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Application of Psyllium, PEG, Agarose and Collagen Based Hydrogels for Controlled *In Vitro* and *In Vivo* Drug (Insulin) Delivery

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UA designed the project and executed this study. SN, UH and TK analysed the data, and wrote manuscript. MA, Atta ur R and Anwar ur R characterized the hydrogels structure and swelling properties.

Key words
PEG, Insulin, Hydrogel, Drug release, Collagen, *In vitro, In vivo*, FTIR, SEM

ABSTRACT

This study delves into the drug release behavior of hydrogels influenced by pH, unveiling a non-Fickian diffusion mechanism and affirming their viability as potent drug carriers through swelling kinetics and FTIR analysis. The primary aim is to explore Psyllium, PEG, Agarose, and Collagen-based hydrogels as a viable alternative for *in-vitro* or *in-vivo* insulin delivery, with the objective of mitigating the associated risks of pain and infection linked to traditional delivery methods. Innovative hydrogels were devised by combining natural substances (Psyllium, PEG, Collagen, and agarose) in a systematic manner tailored for insulin delivery. The synthesis involved blending 0.5 g of psyllium thoroughly with 100ml of distilled water, followed by the integration of PEG, agarose, and collagen into the swelling mixture. The resulting hydrogels were formed after overnight incubation at 37°C. FTIR analysis revealed distinctive bonding patterns, elucidating their physical attributes. Collagen isolation from chicken heart samples was validated through SDS-Page and FTIR analysis, and quantified using the Bradford test. The hydrogels exhibited peak swelling and drug release under alkaline pH conditions, indicating potential for intestinal insulin delivery while resisting stomach acidity. Unconventional swelling kinetics suggested an alternative diffusion pattern, facilitating targeted insulin delivery. Furthermore, these hydrogels showcased antimicrobial properties, evident from observed inhibition zones. In vivo insulin release studies in mice demonstrated a successful reduction in glucose levels, affirming insulin delivery, as measured by glucometer readings. The culmination of various analyses underscores the considerable potential of psyllium, PEG, and collagen-centric hydrogels for *in-vitro* or *in-vivo* insulin delivery, offering a promising avenue for supporting diabetic patients.

INTRODUCTION

Diabetes mellitus (DM) stands as a significant global health concern, rooted in challenges related to insulin production or its efficacy. Insulin, a vital protein hormone constructed from 51 amino acids, plays a pivotal role in regulating glucose metabolism within the body. The alarming surge in DM cases worldwide is forecasted to soar to an estimated 300 million by 2025, presenting grave risks of organ deterioration, including cardiovascular complications, renal issues, visual impairment, and chronic ulcers. DM encompasses two primary categories: Type 1 DM, characterized by limited insulin production, necessitating insulin replacement therapy, and Type 2 DM, where the body generates insulin but encounters resistance, leading to heightened levels of glucose in the bloodstream (Bastaki, 2005).

The typical method of delivering insulin through painful subcutaneous injections multiple times a day remains a challenge for patients. While the end aim is to restore the body's ability to produce and utilize insulin, various approaches have been explored to alleviate the discomfort. One promising avenue involves the innovative use of hydrogels as a potential alternative for orally administering insulin, offering a hopeful solution to improve patient experiences in managing their condition. A hydrogel

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is a three-dimensional network of polymers capable of absorbing substantial amounts of water. Its stability arises from either physical or chemical connections (Campoccia *et al*., 1998). Physical hydrogels involve polymer chains linked via hydrogen bonds, hydrophobic interactions, or entanglement, while chemically linked chains constitute chemical hydrogels (Lin and Anseth, 2009). Both types contain clusters with high cross-linkage but low water retention (Hubbell, 1995). Hydrogels are widely used in drug delivery, biosensor development, tissue engineering, and cell culturing (Zhang and Khademhosseini, 2017). Psyllium, collagen, and PEG-based hydrogels have shown promise in insulin delivery due to their protective nature. Psyllium's ability to prevent glucose absorption and provide mucilage aids in hydrogel formation (Singh and Singh, 2012). Drug release from these hydrogels depends on water absorption and the disconnection of interlinked chains (Zarzycki *et al*., 2010).

Psyllium stands out as a soluble fiber obtained from the seeds of the Plantago ovata plant, renowned for its versatile health benefits. Primarily employed as a dietary supplement, its efficacy lies in supporting digestive processes and fostering regularity in bowel movements. Upon contact with water, psyllium undergoes a transformative process, morphing into a gel-like substance within the digestive tract. This unique attribute aids in various aspects of gastrointestinal health, promoting smoother digestion and facilitating regularity in bowel movements, thereby relieving constipation and promoting gut health. research has suggested that psyllium may have a positive impact on individuals grappling with diabetes (Abutair *et al*., 2016). Its soluble fiber properties may aid in regulating blood sugar levels by slowing down the absorption of glucose in the intestines. This mechanism could potentially offer benefits in managing blood sugar levels for individuals with diabetes, contributing to better glycemic control. The comprehensive exploration of psyllium's multifaceted health benefits in promoting digestive health, potentially lowering cholesterol, and aiding in blood sugar regulation underscores its versatility and potential as a natural remedy for various health concerns. However, while promising, ongoing research endeavors continue to delve deeper into its mechanisms and efficacy to fully comprehend and harness its therapeutic potential (Thakur and Thakur, 2014).

Collagen, often likened to a foundational adhesive, holds significant prominence as a protein found abundantly across mammals and a diverse range of vertebrates and invertebrates. Within mammals, it constitutes a notable portion, comprising 25-35% of the total protein content, and plays a pivotal role predominantly in connective tissues like bones, cartilage, and skin (Sargeant *et al*., 2012). Amidst the expansive family of collagen proteins numbering at 29 identified variants thus far tropocollagen emerges as a crucial entity. This elongated fibril protein assumes a distinctive triple helix structure, defining the core of collagen architecture (Shoulders and Raines, 2009). At its fundamental level, tropocollagen manifests as a monomeric unit designated as (Glycine-X-Y)n. This specific sequence exhibits glycine occupying the third position, a critical characteristic that accommodates smaller side chains due to its unique hydrogen side chain. Meanwhile, proline and hydroxyproline side chains typically orient outward from the core structure (Ramachandran and Ramakrishnan, 1976). This intricate structural arrangement of collagen, characterized by its triple helix and the strategic positioning of glycine, proline, and hydroxyproline, delineates its resilience and functionality in providing structural support, strength, and elasticity to various tissues throughout the body. The multifaceted nature of collagen's structural intricacies continues to captivate scientific inquiry, forming a foundational understanding in various fields, including biomedicine, biomaterials, and tissue engineering.

Polyethylene glycol (PEG), also referred to as polyethylene oxide, stands as a hydrophilic polyether compound sourced from petroleum. Renowned for its biocompatible properties, PEG holds the capability to mimic the intercellular matrix, rendering it an ideal candidate for cell encapsulation and various biomedical applications (Papavasiliou *et al*., 2012). Hydrogels formulated with a combination of PEG, collagen, and agarose present versatile structural characteristics, offering a platform for controlled drug release mechanisms through modifiable crosslinking densities (Sagle *et al*., 2009). Within these hydrogels, PEG plays a pivotal role in augmenting structural integrity and biocompatibility without altering the fundamental chemical composition of the matrix. The process of PEG interacting and integrating with other molecules, especially in these hydrogel systems, is termed "PEGylation" in scientific contexts (Yom-Tov *et al*., 2016). This interaction significantly influences the properties of the resulting hydrogel, enhancing its stability and biocompatibility, crucial factors in biomedical applications. Moreover, the amalgamation of psyllium, collagen, and PEG involves an intricate process wherein these components are interconnected to form a mesh-like arrangement. This interconnection is facilitated by the utilization of a cross-linking agent, glutaraldehyde, which serves to bond the components, creating a structured network within the hydrogel (Tian *et al*., 2016). This mesh-like arrangement derived from fortified by the cross-linker glutaraldehyde, showcases the potential for creating intricate scaffolds or matrices with tailored properties.

These innovative combinations and their ability to engineer specific structural arrangements offer promising avenues in various biomedical fields, particularly in tissue engineering, drug delivery systems, and regenerative medicine (Sohail *et al*., 2019).

In our recent research, we have created hydrogels by blending psyllium, PEG, Agarose, and collagen to explore their capacity for insulin delivery. To establish cross-linkages, we employed glutaraldehyde as a cross-linker. These designed hydrogels aim to shield insulin from degradation in the acidic environment of the gastrointestinal tract, potentially enhancing its delivery efficacy.

MATERIALS AND METHODS

Plantago psyllium husk was obtained from Qarshi Industries located in Lahore, Pakistan. Glutaraldehyde was acquired from Bio Basic Inc. PEG was sourced from Sigma Aldrich Pakistan. Humulin 70-30 (insulin) was purchased from Lilly. Collagen extraction was performed using chicken hearts obtained from the local market in Lahore.

Collagen isolation

Chicken heart samples underwent a rigorous extraction process initiated with treatment using acid and pepsin, followed by subsequent centrifugation steps aimed at isolating the collagen component. The extracted collagen was subjected to further refinement through dialysis and enzymatic treatment, a meticulous procedure designed to purify and enhance its properties. Confirmation of successful collagen extraction and quantification was achieved through a series of analytical tests (Mayne and Zettergren, 1980). SDS-Page analysis, utilizing an 8% gel system as prescribed in the Sambrook and Russel manual, allowed for the separation and characterization of the extracted collagen. Additionally, FTIR (Fourier Transform Infrared) spectroscopy was employed to examine both the original collagen molecule and the structured hydrogel. This analysis utilized the Shimadzu IR Prestige-21 equipment located in Kyoto Prefecture, Japan, with a resolution fine-tuned to 4 cm^{-1} across the 500-4000 cm^{-1} wavenumber range. The choice of these analytical techniques, SDS-Page and FTIR spectroscopy, served distinct purposes in the characterization and validation of the extracted collagen. SDS-Page provided insights into the molecular weight and purity of the isolated collagen, while FTIR spectroscopy offered valuable information on the structural composition and functional groups present in both the initial collagen molecule and its modified form within the hydrogel structure. This comprehensive analysis provided a robust assessment of the successful extraction, purification, and structural alterations of the collagen, forming a crucial foundation for further investigations and applications in various scientific domains.

Swelling characters

In investigating the swelling properties of ispaghula-based hydrogels laden with insulin across different pH solutions, the precise measurement of the hydrogel's weight held significant importance. The data collected from these measurements underwent a detailed analysis utilizing the Korsmeyer-Peppas Equation: $F (\%) = Mt/ M\infty = ktn$. In this equation, F signifies the extent of swelling, while Mt represents the mass at a specific time T. $M\infty$ denotes the stabilized mass of the hydrogel, while constants K and n elucidate the mechanism governing the hydrogel's swelling behavior (Peppas and Franson, 1983). The process of incorporating insulin into the hydrogels involved a carefully orchestrated swelling technique. Initially, the hydrogels were immersed in a solution containing insulin at the desired concentration. This immersion facilitated the migration of insulin into the matrix, enabled by the relaxation of the hydrogel network (Yacob and Hashim, 2014). Consequently, the hydrogels swelled as they absorbed the insulin, a process conducted overnight at a controlled temperature of 37°C. Subsequent to this, the hydrogels were left at room temperature until complete drying, culminating in the creation of the drug-containing device. This methodical process of incorporating insulin into the ispaghula-based hydrogels through controlled swelling mechanisms offers insights into the behavior and properties of the resulting drug-delivery system. The precision in the process, from swelling to drying, holds paramount importance in the fabrication of the drug-containing device; showcasing potential applications in pharmaceutical and biomedical fields (Yacob and Hashim, 2014).

BSA solution

In the process of formulating the drug-loaded hydrogel, the quantification of insulin content was accomplished through a precise methodology. Initially, various solutions containing known concentrations of bovine serum albumin (BSA) were prepared and subjected to analysis using a UV-visible spectrophotometer. This instrument allows for the measurement of light absorbance by a solution across specific wavelengths. By evaluating the absorbance levels of the BSA standard solutions, a detailed graph was constructed, correlating the absorbance values to the known concentrations of BSA (Gonzalez *et al*., 2005). This calibration curve served as a reference, enabling the estimation of insulin concentrations in subsequent

solutions based on their respective absorbance readings. The work conducted by Ostróżka-Cieślik *et al*. (2023) likely presented a robust and reliable approach to determine the concentration of insulin, crucial for formulating precise drug-loaded hydrogels. This method, utilizing UV-visible spectrophotometry alongside standardized calibration graphs, ensures accurate quantification of insulin content, facilitating the development of effective drug delivery systems.

In-vivo *and* in-vitro *analysis*

In the *in-vitro* experiments, hydrogels loaded with insulin were placed separately in buffers at pH 5.4 and pH 7.5, maintaining a temperature of 37°C. Over a period of 6 h, the drug release was monitored by measuring absorbance at 280 nm at 30-min intervals. This allowed for the assessment of how effectively the insulin was being released from the hydrogels under different pH conditions (Kim and Peppas, 2013). For the *in-vivo* evaluation, a total of nine mice underwent a 12-h fasting period to standardize their glucose levels. These mice were then divided into three groups: The control group received no treatment, the negative group received hydrogels without insulin, and the third group received hydrogels loaded with insulin (Krauland *et al*., 2004). Glucose levels in these mice were meticulously monitored at 30-min intervals to evaluate the hydrogels efficacy in delivering insulin and its subsequent impact on glucose regulation (Traitel *et al*., 2000). This comprehensive approach, combining *in-vivo* and *in-vitro* methodologies, provides a thorough understanding of the hydrogels performance in insulin release and its potential therapeutic effects on glucose levels. The study conducted by Krauland *et al*. (2004) likely shed light on the hydrogels suitability as an effective insulin delivery system, with implications for diabetic management.

RESULTS

Psyllium ispaghol (0.5g) was stirred in 100ml of d.H_2O for O/N to swell evenly. It was stirred continuously for 2 h to which 20% glutaraldehyde (2ml) was supplemented as a cross-linker. Further 0.7% agarose (2ml), 4.5% PEG (5ml), and 2ml purified collagen were added. Incubation was done at 37ºC for O/N.

Collagen isolation from chicken hearts was conducted using a modified version of the method outlined by Zhang *et al*. (2007). Initially, 1g of heart sample was suspended in 0.5M acetic acid and 1% pepsin for 24 h at 4°C with nonstop mixing. After multiple centrifugations, the supernatant was wasted, and the sediment was re-dissolved in 5ml of 0.5M acetic acid using a vortex. Dialysis against 0.1M acetic acid buffer was carried out at 4°C with continuous stirring to extract the collagen. These stringent conditions resulted in the denaturation and removal of all functional proteins, ultimately leaving the acid-soluble collagen treated with enzymes. To validate the successful collagen extraction, SDS-page and FTIR analysis were performed, while the quantity of collagen was determined by Bradford test analysis.

We utilized a resolving gel (8%) and a stacking gel (5%), subjecting the gel to a 72V current for 1 h. Following this, the gel underwent staining using Coomassie Brilliant Blue dye R250 and a subsequent de-staining process involving acetic acid and methanol. In the provided image, a distinct single band above the 200kDa mark was observed, solidifying the presence of collagen, a vital structural protein. This band exhibited collagen aggregates with high molecular weight, attributed to the presence of disulfide linkages (Bashey *et al*., 1992). To delve deeper into the collagen's presence and composition, further analysis was conducted using FTIR and the Bradford test.

The FTIR spectrum revealing collagen's distinct bonding patterns. A significant peak at 1705.07 cm^{-1} characterizes the appearance of the Amide I band. Notably, the peaks at 1480 cm^{-1} and 1280 cm^{-1} are designated to the Amide II and Amide III bands, respectively. The presence of these Amide I and Amide II bands provides strong evidence of the existence of the structural protein, collagen, following the pepsin treatment. Additionally, peaks observed at 3425.58 cm^{-1} and 2617.40 cm^{-1} correspond to the N-H stretching and C-H stretching, respectively (Aleem *et al*., 2017).

The process began by creating a calibration curve using bovine serum albumin (BSA), plotting the absorbance readings against various BSA dilution concentrations at a specific wavelength of 595nm. This calibration curve served as a reference standard, allowing for the determination of protein concentrations based on absorbance values (Lorenzen *et al*., 1993). Following this, the absorbance of the solution containing the extracted collagen was measured at the same wavelength, 595nm. By applying the absorbance value obtained from the collagen extract solution to the calibration curve formula previously established using BSA, the corresponding protein concentration was calculated. In this instance, the calculated concentration of collagen in the solution was determined to be 10.5 µg/ml (Lareu *et al*., 2010).

The investigation into the design and surface characteristics of the hydrogel involved a thorough analysis using a scanning electron microscope (SEM), as depicted in the accompanying diagram. Multiple magnification levels, such as X-500 and X-1k at 10KV, were employed to scrutinize the hydrogel's surface morphology. At

lower magnifications (X-500 and X-1K), the hydrogel exhibited a notable porous structure, discernible under the microscope. Upon further magnification, particularly at X-1K, the porous structure became more pronounced and revealed intricate features dispersed across the hydrogel surface. These features appeared dynamic and intricate, attributable to the interactions and cross-linkage between the various components composing the hydrogel, including agarose, collagen, PEG, and agarose (Salerno *et al*., 2011). The detailed examination using SEM and varied magnification levels offered insights into the structural composition and surface morphology of the formulated hydrogel. This revealed the intricate network and interplay between different components, contributing to the porous and dynamic nature of the hydrogel's surface, vital information for understanding its properties in various applications, such as drug delivery or tissue engineering.

To execute the gravimetric analysis, the hydrogels were immersed separately in buffers of different pH levels (5.4 and 7.4). The swelling behavior was monitored by measuring the weight of each hydrogel at regular time intervals, specifically at 10-min intervals, throughout the experimental duration. By obtaining successive weight measurements, researchers were able to track and record the extent of swelling exhibited by the hydrogels in response to the varying pH conditions (Astrini *et al*., 2012). The data collected from these measurements facilitated the construction of a swelling graph, illustrating the swelling behavior of the hydrogels over different time intervals in pH 5.4 and pH 7.4 environments. This methodical approach provided valuable insights into how pH influences the swelling kinetics of the hydrogels, contributing to a comprehensive understanding of their behavior and potential applications in various fields such as drug delivery or biomaterials science (Ray *et al*., 2010).

Type of diffusion in different pH (acidic and basic) followed by the oral formualtion was determined by using the following equation. Whereas value of n tells about the type of diffusion occuring as given in the Table I.

$$\frac{Mt}{Meq} = Kt^n$$

Table I. Diffusion exponent showing type of transport.

Diffusion exponent (n)	Type of transport
n < 0.5	Less fickian diffusion
n = 0.5	Fickian diffusion
0.5 < n < 1	Non-fickian diffusion
n = 1.0	Case II transport
n > 1.0	Super case II transport

Fig. 1. Hydrogel (Psyllium-PEG, collagen and agarose based).

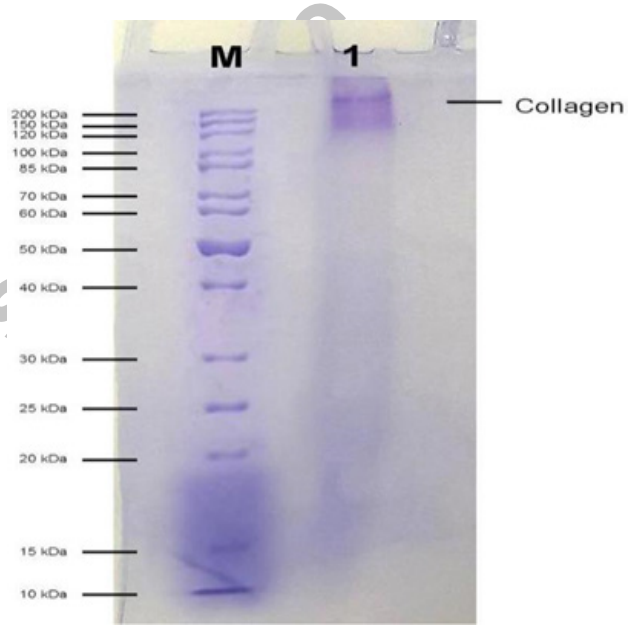

Fig. 2. SDS-page profile of chicken heart collagen (8%) Lane M: Protein Ladder (10-200kDa) Lane 1: Collagen Band (above 200kDa) from Chicken Heart.

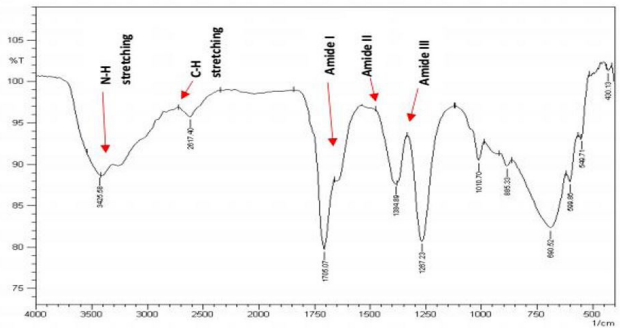

Fig. 3. FTIR spectrum displaying collagen's distinctive peaks.

Hydrogels followed less Fickian diffusion in acidic pH as the value of n was found out to be 0.11 and in alkaline pH as the value of n was found out to be 0.312.

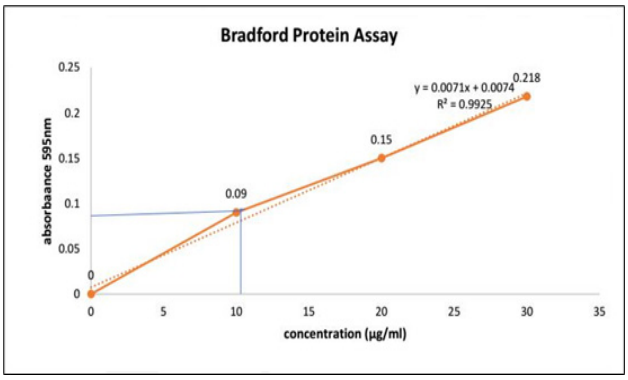

Fig. 4. A BSA standard curve was employed to ascertain the collagen concentration.

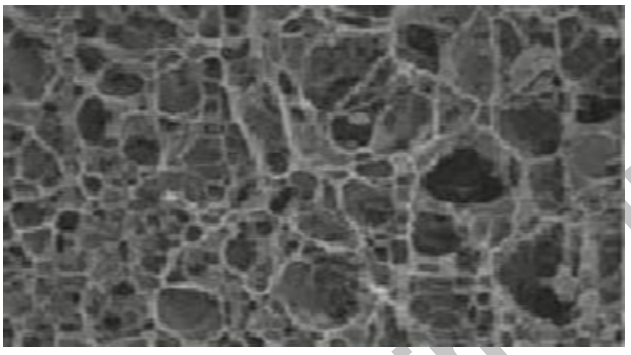

Fig. 5. The morphology of the hydrogel was investigated using SEM at an acceleration voltage of 10.0KV with a magnification of X1.00k.

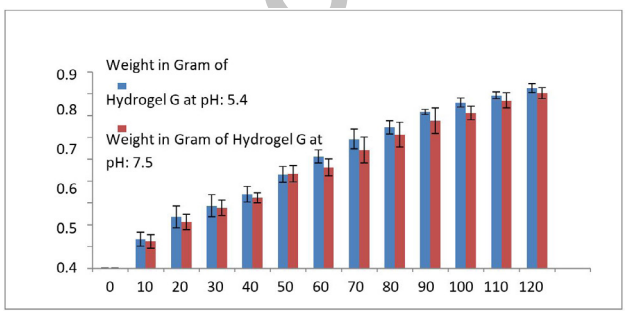

Fig. 6. Swelling properties of hydrogel.

The formulated hydrogel underwent a meticulous sterilization procedure using UV radiation within a controlled laminar flow cabinet for duration of 1 h. This step aimed to ensure the elimination of potential contaminants or microorganisms from the hydrogel surface. Following the sterilization process, the treated hydrogels were positioned at the center of plates, and a streaking technique was employed around these hydrogels. This involved spreading a bacterial culture, likely *E. coli* strain, around the perimeter of the hydrogels on the plates (Zaidan *et al*., 2005). The plates were then securely sealed with parafilm to prevent contamination and incubated overnight at a constant temperature of 37°C. Upon the subsequent day, the hydrogels were meticulously examined and evaluated for their ability to resist or inhibit the growth of the streaked *E. coli* strain. This assessment, conducted by Wu *et al*. (2019), likely aimed to ascertain the hydrogels antimicrobial or bacteriostatic properties, essential considerations for potential applications in biomedical or pharmaceutical settings.

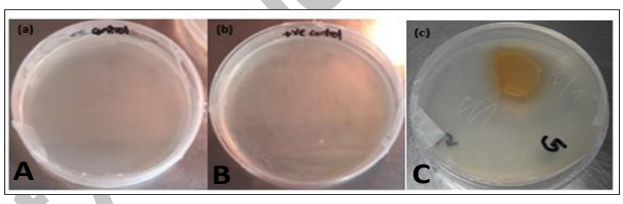

Fig. 7. On MH agar plate, hydrogel G resistivity against microbial activity (a) +ve control (strain), (b) –ve control (no strain) and (c) no growth of strain on hydrogel.

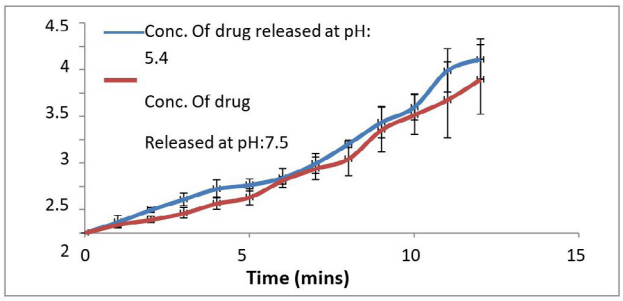

Fig. 8. Conc. of drug release at different pH.

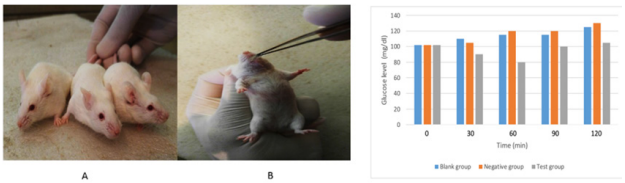

Fig. 9. Graph depicting the blood glucose levels over specified time: Blank (Blue), Negative (Red), and Test (Grey).

The *in-vitro* release study involved immersing the hydrogels in distinct buffers-phosphate buffers with varying pH levels of 5.4 and 7.4, totaling 15ml each.

These setups were placed on an orbital shaker rotating at 300 RPM, maintaining a constant temperature of 37°C (Kim and Peppas, 2003). At regular 10-min intervals, 2ml samples were meticulously withdrawn from the solutions. The absorbance of these samples was precisely measured at a wavelength of 280 nm. To ensure accuracy, a blank measurement using fresh aqueous solution was initially established as a reference point for absorbance measurements. Subsequent to absorbance recording, the sampled solution was carefully returned to its original container on the orbital shaker. This cyclic process of sample extraction, absorbance measurement, and reintegration into the shaker setup continued iteratively until equilibrium was attained (Khodaverdi *et al*., 2012). The determination of the drug's release quantity was achieved using a standard curve, most likely developed based on known concentrations of the drug. This systematic approach, detailed by Agarwal and Khan (2001), provided crucial insights into the controlled release dynamics of the drug from the hydrogels under specific pH conditions, essential for assessing their viability as drug delivery systems.

Following the application of insulin-loaded hydrogels crafted with PED, collagen, agarose, and psyllium, an *in-vivo* assessment of drug release was conducted by monitoring the blood glucose levels of mice at 30-min intervals. This monitoring aimed to evaluate the hydrogels' efficacy in releasing insulin within a biological setting (Traitel *et al*., 2000). The study, conducted by Wood *et al*. (2010), likely presented a comparative figure illustrating the fluctuations in blood glucose levels among three distinct groups of mice. These groups possibly included a control group, a negative control group (receiving hydrogels without insulin), and a third group administered with insulin-loaded hydrogels (Yang *et al*., 2021). The figure likely depicted the variations in blood glucose levels over time, showcasing the impact of the administered hydrogels on regulating blood sugar levels. Such comparative analysis aids in assessing the effectiveness of the insulin release from the hydrogels and its subsequent influence on glucose regulation *in vivo*, providing critical insights into the potential therapeutic applications of these drug delivery systems (Mansoor *et al*., 2021).

DISCUSSION

This study focuses on harnessing hydrogels as potential carriers for drug delivery, specifically exploring their fascinating attribute known as pH sensitivity. The study aims to develop and evaluate a hydrogel tailored for oral insulin delivery. This particular hydrogel combines psyllium, PEG, and collagen, renowned for their ability to create robust and biocompatible gels. What distinguishes this hydrogel is its responsiveness to pH changes within the gastrointestinal tract, enabling a controlled and targeted release of insulin (Shaikh *et al*., 2021). The preference for oral insulin administration lies in its non-invasive nature and enhanced patient adherence. However, delivering insulin orally faces challenges due to its degradation in the harsh gastrointestinal environment. This study addresses this challenge. To analyze insulin release under different pH conditions, UV spectroscopy with BSA as a standard curve was utilized. The findings showcased minimal drug release in acidic conditions, contrasted sharply with a significant release observed under alkaline pH, as indicated by the changing absorbance values over time (Mansoor *et al*., 2021). Precise quantification of the released drug was achieved by referencing the established BSA standard curve. This study's insights hold promise for overcoming obstacles in oral insulin delivery, potentially revolutionizing diabetes management strategies.

The *in-vitro* study involved submerging the hydrogels in phosphate buffers of pH 5.4 and 7.4 (15ml each). They were placed on an orbital shaker (300 RPM) at 37°C. Samples (2ml) were withdrawn every 10 min, their absorbance measured at 280 nm. Fresh aqueous solution served as a reference. This process continued until equilibrium. Drug release quantity was determined using a standard curve, crucial for evaluating the hydrogels' drug delivery potential (Kim and Peppas, 2003; Khodaverdi *et al*., 2012; Agarwal and Khan, 2001)

In the transition to the *in-vivo* phase involving mice, the drug delivery system utilizing hydrogels demonstrated encouraging behavior concerning insulin release triggered by distinct pH environments. The experimental procedure commenced with a standardized 12-h fasting period for all nine mice. This fasting duration was crucial to establish a consistent and stable baseline for their glucose levels before the experiment. Following this fasting period, the mice were orally administered insulin-loaded hydrogels (Traitel *et al*., 2000). This approach aligns with prior research methods and aims to evaluate the hydrogel's efficacy in delivering insulin within the biological systems of living organisms, specifically mice in this case.

The monitoring of glucose levels at 30-min intervals using a glucometer provided valuable insights into the dynamics of insulin release. The observed gradual decrease in blood glucose levels indicated a controlled and steady release of insulin from the hydrogel within the intestinal environment. This pattern was quite noteworthy as it suggested that the hydrogel facilitated a gradual and regulated release of insulin over time. One remarkable aspect was the hydrogel's protective function, notably in the stomach's harsh acidic conditions. Despite encountering

this challenging environment, the hydrogel effectively preserved the integrity of the insulin. This finding indicates the hydrogel's potential to shield insulin from degradation, ensuring its stability until it reached the targeted site in the intestine. The gradual recovery of blood glucose levels in mice throughout the experiment further supported the idea of a consistent and incremental release of the drug. This progressive decline in glucose levels reflected a sustained and controlled release of insulin, emphasizing the hydrogel's effectiveness in modulating and regulating drug delivery. The study's outcomes, as documented by Yang *et al*. (2021), underscore the promising capability of the hydrogel in precisely controlling insulin release, holding potential for advanced drug delivery systems.

CONCLUSION

Psyllium possesses a dual advantage when incorporated into drug transport systems, leveraging its sugar-dropping assets and gel-forming features. This makes it an excellent candidate for managing diabetes mellitus, a fact substantiated by the drug release dynamics observed in various release media and mice. The drug release mechanism at pH 5.4 and 7.5 buffer systems follows the Fickian diffusion, indicating that water influx into the hydrogel occurs in tandem with drug release, showcasing impressive swelling properties. Moreover, the hydrogels demonstrate anti-albumin denaturation activity, underscoring that the biological activity of the released drug remains unaltered. This paves the way for psyllium-based insulin-loaded hydrogels to be considered safe, biocompatible, and highly efficient in drug delivery systems.

DECLARATIONS

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Ethical statement and IRB approval

The study receives ethical approval from ethics committee School of Chemistry (D/1254/PUSC).

Statement of conflict of interest

The authors have declared no conflict of interest.

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