Advances in Bioresearch
Adv. Biores., Vol 14 (6) November 2023: 272-279
©2023 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.14.6.272279

Advances in Bioresearch

ORIGINAL ARTICLE

Gellan Gum-Based Hydrogel for The Transdermal Delivery of Naproxen Sodium: Statistical Optimization and *In-Vitro* Evaluation

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ABSTRACT
Naproxen sodium is a non-steroidal anti-inflammatory drug (NSAID) often used for the symptomatic management of inflammatory conditions such rheumatoid arthritis and ankylosing spondylitis. The present study set out to prepare Naproxen Sodium based hydrogel and optimise it using Box behnken design (BBD). Using different concentrations of gellan gum, carbopol, and polyethylene glycol formulations F1–F17 were made. F9 was chosen and seems to have optimal physicochemical parameters for transdermal administration. After 12 hours, the drug release from the optimised formulation was still above 90%. Rheological measurements confirmed the formulation's viscoelastic behaviour and long-term stability. Carbopol concentration was shown to have a larger effect on gel viscosity than gellan gum concentration. F9 was shown to have a higher rate of drug release compared to other pure drug solutions.
Key words: *Naproxen Sodium, Gellan Gum, Carbopol, Box behnken design, Hydrogel.*

Received 24.05.2023 Revised 08.09.2023 Accepted 23.10.2023

How to cite this article:
Sandip H, Neelam K. Novel Homozygous missense variant in calcium and integrin-binding protein 2 (CIB2) underlies autosomal recessive non-syndromic hearing loss in an Indian family. Adv. Biores., Vol 12 (6) November 2023: 272-279.

INTRODUCTION

Rheumatoid arthritis (RA) is an inflammatory disease associated with severe pain, stiffness, and peripheral joint swelling [1]. Naproxen is generally used to treat pain, pyrexia, inflammation, and stiffness produced by osteoarthritis, rheumatoid arthritis, injuries, tendinitis, bursitis, and psoriatic arthritis. From the perspective of the side effects related to naproxen's oral route, it can be administered safely via topical drug delivery with minimal side effects [2]. Additionally, the topical approach offers certain distinct advantages: a practically larger surface area of skin for absorption, local drug delivery to affected tissues, a non-invasive route, eliminated side effects, maintenance of plasma–drug concentration, ease of removal or replacement, and the avoidance of presystemic metabolism. Being a BCS class II drug, a suitable drug delivery system which can load an adequate amount of Naproxen and release it on the skin surface is good for its transdermal therapy [3]. Hydrogel, a versatile drug delivery system, has emerged as an attractive delivery mode for transdermal therapy of drugs because of the physicochemical and biological characteristics it possesses, which includes controlled drug release, good stability, higher percutaneous absorption, and nontoxic nature [4]. In addition, hydrogels can offer the advantages of flexibility in dosing, ease of application, reduce skin irritation, promote skin hydration, improve drug diffusion, and improve patient compliance. Among various polymers studied, gellan gum, an anionic polysaccharide, has gained much attention in recent years owing to its excellent gelling and tunable mechanical properties [5].

Optimization was performed by examining the effect of various formulation components (gellan gum, carbopol, and PEG 400) on viscosity, in vitro release, and ex vivo permeation. The optimized gel (F9)

contained Naproxen (100 mg), gellan gum (250 mg), carbopol (150 mg), PEG 400 (15 ml), tween 80 (1 ml), ethanol (10 ml), and water (up to 30 ml).

MATERIAL AND METHODS

Naproxen Sodium was purchased by Hetero Pvt Ltd. Hyderabad. Gellan gum, PEG 400, ethanol, and tween 80 was purchased from Sigma Aldrich. Carbopol 940P, Triethanolamine, was purchased from Research lab Fine chem. industries, Mumbai, India**.**

Experimental Studies

Formulation of NPX topical hydrogel

Hydrogels were fabricated using different concentrations of polymeric dispersions. 0.1, 0.5, 0.75, 1% concentrations of carbopol 940 colloidal dispersions were prepared using distilled water. 0.1, 0.5, 0.75, 1% concentrations of gellan gum colloidal dispersions were prepared using distilled water [6]. After complete dispersion, both the polymer solutions were kept in dark for 24 h for complete swelling. Polymer dispersions were prepared using a magnetic stirrer at 500 rpm. Carbopol 940 was dissolved in distilled water, and then gellan gum was added as a colloidal dispersion while the mixture was stirred magnetically. The polymeric dispersion was then treated with sodium hydroxide solution before being combined with the aqueous drug solution. The remaining distilled water was then added while the mixture was being stirred magnetically, and the result was a uniform gel dispersion.

Formulation of Design Batches Using 3^3 Full Factorial

3^3-factor Box-Behnken design (BBD) was run and identify the effects of gellan gum (X1), Carbopol (X2), and PEG 400 (X3) as well as their interactions using a statistical analysis program (Design-Expert® DX 12.0.3.0 Software, StatEase Design Expert, MN, USA). Viscosity, swelling index, Tensile strength and WVTR were treated as dependent factors with 17 experimental runs A model, shown in Equation (1) below, was fitted to the data obtained from the experimental runs.

$$Y= \beta0 + \beta1A+ \beta2B+ \beta3C+ \beta11A2+ \beta22B2+ \beta33C2+ \beta12AB + \beta13AC+ \beta23BC \dots (1)$$

Y is the response, the constant 0 represents the intercept coefficient (regression coefficient at the center point); 1, 2, and 3 are the single variable (linear coefficients); 11, 22, and 33 are quadratic coefficients that predict the double actions of each factor; and 12, 13, and 23 are second order interaction coefficients that show the extent to which the variables studied interact [7].

Evaluation of Gel Formulation

Gels were examined visually to determine their degree of transperency. At room temperature, a digital pH meter (pHep, Hanna Instruments, Woonsocket, RI, USA) was used to measure the pH of the gels. Using a viscometer (Brookfield LVDV-I Prime, Brookfield Engineering, Middleboro, MA, USA), with spindle number S96 and varying rotation at 25 °C, the viscosity of the created gel compositions was determined [8].

Drug content

The produced gel, at 1gm, was blended with 100 ml of the appropriate solvent. The drug concentration was estimated by filtering the stock solution and then making appropriate dilutions before measuring the absorbance at 272 nm using a UV/Vis spectrophotometer (Shimadzu UV 1700).

Swelling index (SI)

Hydrogel samples were dried at 60 ^{0}C (Wa) for 12 hours after being sliced into 1cm1cm squares [9]. The samples were then removed from the oven and incubated in 37°C (Ws) phosphate buffer saline (PBS) at a pH of 7.2. To get the proportion of swelling, the following equation (2) was used:

$$SI = (Ws/Wa) \times 100 \dots\dots\dots (2)$$

Where Wa is the weight of the hydrogel samples after they have dried for 12 hours at 60C, and Ws is the weight of the hydrogel samples after they have been soaked in PBS until they have attained a consistent weight.

Water vapor transmission rate

Round pieces of hydrogel were cut and affixed to the top of a glass container containing 8 grams of $CaCl_2$ [10]. After that, the bottle spent 24 hours in an incubator at 75% RH and 40°C. According to JIS 1099A standard method18, the water vapour transfer rate (WVTR, g/m2/day) was determined using the following (equation 3):

$$WVTR = ((W2-W1)/S) \times 24 \dots\dots\dots\dots (3)$$

Where W1 is the weight of the $CaCl_2$ bottle before being placed in the incubator, W2 is the weight of the bottle after being placed in the incubator, and S is the area across which the sample was transmitted.

Solid state characterization

Fourier Transform Infrared Analysis

To analyze the drug, NPX, Gellan gum, Carbopol, PEG, and Hydrogel formulations FT-IR spectrometer (PerkinElmer, France) was used. The samples were scanned at a resolution of 4 cm between 4000 and 6000 cm^{-1} [11].

Differential scanning calorimetry

At a drug-to-lipid ratio of 1:1, 4000 model from Waltham, Massachusetts-based company Perkin Elmer to conduct thermal analysis of NPX, Gellan gum, Carbopol, PEG, and Hydrogel to achieve optimal hydrogel performance was used. These thermograms were taken at temperatures ranging from 20 to 200 degrees Celsius, with an increase of 20 degrees Celsius each minute.

Powder X-ray diffractometry (PXRD)

Throughout the PXRD tests, the samples were exposed to nickel-filtered Cu Ka radiation (40 kV, 30 mA) and scanned from 20 to 700, 20 with a step size of 0.0450 and a step length of 0.5 s.

Morphology by Scanning electron microscopy (SEM)

A drop of NPX hydrogel was diluted in double distilled water and then air-dried on a sample holder (SEM, Hitachi, Tokyo, Japan). The sample was then analyzed using a number of different magnifications and accelerating voltages, reaching as high as 15,000. [12].

Drug Release

Franz diffusion cell (Electro Lab, Mumbai, India) was used for in vitro NPX release from gels, with drug-releasing surface area of 1.13 cm^2. The donor and receptor compartments were separated by a dialysis membrane with a pore size of 2.4 nm [13]. Phosphate-buffered saline (PBS; pH 7.4) and tween 80 (10% w/v) were used as surfactants in the receiver chamber's 20 mL volume to improve NPX solubility. Receptor media were maintained at 32±0.5 °C with continual stirring (50 rpm). The diffusion membrane was coated with a gel formulation (1 g) and then covered with Parafilm. At intervals of 0.5, 1, 2, 3, 4, 8, 12 and 24 hours, 5 mL aliquot samples were taken and replaced with fresh media and the drug content was measured using HPLC.

Ex Vivo Permeation

The donor compartment has skin that has been fastened with the stratum corneum side up. The diffusion membrane was coated with a gel formulation (1 g) and then covered with Parafilm. Receptor media were maintained at 37±0.5 °C with continual stirring (50 rpm). At 1, 2, 4, and 6 hours, 5 mL samples were taken out of the media and replaced with a fresh volume. HPLC was used to calculate the concentration of NPX in the receiver solution. Slope of a linear plot of NPX penetrated through 1 cm2 of skin over time [14] was used to get the steady-state flux, Jss (g/cm2/h).

Stability Studies

Optimized formulation was kept in a stability chamber for three months at 25±0.2 °C/75±5% relative humidity [15]. Physical appearance, pH, viscosity, assay, and release rate were assessed weekly using gathered samples.

RESULTS AND DISCUSSION

Optimization of the hydrogel formulation

Table 1: Experimental runs of Box-Behnken design for hydrogel formulations

Run	X1	X2	X3	Y1	Y2	Y3	Y4
1	375	200	22.5	15168.5 ±185	3.65±0.01	154.36±1.25	956.43±2.31
2	500	200	15	10453.3±224	0.96±0.03	185.46±3.42	1964.25±0.05
3	250	200	30	14589.3±165	2.51±0.04	362.57±1.62	1059.34±0.69
4	375	200	22.5	14518.4±135	3.26±0.02	160.48±1.38	865.27±1.42
5	500	300	22.5	11854.4±248	4.52±0.11	264.81±2.49	1857.26±2.01
6	500	200	30	18752.4±216	3.62±0.26	325.46±2.01	895.67±0.38
7	500	100	22.5	14957.9±159	4.25±0.34	425.13±1.37	1324.05±1.26
8	250	200	15	16258.9±243	5.62±0.13	269.81±2.05	1259.75±0.49
9	250	100	22.5	8964.75±169	6.92±0.02	523.41±1.05	1124.61±1.32
10	375	300	15	10754.3±258	3.05±0.04	112.48±1.24	1857.43±2.04
11	375	100	30	9846.28±143	3.02±0.09	395.82±1.03	1027.43±1.25
12	375	200	22.5	15486.4±165	3.26±0.08	285.64±2.04	1254.76±0.69
13	375	100	15	10324.5±189	4.52±0.05	195.67±2.36	1952.34±0.83
14	375	200	22.5	14832.2±211	3.62±0.04	190.42±3.51	1042.31±0.35
15	375	200	22.5	14562.3±216	3.46±0.13	175.64±3.28	984.36±2.14
16	375	300	30	16859.1±247	2.86±0.04	165.82±2.51	1854.06±2.13
17	250	300	22.5	18965.4±163	5.19±0.02	295.86±1.49	1124.05±2.18

The Effect of Formulation Variables on Viscosity

VISCOSITY = +14913.54 -345.05A +1792.47B +1532.01C -3276.05AB +2492.19AC +1645.75BC +919.75A2 -2147.68B2 -819.81C2

The coefficient's positive value indicates a favorable influence on viscosity, while its negative sign suggests the opposite effect on the viscosity of the intended batches. As a result, gellan gum and carbopol have a beneficial impact (raise viscosity), whereas PEG 400 has a detrimental effect (reduce viscosity).

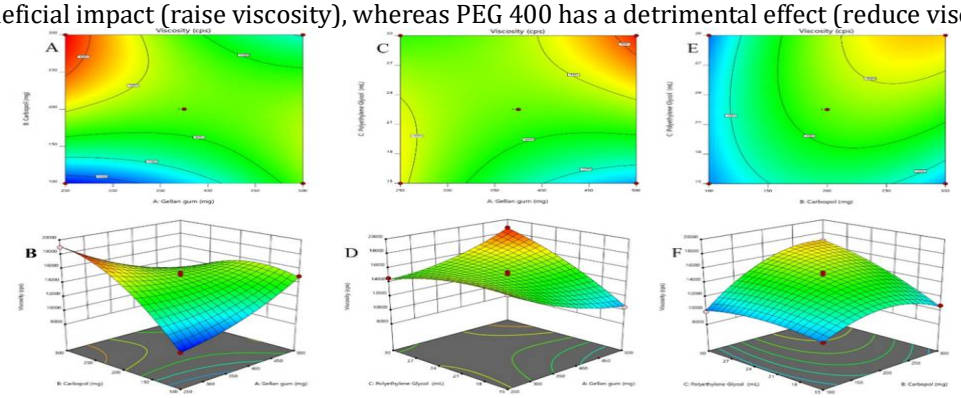

Figure 2: 2D contour graph, 3D response surface plot of independent variables on viscosity (Y1)

Optimization of the formulation based on the swelling index

SWELLING INDEX = +3.45 -0.8612A -0.3863B -0.2675C +0.5000AB +1.44AC +0.3275BC +0.7925A2 +0.9775B2 -1.06C2

Maximum and lowest values of the swelling index percentage were 0.96±0.03% and 6.92±0.02%, respectively. Increased cross-linking at higher polymer concentrations reduces porosity and swelling index. However, it was found that the swelling ratio could be improved by adding PEG 400 to the Carbopol solution. Therefore, it was determined that the hydrogels developed had sufficient swelling capacity.

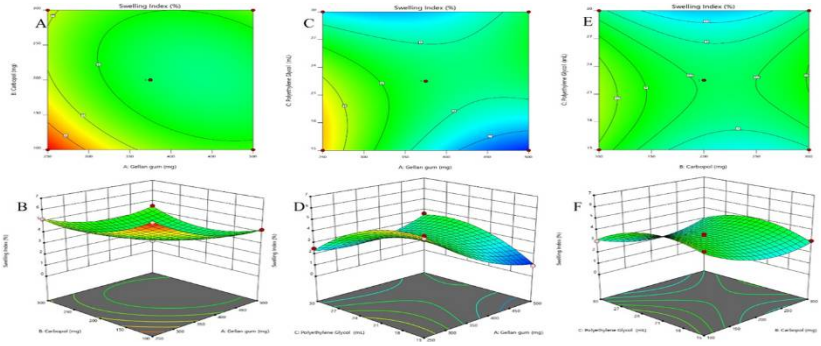

Figure 3: 2D contour graph, 3D response surface plot of independent variables on swelling index (Y2)

Optimization of the formulation based on the tensile strength

Tensile strength = +193.31 -31.35A -87.63B +60.78C +16.81AB +11.81AC -36.70BC +126.19A2 +57.81B2 -33.67C2

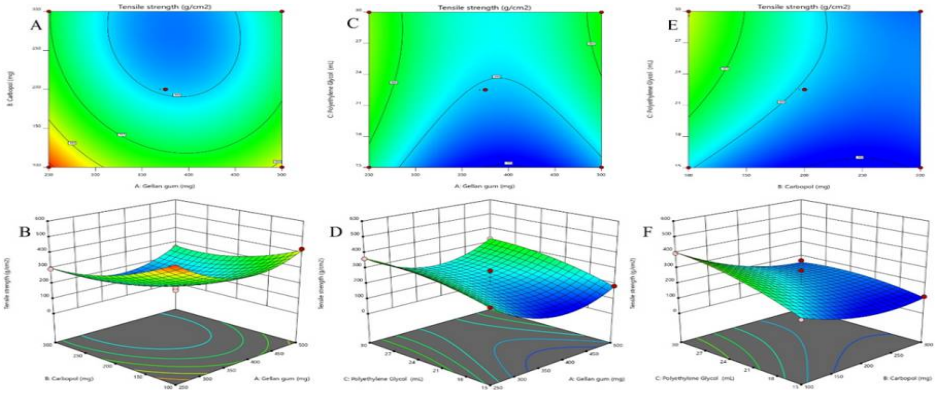

Figure 4: 2D contour graph, 3D response surface plot of independent on Tensile strength (Y3)

Optimization of the formulation based on water vapor transmission rate

WVTR = +1020.63 +184.19A +158.05B -274.66C +133.44AB -217.04AC +230.38BC -20.60A2 +357.46B2 +294.72C2

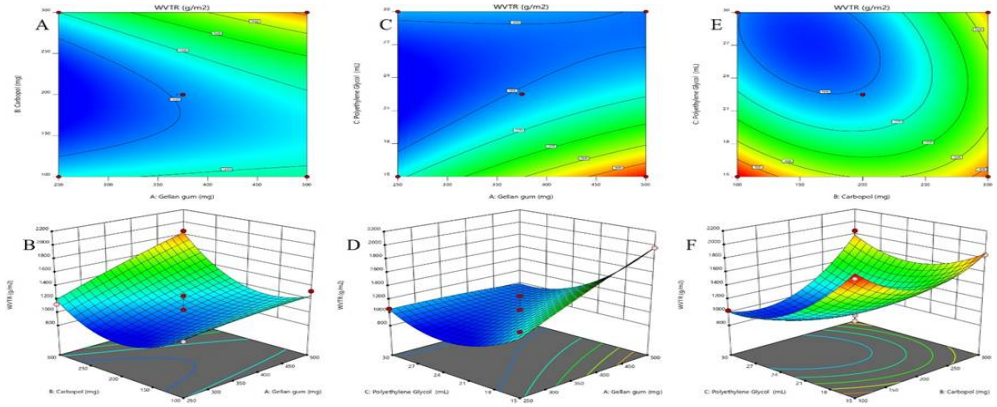

Figure 5: 2D contour graph, 3D response surface plot of independent variables on water vapor transmission rate (Y4).

Selection of optimized formulation

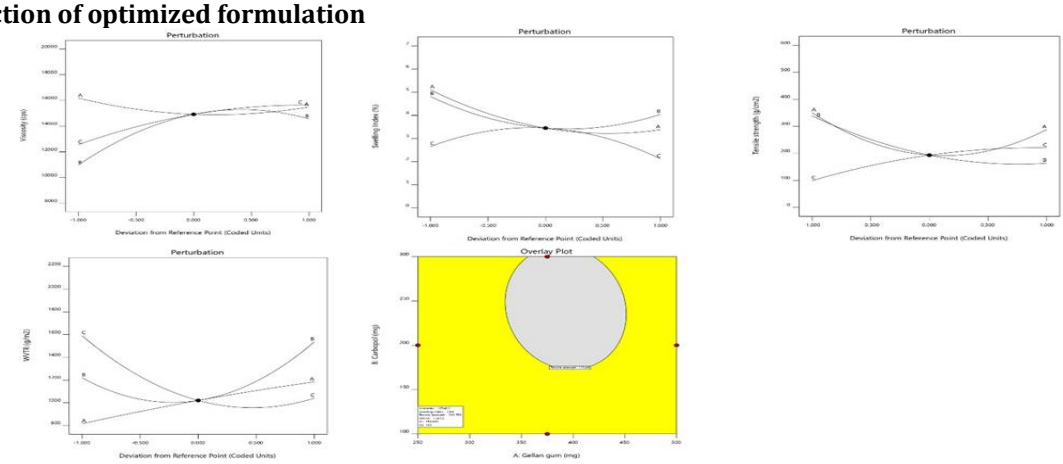

Figure 6: Perturbation plots of optimized formulation

For more research, the software recommended the formulation containing 250 mg gellan gum, 100 mg Carbopol, and 22.5 mg PEG 400. Gel viscosity was 8964.75± 169 Pa.s, swelling index was 6.92± 0.02%, water vapor transmission rate was 1124.61± 1.32 g/m2/day, and tensile strength was 523.41 ±1.057 g/cm^2.

Physicochemical Properties of Gels

F2 and F9 were found to have a stickiness, due to the increased amounts of gellan gum and carbopol. F1, F3, F6, and F8 with a low total amount of gellan gum and/or carbopol were found to have a grainy texture. It was discovered that two gels (F5 and F7) were clean and sticky-free. F1-F8 have pH values (between 5.5 and 7.2) that are optimal for transdermal administration and are unlikely to induce skin irritations. In F1-F17, the assay results in gels were between 95% - 105%.

Solid state Characterization

Fourier Transform Infra-Red Spectroscopy (FTIR)

Absorption bands at 1028, 854 cm^{-1}, 1630 cm^{-1}, and 1727 cm^{-1} were seen in FTIR spectra of NPX. NPX's C-O-C bond-parallel vibration modes were detected at 1028.96 and 854.78 cm^{-1}. The 1733.52 cm^{-1} band, meanwhile, was on a plane that coincided with the carbonyl stretching area.

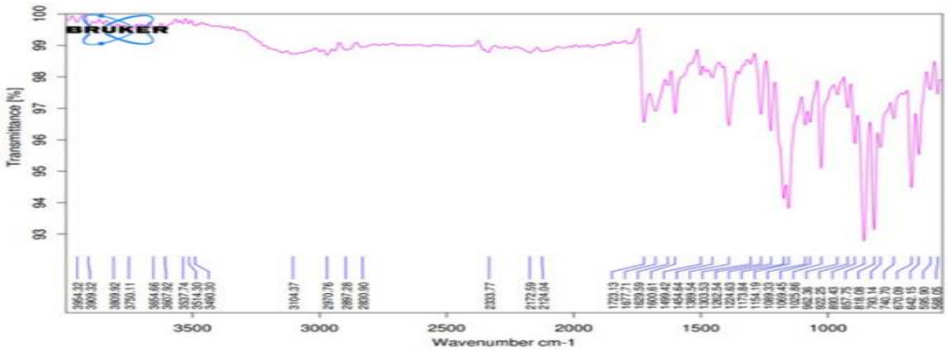

Figure 7: FTIR spectrum of Naproxen Sodium

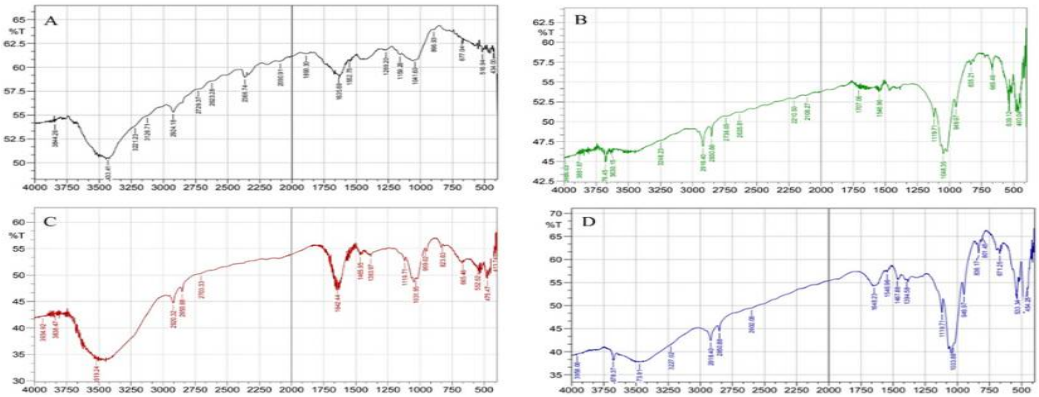

Figure 8: FTIR spectrum of A) Gellan Gum, B) Carbopol 940, C) Physical Mixture and D) optimized Hydrogel formulation

Pure gellan gum's FTIR spectrum reveals bands at 1627.81 and 1409.87 cm $^{-1}$, which originate from the asymmetric and symmetric stretching of the carboxylate group, respectively. In the instance of Carbopol-940, the O-H and intramolecular hydrogen bonding was indicated by the OH stretching vibration in the FTIR spectra with a peak between 3000 and 2950 cm^{-1}. The carbonyl C=O stretching vibration, i.e. C=O, was identified between 1750 and 1700 cm^{-1}. C-O-H peak at 1450-1400 cm^{-1}. The results showed that there were no chemical reactions, and the formulations held up well.

Differential scanning calorimetry

The endotherm of melting Naproxen sodium at a heating rate of 2^0C min^{-1}. The graph displays melting temperatures of 150.37 °C, onset, peak, and endset temperatures of 164.61°C and 228.16 °C, respectively, and a specific heat capacity of -47.37 J/gm for the sample indicating its crystalline nature. This peak was also visible in the thermogram of the physical combination. In the thermogram of the drug's physical combination, its characteristic peak stands out. The absence of this peak in the thermogram of the lyophilized mucoadhesive in situ gelling system is suggestive of successful preservation of the drug-loaded bilosomal structure throughout the gelling process.

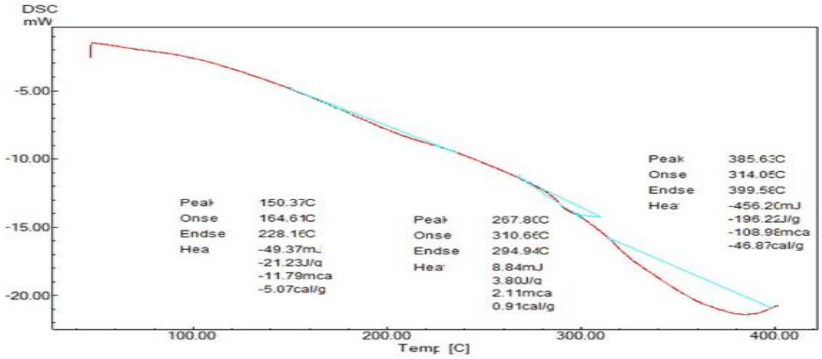

Figure 9: DSC thermogram of Naproxen sodium

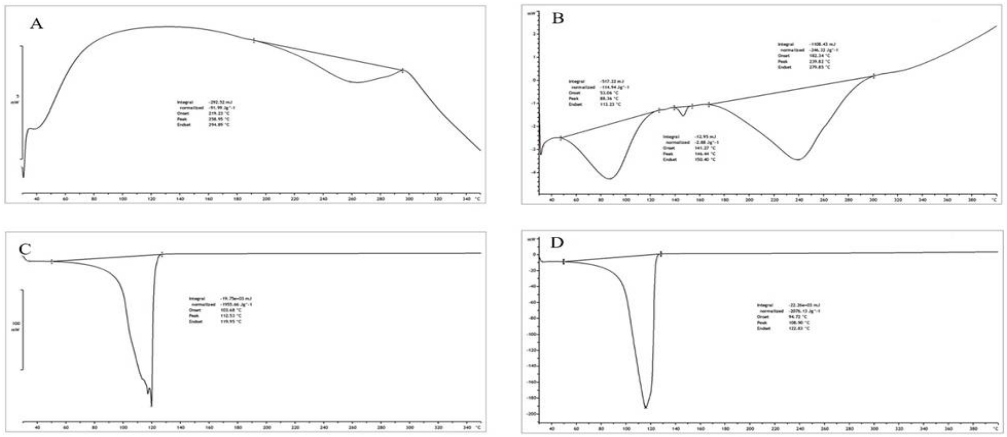

Figure 10: DSC thermogram of A) Gellan Gum, B) Carbopol 940, C) Physical Mixture and D) optimized Hydrogel formulation

Powder X-ray diffraction (P-XRD) analysis

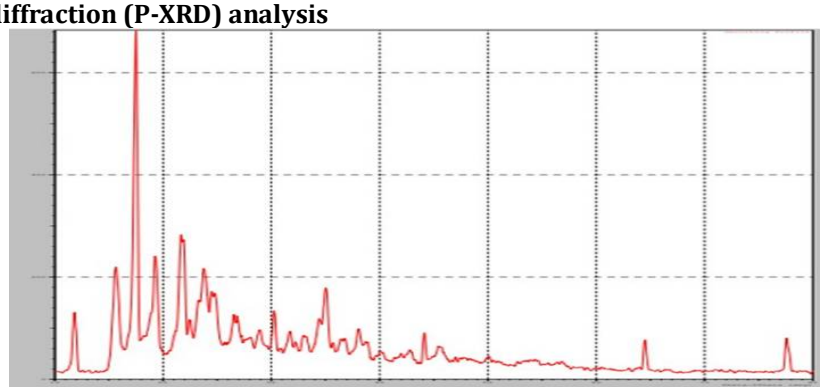

Figure 11: Powder X-ray diffractogram of Naproxen sodium

The two peaked at a similar 21 degrees. The drug's crystalline nature is shown by the sharp peak in the XRD spectra. Sodium Naproxen powder was analyzed for its structure by XRD analysis. It is easy to see from this diagram that there are 10 prominent peaks between 10 and 30.

Scanning electron microscopy (SEM)

The gellan gum was very porous and had a well-organized three-dimensional network. Figure 12 shows that after 24 hours of in vitro dissolving investigation, hydrogel sheets had a more porous structure than the intact sample that had not been subjected to the test.

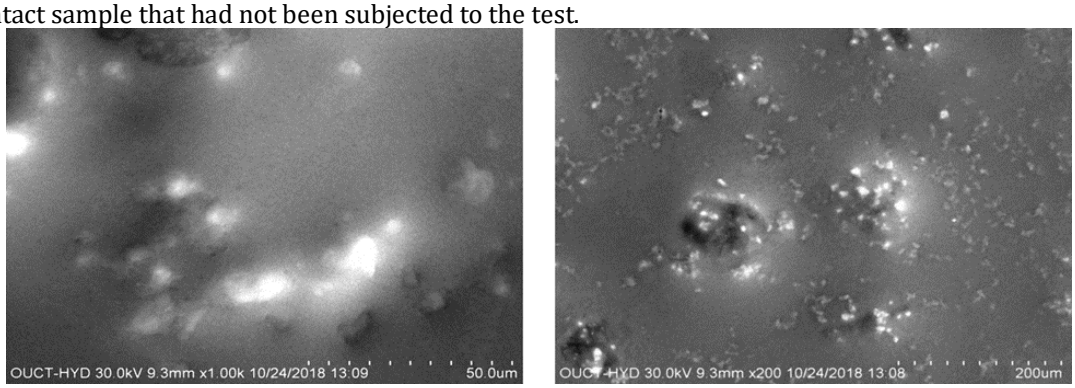

Figure 12: SEM images of Optimized hydrogel with different magnifications

Drug Release

The drug release rate from the produced gels (F9) was more than 90% after 12 hours. The release of naproxen sodium from the produced gels is biphasic, with the largest quantity being released during the first hour. When compared to the pure drug solution that was prepared with a higher concentration of gellan gum, the drug release from gels (F9) containing a lower concentration of gellan gum was relatively rapid (~200% in 1 h).

Ex Vivo Permeation

Naproxen sodium's ability to penetrate full-thickness rat skin was studied. Figure 13 shows that after one hour, the receiver fluid has absorbed a particular concentration of nebivolol (F9; 23.58 µg/cm2 and pure drug solution; 10.48µ g/cm2).

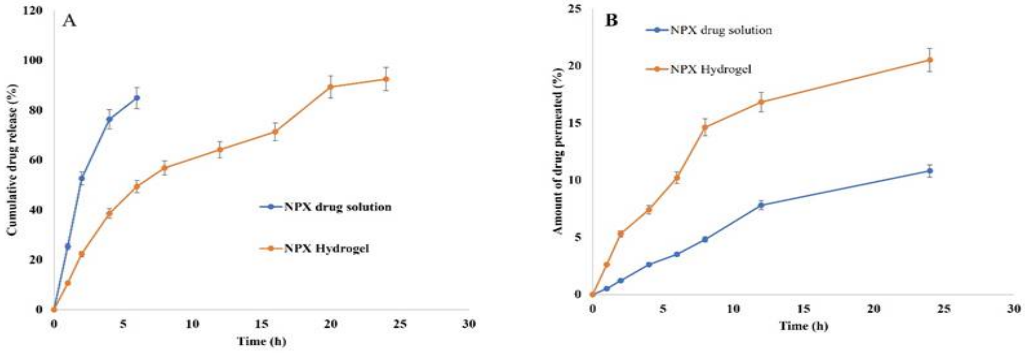

Figure 13: Comparison between the optimized hydrogel and pure drug solution of Invitro and ex-vivo drug release studies

Stability

Stability data demonstrates that there are no discernible changes to F9's outward appearance, pH, viscosity, or drug content over the course of three months of storage.

CONCLUSION

3^3-factor experimental design was used to study the effects of gellan gum (X1), Carbopol (X2), and PEG 400 (X3) on viscosity, in vitro release (12 h), and ex vivo permeation (12 h) serving as dependent variables. Gellan gum was chosen as the gelling agent, carbopol as the viscosity modifier, PEG 400 as the co-solvent. That formulation's rheological characteristics displayed viscoelastic behavior and excellent storage stability. The SEM analysis verified the well-organized three-dimensional network structure of the gellan gum/PEG hydrogel, which included a sizable number of pores. Maximum drug release was seen with the F9 formulation, with an in vitro drug release in 24 hours from a formulation including carbopol and gellan gum.

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