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Original Article

Design Hybrid Nanogel of Prednisolone for Topical Application, Preparation, Characterization, In-vitro and Ex-vivo Evaluation

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Abstract

The prednisolone was very slightly soluble in water. It was a curative agent against oral recurrent aphthous stomatitis. The main objective of this study is to design, prepare, and evaluate a hybrid nanogel of prednisolone as a topical dosage form to increase prednisolone solubility, stability, and therapeutic activity. The microwave-based method prepared nine prednisolone lipid polymer hybrid nanocarriers LPHNs formulations (H1-H9). The conventional prednisolone gel (G) was prepared by solvent diffusion. The H1-H9 was evaluated thermodynamically and entered into characterization processes. The hybrid nanogels HN1-HN9 formulations were tested for various evaluations. All the H1-H9 formulations showed high thermodynamic stability and nanosized globules, low polydispersity index, acceptable surface charge, entrapment efficiency, and drug loading. The evaluation processes indicate stable organoleptic properties, high homogeneity, fair pH and spreadability coefficient values with plastic viscosity and no erythemic reaction. The profile of prednisolone release and permeability coefficient (cm/min) was significantly higher (p-value <0.05) for HN3 and significantly lower (p-value < 0.05) for conventional prednisolone gel (G). The optimized HN1-HN9 formulations were promised drug delivery systems for treating recurrent aphthous stomatitis and a wide variety of oral lesions in addition to local and transdermal delivery of various therapeutic agents and cosmetics.

Keywords: Recurrent aphthous stomatitis; Microwave-based method; Prednisolone; Chitosan; Cardamom oil; Topical pharmaceutical dosage form.

1. Introduction

With the wide spread of diseases and epidemics in our time, it has become imperative for researchers and industrial pharmacologists to find the necessary drug designs to limit the spread of many diseases that may affect the quality of human life and everyday life. The oral lesion is one of the important diseases related to human comfort and permanence of productivity. Recurrent aphthous stomatitis (canker) is the most famous among differential diagnoses of oral lesions. It is chronic mucosal layer inflammation of the mouth. The patient shows a painful burn sensation in the affected

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part of the mouth that can develop into an ulcer within 2 to 48 hours [1].

The oral aphthae etiology may include local trauma, smoking cessation, genetic factors, food allergens, endocrine alterations such as the menstrual cycle), specific microbial agents and chemical products, stress, and anxiety [2]. Several approaches to canker treatment include pain alleviation, accelerated healing of ulcers, and inhibition of aggravation [3]. These can be achieved by inhibition of inflammatory processes. Topical corticosteroids greatly limited the inflammatory responses associated with canker development. The role of glucocorticoids in oral lesion control was vital because they acted as potent anti-inflammatory actions, analgesic, and immunomodulators, including the decrease in the number and function of different immune cells, such as eosinophils, T and B lymphocytes, monocytes, neutrophils and decrease the production of chemokines, cytokines and eicosanoids and promote the inhibitory factor production of macrophage migration at sites of inflammation which can control canker and contribute to the speedy recovery process [4, 5].

Prednisolone is a potent synthetic glucocorticosteroid highly employed for its anti-inflammatory and immunosuppressive attributes. It was belonging to class II according to the biopharmaceutical classification system. It is a hydrophobic therapeutic agent and is slightly soluble in water. The solubility factor of drugs is an important pillar in the pharmaceutical manufacturing process [6, 7].

The topical pharmaceutical dosage forms such as nanogels represent the first choice for canker treatment because they are effective, safe, and provide high local drug concentration. Nanogels are efficient nanocarriers that have three-dimensional cross-linked network chassis. It has suitable bioadhesive and biocompatible attributes. The main obstacles of nanogels are hydrophilicity and poor control of drug release that restrict the delivery of hydrophobic therapeutic agents [8-10]. A more recent delivery system, hybrid nanogels (HNs), has emerged to overcome this demerit. The HNs is a drug delivery system that protects the encapsulated hydrophilic and hydrophobic drugs. The main constituents of HNs are lipid-based nanoparticles, polymer, and gel bases that give combined features of polymeric nanocarriers and lipid-based nanocarriers [11-13]. The lipid enhances solubility and increases entrapment efficiency of hydrophobic drugs, whereas the polymer provides great control to therapeutic agent release, compared to lipid base nanocarriers and polymeric nanoparticles alone [14]. Hybridizing polymeric and lipid-based nanoparticles provides a drug delivery system that gives nanoscale particle size, furiousness, higher drug payload, sustained drug delivery, and high stability during formulation storage [15, 16]. The present research aims to prepare and characterize prednisolone hybrid nanogel (HN) to increase the solubility and permeability of prednisolone and sustain drug release that leads to increased local bioavailability and effectiveness.

2. Materials and Methods

2.1. Materials

Prednisolone, chitosan, and triethanolamine were purchased from Beijing Yibai Biotechnology Co., Ltd. (China). Polyacrylic acid (PAA)-940,

polyethylene glycol (PEG)-laurate, and lauric acid were purchased from Nanjing Duly Biotech Co., Ltd. (China). Hemani International KEPZ (Karachi, Pakistan) purchased the coconut and cardamom oil. The ethanol, methanol, potassium chloride, disodium hydrogen phosphate, sodium hydroxide, and potassium dihydrogen phosphate were purchased from Grin Land Chemical Company (United Kingdom). All solvents and reagents used in the experiments were of analytical grade.

2.2. Methods

2.2.1. Preparation of prednisolone LPHN formulations (H1-H9)

The microwave-based method prepared nine prednisolone LPHN formulations (H1-H9). The first step was preparing a hydrophobic blend by dissolving prednisolone, chitosan polymer, and lauric acid in cardamom oil and coconut oil using a magnetic stirrer device at 1000 rpm for 5 minutes. The second step was a hydrophilic blend that contained PEG-laurate, and double distilled water was prepared under a magnetic stirrer at 1000 rpm for 5 minutes. The third step involves mixing hydrophobic and hydrophilic blends according to the optimized concentrations described in **Table 1**. The mixture was inserted in a microwave instrument, Denka YMO-G30LR-30L model, for less than 10 seconds, then subjected to a magnetic stirring of 1000 rpm to form a colloidal dispersion system of prednisolone LPHN [13,15].

2.2.2. Preparation of prednisolone hybrid nanogel formulations (HN1-HN9)

It was formulated by PAA-940 dissolving in double distilled water with continuous stirring using a magnetic stirrer. A few drops of triethanolamine were added until a pH of about (6.2-7.4) was obtained. Prednisolone LPHNs were mixed with newly prepared gel in a 1:1 ratio using an electric homogenizer to get a homogeneous prednisolone hybrid nanogel (HN).

Table 1: The selected prednisolone hybrid nanogel formulations (HN1-HN9) for characterization and optimization.

Formulation code	Prednisolone %(w/w)	Cardamom %(w/w)	Coconut oil %(w/w)	Lauric acid %(w/w)	Chitosan %(w/w)	PAA-940 %(w/w)	PEG-laurate %(w/w)	Distilled water % (w/w) Up to
HN1	1	3	1	1	0.5	0.2	30	100
HN2	1	3	1	1	0.5	0.2	34	100
HN3	1	3	1	1	0.5	0.2	38	100
HN4	1	3	1	1	0.5	0.4	36	100
HN5	1	4.5	1.5	1.5	0.5	0.4	36	100
HN6	1	6	2	2	0.5	0.4	36	100
HN7	1	4.5	1.5	1.5	0.2	0.2	38	100
HN8	1	4.5	1.5	1.5	0.4	0.4	38	100
HN9	1	4.5	1.5	1.5	0.6	0.6	38	100
G	1	--	--	--	--	0.4	--	100

The prednisolone hybrid nanogel formulations (HN1-HN9) were stored in a tightly closed container at 25 ^{O}C temperatures for evaluation [17- 19]. The conventional gel of prednisolone (G) was prepared by dissolving 1 gram of the drug in 5ml of ethanol and then incorporating to PAA-940 base gel to create prednisolone gel that was kept in the container open for 24 hours to get gel ready for laboratory work [18].

2.3. Thermodynamic stability tests of prednisolone LPHNs formulations (H1-H9)

The thermodynamic stability experiments [15] were achieved to evaluate physical stability as follows:

2.3.1. Centrifugation test

It was achieved through VS-18000 M, Vision Scientific Co. LTD-Korea at 5000 rounds per minute (rpm) for about 30 minutes, and the physical appearance of prednisolone LPHN formulations (H1-H9) was checked through a centrifugation process.

2.3.2. Heating-cooling test

For about 48 hours, prednisolone LPHN formulations (H1-H9) were stored at 45^{o}C and 0^{o}C temperatures using a refrigerator for each temperature.

2.3.3. Freezing–thawing test

Under two temperatures of -21^{o}C and 21^{o}C, prednisolone LPHN formulations (H1-H9) were tested for physical, not less than 24 hours for each temperature. The prednisolone LPHN formulations (H1-H9) with maximum physical stability were selected for investigation.

2.4. Characterization of the prednisolone LPHNs (H1-H9)

2.4.1. Particle size determination

Five milliliters of the prednisolone LPHNs (H1-H9) were sonicated at 37^{o}C for 30 min and measured using the Horiba instrument, Ltd. Kyoto, Japan analyzer. Photon correlation spectroscopy (PCS) was an experimental technique used to determine the particle size of prednisolone LPHNs (H1-H9). The experiment was performed in three trials [14-16].

2.4.2. Polydispersity index (PDI) determination

PDI of the prepared prednisolone LPHNs (H1-H9) was achieved to determine the distribution pattern of nanoparticles within the colloidal system. Samples of five milliliters were sonicated at 37^{o}C for 30 min and measured using Horiba Instrument, Ltd. Kyoto, Japan. The PCS technique had been used in the experiment. The higher PDI value indicates the lower uniformity of particle size. The measurement was performed in three trials [14-16].

2.4.3. Measurement of zeta potential (ZP)

The PCS technique was used to measure ZP. The ZP is an index that gives us information about the surface charge of nanoparticles. Samples of five milliliters were sonicated at 37^{o}C for 30 min and measured using Horiba Instrument, Ltd. Kyoto, Japan. The experiment was achieved in three trials [14-16].

2.4.4. Entrapment efficiency (EE) and drug loading (DL)

The EE expressed in percentage (%) is a parameter to reveal the therapeutic agents'

encapsulation process. The indirect method was achieved to determine EE by calculating the free prednisolone drug in the super layer after applying the centrifugation process. Then, apply the following equation 1:

EE (%) = [(Total prednisolone amount – Free prednisolone amount / Total prednisolone amount)] ×100 Equation 1

The drug loading (DL) parameter expressed in percentage (%) is the prednisolone quantity found in the nanoparticles divided by the total quantity of lipids employed. It is determined by equation 2:

DL (%) = [(Total prednisolone amount − Free prednisolone amount / Total lipid amount)] × 100 Equation 2

The experiments were performed in triplicate for EE and DL [15].

2.5. Evaluation of prednisolone hybrid nanogel formulations (HN1-HN9)

2.5.1. Organoleptic Test

It is performed by naked eye observations of the odor's shape, color, and smell that can take place in prednisolone hybrid nanogel formulations (HN1-HN9) at 0, 7, 14,21and 28 days. The tests occurred in triplicate [20, 21].

2.5.2. Homogeneity measurement

A homogeneity test is performed by application of 0.5g of prednisolone hybrid nanogel formulations (HN1-HN9) to transparent material such as glass pieces.

2.5.3. Measurement of pH

The pH test is achieved by employing a digital pH meter of Biobase Meihua Trading Co., Ltd., China. The samples were taken from prednisolone hybrid nanogel formulations (HN1-HN9) of 10 g. The skin criteria pH is in the range of 4.5 - 6.5. The measurements were done in triplicate [20, 21].

2.5.4. Spreadability studies

The spreadability test is one of the important measures for prednisolone hybrid nanogel formulations (HN1-HN9). Two separated glass slides with dimensions (7.5×2.5 cm) performed it. The first lower slide that contains 0.5 g of (HN1-HN9) was tied with a wooden base. When the thread and 100 g weight tied to the second glass slide were applied to the first slide, the pulling process to the distance of 7.0 cm was achieved. The time in seconds and weight in grams that required moving the second glass slide was recorded. The spreadability process can be calculated from the following equation 3:

S=M×L / T Equation 3

S = Spreadability, M = Weight that tide to upper glass slide, L = Length of glass slide T = T is the time to separate two glass sides. The study was achieved in three trials [21].

2.5.5. Viscosity measurement

The viscosity of prednisolone hybrid nanogel formulations (HN1-HN9) was tested using a rotational digital rheometer with a spindle number (2) from Biobase Meihua Trading Co., Ltd. at 25°C. The samples of the hybrid nanogel were exposed to different rotating speeds in RPM, which are (0.1, 0.3, 0.6, 1.5, 3, 6, 12, 30, and 60). The experiments were achieved in three trials [15, 21].

2.5.6. In-vitro diffusion studies

The in-vitro diffusion experiments were achieved using a Franz diffusion cell. The samples of prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G) were applied onto the dialysis membrane surface inserted between donor and receptor chambers. The phosphate buffer solution pH 7.4 was 200 mL, filled the receptor compartment, and treadled constantly using magnetic beads at 25°C. Samples of 0.1 gram from the receptor compartment were collected at predetermined time intervals (0, 0.25, 0.3, 1, 2, 4, 6, 8, and 12 h) with the replacement of the same volume of fresh diffuse solution to conserve constant volume. The samples were analyzed using an ultraviolet-visible spectrophotometer (Biobase Meihua Trading Co., Ltd) at 243nm [21-23].

2.5.7. Skin irritation test

Before the skin irritation test, approval was obtained from the Institutional Ethics Committee in Ali Obais Hospital (approval number 239-12.1.2023). The fasted male sheep weighing about 16 kg were used to test skin irritation. The male sheep were kept on standard feed and had free access to food and water. Hair was removed from the skin surface of sheep and divided into three places of an area of 4 cm^2 (2cm x 2cm) for application of 500 mg samples of prednisolone hybrid nanogel (HN1-HN9), hybrid nanogel (without drug) and conventional prednisolone gel (G), where the application process twice daily. The testing area was observed for any erythemic or edema reaction after 1, 24, 48, and 72 h of sample application. The sensitivity was graded as 0, 1, 2, and 3 for no reaction, slight patchy erythema, patchy erythema, and severe erythema with or without edema, respectively [21].

2.5.8. Ex-vivo skin permeation study

An ex vivo study was achieved on fasted male sheep weighing about 16 kg. The animal was slew and anatomized according to the ethics committee's approval. The hairless abdominal skin was surgically isolated, and carefully removed subcutaneous fat by cold normal saline solution. The abdominal skin was cut into segments of 4 cm^2 (2cm x 2cm) to be a biological membrane inserted between the donor and receptor compartment of a Franz-type diffusion cell containing 200 mL of phosphate-buffered saline pH 7.4 in the receptor chamber. Samples of prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G) were 0.1 grams and placed in the donor chamber of the Franz cell. Samples from the receptor chamber (200 mL) were withdrawn at periodic intervals and analyzed for the quantity of prednisolone permeated using an ultraviolet-visible spectrophotometer (Biobase Meihua Trading Co., Ltd) at 243 nm. The experiments were achieved in triplicate. The permeability coefficients were determined using Equation 4.

$$M = Peff\ S\ Cd\ tres \qquad \text{Equation 4}$$

Where:

M = quantity of therapeutic agent absorbed

Peff = effective membrane permeability (cm/min)

Cd = apparent luminal drug concentration (initial concentration. or C donor)

tres = residence time of drug in GI lumen.

S = surface area available for absorption [14,15].

2.6. Statistical analysis

The experimental data was obtained as the mean of three samples with the application of standard deviation. Statistical analysis was achieved by the Microsoft Excel program 2010. The statistical test was an analysis of variance (ANOVA), where the level (P>0.05) was kept as not significant [14, 15].

All experiments were performed under the approval of the ethics committee of scientific research in Babil Health directorate, Ali Obais Hospital, where the approval number was 239-12.1.2023.

3. Results and Discussion

3.1. Formulation of prednisolone lipid-polymer hybrid nanocarriers LPHN (H1-H9) and prednisolone hybrid nanogel (HN1-HN9)

The prednisolone LPHN (H1-H9) were prepared successfully using the microwaves-based method according to experimental components, as shown in **Table 1**. All the formulated LPHNs (H1-H9) enter the thermodynamic stability analysis to check the physical stability. It was found that all the prednisolone LPHN formulations (H1-H9) had a stable physical constancy where there was no phase separation with constant appearance and color that indicate pharmaceutical physical stability. These prednisolone LPHNs (H1-H9) formulations were entered into the characterization processes and used to formulate prednisolone hybrid nanogel (HN1-HN9).

3.2. Characterization of the prednisolone LPHNs (H1-H9)

3.2.1. Particle size determination

The study of the average size of prednisolone LPHNs (H1-H9) formulations was determined by z-average using the DLS technique. Particle size is an important parameter affecting stability, drug release profile, efficiency, muco-adhesion, cellular uptake, and bio-distribution [15]. The outcomes of particle size analysis were H1 (101 nm); H2 (94 nm); H3 (92 nm); H4 (96 nm); H5 (110 nm); H6 (146 nm); H7 (109 nm); H8 (120 nm) and H9 (131 nm) as shown in **Table 2**. The variance analysis showed a significant correlation between particle size and the independent variables, PEG-laurate, lipid content, and chitosan at the level ($p<0.05$).

3.2.2. Polydispersity index (PDI) determination

PDI is a parameter for measuring prednisolone LPHN (H1-H9) formulations homogeneity. It is a dimensionless test with values from 0 to 1.

The smaller values show a finer particle size distribution, narrower and more homogenous, while the uniformity of the particle size in the formulation decreases as the PDI increases. PDI was from (0.317 to 0.505) as shown in **Table 2**. The result of the ANOVA indicated a significant correlation between PDI as a dependent variable and PEG-laurate, lipid content, and chitosan at level ($P < 0.05$).

3.2.3. Measurement of zeta potential (ZP)

The diffuse layer of charges in a shear plane around nanocarriers was expressed as zeta potential. It is used as a parameter related to nanoparticles' physical stability. The outcome of the mean absolute zeta potential was (18 to 43 mV) as shown in **Table 2**. There was a significant relationship ($p<0.05$) between zeta potential and independent variables.

Table 2: Summary of characterization results of prednisolone LPHNs formulations (H1-H9).

Formulation Code	Globule size (nm)*	PDI*	Zeta potential*	Entrapment efficiency % (w/w)*	Drug loading % (w/w)*
H1	101±3.214	0.409±0.002	35±1.527	87.3±1.5	19.6±2.081
H2	94±2.309	0.389±0.001	36±1.5	86.6±1.52	25.6±1.527
H3	92±3.214	0.317±0.001	43±1.52	81.6±1.527	26.6±2.081
H4	96±2.081	0.405±0.003	35±2.516	86.8±2.645	25.3±2.516
H5	110±1.527	0.455±0.003	26±1.527	88.3±1.35	18.03±1.950
H6	146±1.527	0.51±0.002	15±1.527	89.1±0.680	15.033±1.703
H7	109±3.511	0.434±0.003	32±1.527	88.2±1.365	18.66±2.081
H8	120±1.527	0.468±0.001	25±2.081	88.4±1.252	17.8±1.743
H9	131±1.527	0.505±0.002	18±1.527	88.8±1.001	17.33±2.081

3.2.4. Entrapment efficiency (EE) and drug loading (DL)

The entrapment efficiency and drug loading gave information about the ability of drug encapsulation for nanocarriers. The outcomes of EE (w/w %) were H1 (87.3%), H2 (86.67%), H3 (81.66%), H4 (86.8%); H5 (88.37%), H6 (89.13%), H7 (88.2%), H8 (88.4%) and H9 (88.86%) as shown in **Table 2**. The results of drug loading (w/w %) were H1 (19.6%); H2 (25.6%); H3 (26.67%); H4 (25.3%); H5 (18.03%); H6 (15.03%); H7 (18.6%); H8 (17.8%) and H9 (17.3%) as shown in **Table 2**. The clear values of the data confirm the ability of the prednisolone LPHN (H1-H9) formulations to accommodate therapeutic agents. The analysis of variance confirmed a significant relationship between dependent variables (entrapment efficiency and prednisolone loading) and PEG-laurate, lipid content, and chitosan at level ($p<0.05$). Therefore, the null hypothesis was rejected and accepted the alternative hypothesis.

3.3. Evaluation of prednisolone hybrid nanogel formulations (HN1-HN9)

3.3.1. Organoleptic Test

It was found that prednisolone hybrid nanogel formulations (HN1-HN9) show acceptable physical properties, as shown in **Table 3.**

Table 3: The organoleptic properties of prednisolone hybrid nanogel formulations (HN1-HN9).

Formulation code	Color	Odor	Phase separation	Homogeneity
HN1-9	Colorless	Odorless	No	Homogeneous

3.3.2. Homogeneity measurement

Homogeneity was considered one of the most important physical attributes that can be used to evaluate the prednisolone hybrid nanogel formulations (HN1-HN9), as shown in **Table 3,** which indicates the physical stability of all formulations [20, 21].

3.3.3. Measurement of pH

The outcome indicated that pH value lies in the range (3.936-4.343) as shown in **Table 4** is nearly suitable for topical application [20, 21].

Table 4: Slope and permeation coefficient for prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G) through experimental skin membrane.

Formulation code	Slope (µg/mL)	Permeability coefficient (cm/min)
HN1	0.0254	0.00000635±4.93E-09
HN2	0.0257	0.00000642±3.51E-09
HN3	0.0258	0.000006453±3.6E-09
HN4	0.0250	0.00000625±4.5E-09
HN5	0.0241	0.00000626±3.05E-07
HN6	0.0239	0.000005975±1.9E-09
HN7	0.0253	0.00000632±2.5E-09
HN8	0.0251	0.00000627±2.08E-09
HN9	0.0212	0.00000531±9.9E-09
G	0.0179	0.00000635±4.93E-09

3.3.4. Spreadability studies

The spreadability parameter of prednisolone hybrid nanogel formulations (HN1-HN9) is related mainly to the concentration of lipid content, polymeric materials, and PAA-940. It was an important factor associated with the ability of the gel to spread through affected skin and patient compliance. It depended on the rheology of HN1-HN9. The HN3 formulation was significantly higher (p-value<0.05) in spreadability and was significantly lower (p-value<0.05) in spreadability for (HN9), as shown in **Table 5**.

3.3.5. Viscosity measurement

A rotational digital rheometer with a spindle number (2) from Biobase Meihua Trading Co., Ltd. measured viscosity successfully, as shown in **Table 6**.

Table 5: Summary of evaluation results of prednisolone hybrid nanogel formulations (HN1-HN9).

Formulation code	pH*	Spreadability (g*cm/sec) *	Viscosity (1.5 RPM)* (mP.s)	Release of prednisolone**	Permeability coefficient* (cm/min)
HN1	4.343±0.045	125.366±0.251	19305±1.527	80.836±0.03	0.00000635±4.93E-09
HN2	4.256±0.025	113.652±0.02	19307±1.527	81.45±0.036	0.00000642±3.51E-09
HN3	4.153±0.025	104.162±0.035	19310±2.081	88.025±0.003	0.000006453±3.6E-09
HN4	4.046±0.045	62.54±0.026	19327±1.527	74.846±0.035	0.00000625±4.5E-09
HN5	4.246±0.041	59.528±0.005	19334±1.527	73.44±0.036	0.00000626±3.05E-07
HN6	4.15±0.04	56.828±0.01	19345±1.527	72.63±0.02	0.000005975±1.9E-09
HN7	3.936±0.04	92.582±0.009	19347±1.527	76.733±0.0208	0.00000632±2.5E-09
HN8	3.943±0.02	52.089±0.004	19347±2.081	75.064±0.004	0.00000627±2.08E-09
HN9	4.163±0.025	35.718±0.005	19364±2.645	70.195±0.004	0.00000531±9.9E-09

* at 12 hours Values are expressed as mean ± SD (n=3). ** Release of prednisolone as cumulative persent in phosphate buffer pH 7.4 + 0.3 % polysorbate 80 solutions.

Various parameters were obtained: viscosity, shear rate, and shear stress, using different rotating speeds. When the shear rate was plotted against shear stress, a rheogram chart was achieved, as shown in **Figure 1.**

All prednisolone hybrid nanogel formulations (HN1-HN9) show non-Newtonian plastic flow due to no gel flowing related to shear stress until it reaches a specific transition point. The ANOVA confirmed a significant relationship ($p<0.05$) between viscosity and PEG-laurate, lipid content, and chitosan.

Table 6: Spreadability coefficient for prednisolone hybrid nanogel formulations (HN1-HN9).

Formulation code	Time (sec)	Spreadability coefficient (g * cm/sec)
HN1	2	125.366±0.251
HN2	2.2	113.652±0.02
HN3	2.4	104.162±0.035
HN4	4	62.54±0.026
HN5	4.2	59.528±0.005
HN6	4.4	56.828±0.01
HN7	2.7	92.582±0.009
HN8	4.8	52.089±0.004
HN9	7	35.718±0.005

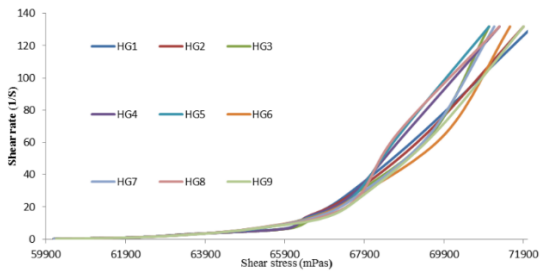

Figure 1. Graph representing proximate analysis of marve seed with the chia seed.

3.3.6. In-vitro diffusion studies

The release of prednisolone forms the prednisolone hybrid nanogel formulations (HN1-HN9), and conventional prednisolone gel (G) was studied by the Franz diffusion cell method using a dialysis bag as a diffusion membrane.

The diffusion media was phosphate buffer pH 7.4+0.3% polysorbate 80 solutions. According to the experimental data, there is no bust diffusion from all the prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G). There was a sustained release process over 24 hours from all formulations. The profile of prednisolone release was significantly higher (p-value < 0.05) in the dissolution rate for HN3 and was significantly lower (p-value < 0.05) in the dissolution rate of (G), as shown in **Figure 2.**

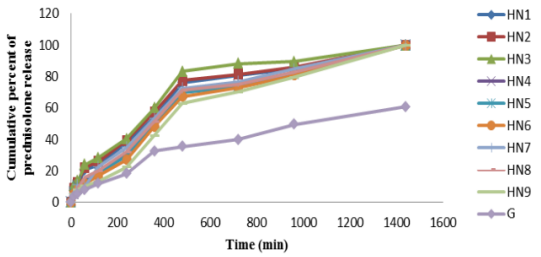

Figure 2. In vitro drug release profile from prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G) at phosphate buffer pH 7.4 + 0.3 % polysorbate 80 solution.

The comparability profile of the prednisolone release from the prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G) was in the following descending order: HN3 > HN2> HN1 > HN7 > HN8 > HN4 > HN5 > HN6 > HN9 > G. The ANOVA has confirmed a significant relationship ($p<0.05$) between diffusion of prednisolone and independent variables.

3.3.7. Skin irritation test

The skin irritation experiment was achieved successfully. There was no erythemic reaction at the site of sheep skin application. It ascertains the safety of optimized HN1-HN9 formulations on biological membranes [21].

3.3.8. Ex-vivo skin permeation study

The permeability coefficient (cm/min) was calculated after obtaining prednisolone flux (µg/mL) from the data of an experiment. The experimental results of the ex-vivo skin permeation parameter indicated that the permeability coefficient (cm/min) of prednisolone was significantly higher (p-value <0.05) for HN3 and was significantly lower (p-value < 0.05) for conventional prednisolone gel (G) as shown in **Table 5**. The comparability profile of the prednisolone release from the prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G) was in the following descending order: HN3>HN2>HN1>HN7>HN8>HN4>HN5>HN6>HN9>G as shown in F**igure 3**.

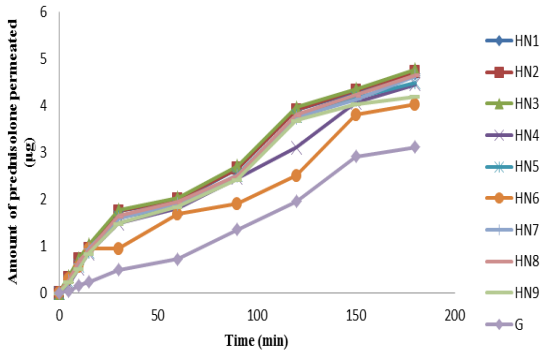

Figure 3. Permeation of bioactive agent from prednisolone hybrid nanogel formulations (HN1-HN9) and conventional prednisolone gel (G) through the experimental skin membrane.

The conventional prednisolone gel (G) gave a lower permeation coefficient than all prednisolone hybrid nanogel formulations (HN1-HN9) because HN1-HN9 contained nanoparticles loaded with prednisolone. The ANOVA explained a significant (p-value <0.05) relationship between ex-vivo intestinal permeation parameters and independent factors. A summary of evaluation results of prednisolone hybrid nanogel formulations (HN1-HN9) is shown in **Table 6**.

The physical hybridization process between polymeric nanocarriers and lipid-based nanocarriers gave a drug delivery system that was rigid in composition but very flexible regarding prednisolone release. The outcomes of characterization indicate that all globules of prednisolone LPHN (H1-H9) formulations had been distributed in nanometer size, indicating a colloidal disperse system. The PDI indicated that prednisolone LPHN (H1-H9) formulations are homogenous. According to the thumb rule, the zeta potential absolute values are: 5 mV show fast aggregation, about 20 mV supply only short-term stability, above 30 mV offer good stability, and 60 mV excellent stability. Theoretically, the prednisolone LPHN (H1-H9) formulations should have a high surface charge to avoid aggregation during the collision in the colloidal solutions. This rule applies to nanoparticulated systems that depend on DLVO forces to evaluate the physical stability. Prednisolone LPHN (H1-H9) formulations depend on non-DLVO forces, which are steric forces and hydration forces, to achieve pharmaceutical physical stability; therefore, despite the low value of zeta potential, it was shown that non-DLVO forces achieved high stability due to the stabilization process. Also, the thumb rule applies to the electric stabilization of

small molecular weight particles but not to large molecular weight particles such as PEG-laurate, which are nonionic stabilizers that present in prednisolone LPHN (H1-H9) formulations [15, 23].

In entrapment efficiency, it was found that an increase in the quantity of lipid contents, which are cardamom oil, coconut oil, and lauric acid (3:1:1), leads to an increase in entrapment efficiency and a decrease in felodipine loading at a constant concentration of surface-active agents: co-surfactant blend. It is due to an increment in the lipid content area available for prednisolone encapsulation [15]. In evaluating prednisolone hybrid nanogel formulations (HN1-HN9), the organoleptic properties reflect the colloidal structure of the nanosystem and indicate high physical stability [20, 21]. The outcome of pH indicated that the pH value was suitable for topical application [20, 21]. The spreadability parameter decreases as the viscosity of formulations increases [21]. The plastic flow of HN1-HN9 provided easy wiping on the ulcerated and infected skin or membranes. It was found that the concentration of lipid increases leads to increased viscosity due to increased volume concentration of nanoparticles that make HN1-HN9 more flow resistant. Also, as gel base polymer increases, it leads to increased viscosity due to decreased distilled water content and increased carbomer-940 concentration that intertwines with chains of PEG to provide great flow resistance [15, 21]. It was observed that the conventional prednisolone gel (G) gave a lower dissolution rate of felodipine profile in comparison to all prednisolone hybrid nanogel formulations (HN1-HN9) due to that HN1-HN9 formulations have nanocarriers which provide a large surface area in contact to the phosphate buffer pH 7.4 + 0.3 % polysorbate 80 solutions and this was permitted a higher interaction area with the diffusion medium that increases rate of dissolution [21, 23]. There was no erythemic reaction at the site of sheep skin application. Spireas S and Srinivas S (1998) found that prednisolone loaded to liquisolid compacts exhibited higher medication discharge rates in various disintegration media and volumes, contrasted with tablets arranged by the direct compression technique. Zakeri P et al. (2011) prepare solid dispersion of an ineffectively water-dissolvable medication, prednisolone, to enhance the dissolution rate. Parikh Harshil et al. (2022) state that these nanoparticles offer enormous potential for accomplishing the target of controlled and site-explicit prescription. It can reduce undesirable consequences and toxic effects of medication methods compared to polymer counterparts. The literature review provided the different techniques for enhancing prednisolone solubility. The conducted research was designed and prepared prednisolone LPHN (H1-H9) formulations that used to create hybrid nanogels (HN1-HN9) formulations to improve prednisolone solubility and hydrophobicity to facilitate the passage of therapeutic agents across the biological membrane that led to increase in therapeutic activity [14, 15].

4. Conclusion

The hybrid nanogel was designed and prepared successfully. The hybrid gel depended on LPHNs as a preparation base. All optimized HN1- HN9 formulations exhibited good physical stability due to passage through thermodynamics tests. The outcomes of

characterization processes for (H1-H9) formulation were nanosize particle, accepted PDI, good zeta potential, entrapment efficiency, and drug loading that reflect the success of nano delivery system as a structural base for hybrid nanogel. The evaluation process for (HN1-HN9) formulations shows good organoleptic properties, homogenous, optimum pH, easy spreadability, plastic program, no burst release with sustained drug release, no irritation on experimental skin membrane, and enhanced permeability coefficient. The characterization and evaluation processes emphasized that optimized HN1-HN9 formulations were promised drug delivery systems for treating canker and other oral lesions in addition to local and transdermal delivery of various therapeutic agents and cosmetics.

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None.

Conflict of interest

The authors declare to have no conflict of interest.

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