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Formulation and Evaluation of PVA Loaded Nano Fibrous in Situ Gel.

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Abstrat: The comprehensive Preformulation study conducted on Bovine Insulin-loaded nanofibers involved an array of analytical techniques, each shedding light on critical aspects of the formulation's composition, stability, and performance. Fourier Transform Infrared (FTIR) spectroscopy revealed distinct peaks characteristic of Bovine Insulin, chitosan, sodium alginate, and other excipients, affirming the purity and compatibility of the components within the nanofibers. Differential Scanning Calorimetry (DSC) provided insights into the thermal behavior of Bovine Insulin and its interactions with polymers, crucial for understanding formulation stability. X-ray Diffraction (XRD) analysis highlighted a shift towards amorphous nature in Bovine Insulin powder when incorporated into nanofibers, indicating enhanced solubility. Additionally, determination of thiol groups elucidated the formation of bonds between chitosan and sodium alginate, contributing to the structural integrity of the nanofibers. Characterization studies, including SEM imaging, rheological analysis, and evaluation of spreadability and extrudability, provided valuable insights into the physical properties and applicability of the nanofiber gel, culminating in the identification of an optimized formulation, NGA5, exhibiting superior drug content uniformity, viscosity, and extrudability, thus holding promise for effective nasal delivery of Insulin.

***Keyword*:** Electrospun nanofibres, Polymer Conjugates (Thiomers), Central composite design, Stability study, In-vivo activity.

Introduction

Nanofibers are long, thread-like structures with sizes in the nanoscale range; they are also called ultrafine or superfine fibers. Depending on the substance it is made of, a single nanofibers diameter may be anywhere from a few nanometers to a few micrometers. Numerous methods for producing nanofibers have emerged in response to developments in nanomaterial processing. [1-2] Electrospinning has been the most popular fiber processing method because of its low cost, high versatility, ease of production, and relatively simple setup compared to other methods like direct drawing, self-assembly, template synthesis, phase separation, melt blowing, and hydrothermal processing. [3-4] Electrospinning is a technique that uses electric force to extract nanofibers out of liquids or molten materials. Although the process was first invented in 1900, electrospinning did not begin to get traction until the early 1990s, thanks to developments in Nanoscience and nanotechnology. The Reneker lab stands out among the field's prominent research groups because of the significant role it played in the advancement of the technology. The acronym "electrospinning" (meaning "electrostatic spinning") was also

popularized by them. [5] The process of electrospinning can create nanofibers from a variety of materials, including composites, multi-component, core-sheath, hollow, and porous fibers. These nanofibers have unique properties like a high surface-to-volume ratio, a controllable structure, and good tensile strength. [6-10]Electrospinning often makes use of polymers including polyacrylonitrile (PAN), Polyvinylpyrrolidone (PVP), polyethylene oxide (PEO), and polyvinyl alcohol (PVA). In order to prepare these polymers for electrospinning, they are either melted or dissolved in the correct solvents. Ceramics, carbon, and a wide variety of composites are among the many other materials that may be electrospun. Numerous fields have found uses for electrospun nanofibers, such as biomedicine, energy devices, sensors, protective apparel, filtration and separation, and more. [11-16] For local infections affecting the nose and paranasal sinuses, such as rhinitis, sinusitis, rhino-sinusitis, and nasal epithelial lesions, the best way to deliver topical treatments is via the nose. An additional non-invasive option for systemic delivery of drugs with low bioavailability is the nasal mucosa. Drugs that normally undergo significant first-pass metabolism and/or gastric degradation after oral administration can instead be rapidly absorbed via the nasal epithelium due to its high vascularization. The nasal route has also shown potential for pharmaceutical delivery to the brain as it circumvents the blood-brain barrier (BBB), which restricts the diffusional transport mechanisms of several therapeutic medications after oral or parenteral administration. When drugs are administered nose-to-brain, the olfactory neuroepithelium transfers them directly and quickly to the central nervous system (CNS). [17-18]

Materials and Methods:

MATRIALS:

Bovine Insulin, chitosan, EDAC, cysteine, sodium alginate, sodium hydroxide, sodium acetate, 5,5'-dithiobis (2-nitrobenzoic acid), sodium carboxymethyl cellulose, methanol, acetonitrile, hydrochloric acid (HCl) 32%, o-phosphoric acid 85%, phenol-extra pure, m-cresol – pure, o-nitrophenol 99%, and potassium Dihydrogen phosphate were purchased from Cosmo Chem. Pvt. Ltd. Tris (2-carboxyethyl) phosphine hydrochloride was purchased from Merck Pvt. Ltd. Polyvinyl alcohol (PVA) was purchased from Akshar Chem. Pvt. Ltd.

Methods:

Formulation and Development

Preparation of Polymer Conjugates (Thiomers)

Synthesis of chitosan conjugate [19]

Chitosan (500 mg) was dissolved in 4 mL of 1M HCl and then diluted with demineralized water to obtain a 1% (w/v) polymer solution. Ethyl (dimethylaminopropyl) carbodiimide (EDAC) was added to achieve a final concentration of 200 mM. After 20 minutes, varying amounts of N-acetyl cysteine (4 gm) were slowly added under stirring. For reactions involving aromatic ligands, tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was added at a final concentration of 10 mM as a reducing agent. The pH was adjusted to 5 with 1M NaOH, and the reaction proceeded for 5 hours at room temperature with vigorous stirring. After dialysis, the pH was readjusted to 4, and the solutions were freeze-dried at 77°C and 0.01 mbar, then stored at 4°C until further use.

Synthesis of sodium alginate conjugate-[20]

Sodium alginate (500 mg) was dissolved in 4 mL of 1M HCl and diluted with demineralized water to create a 1% (w/v) polymer solution. Ethyl (dimethylaminopropyl) carbodiimide (EDAC) was then added to achieve a final concentration of 200 mM. After 20 minutes, varying amounts of N-acetyl cysteine (4 gm) were slowly added under stirring. For reactions involving aromatic ligands, tris (2-carboxyethyl) phosphine hydrochloride (TCEP) was added at a final concentration of 10 mM as a reducing agent. The pH was adjusted to 5 with 1M NaOH, and the reaction proceeded for 5 hours at room temperature with vigorous stirring. After dialysis, the pH was readjusted to 4, and the solutions were freeze-dried at 77°C and 0.01 mbar, then stored at 4°C until further use.

Determination of Thiol Group Contents with Ellman's Reagent method.

Content of free thiol groups immobilized on chitosan can be determined by spectrophotometry using Ellman reagent [5.5 '-dithiobis (2-nitrobenzoic acid)] (DTNB). Before determining the content of thiol groups in the sample must be made the solution of cysteine standard curve:

For Chitosan conjugate

Preparation of standard calibration curve of N-Acetyl Cysteine [21]

A DTNB stock solution containing 50 mM sodium acetate and 2 mM DTNB was prepared using molecular biology grade water. A 1 M Tris solution with pH adjusted to 8.0 was then made. Cysteine standard solutions starting at a concentration of 10 µM were prepared. For each measurement, 10 µL of cysteine standard solution was combined with 50 µL of DTNB solution, 100 µL of Tris solution, and aqua bidestilata to reach a final volume of 1000 µL. After mixing and a 5-minute incubation at room temperature, absorbance was measured at 412 nm. Dilutions were made from the final volume to prepare 100 µL of solution, and solutions ranging from 10-60 µL were prepared. Absorbance was measured for each to generate a regression equation.

Preparation of sample solution

Sample solution prepared by dissolving 50 mg of chitosan conjugate in 25 mL of Nuclease-Free Water. Amount of 10 µL of sample solution is inserted into a measuring flask, then added 50 µL DTNB solution, 100 µL solution of Tris, and of Nuclease-Free Water until the final volume of 1000 µL. Solution was mixed well and incubated at room temperature for 5 minutes, then absorbance was measured at a wavelength of 412 nm. Absorbance obtained subsequently incorporated into the regression equation of standard solution of cysteine thus obtained concentration of the sample being examined.

For sodium alginate

Solution of cysteine standard curve: [21]

DTNB stock solution containing 50 mM sodium acetate and 2 mM DTNB prepared using molecular biology degree water (aqua bidestilata). Tris solution was then made with a final concentration of 1 M and its pH adjusted to 8.0. A series of cysteine standard solution was made starting at a concentration of 10 µM. Amount of 10 µL standard solution of cysteine inserted into the measuring flask, then added 50 µL DTNB solution, 100 µL solution of Tris, and aqua bidestilata until the final volume of 1000 µL. Solution was mixed well and incubated at room temperature for 5 minutes, then absorbance was measured at a wavelength of 412 nm. Absorbance obtained and graphed against concentration to obtain the regression equation.

Preparation of calibration curve

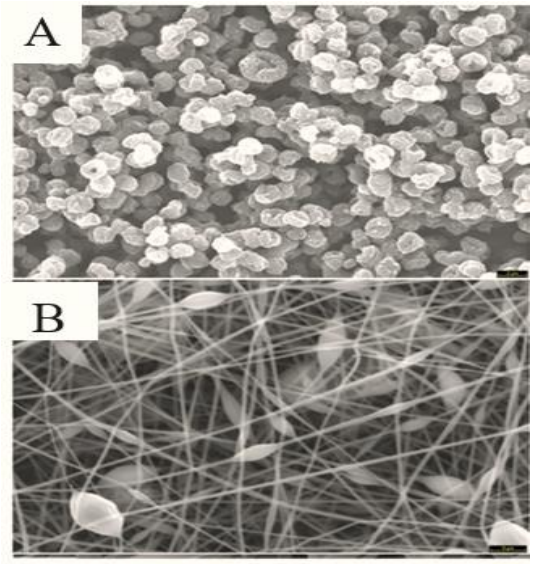
From the final volume of 1000 µL, dilutions were made to prepare 100 µL of the solution and simultaneously 10-60 µL solutions were made. The absorbances were obtained to generate regression equation.

Preparation of sample solution

Sample solution prepared by dissolving 50 mg of sodium alginate conjugate in 25 mL of Nuclease-Free Water. Amount of 10 µL of sample solution is inserted into a measuring flask, then added 50 µL DTNB solution, 100 µL solution of Tris, and of Nuclease-Free Water until the final volume of 1000 µL. Solution was mixed well and incubated at room temperature for 5 minutes, then absorbance was measured at a wavelength of 412 nm. Absorbance obtained subsequently incorporated into the regression equation of standard solution of cysteine thus obtained concentration of the sample being examined.

Preparation of Nanofiber [22]

PVA and PEO were dissolved in chitosan and sodium alginate conjugate thiomer solution at the concentrations of 8mg/ml. The solution was stirred for 72 hours at room temperature, allowing all solid components to completely solubilize. Bovine Insulin powder was then added at a final concentration of 2.8 mg/ml. At the given concentration, Bovine Insulin dissolved rapidly to produce a translucent solution of uniform color and viscosity. Many trials on following conditions were studied to Optimized the nanofiber formation method. 10, 15, 20Kv DC offset, 13,15 and 17 cm air gap and 1,3 and 5ml/h flow rate studied, of which at 15 kV DC offset, 17 cm air gap, 18 ga. blunted needle, and 3 ml/h flow rate the desired size nanofibers were formed.



The above figures shows, A) the nanofibers formed at 10 Kv DC offset, 13 cm air gap and 1ml/h flow rate whereas B) the nanofibers formed at 20 Kv DC offset, 15 cm air gap and 5ml/h flow rate, which confirms that there is no proper formation of nanofibers at set conditions. Whereas the nanofibers formed at 15 kV DC offset, 17 cm air gap, 18 ga. blunted needle, and 3 ml/h flow rate showed good size nanofibers formation, so the above condition was considered as Optimized condition. The solution was electrospun into solid fiber mats under the following conditions: 15 kV DC offset, 17 cm air gap, 18 ga. blunted needle, and 3 ml/h flow rate. A typical spinning run used 5 ml of solution and a round collecting mandrel (6.5 mm diameter). Scaffolds were dried under vacuum 3 hours to remove residual solvent before any further testing.

Table 1: Optimized Formulation Table of Nanofibre Synthesis:

Sr. No.	Batch Name	Chitosan Thiomer (ml)	Sodium alginate Thiomer (ml)	PEO (mg/ml)	PVA (mg/ml)	Bovine Insulin Powder (mg)
1.	NF1	-	20	8	-	2.8
2.	NF2	-	20	-	8	2.8
3.	NF3	20	-	8	-	2.8
4.	NF4	20	-	-	8	2.8

Preparation of In-Situ Gel Containing Electrospun Nanofibres

To prepare the gel, started by dissolving 0.4 g of sodium carboxy methyl cellulose (CMC) in a small amount of a buffer solution with a pH of 7.4. Ensured thorough dissolution by continuous stirring. Separately, dispersed 4.0 mg of drug-loaded nanofibers into the CMC solution, achieving a uniform mixture by employing a

homogenizer to ensure the nanofibers are well-distributed. Adjusted the gel's consistency by adding small amounts of either CMC / buffer solution until the desired texture is reached, stirring after each addition. Verified the pH of the final gel, making adjustments with additional buffer solution.

Experimental design for in-situ gel formulation of PVA using central composite design (CCD) [23]

In the current study, a Response Surface Methodology (RSM) known as Central Composite Design (CCD) was employed using Design Expert® software (Version 13.0). The CCD was applied with three independent variables: the amount of PVA+Sodium alginate (A), PVA+Chitosan (B), and Sodium carboxymethyl cellulose (C).The dependent variables under investigation in this study are pH and spreadability. This CCD design incorporated factorial points, a center point, and axial points, resulting in a total of 15 runs. The details of the independent variables, their coded levels, and the scheme matrix of the CCD can be found in the provided table.

Table 2: DOE Suggested Batches of PVA Loaded Nasal Gel

Formulation Code	PVA + Sodium Alginate Nanofiber (mg)	PVA + Chitosan Nanofiber (mg)	Sodium Carboxy Methyl Cellulose (mg)	Buffer pH 7.4 Q.S. to (gm)
NGA 1	2	2	0.4	10
NGA 2	2	4	0.2	10
NGA 3	3	3	0.131821	10
NGA 4	4	2	0.4	10
NGA 5	4	2	0.2	10
NGA 6	1.31821	3	0.3	10
NGA 7	3	4.68179	0.3	10
NGA 8	4	4	0.4	10
NGA 9	3	3	0.3	10
NGA 10	4	4	0.2	10
NGA 11	4.68179	3	0.3	10
NGA 12	2	2	0.2	10
NGA 13	3	3	0.468179	10
NGA 14	2	4	0.4	10
NGA 15	3	1.31821	0.3	10

Preformulation Study

Fourier-transform infrared spectroscopy (FTIR) [24]

Using Fourier transform infrared spectroscopy, the IR spectrum of Bovine Insulin was recorded and compared to drug reference spectra. Sample was placed on the sample platform and squeezed between the knob and sample platform. The spectrum of infrared light was measured between 500 and 3500 cm-1.

Differential Scanning Calorimetry (DSC) [25]

Differential scanning calorimetric (DSC) measurements were carried out on a modulated DSC (Mettler Toledo, SW STARe, USA). Samples were weighed (2-8mg), the aluminum pans were used and hermetically covered with lead. The heating rage was 50-250 °C for sample with constant increasing rate of temperature at 10°C /min under nitrogen atmosphere (50-60ml/min). The resultant thermograms of formulation was obtained. The Bovine Insulin powder, Chitosan loaded-NF3 and Sodium alginate loaded –NF1 formulation, these samples were studied for DSC.

X- ray diffraction study of Bovine Insulin powder (XRD) [26]

The structural composition can be studied using X-ray diffraction method. Bovine Insulin powder samples were ground to fine powder and spectra were recorded on Bruker XRD Instrument using the source Copper K alpha.X-ray Powder diffraction measurements is used to confirm the crystalline nature and glass formation of the sample. A XPERT-PRO diffractometer system with a rotating anode Cu Ka was used and scans are taken between 5 °C and 100 °C.

Evaluation and Characterization of nanofiber

Evaluation of nanofiber

Drug entrapment efficiency [27]

The ultra-centrifugation technique was used to assess the drug entrapment effectiveness of nanofiber formulations. Using ultracentrifugation at 10,000 rpm for 30 minutes. The pellets were re-dissolved in distilled water, and the supernatant was scanned with a UV-visible spectrophotometer in this parameter.

The drug encapsulation efficiency was determined by using the relation in this equation.

% Drug entrapment efficiency= experimental drug content x 100 / Theoretical drug content

Swelling index [28]

Swelling capacity of the nanofibres samples was investigated by direct immersion of formulations in PBS (pH 7.4) to simulate medium conditions. The swelling degree (S) was calculated according to

$$S\ (\%) = \{(wt - wd)/wd\}\ x100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Where wt. is the weight of the swollen sample at time t and Wd is the initial weight of the dry sample.

Characterization of nanofiber

FTIR [24]

FTIR spectroscopy was used in the range 400-4000 cm-1 to determine the type of functional groups on the Bovine Insulin and prepared nanofibre formulation. Bovine Insulin and dried nanofibers were mixed with KBr powder and pelletized. The IR characterizations were performed using a Perkin-Elmer Spectrum GX FTIR spectrometer.

DSC Compatibility study (Drug-Excipients) [25]

Differential scanning calorimetric (DSC) measurements were carried out on a modulated DSC (Mettler Toledo, SW STARe, USA). Samples were weighed (2-8mg), the aluminum pans were used and hermetically covered with lead. The heating rage was 50-250 °C for sample with constant increasing rate of temperature at 10°C /min under nitrogen atmosphere (50-60ml/min). The resultant thermograms of formulation was obtained. The Bovine Insulin powder, Chitosan loaded-NF3 and Sodium alginate loaded –NF1 formulation, these samples were studied for DSC. [307]

Scanning electron microscope (SEM) [29]

The Scanning electron microscope (SEM, Zeiss, Japan) was used to observe the morphology and diameter of the NF1 nanofibers, NF2 nanofibers, NF3 nanofibers and NF4 nanofibers electrospun nanofibers. In this study the samples image were captured at the accelerating voltage of 5 kV under the magnification of 17.42 X. The synthesized nanofibers were cut into small pieces and coated on a gold sputter (sputter coater: Emitech SC7620) in order to prevent charging and improve the resolution of the image. The average fiber diameter was measured with the SEM images.

TGA [30]

The thermal stability and the fraction of the volatile components of the synthesized nanofiber NF1, NF2, NF3, NF4 were analyzed with the help of Thermo Gravimetric Analyzer (TGA) (PERKIN – ELMER TG – DTA). The samples were heated at constant heating rate at 10°C / min from room temperature to 800°C under nitrogen (N2) atmosphere at a purging rate of 100 ml / min. During the process the weight of the samples were measured, it was not degraded, no change in weight during the desired temperature of the polymer and also no slope was observed in the TGA graph with negligible changes of the samples. If the samples were degraded the weight will decrease and if the weight will increase because the samples were reacted with oxygen.

Characterization of In-Situ Gel Containing Electrospun Nanofibres

Determination of pH [31]

The pH of the Nanogels was determined by using digital pH meter which was previously calibrated by standard solution prepared by standard capsules of pH 4, 7 and 9.2 respectively. pH measurement of the gels was carried out by dipping the pH-electrode of a digital pH meter completely into the gel formulation for 10 min prior to taking the readings in order to allow the pH values to stabilize. The measurement was carried out in triplicate, and the average of the three readings was recorded. The electrode was washed thoroughly between each reading.

Determination of Viscosity [32]

The viscosity of the Nanogels was determined by using Brookfield viscometer with spindle no. 64, rotated at 5 rpm for 5 min at 25 °C temperature.

Determination of Spreadability [33]

The spreadability of Nanogels was determined using the "maximum slip and minimum drag" principle. Excess gel formulation was placed between two glass slides, and a 1000 g weight was applied for 1 minute to expel air and ensure uniform distribution. After removing the weight, excess gel was scraped off the edges. The lower slide was fixed, while the upper slide was attached to a string connected to a pan with an 80 g weight. Pulling the upper slide measured the time it took to separate the two slides, indicating spreadability.

The experiments were done in triplicate.

The following formula is used to calculate the spreadability.

$$S = m \times l\, t \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where, S is the Spreadability, m is that the weight tied to the upper slide (g), l is the length of a glass slide (cm), t is the time taken to separate the slide completely from each other (s).

Extrudability [34]

The extrudability of Nanogels was determined by the amount of gel extruded from the tube on the application of pressure. The formulation was filled in a clean lacquered collapsible aluminum tube of capacity 5 g with 5 mm orifice, and the tube is pressed firmly at the crimped end, and the clamp was applied to prevent any rollback. The amount of extruded gel was collected carefully and weighed accurately. Extrudability was then determined

by measuring the amount of gel extruded (in percentage) through the orifice when a pressure was applied to the tube. The experiment was performed in triplicate.

Drug Content Uniformity [35]

To ensure uniform distribution of the drug entrapped in nanofibers within the gel, samples were collected from different locations (top, middle, and bottom) of the tube. Each sample weighing 0.250 g was accurately transferred to a 250 ml volumetric flask and diluted with 100 ml methanol to break the nanofiber structure. The flask was vigorously shaken for 30 minutes on a mechanical shaker to disperse the gel, followed by 10-15 minutes of sonication for complete drug extraction. The solutions were then filtered and analyzed by UV-Vis spectrophotometer to determine drug content using a standard calibration curve.

In vitro drug diffusion study [36]

Cellophane membrane diffusion technique was used to study in-vitro diffusion of drug from the prepared nanogel formulations. The receptor medium used was freshly prepared phosphate buffer pH 7.5. Cellophane membrane soaked overnight in the receptor medium was on the Franz's Diffusion cell assembly 0.5 g of formulation was placed in the donor compartment and the assembly was kept on the multistation diffusion study apparatus (make Orchid Scientific) at $37^0C \pm 2^0C$ and stirred at 700 RPM. Aliquots of 0.5 ml were withdrawn at pre-determined time intervals (0.5, 1, 2, 4, 8, and 12 hrs.) and immediately replaced by same volume of the fresh medium. The aliquots were suitably diluted with the dissolution medium and analyzed by UV-Vis Spectrophotometer at 276nm (λmax). The data obtained from the in-vitro diffusion studies were fitted to various kinetic equations to find out the mechanism of drug release from the Nanogels.

In-vivo activity [38] 325-326

Streptozotocin (STZ) Induced Antidiabetic Activity:

Animal Selection:

Wistar albino rats weighing 220 to 250g were chosen for the study.

Preparation of Trisodium Citrate:

Weighed accurately 8.4gm of Trisodium Citrate Dihydrate (TCD) using a weighing balance. Then measured TCD was added to a clean container and in this container 200ml of distilled water was poured and stirred the mixture until the TCD is completely dissolved. Afterwards to adjust the PH 4.5, started by adding a small amount of citric acid (around 0.5gms) to the solution. Then stirred and checked the pH using PH meter. Repeated this process until the pH reaches to 4.5. After adjusting the pH of 4.5 diluted the solution with additional distilled water to make up the final volume 250ml with thorough mixing.

Diabetes Induction:

Diabetes was induced in overnight fasted rats by injecting 0.5ml streptozotocin (50mg/kg) intraperitoneally (i.p). Streptozotocin is a compound known for inducing diabetes in experimental animals.

Selection Criteria for Diabetic Rats:

Seven days after streptozotocin injection, rats with blood glucose levels more than 200 mg/dL were selected.

Group Division:

After an overnight fast, rats were divided into five groups (n = 6).

Group 1: Control rats orally administered with distilled water (10ml /kg) (p.o.).

Group 2: Streptozotocin-induced diabetic rats administered orally with distilled water.

(50 mg/kg) (p.o.)

Group 3: Streptozotocin-induced diabetic rats administered orally with Metformin dissolved in distilled water (20 mg/kg).

Group 4: Streptozotocin-induced diabetic rats received nanofiber loaded intranasal in gel (NGA 5) form (1.5 IU/kg) using a micro syringe attached to a blunt needle with a 0.5-inch polyethylene tube at the end.

Group 5: Streptozotocin-induced diabetic rats received nanofiber loaded intranasal in gel (NGB3) form (1.5 IU/kg) using a micro syringe attached to a blunt needle with a 0.5-inch polyethylene tube at the end.

Blood Collection and Glucose Measurement:

At specified time intervals (On day 0, 7, 14, 28 after treatment) blood was collected from orbital sinuses. Blood glucose levels were determined using a digital glucometer.

Stability Study [39] 327

Accelerated stability studies of optimized nanogel was carried out according to ICH Q1A (R2) guidelines. The stability study was performed at 25 ±2°C and 60 ±5% RH in an environmental stability chamber over a period of three months to assess the stability of nanogel. The nanogel was transferred to amber-colored glass vials, which were plugged and kept in the stability chamber. The drug content, viscosity and Entrapment efficiency measured after three months.

Results and Discussion

Preformulation Study

FTIR spectra of Bovine Insulin

The FTIR studies showed that the significant peaks of Bovine Insulin are O-H stretching at 1390.32 cm-1, C=O vibration at 1642.49 cm1 and C-H [CH2] bending at 1445.45 cm1 and C-O-C at 1079.93 cm1, NH cm-1 at 3268.41 cm-1. Based on that FTIR spectrum of Bovine Insulin functional groups peak was coincided with standard Insulin pure drug. Based on this result the drug was confirmed as in its pure form without by-products.

FTIR Spectrum of Bovine Insulin and chitosan loaded nanofibres solution

The FTIR studies showed that the significant peaks of Bovine Insulin are C=O vibration at 1641.84 cm1 and C-H [CH2] bending at 1415.78 cm1 and C-O –C at 1022.72 cm1, NH cm-1 at 3265.11 cm-1. Based on that FTIR spectrum of Bovine Insulin functional groups peak was coincided with standard Insulin pure drug. The IR spectra did not show any difference in wavelength from those obtained for their physical mixture with polymers as compared to drug. These obtained results indicate that there was no interaction between Bovine Insulin and chitosan polymers and the other excipients. Hence they are compatible with each other. Thus, Bovine Insulin, can be used in combination for the preparation gel.

FTIR Spectrum of Bovine Insulin and sodium alginate loaded nanofiber solution

The FTIR studies showed that the significant peaks of Bovine Insulin are C=O vibration at 1644.42 cm1 and C-H [CH2] bending at 1463.21 cm1 and C-O-C at 1049.51 cm1, NH cm-1 at 3309.47 cm-1. Based on that FTIR spectrum of Bovine Insulin functional groups peak was coincided with standard Insulin pure drug. The IR spectra did not show any difference in wavelength from those obtained for their physical mixture with polymers as compared to drug. These obtained results indicate that there was no interaction between Bovine Insulin and sodium alginate polymers and the other excipients. Hence they are compatible with each other. Thus, Bovine Insulin can be used in combination for the preparation gel.

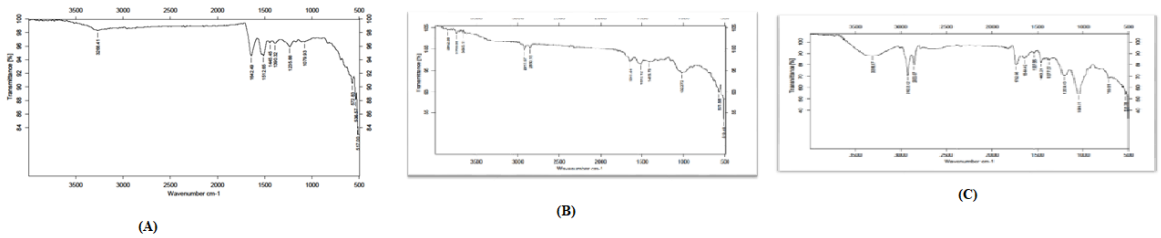


Figure 1: (A) FTIR Spectrum of Bovine Insulin and chitosan loaded nanofibres solution, (B) FTIR Spectrum of Bovine Insulin and chitosan loaded nanofibres solution, (C) FTIR Spectrum of Bovine Insulin and sodium alginate loaded nanofiber solution

DSC Compatibility study (Drug-Excipients)

The observation of a peak at 51.98°C in the DSC (Differential Scanning Calorimetry) thermograms of Bovine Insulin powder provides valuable insights into its thermal behavior and stability. The peak observed at 51.98°C indicates a thermal event or transition occurring within the sample at this temperature.

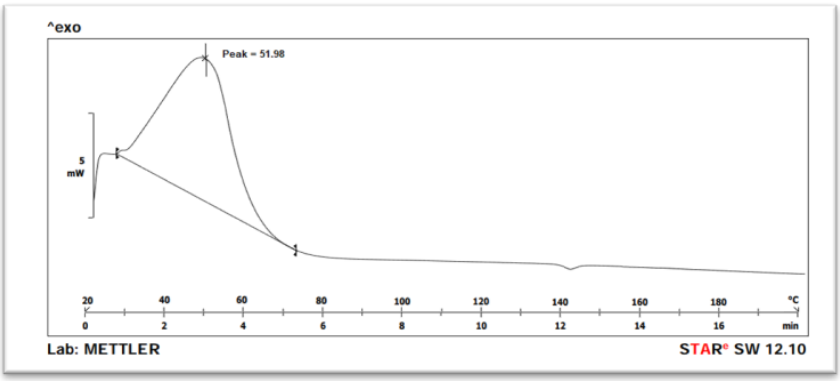


Figure 2: DSC Spectrum of Bovine Insulin powder

X- ray diffraction study of bovine Insulin powder

The characteristic peaks of Bovine Insulin powder were centered at 2ϴ = 26.0783 corresponding to the crystallographic planes respectively. The XRD analysis of Bovine Insulin powder indicates a reduction in peak intensity, suggesting an increase in solubility and a conversion from crystalline to amorphous nature.

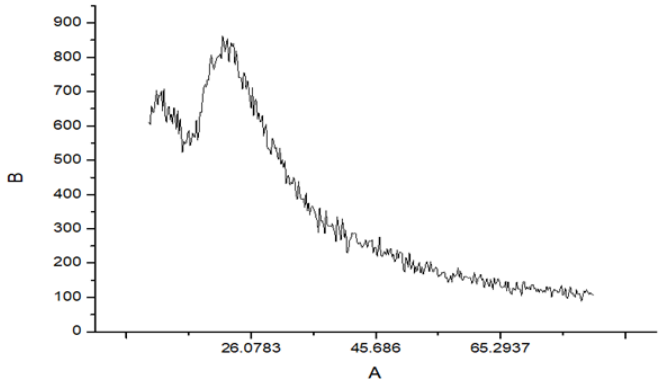


Figure 3: X-ray diffraction patterns of Bovine Insulin powder

Determination of Thiol Grou

Preparation of Standard Calibration Curve of N-Acetyl Cysteine:

Table 3: Calibration Curve of N-Acetyl Cysteine

Concentration (µg/ml)	Absorbance
0	0.0000
10	0.03320
20	0.06269
30	0.09654
40	0.12010
50	0.15020

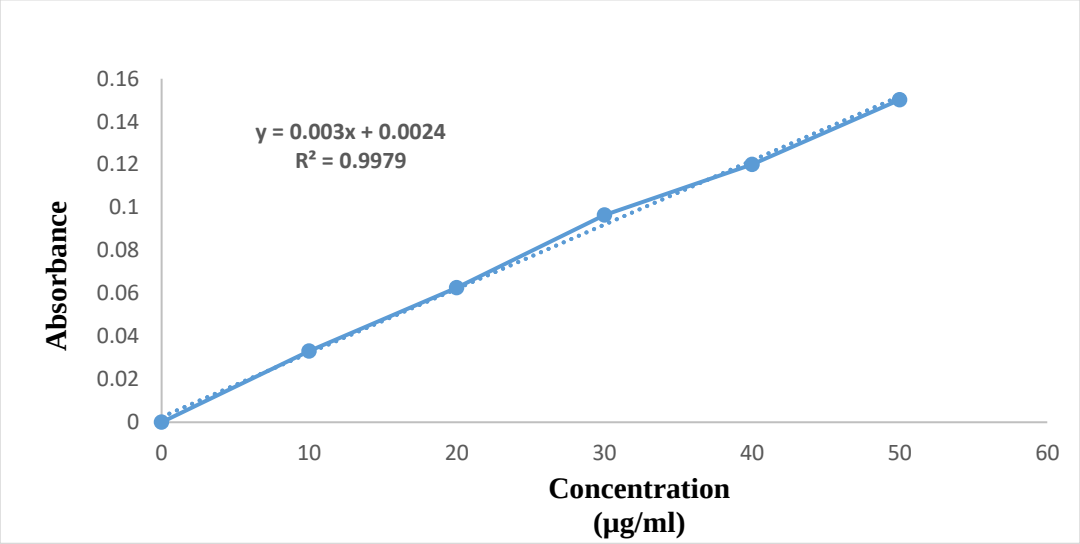


Figure 4: Calibration curve of cysteine

For Chitosan conjugate

Contents of thiol groups in the chitosan conjugate is calculated by inserting the sample absorbance of chitosan conjugate were read in the Uv-vis spectrophotometer into the regression equation curve standard solution of cysteine, i.e. $y = 0.003 x + 0.0024$; $R^2 = 0.9979$. Absorbance data and concentration of free thiol groups in the sodium alginate conjugate can be seen in Table.

Table 4: Absorbance and content of free thiol groups in the 50 mg of chitosan conjugate

Sr. No.	Sample name	Absorbance of sample	Concentration of sample (Unit)
1	Chitosan conjugate (without EDAC)	0.017	4.867
3	Chitosan conjugate (with EDAC) 200mM	0.5986	198.733

The results showed that there are free thiol groups present, which are formed. Chitosan and sodium alginate conjugates by the addition of EDAC 200mM has the highest content of thiol groups of conjugates. EDAC acts as a catalyst in the formulation of chitosan conjugates. The results showed that chitosan and sodium alginate conjugates have a thiol group, it became evident that the amide bond formed between chitosan and sodium alginate.

For Sodium alginate conjugate

Contents of thiol groups in the sodium alginate conjugate is calculated by inserting the sample absorbance of sodium alginate conjugate were read in the uv-vis spectrophotometer into the regression equation curve standard solution of cysteine, i.e. $y = 0.003 x + 0.0024$; $R^2 = 0.9979$. Absorbance data and concentration of free thiol groups in the sodium alginate conjugate can be seen in Table.

Table 5: Absorbance and content of free thiol groups in the 50 mg of sodium alginate conjugate

Sr. No.	Sample name	Absorbance of sample	Concentration of sample (Unit)
1	Sodium alginate conjugate (without EDAC)	0.008	1.887
2	Sodium alginate conjugate (with EDAC) 200mM	0.5986	198.733

The results showed that there are free thiol groups present, which are formed. Chitosan and sodium alginate conjugates by the addition of EDAC 200mM has the highest content of thiol groups of conjugates. EDAC acts as a catalyst in the formulation of chitosan conjugates. The results showed that chitosan and sodium alginate conjugates have a thiol group, it became evident that the amide bond formed between chitosan and sodium alginate.

Evaluation and Characterization of Nanofibers:

Evaluation of Nanofibers:

Drug entrapment efficiency

The entrapped efficiency of different formulations (NF1, NF2, NF3, NF4):

Table 6: Determination of Entrapment efficiency (%) of nanofiber NF1-NF4

Sr. no	Formulation	Entrapment efficiency (%)
1.	NF1	75.22±077
2.	NF2	77.20±0.56
3.	NF3	78.45±0.14
4.	NF4	85.12±0.89

Swelling index

Table 7: Swelling index

Sr. no	Formulation	Swelling (%)
1.	NF1	64.44±0.01
2.	NF2	78.59±0.02
3.	NF3	76.06±0.12
4.	NF4	88.62±0.05

The swelling index of the electrospun nanofiber materials was determined using a gravimetric method. The swelling index of the nanofiber plays an important role in the loading and release behavior of a drug. Above figure shows the degree of swelling of drug loaded nanofiber gel at different time intervals. The degree of swelling of Nano fibrous gel (NF1-NF4) in PBS pH 7.4 was 64.44, 78.59, 76.06 and 88.62for the time intervals of 1, 2, 4, 6, 8, 10, and 12 h, respectively.

Characterization of Nanofibers:

IR spectrum

IR spectrum of prepared nanofibers batch NF1

The FTIR studies showed that the significant peaks of Bovine Insulin are C=O vibration at 1735.84 cm1 and C-H [CH2] bending at 1464.24 cm1 and C-O-C at 1010.35cm1, O-H Stretch cm-1 at 3648.07 cm-1. Based on that FTIR spectrum of Bovine Insulin functional groups peak was coincided with standard Insulin pure drug. The IR spectra did not show any difference in wavelength from those obtained for their nanofiber formulation as compared to drug.

IR spectrum of prepared nanofiber batch NF2

The FTIR studies showed that the significant peaks of Bovine Insulin are C=O vibration at 1731.49cm1 and C-H [CH2] bending at 1468.78 cm1 and C-O-C at 1056.73cm1, O-H Stretch cm-1 at 3666.14 cm-1. Based on that FTIR spectrum of Bovine Insulin functional groups peak was coincided with standard Insulin pure drug. The IR spectra did not show any difference in wavelength from those obtained for their nanofiber formulation as compared to drug.

IR spectrum of prepared nanofibers batch NF3

The FTIR studies showed that the significant peaks of Bovine Insulin are C=O vibration at 1789.57cm1 and C-H [CH2] bending at 1515.02 cm1 and C-O-C at 1053.61cm1, O-H Stretch cm-1 at 3608.89 cm-1. Based on that FTIR spectrum of Bovine Insulin functional groups peak was coincided with standard Insulin pure drug. The IR spectra did not show any difference in wavelength from those obtained for their nanofiber formulation as compared to drug.

IR spectrum of prepared nanofiber batch NF4

The FTIR studies showed that the significant peaks of Bovine Insulin are C=O vibration at 1682.50cm1 and C-H [CH2] bending at 1560.81 cm1 and C-O-C at 1031.78cm1, O-H Stretch cm-1 at 3739.89 cm-1. Based on that FTIR spectrum of Bovine Insulin functional groups peak was coincided with standard Insulin pure drug. The IR spectra did not show any difference in wavelength from those obtained for their nanofiber formulation as compared to drug.

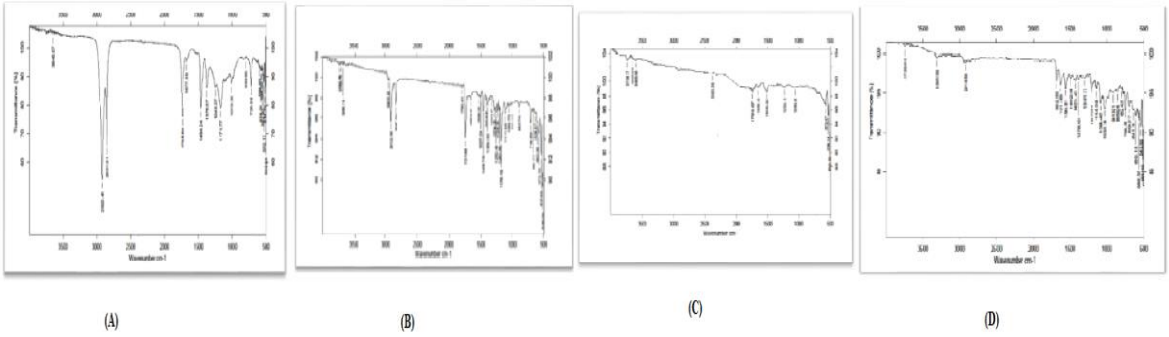
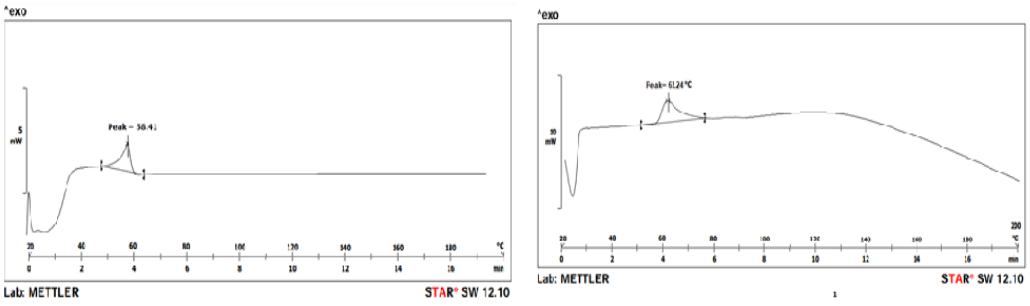


Figure 5:(A) IR spectrum of prepared nanofibers batch NF1, (B) IR spectrum of prepared nanofibers batch NF2, (C) IR spectrum of prepared nanofibers batch NF3, (D) IR spectrum of prepared nanofibers batch NF4

DSC compatibility study

The DSC thermograms of sodium alginate (NF1) exhibits one endothermic band at 58.41℃ .The first endothermic band corresponds to the evaporation of hydration water molecules while the exothermic one indicates the oxidative degradation of alginate polymers

The observation of a peak at 63.24°C in the DSC (Differential Scanning Calorimetry) spectrum of the formulation blend (Chitosan loaded-NF3) indicates a thermal event or transition occurring within the sample at this temperature. The peak observed at 63.24°C could potentially correspond to various phenomena depending on the specific composition and characteristics of the formulation blend:

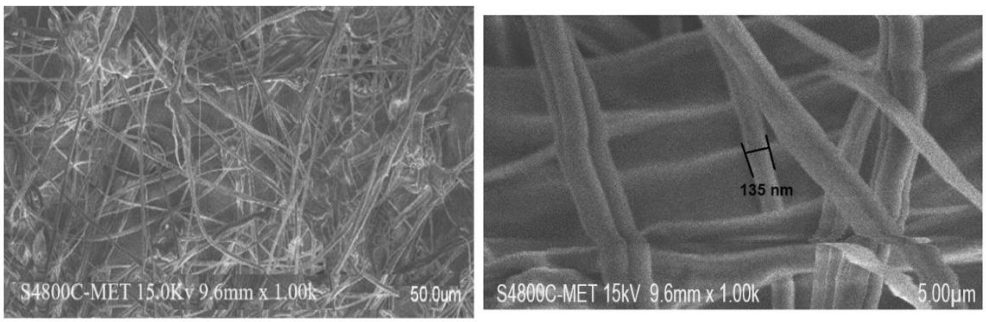


(A) (B)

Figure 6 :(A) DSC spectrum of formulation blend (Sodium alginate loaded –NF1) ,(B) DSC spectrum of formulation blend (Chitosan loaded-NF3)

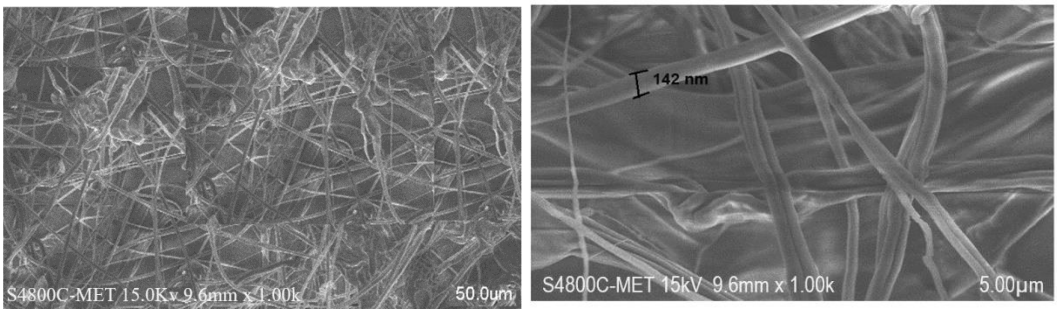
Scanning electron microscope (SEM)

Particle shape and its arrangement inside the formulation can be unfolded by scanning electron microscopy (SEM). Scanning electron microscopy was used to examine the surface morphology of silver nanofibers. The SEM graphs are represented below. The morphology for plain and prepared nanofiber patches was analyses by using a Hitachi S-4700 SEM (scanning electronin Hitachi Company, Japan). Before being considered, samples were placed on metal ends using double-sided adhesive tape and vacuum-coated with a gold sputter layer. A mixed population of sub-micron (~200 nm) and larger (2–4μm) fibers were observed for all the formulated nanofibers.



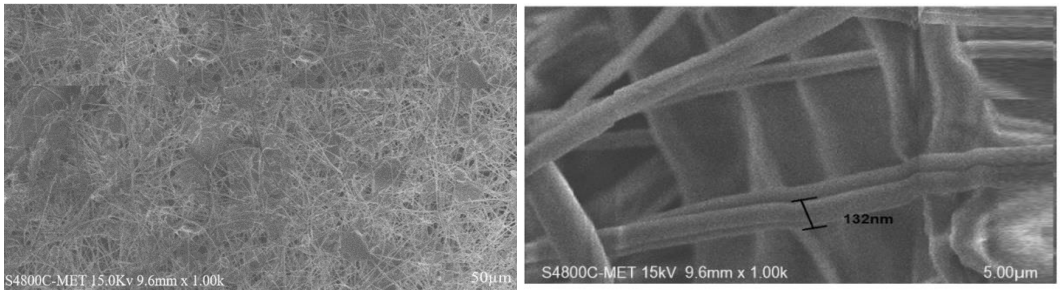
(A) (B)

Figure 7: Scanning electron microscopy (SEM) images of NF1 nanofibers containing sodium alginate (NF1). A) SEM image of spunbond fibers
B) SEM image of electrospun fibers



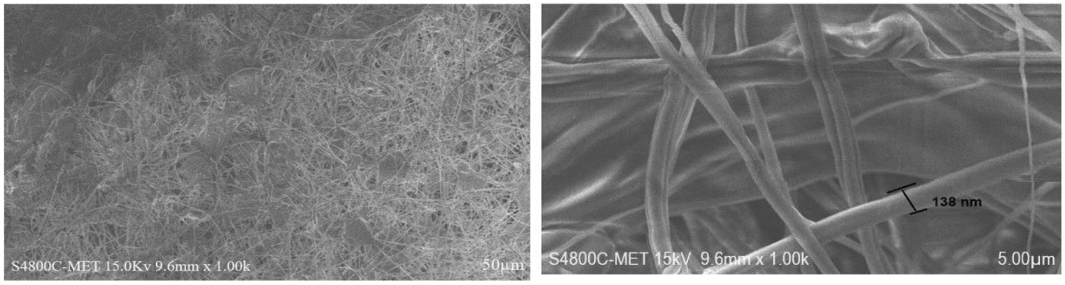
(A) **(B)**

Figure 8: Scanning electron microscopy of NF2 a) SEM image of spunbond fibers B) SEM image of electrospun fibers)



(A) **(B)**

Figure 9: Scanning electron microscopy of NF3 A) SEM image of spunbond fiber B) SEM image of electrospun fibers



(A) **(B)**

Figure 10: Scanning electron microscopy of NF4 A) SEM image of spunbond fibers, B) SEM image of electrospun fibers

TGA (Thermogravimetric Analysis) of Prepared Nanofibres:

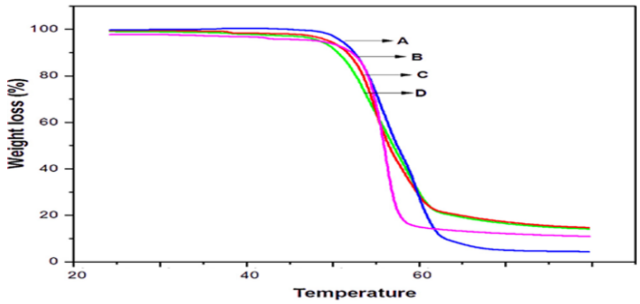


Figure 11: TGA Thermograms of A) NF1 B) NF2 C) NF3 D) NF4

Thermo gravimetric Analysis (TGA) is pivotal for understanding materials' thermal behavior and stability. Figure No.11 displays TGA thermograms of samples NF1, NF2, NF3, and NF4, revealing their decomposition patterns. TGA subjects samples to controlled temperature ramps, monitoring weight changes. Initial thermograms portions show moisture or volatile components, followed by significant weight loss during main decomposition. Onset and peak temperatures of decomposition offer insights into thermal stability and composition. Thermograms may also indicate residue formation, reflecting sample purity or inorganic filler presence.

Optimization OF PVA (Polyvinyl Alcohol) Loaded In-Situ Nanofiber Gel

Characterization of PVA (Polyvinyl Alcohol) Loaded In-Situ Nanofiber Gel:

Determination of pH

The optimized batch for nasal application, NGA5, has a pH of 6.3, making it ideal for this specific application. This pH value serves as a reference point for the desired pH range for nasal formulations, indicating that formulations close to pH 6.3 may be suitable for nasal products. However, additional considerations and adjustments are necessary to ensure that formulations meet all other requirements for nasal delivery.

Table 8: Determination of pH of Nanofibrous gel

Formulation	pH
NGA1	6.2±0.120
NGA2	5.0±0.020
NGA3	5.5±0.050
NGA4	5.4±0.060
NGA5	6.3±0.120
NGA6	6.1±0.005
NGA7	6.8±0.030
NGA8	6.7±0.210
NGA9	6.9±0.320
NGA10	7.2±0.641
NGA11	6.4±0.060
NGA12	6.2±0.050
NGA13	6.6±0.020
NGA14	6.1±0.014
NGA15	6.0±0.324

Values are expressed as Mean±S.D.

ANOVA for 2FI model

Response 1: PH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.71	6	0.6178	4.00	0.0377	significant
A-PVA+Sodium alginate	0.5021	1	0.5021	3.25	0.1091	

B-PVA+Chitosan	0.3594	1	0.3594	2.33	0.1658	
C-sodium carboxymethyl cellulose	0.1922	1	0.1922	1.24	0.2972	
AB	1.56	1	1.56	10.08	0.0131	
AC	0.8256	1	0.8256	5.34	0.0496	
BC	0.2701	1	0.2701	1.75	0.2227	
Residual	1.24	8	0.1545			
Cor Total	4.94	14				

Factor coding is **coded**.
Sum of squares is **Type III - Partial**

The **Model F-value** of 4.00 implies the model is significant. There is only a 3.77% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case AB, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	0.3931		**R^2**	0.7499
Mean	6.23		**Adjusted R^2**	0.5623
C.V. %	6.31		**Predicted R^2**	0.1152
			Adeq Precision	7.1065

The **Predicted R^2** of 0.1152 is not as close to the **Adjusted R^2** of 0.5623 as one might normally expect; i.e. the difference is more than 0.2. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response transformation, outliers, etc. All empirical models should be tested by doing confirmation runs.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 7.107 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

PH =+6.23+0.1917* A++0.1622 * B+0.1186* C+0.4413* AB-0.3212* AC+0.1838* BC

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors

PH =+7.55066-0.168255 PVA+Sodium alginate-1.71278 PVA+Chitosan+5.31120sodium carboxymethyl cellulose+0.441250PVA+Sodium alginate * PVA+Chitosan-3.21250 PVA+Sodium alginate * sodium carboxymethyl cellulose+1.83750PVA+Chitosan * sodium carboxymethyl cellulose

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

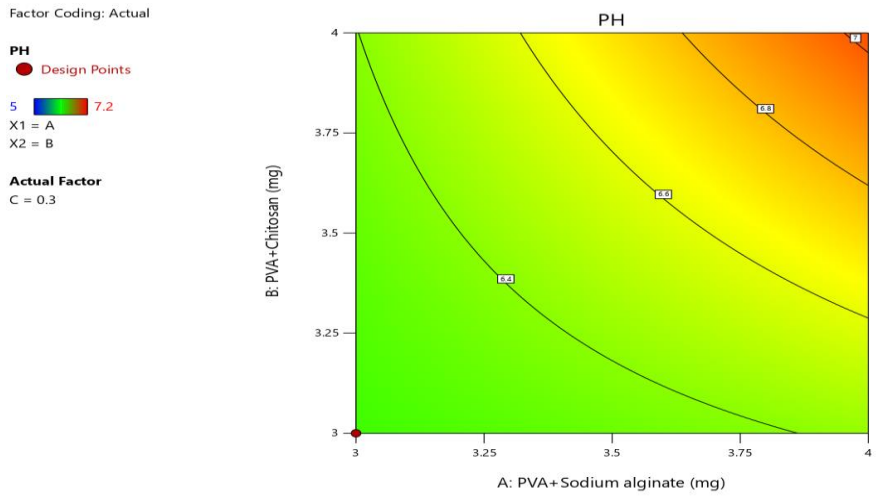


Figure 12: 2D Counter Plot of pH for (PVA Loaded) Nanofibrous Gel

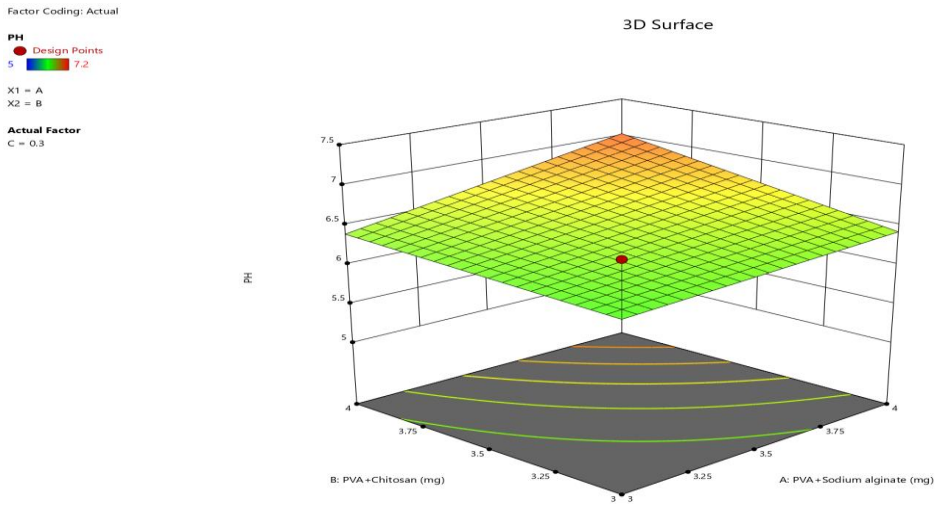


Figure 13: 3D Surface Plot of pH for (PVA Loaded) Nanofibrous Gel

Determination of Viscosity

The data suggests that NGA5 is the optimized nasal formulation with a viscosity of 457.87Cps. Future research should focus on understanding how this optimized viscosity affects drug release, stability, and patient acceptance, with the goal of further improving the formulation's efficacy and performance."

Table 9: Rheological study of Nano fibrous gel

Sr. No.	Formulation Code	Viscosity (cPs)
1	NGA1	169.33±0.150
2	NGA2	195.83±0.050
3	NGA3	305.80±0.100
4	NGA4	225.56±0.150
5	NGA5	457.87±0.100
6	NGA6	215.43±0.150
7	NGA7	298.66±0.020
8	NGA8	315.78±0.020
9	NGA9	324.91±0.030
10	NGA10	245.79±0.002
11	NGA11	278.92±0.030
12	NGA12	215.46±0.002
13	NGA13	200.063±0.010
14	NGA14	319.463±0.020
15	NGA15	245.877±1.700

Values are expressed as Mean±S.D.

Determination of Spreadability

Optimal formulation in a spreadability study involves careful consideration of the desired properties for the intended application. In this case, if the objective is to achieve lower spreadability, NGA5 emerges as the preferred choice with its lowest spreadability value of 12.56 g.cm/sec. A lower spreadability value suggests that the gel may exhibit greater cohesion, maintaining its position upon application rather than spreading extensively.

Sr. No.	Formulation Code	Spreadability (g .cm/sec.)
1.	NGA1	13.71±0.010
2.	NGA2	17.85±0.050
3.	NGA3	16.64±0.247
4.	NGA4	19.46±0.050
5.	NGA5	12.56±0.040
6.	NGA6	14.99±0.264
7.	NGA7	13.50±0.120
8.	NGA8	12.99±0.070
9.	NGA9	15.46±0.641
10.	NGA10	15.99±0.030
11.	NGA11	17.58±0.010
12.	NGA12	15.36±0.005
13.	NGA13	14.25±0.010
14.	NGA14	13.25±0.230
15.	NGA15	16.94±0.310

Table 10: Spreadability study of Nano fibrous gel

Values are expressed as Mean±S.D.

ANOVA for 2FI model

Response 2: Spreadability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	45.05	6	7.51	4.66	0.0249	significant
A-PVA+Sodium alginate	1.97	1	1.97	1.22	0.3010	
B-PVA+Chitosan	3.38	1	3.38	2.10	0.1854	
C-sodium carboxymethyl cellulose	2.97	1	2.97	1.84	0.2115	
AB	3.21	1	3.21	1.99	0.1956	
AC	12.88	1	12.88	7.99	0.0222	
BC	20.64	1	20.64	12.81	0.0072	
Residual	12.89	8	1.61			
Cor Total	57.94	14				

Factor coding is **coded**.
Sum of squares is **Type III - Partial**

The **Model F-value** of 4.66 implies the model is significant. There is only a 2.49% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case AC, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Fit Statistics

Std. Dev.	1.27		**R^2**	0.7776
Mean	15.37		**Adjusted R^2**	0.6108
C.V. %	8.26		**Predicted R^2**	-0.4090
			Adeq Precision	8.6556

A negative **Predicted R^2** implies that the overall mean may be a better predictor of your response than the current model. In some cases, a higher order model may also predict better.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 8.656 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

Spreadability=+15.37+0.3797* A-0.4976*B-0.4664*C-0.6338*AB+1.27*AC-1.61*BC

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors

Spreadability=+8.38016-1.52528* PVA+Sodiumalginate+6.22242*PVA+Chitosan+5.46105*sodium carboxymethyl cellulose-0.633750* PVA+Sodium alginate * PVA+Chitosan+12.68750* PVA+Sodium alginate * sodium carboxymethyl cellulose-16.06250*PVA+Chitosan * sodium carboxymethyl cellulose

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

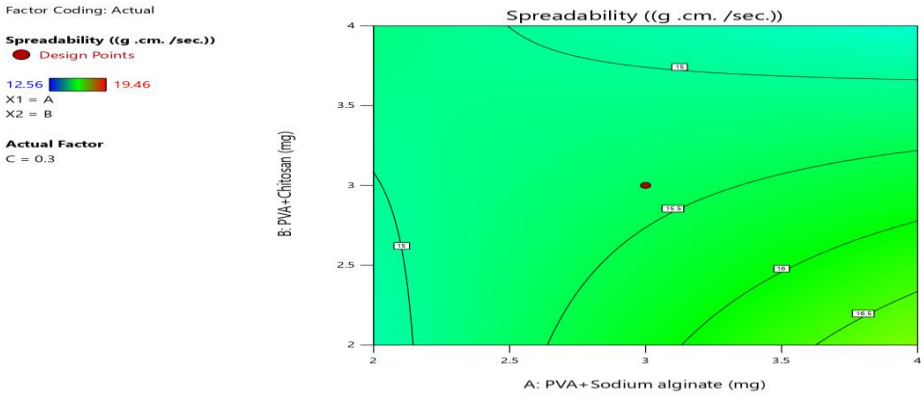


Figure 14: 2D Counter Plot of Spreadability for (PVA Loaded) Nanofibrous Gel

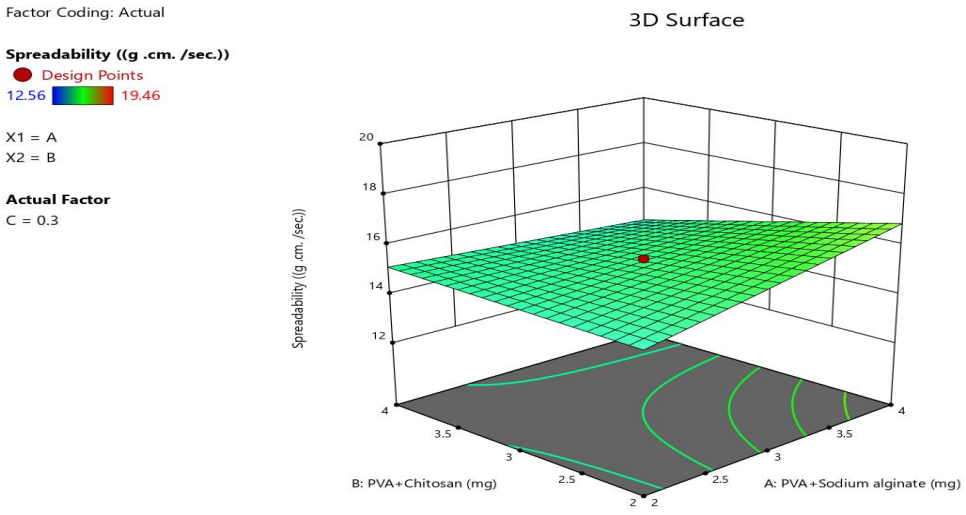


Figure 15: 3D Surface Plot of Spreadability for (PVA Loaded) Nanofibrous Gel

Extrudability

The various formulations tested, NGA5 emerges as the optimal choice in terms of extrudability, boasting the highest value of 21.48 gm. /cm2. This indicates that NGA5 exhibits superior ease of extrusion from the collapsible tube compared to the other formulations. The significance of extrudability lies in its direct impact on user experience and product usability, particularly in applications where consistent and effortless dispensing is

paramount. Therefore, NGA5 stands out as the preferred formulation, offering practical advantages and ensuring smooth application, thus enhancing overall product satisfaction and usability.

Table 11: Determination of Extrudability of Nano fibrous gel

Sr. No.	Formulation Code	Extrudability (gm /cm^2)
1	NGA1	14.86±0.030
2	NGA2	16.87±0.050
3	NGA3	12.36±0.120
4	NGA4	17.89±0.060
5	NGA5	21.48±0.005
6	NGA6	21.44±0.141
7	NGA7	13.44±0.246
8	NGA8	15.46±0.050
9	NGA9	18.89±0.240
10	NGA10	21.35±0.030
11	NGA11	20.16±0.010
12	NGA12	14.87±0.040
13	NGA13	16.44±0.050
14	NGA14	18.49±0.314
15	NGA15	17.65±0.050

Values are expressed as Mean±S.D.

Drug Content Uniformity

The optimized batch for nasal application, NGA5, has a drug content of 98%, making it ideal for efficacy and batch-to-batch uniformity. Other formulations range from 78% to 92.01% drug content, with NGA5 standing out for its high drug content. Comparing formulations to NGA5 helps identify alternatives or assess performance. Formulations close to NGA5's drug content may also be suitable candidates. Overall, NGA5's drug content of 98% for optimal drug content in nasal applications, guiding formulation selection.

Table 12: Determination of drug content of Nano fibrous gel

Sr. No.	Formulation Code	Drug Content (%)
1.	NGA1	89.13 ± 1.020
2.	NGA2	90.06 ± 1.330

3.	NGA3	92.01 ± 1.040
4.	NGA4	96.01 ± 1.120
5.	NGA5	98.00 ± 0.006
6.	NGA6	88.00 ± 0.150
7.	NGA7	90.00 ± 0.069
8.	NGA8	87.00 ± 0.081
9.	NGA9	85.00 ± 0.289
10.	NGA10	78.00 ± 0.265
11.	NGA11	87.00 ± 0.115
12.	NGA12	85.00 ± 0.260
13.	NGA13	87.40 ± 0.115
14.	NGA14	92.00 ± 1.135
15.	NGA15	82.14 ± 0.078

Values are expressed as Mean±S.D.

In vitro drug diffusion study

The release of the active ingredient increases gradually over time. The most significant increase occurs between 8 to 12 hours, with a jump from 88.57% to 92.78%. This indicates that the formulation continues to release the active ingredient even after 8 hours, with a notable increase by the 12th hour. Therefore, the optimized batch NGA5 shows a sustained release profile, which is desirable for certain pharmaceutical formulations, especially for drugs that require a prolonged duration of action or a steady release over time to maintain therapeutic efficacy.

Table 13: Drug release profile of nanofiber gel

Formulation Code / Time in Hrs.	0.5	1	2	4	8	12
NGA1	34.17±0.230	34.17±0.140	42.83±1.450	64.50±0.990	74.51±1.020	84.56±0.560
NGA2	52.83±0.450	60.33±0.870	63.33±1.050	64.50±0.440	72.56±0.77	88.74±0.160
NGA3	42.17±0.130	47.14±0.330	56.03±0.530	66.66±0.330	70.14±0.930	89.15±0.330
NGA4	45.14±0.780	49.13±0.400	58.15±0.520	59.88±1.040	71.85±1.360	85.54±0.580
NGA5	35.23±0.010	46.28±0.005	48.23±0.010	55.68±0.023	88.57±0.000	92.78±0.450
NGA6	32.12±0.010	35.99±0.026	45.58±0.005	50.47±0.005	55.64±0.010	75.45±0.005
NGA7	45.23±0.020	49.53±0.010	52.14±0.020	55.67±0.010	60.17±0.010	62.49±0.010
NGA8	49.58±0.011	50.47±0.0057	52.36±0.012	54.88±0.570	55.88±0.010	63.47±0.010
NGA9	53.14±0.005	55.56±0.005	57.45±0.017	59.54±0.005	60.14±0.011	62.23±0.063
NGA10	50.79±0.005	52.47±0.005	55.49±0.011	62.78±0.010	69.36±0.025	70.69±0.037
NGA11	61.4±0.0057	62.77±0.0057	65.76±0.012	70.14±0.010	72.33±0.020	75.87±0.010
NGA12	58.46±0.110	59.99±0.010	64.89±0.020	68.74±0.010	70.02±0.020	71.35±0.010
NGA13	60.74±0.010	62.78±0.011	64.25±0.011	65.06±0.010	72.44±0.015	75.68±0.010

NGA14	71.58±0.020	72.16±0.006	75.64±0.020	79.87±0.010	82.16±0.015	85.47±0.020
NGA15	67.47±0.010	74.56±0.010	78.49±0.040	82.47±0.010	85.47±0.020	87.98±0.005

Values expressed as Mean ± S.D.

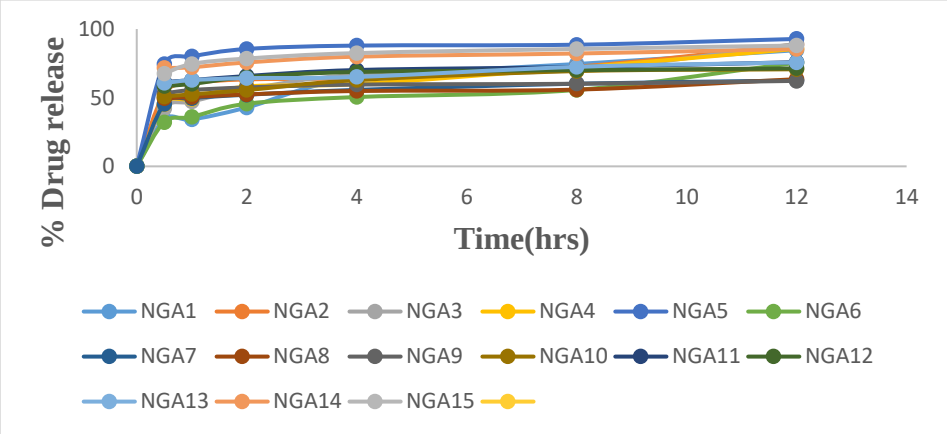


Figure 16: Drug Release Profile of (PVA Loaded) Nanofibrous Gel (NG1-NG15)

Release kinetic modeling:

In order to define the release mechanism of the drug substance that gives the best description of the release pattern; the in vitro release data for all optimized batches were fitted to kinetic equations models. The kinetic equations were used i.e., zero, first-order and Higuchi model. Both the kinetic rate constant (k) and the determination coefficient (R2) were calculated and presented in below graphs. The best fit model with the highest determination coefficient (R^2) value for optimized batch was Higuchi model.

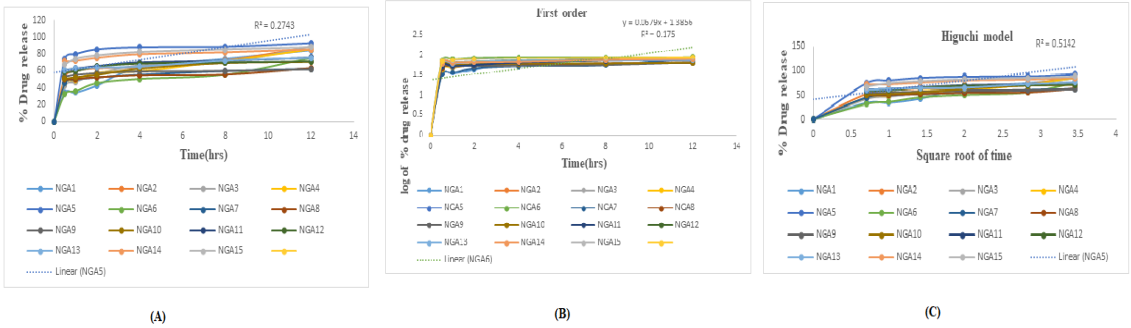


Figure 17 :(A) Zero order of optimized batches, (B), First order of optimized batches (C) Higuchi model of optimized batches

Table 14: R2 value of kinetic models

Models	Optimized Batches	R2 Value
Zero order	NGA5	0.2743
First order	NGA5	0.1750

Higuchi model	NGA5	0.5142

In-vivo activity

Streptozotocin induced antidiabetic activity

In vivo anti-diabetic study was performed for formulated PVA Loaded Nanogels.

The blood glucose levels are tabulated in Table: 15.

Table 15: Initial and final blood glucose level before and after streptozotocin administration PVA Loaded Nanofibrous Gel

GROUPS	Glucose Level on Day 0 (mg/dl)	Glucose Level on Day 7 (mg/dl)	Glucose Level on Day 14 (mg/dl)	Glucose Level on Day 28 (mg/dl)
Positive Control	124.23±3.68	128.56±4.38	138.58±2.56	132.47±1.96
Disease Inducer Control	129.24±3.45^{ns}	227.29±4.28$^{@}$	242.78±5.68$^{@}$	236.24±2.75$^{@}$
Metformin Standard	122.39±6.15^{ns}	236.19±3.21^{ns}	162.69±3.64**	129.53±4.93**
Nanogel NGA-5	128.25±7.63^{ns}	222.23±8.32^{ns}	178.28±4.39**	151.47±2.38**

The results were expressed as mean SD (n = 6),
ns p>0.05, non-significant; **p<0.01, very significant; when compared to Negative Control group. @ p<0.01, when compared with Positive control group
Based on the provided data on glucose levels over the course of the experiment, here is a conclusion drawn regarding the anti-diabetic activity:

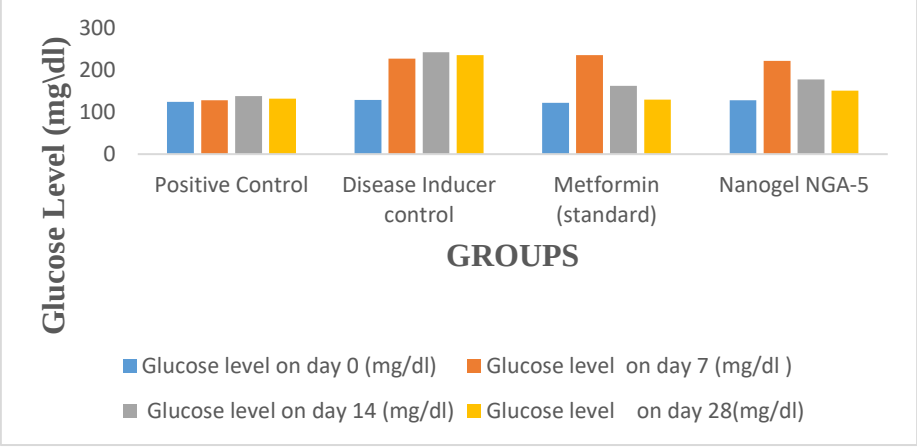


Figure 18: Initial and final blood glucose level before and after streptozotocin administration PVA Loaded nanofibrous gel.

Stability Study

Stability study of PVA loaded Nano fibrous gel

The NGA5 nasal formulation demonstrates some variability in spreadability, viscosity, and drug content over a three-month storage period. The changes in spreadability and viscosity are relatively small and might still be considered acceptable for pharmaceutical formulations. However, the noticeable variations in drug content, particularly in the third month, raise some concerns about its stability. Further investigation and potential reformulation may be necessary to ensure consistent drug content over an extended storage period.

Table 16: Stability study of Nano fibrous gel (PVA)

Storage Duration	Formulation Code	Spreadability (g.cm./sec.)	Viscosity (Cp)	Drug Content (%)
"0" Month	**NGA5**	12.56±0.040	457.87±0.100	98.00±0.006
"1" Month	**NGA5**	12.56±0.020	457.87±0.010	98.00±0.001
"2" Month	**NGA5**	12.55±0.026	457.86±0.020	97.99±0.005
"3" Month	**NGA5**	12.55±0.023	457.86±0.020	97.98±0.017

Values expressed as Mean ± S.D.

Conclusion:

The study showcases meticulous Preformulation examination and optimization of nanofibers loaded with Bovine Insulin for nasal gel formulation. Analysis via FTIR spectroscopy confirms intact functional groups of Bovine Insulin in chitosan and sodium alginate-loaded nanofibers, indicating polymer compatibility. DSC and X-ray diffraction studies reveal increased solubility and a transition to amorphous states in powder form. Thiol group determination indicates successful conjugation, while nanofiber characterization shows satisfactory drug entrapment efficiency and swelling behavior. Further optimization with PVA-loaded in-situ nanofiber gel yields promising results in pH, viscosity, spreadability, and drug diffusion. Optimized batch NGA5 exhibits desirable properties for nasal application, potentially advancing drug delivery systems. In vivo antidiabetic testing of NGA5 in diabetic animal models could signify its efficacy in lowering blood glucose levels and enhancing Insulin sensitivity, suggesting its potential as a diabetic treatment.

Conflict of Interest

The authors declare no conflict of interest.

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