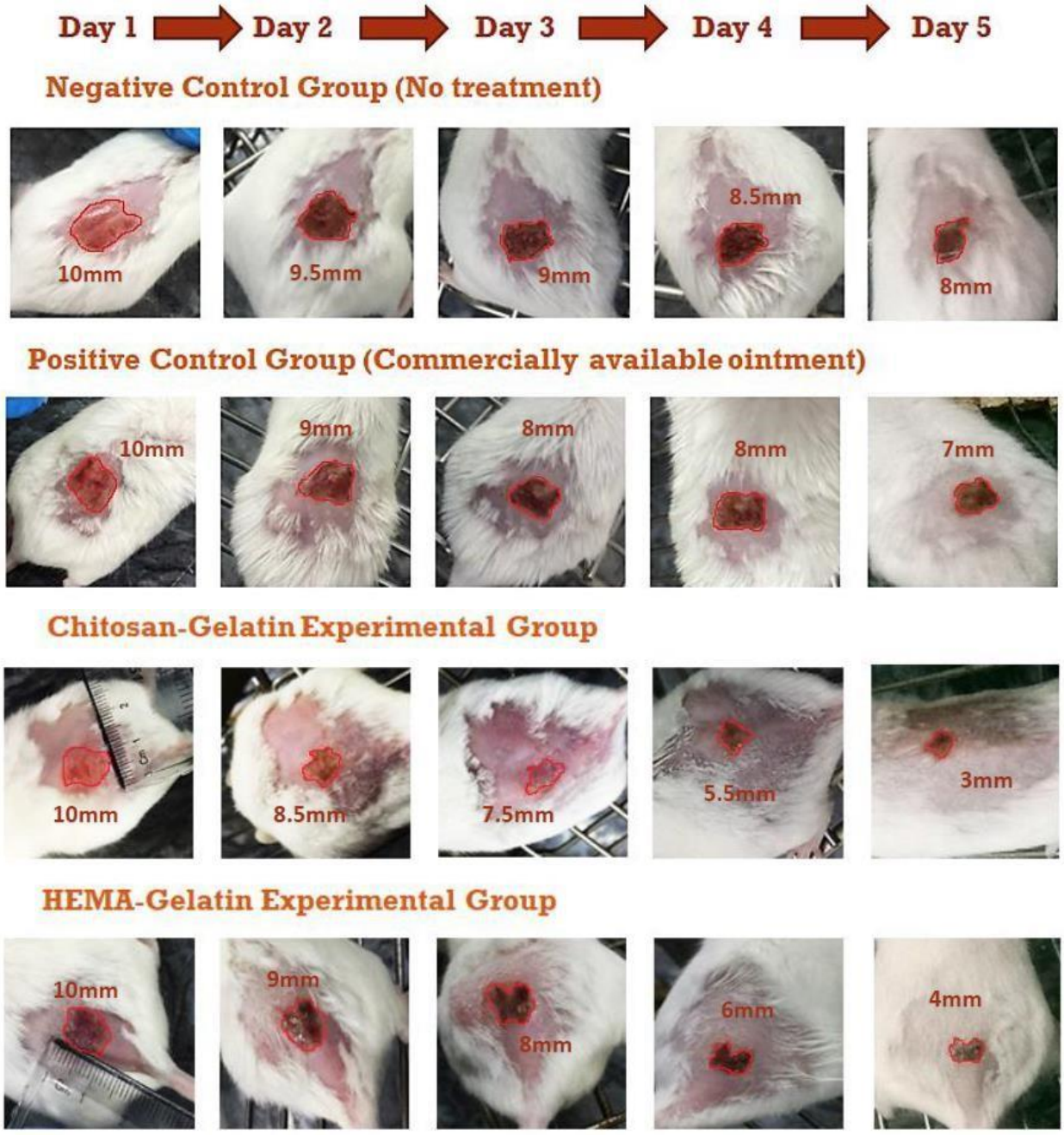


Figure 30. Pictorial representation of wound healing in control and experimental groups.

The wound condition was checked daily, and the diameter wound was recorded, for five days after formation of wound. The study was carried out in triplicates for each group; positive control, negative control, experimental (CG) and experimental (HG). The average results are described pictorially **(Figure 30)** as well as in graphical form **(Figure 31)** to compare the difference in healing.

The initial wound diameter was 10mm in all groups, which gradually decreased after healing. The negative control group in which no treatment was given, the wound healing was very slow and did not change much within five days. As for positive control group, the commercially renowned ointment was applied daily, but the wound healed at a slower pace than experimental groups. Between the two experimental groups, results varied each day but in the final outcome (day 5), CG hydrogel showed a little better result than HG hydrogel. The final diameter of CG was 3.33mm whereas in HG it was 4mm.

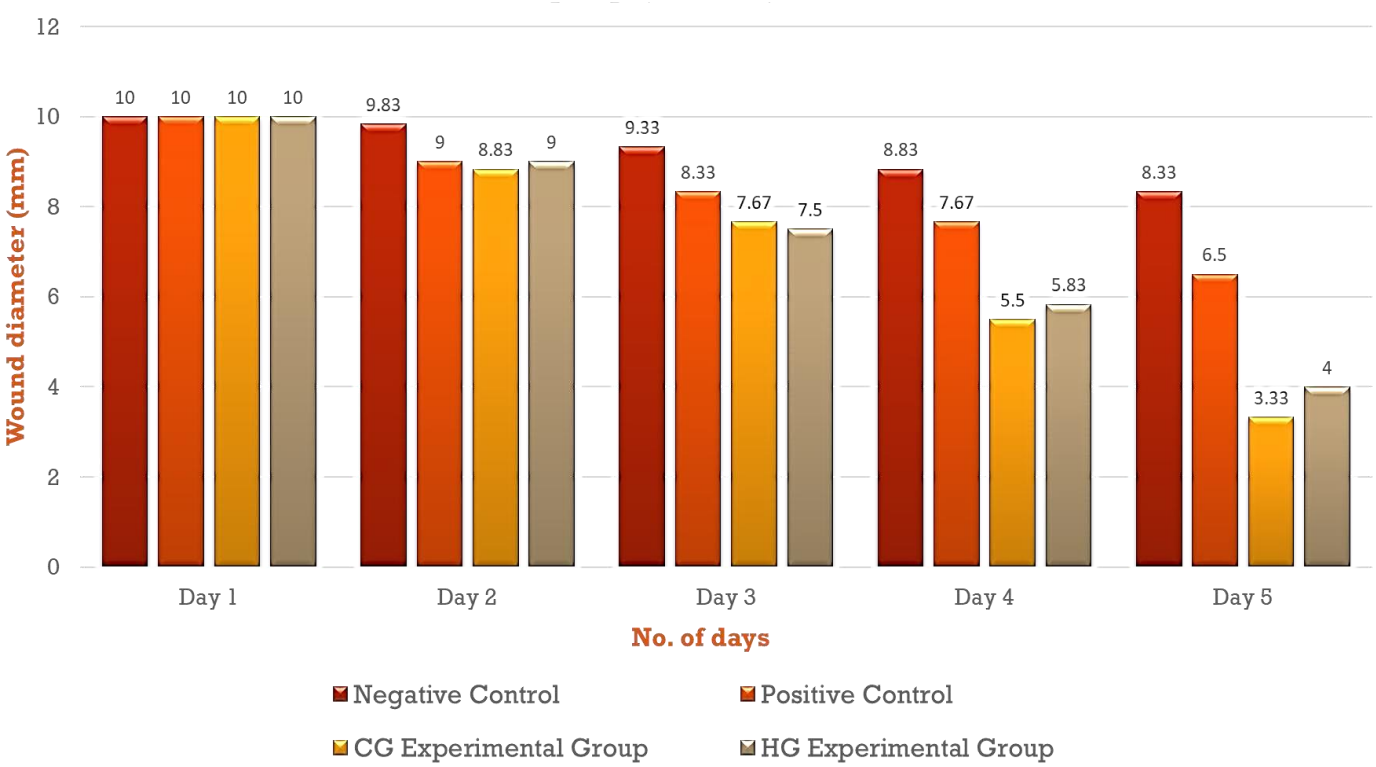


Figure 31. Average change in diameter of wounds in each group during evaluation for five days.

Chapter 4 Conclusions and Future Perspectives

The final comparison of the two hydrogel composites, one natural and the other semisynthetic, deduced by this research is as follows,

Chitosan-Gelatin	HEMA-Gelatin
• Transparent	• Opaque
• Easy to fabricate	• Requires more effort in fabrication
• Greater mechanical strength	• Less mechanical strength
• Flexible in dry form	• Brittle in dry form
• Thin film	• Thick film
• Smooth surface	• A little rough surface
• Consistent drug release at all pH	• Irregular drug release profile at some pH
• Equilibrium Water Capacity = 94%	• Equilibrium Water Capacity = 69%

Research data depicts that moist wound healing is three to five times quicker as compared to the wounds that are left to dry out, therefore, hydrogels showed promising results in wound healing. About comparison between two composites, by keeping in view the results of material characterization, in-vitro and in-vivo results carried out till now, it can be proposed that **Chitosan-Gelatin is a better candidate** to be used as a wound dressing as compared to HEMA-Gelatin.

The drug incorporated in the hydrogels is also present in the Polyfax (used for the treatment of positive control group), in fact Polyfax also contains Polymyxin B sulphate but still better results were shown by drug loaded hydrogels in in-vivo studies. From drug release studies, it has also been established that polymers-based dressings in contrast to hydrocolloids, facilitate and support bacitracin release into wound effectively. Wound dressings made up of natural polymers (CG) evidently provided adequate wound recovery and non-toxicity. No debris from the dressing was left in the wound, eliminating the need for extensive cleaning between dressings.

In conclusion, this formulation has the potential to be used as an anti-bacterial and moist film dressing for drug delivery in wound management.

Limitations and Future Aspects

In many scenarios, an additional cover dressing will be needed since the single dressing can lose moisture easily and is difficult to secure, if not covered properly. The hydrogel dressings are required for the wounds with normal exudate, therefore in case of extremely moist wounds or those displaying heavy exudate, hydrogel dressings should be avoided.

The crosslinking of hydrogel composites was chemical in nature, the mechanical strength of hydrogels can be further increased by inducing physical crosslinking along with chemical crosslinking. Other types of natural and synthetic polymers can also be tested to be used as an antimicrobial wound dressing.

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