RESEARCH ARTICLE

4-Hydroxyisoleucine-Loaded Nanogel: Development from an Optimized Nanoemulsion for Antidiabetic Therapy

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ABSTRACT

This work focuses on the creation and assessment of a nanogel system produced from an optimal nanoemulsion formulation infused with 4-hydroxyisoleucine, a powerful antidiabetic bioactive agent. Diabetes mellitus affects 425 million people globally, with 82 million in Southeast Asia. The pandemic could lead to cardiovascular disorders, strokes, renal failures, and blindness. Fenugreek has anti-diabetic properties and reduced cholesterol levels in type II diabetes patients. Nano-carriers improve drug bioavailability.

Method: Nanoemulsions were created with an aqueous titration emulsification technique and tailored according to droplet size, polydispersity index (PDI), zeta potential, and entrapment efficiency. The improved nanoemulsion demonstrated advantageous physicochemical characteristics, including nanoscale droplet dimensions and steady dispersion. The optimized nanoemulsion was integrated into a biocompatible gel matrix, resulting in a nanogel with improved stability and regulated drug release properties. The synthesized nanogel was assessed for rheological characteristics, pH, Spreadability, *in vitro* drug release, and ex vivo skin penetration. The results demonstrated a prolonged release profile and enhanced penetration efficiency, indicating increased transdermal administration of 4-hydroxyisoleucine.

Conclusion: The research investigates the synthesis and characterisation of 4-hydroxy isoleucine (4HILCN), a medication of 99% purity. FTIR spectroscopy was used to identify and describe the medication, assess its purity, and conduct quantitative analysis. The medication was formulated using an optimum composition of three mixtures including oil and Smix and water. The nanoemulsion was transformed into a hydrogel for dermal application using Carbopol-934. The uniformity of the compositions remained stable after being transferred into flint-colored glass containers. The ideal pH range for cosmeceutical assessment is 5.0-6.0. Stability experiments indicated no any variation in parameters which are indicative of the instability. *In vitro* investigation of drug release kinetics shown enhanced solubility and diffusion rates. Ex-vivo permeation experiments demonstrated superior penetration of drug from 4HILCN-NG compared to Neat Formulation gel of drug and 4HILCN-NEO. The research indicates that the nanogel technology serves as a potential transdermal delivery system for Antidiabetic treatment, providing improved bioavailability and patient adherence.

Keywords: Nanoemulsion, Nano-gel, Permeability, correlation coefficient, Z-average.

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INTRODUCTION

Diabetes is a chronic disease in which the body either does not create enough insulin or is unable to utilize the insulin that it does make with the appropriate amount of efficiency.[1] In the long run, this might result in major health concerns because to the increased levels of blood sugar that are caused by this.[2] Type 2 diabetes mellitus (T2DM) is the most common kind, significantly affected by lifestyle and dietary practices. Although several oral hypoglycemic treatments exist, many are hindered by constraints like limited absorption, gastrointestinal adverse effects, and suboptimal patient adherence. Consequently, there is an increasing interest in investigating alternate drug delivery methods and bioactive molecules with significant Antidiabetic efficacy.[3]

Diabetes mellitus is a hyperglycaemic condition characterized by elevated blood glucose levels due to insulin production, blood concentration, or malfunction.[4] It has caused a global burden of illness, with $9.5X10^6$ cases till 2014, and an increase in prevalence among adults. The International Diabetes Federation reports 425 million people globally are diabetic, with 82 million in Southeast Asia. The pandemic scenario of diabetes is concerning, as it could lead to cardiovascular disorders, heart attacks, strokes, renal failures, and blindness.[5]

There is reported anti-diabetic attributes of fenugreek which has shown promise for decreasing blood glucose levels. The alcoholic extracts have shown the ability to normalize the activity of essential carbohydrate and lipid metabolism enzymes so serve as effective medicines for the management of diabetes.[6,7]

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Table 1: Composition of three NEOs formulations one NEO with 2 more compositions with +10% and -10% oil & Smix

Smix (2:1)	A: %OIL	B: %Smix	C: %Water	C/f	F/Th	H/C	Droplet Size	PDI	Zeta Potential	EE
Run	%	%	%				nm±SD	n±SD	mV±SD	%
MEO2	24.52	21.43	54.05	√	√	√	98.5±1.34	0.405±0.11	0.6328±0.82	88.34±2.43
NEO3	27.24	23.81	48.95	√	√	√	103.4±1.33	0.404±0.06	0.6333±0.64	92.34±1.62
NEO4	29.96	26.19	43.85	√	√	√	109.9±1.45	0.403±0.66	0.6335±0.92	89.13±1.88

An alternative to metformin therapy, a solution of Trigonella foenum-graecum seeds considerably reduced total cholesterol levels in 114 people with type II diabetes, according to the study. According to [8], Trigonella foenum-graecum seeds might be a new option for managing diabetes, as they increased HDL cholesterol levels in the treatment group. Problems with solubility, digestive reactions, and cellular transport and absorption restrict the use of phyto-bioactive antidiabetic medications. Herbal medicines may have their bioavailability increased with innovative systems like nano-carriers.[3,9-11] The purpose of use of Nano-gel via transdermal route in current study is to make and increase the therapeutic precision and efficiency of the drug while simultaneously limiting its adverse effects and side effects.[12,13]

MATERIALS AND METHODS

Chemicals and Excipients

4Hydroxy isoleucine (4HILCN), obtained from Sigma-Aldrich Chemicals, Bangalore, was tested at 99% purity. Sefsol-218®, Carbopol-934 & Carbopol-940 polymers was provided as complimentary samples. Tween 80, Transcutol, Triethanolamine (TEA) from R. V. N. I. The remaining reagents used in the investigation were of Analytical Reagent Grade.[14]

Prior to Formation Screening

Identification & Marking of Drug

The initial step involved the screening of the drug based on its physicochemical properties. The color, odor, and taste were documented and analyzed in comparison to existing data. UV-spectrophotometry was conducted as The pharmaceutical solution, prepared in a buffer of 0.01% w/v, was subjected to UV scanning within the range of 200-400 nm using the UV-1700 Corporation instrument from Kyoto, Japan, of Brand Shimadzu and the results were duly documented. The Fourier transforms infrared (FTIR)

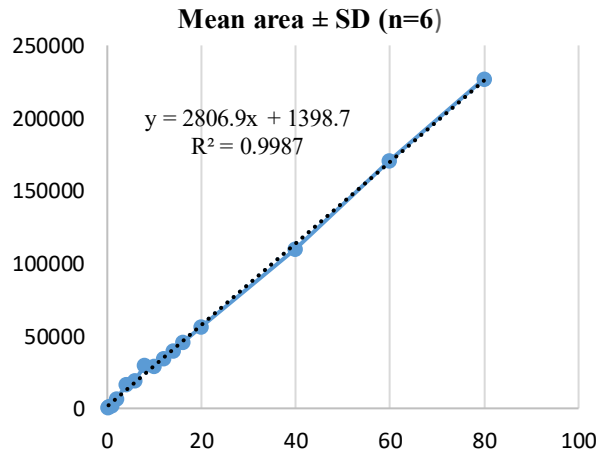

Figure 1: Calibration curve of 4HILCN

Table 2: ANOVA test results

ANOVA summary	Droplet Size	PDI	Zeta Potential
F	2.008	2.079	0.09972
P value	0.1687	0.1597	0.9057
P value summary	ns	ns	ns
Significant diff. among means (P < 0.05)?	No	No	No
R squared	0.2112	0.2170	0.01312

analysis involved pressing a mixture of KBr and 4HILCN in a ratio of described to create pellets. The spectrum results were documented. (Shimadzu, Japan).[15]

HPLC of RP used for Calibration Curve of Drug (4HILCN)

Equipment: A Model BrandShimadzu of RPHPLC was utilized. It featured Q.LC10AVP pumps. A UV/VIS. featuring variable wavelength Detector, SPD.10AVP.Column oven of Brand.Shimadzu, and a system controller of Brand.Shimadzu SCL.10AVP, along with an injector equipped with a loop characterised as Rheodyne, was utilized. The set included Class.VP 5.032 Program.

Initially, various dil. of 4-hydroxyisoleucine were prepared in DM water. Subsequently, 2 µL was subjected to derivatization with OPA (Ortho-Phthalaldehyde) from each dil, and the resulting solution was applied in RPHPLC and (Time of R = 58 min, FR = 0.9 ml/min, λ = 330nm- 440 nm). The mobile phase, transitioning from solvent A to solvent B (methanol: tetrahydrofuran; 97.5:2.5). 2 µl of the sample solution (5 mg/ml) was subjected to derivatization, after the injection of SS, adhering to the same procedure used for the standard solutions.[16]

Development of Nanoemulsion

Preparation of Drug Loaded Nano Emulsion from Optimised Formulation

The optimised formulation and 2 more compositions with +10% and -10% oil & Smix were taken from previous study of same project. Drug was dissolved in oil phase (sefsol218) in appropriate quantity. Then Smix was added to the oily phase and thoroughly mixed under continuous stirring for 20 min. now required quantity of DM water was added and used the vertex to get homogenous and transparent nanoemulsion.[17]

Drug–Excipient Compatibility Evaluation

Analysis was conducted using an FTIR spectrophotometer to examine the interactions among the various components of the enhanced 4HILCN-NEO formulation. FTIR profiling was conducted on the formulation excipients, Blank NE, and the optimized 4HILCN-NEO formulation. The analytics were positioned in the spectrophotometer was used in the ATR (Attenuated Total Reflectance) mode. The range of 3500-600 cm^{-1} was used to detect and assess the spectral area.

Entrapment Efficiency (EE)

The entrapment efficiency percentage of the formulations was

Table 3: Nanogel formulations analysis for its marking

Nanogel	Evaluation Parameter				
	pH	Texture of NG	Spreadability D (cm)	Viscosity (cP) 26 ± 0.5 ℃	Drug Content (%)
NG1 (1%)	6.3	Good	3.2±0.65	207.4±12.5	86.60±0.65
NG2 (2%)	6.9	Good	2.7±0.45	460.2±10.6	87.00±0.65
NG3 (3%)	6.6	Good	2.3±0.76	904.5±12.6	90.47±0.65

Table 4: 4HILCN-NG investigated for stability as

Parameters	Optimised Nanogel formulation (CBT-NG) containing 2% w/v of Drug														
Storage condition	25 °C ± 2 °C/60% RH ± 5% RH					32 °C ± 2 °C/60% RH ± 5% RH					40 °C ± 2 °C/75% RH ± 5% RH				
Months	0	1	2	3	6	0	1	2	3	6	0	1	2	3	6
pH	5.45	5.43	5.41	5.37	5.33	5.43	5.42	5.41	5.38	5.36	5.46	5.43	5.41	5.38	5.3
Viscosity (cP)	384	384	381	377	375	384	382	379	376	371	384	381	376	371	361
Net Content	99	98	98	98	97	99	99	98	98	97	99	98	97	95	95
Homogeneity	Smooth					Smooth					Smooth				
Colour	No change in colour					No change in colour					No change in colour				
Odour	No change in odour					No change in odour					No change in odour				
Microbial load (Bacteria & Fungi)	No microbial growth was observed at 24, 48 and 72 h					No microbial growth was observed at 24, 48 and 72 h					No microbial growth was observed at 24, 48 and 72 h				
Sterility test	No microbial growth was observed at 24, 48 and 72 h					No microbial growth was observed at 24, 48 and 72 h					No microbial growth was observed at 24, 48 and 72 h				

determined using a previously described indirect approach with some changes. Nanoemulsion formulations were subjected to ultracentrifugation at 12000 rpm for 1 hour, after which the upper liquid was collected and diluted. For the same the used solution freshly prepared was phosphate-buffered saline (PBS). By using this it was determined the concentration of the free drug.[18] The samples applied again for the aforementioned procedure for three times. The (EE) percentage was computed as:

$$\% EE = \frac{Total\ Drug\ Added - Free\ Drug\ in\ DM}{Total\ Drug\ Added} X100$$

DM: Dispersion Medium of emulsion

Nanogel Formulation

Preparation of Nano Gel

The NEO was converted into a hydrogel to impart the necessary traits into to the final formulation for over the skin application. The emulsion exhibits fluidity more, so facilitating application on the skin, and its rapid removal may necessitate adjustments in the required dosage. The optimized nanoemulsion 4HILCN-NEO was converted into a nanogel utilizing specifically Carbopol polymers. These materials were utilized due to their biocompatibility, biodegradability, bioadhesive properties, non-irritating nature, and lack of absorption into the body.[19] Selection was done on the basis of required characteristics of NG.

A precisely measured quantity of polymer was dissolved in a suitable volume of DMwater to create a base of varying concentrations (1% to 3% w/v) as gel. The pH of the prepared gel was modified to 6.0 with the incorporation of TEA (Triethanolamine). The 4HILCN-NEO formulation was integrated into each gel basis at a 1:1 ratio and meticulously homogenized to produce 4HILCN-NG, which was then kept for further analysis. Plain gel with 4HILCN was also prepared in the same way coded as (4HILCN-NF).[19]

Screening of the Optimised NG

PH Profiling

The pH of 4HILCN-NG and 4HILCN-NF was calibrated to the standard skin pH near to 6.0. Subsequently, the pH was assessed in three replicates. Under specified temperature and RH conditions, utilizing a digital pH meter (WTW pH -197i, Germany) over a duration of 90 days.

Size of Globules

The frequency distribution of droplets concerning size was assessed using Photon Correlation Spectroscopy (P.C.S.). It examines the variations in light scattering anticipated as a result of the particles motion. The Zetasizer 1000 HS, is utilized for this purpose, with light scattering monitored at a 90-degree angle.

Spreadability

It was assessed to evaluate the capacity to evenly distribute following application to the skin surface. The synthesized 4HILCN-NG and 4HILCN-NF were assessed at many time intervals under varying conditions of experiment like temperature and relative humidity. For this assessment, 0.55 g of newly created gel was positioned in-between glass slides for 1.5 minute, after which the diameter of the occupied space by the gel was evaluated.[20]

Rheological Studies

The viscosity of the NG was assessed utilizing a (Brookfield DV III ultra V6.0 RV cone and plate) at a temperature of 25 °C ± 0.3 °C using Rheometer. The chosen 4HILCN-NE, NG and their respective NF were assessed for their rheological properties. The used instrument was of brand name of Anton.Paar, of company Modular Compact Rheometer-MCR 102) with a good temperature maintaining to (25 ± 0.5°C), using a 4°/40 mm cone and plate geometry with a gap of 0.100 mm to assess the rheological properties of NEs. The steady-state rheology of 4HILCN-NEs was observed after allowing the NEs to rest for about 10 minutes post-loading onto the plate, with regulated rates increasing from 0.001 to 100 and 0.0001 to 100 s^{-1}, respectively.[21,22]

Evaluation of NG

Stability Studies

Various temperatures and relative humidity levels were used to test the organoleptic properties of 4HILCN-NG and 4HILCN-NF, such as smell, color and phaseseparation:

Table 5: 4HILCN-NG, investigation of the release kinetics

Formulation	(R^2) Zero Order	(R^2) First Order	(R^2) Higuchi Model	(R^2) Pappas Model
4HILCN-NG2	0.8638	0.7654	0.9825	0.7654
4HILCN-NEO	0.8576	0.7422	0.9808	0.7422
4HILCN-NF	0.8934	0.8629	0.9919	0.8629

conditions for 90 days: 8 °C ± 1, 25 °C ± 1, 40 °C ± 1, and 40 °C at 75% RH± 1. The (ICH) criteria (2003) were followed in conducting the research. In order to determine the formulations' physical stability and shelf life, both gels were tested for organoleptic characteristics over this period.

Drug Release Study from NanoGel

The 4HILCN released over time from gel was quantified using the US dissolution apparatus. The specifications of the instrument was Pharmacopeia XXIV dissolution device II, it was from (DS 8000, Lab India, India). *In-vitro* dissolution studies are conducted on this dissolving paddle assembly to create release kinetics models from each gel groups. Packets of packed with a nanoemulsion containing a medicine dosage made up of Dialysis membrane are placed in a flask containing buffer. This experiment employs a 37±0.5° C and at speed paddle of 50 rpm. A 5 mL sample was extracted for testing at predetermined time intervals, and an equivalent volume of new dissolving medium is consistently replaced. Drug release at different time periods is determined in percentage. By RPHPLC technique at 230 nm.[23]

Drug Permeation from Nanogel

It was Ex-vivo study. An investigation was conducted using hairless abdomen skin which was positioned in the diffusion cell with the stratum corneum oriented upper donor chamber and the inner side of skin i.e. dermal side directed towards the lower receiver compartment. Area available for diffusion was about 1.8 cm². PBS - pH 7.4 was used as the medium in the recever compartment, maintained at 37 ± 0.5°C using a continuous stirring mechanism. 4HILCN-NE, 4HILCN-NG and 4HILCN-NF were separately administered in the upper chamber over skin for their penetration. To provide occlusive conditions, paraffin film was used to seal the assembly. The sampling done from receptor chamber by removing 200 µL at predetermined point of time for a duration of 24 hours. The withdrawals were counterbalanced by an equal amount of new PBS this will be useful to sustain sink conditions. Each trial was conducted in duplicate. All obtained samples were held at requisite conditions post-withdrawal, filtered, and appropriately diluted. The concentration of 4HILCN was analyzed by RP-HPLC.[24,25]

Table 6: Flux (CAP) of Nanogel (4HILCN-NG), (4HILCN- NEO) & (NF). (Mean ± SD, n=6)*

Codes	CAP (µg/cm^2)	Jss±SD (mg/cm^2/h)
4HILCN-NEO	7636.67	0.318 ± 0.048
4HILCN-NG	7903.33	0.329 ± 0.067
NFCN	523.33	0.022 ± 0.005

The data that was here obtained from this Ex-vivo investigation represents the total quantity of medication that was penetrated. It was through the skin mounted over the cell during a certain duration of time. The data was represented as a f(x) of time (hours). The permeation was determined by the slope of the aforementioned figure during the steady state named as flux (Jss, µg/cm²/h). The ER It is computed based on the equation shown below.

$$ER(\text{denotes the enhancement ratio}) = \frac{Jss(Formulation)}{Jss(Control)}$$

Statistical Analysis

Statistical analysis was conducted using ANOVA in software, Dunnett's post hoc test and this was used In the Graph Pad Prism Software 5.0 which was of the company (San Diego, CA, USA).

RESULTS AND DISCUSSION

Chemicals and Excipients

4Hydroxy isoleucine (4HILCN), was tested to be 99% pure side by side the excipients and remaining reagents used in the investigation were and it was found to be of grade of lab analysis.

Prior to Formulation Studies

Identification and Marking of Drug

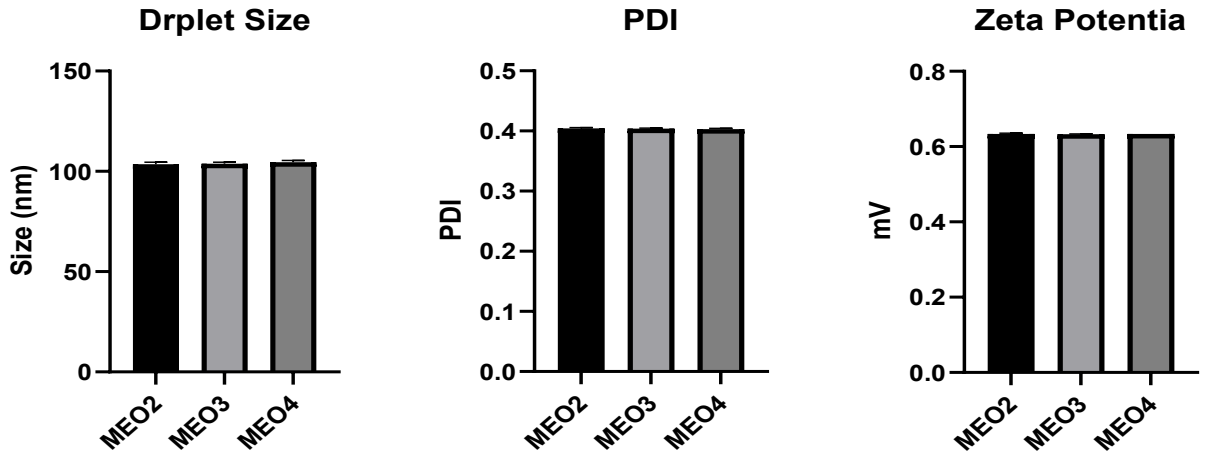

Figure 2: Graphs showing No significant difference among the three formulations

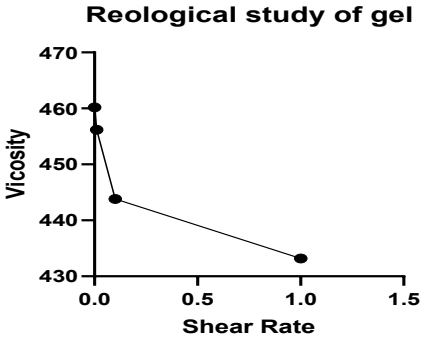

Figure 3: Graph

FTIR spectroscopy is used to detect and characterize 4-hydroxyisoleucine, evaluate purity, and perform quantitative analysis by comparing particular bands with established standards. C=O vibrational stretching A significant band at 1660 cm^{-1} signifies the presence of the carbonyl group (C=O) inside the peptide backbone. A band about 1530 cm^{-1} corresponds to the N-H bending and C-N stretching vibrations of the amino acid chain. C-N stretching absorption bands between 1250-1050 cm^{-1} indicate the presence of C-N bonds. The stretching of the O-H bond is shown by a broad band in the 3600-3000 cm^{-1} range.

RP-HPLC Calibration Curve Drug (4HILCN)

Representative HPLC chromatogram of Cucurbitacin was developed with Retention Time=8.13). The quantitative determination was conducted utilizing a calibration curve for 4-hydroxyisoleucine, described by the equation (Retention time (min) 8.09 minutes).

$y = 2806.9x + 1398.7$

$r^2 = 0.9987$

A good linear CV for (0.25-80 µg /mL) found and (r^2) of 0.9987 ± 0.01[26]

Development of Nanoemulsion

Preparation of Drug Loaded Nano Emulsion from Optimised Formulation

The optimised formulation and 2 more compositions with +10% and -10% oil & Smix were taken from previous study of same project. Drug was dissolved in oil phase (sefsol218) in appropriate quantity. Then with added Smix, thoroughly mixed under continuous stirring for 20 min in oil. now required quantity of DM water was added and used the vertex to get homogenous and transparent nanoemulsion.

Lowering oil and surfactant concentrations leads to smaller globule sizes due to fewer oil droplets and fewer molecules available to stabilize the oil-water interface. This synergistic effect results in smaller globule sizes due to the interaction between oil and surfactant.

Decreasing the concentrations of oil and surfactant in a nanoemulsion leads to an elevated Polydispersity Index (PDI) and possibly greater globule size, attributed to less stability and an increased propensity for aggregation. Surfactants stabilize the interface between oil and water, inhibiting droplet coalescence. Reduced oil concentrations diminish droplet formation, yielding smaller, more uniform droplets, although with possibly diminished stability. An elevated PDI indicates a broader range of globule sizes. It is coded as NEO for further study.

Drug Excipient Interaction Analysis

A significant characteristic bands at 1660 cm^{-1}, 1530 cm^{-1}, 1250-1050 & 3600-3000 cm^{-1} range indicate the presence drug unreacted with excipients.

Entrapment Efficiency (EE)

For the studied nanoemulsion To determine how well the nanoemulsion entraps the active component and delivers it to the desired location, we measure its entrapment efficiency. The entrapment efficiency values for the nanoemulsion formulations that were investigated varied between 88.34%±2.43 % and 92.34%±1.62%, as shown in Table: 1. with an entrapment efficiency value of 92.34%±1.62%, NEO3 stood out among all the nanoemulsion formulations. Important factors that greatly affect the entrapment efficiency are the formulation's composition and the kind of phytochemicals trapped in the vesicles. Cosurfactants are used in nanoemulsion formulations to improve solubility, according to the literature. This has an effect on the bioactive ingredient partitioning and, by extension, on the entrapment efficiency. However, It is possible that the increased of Tween-80 and Transcutol in this gel is responsible for the better entrapment

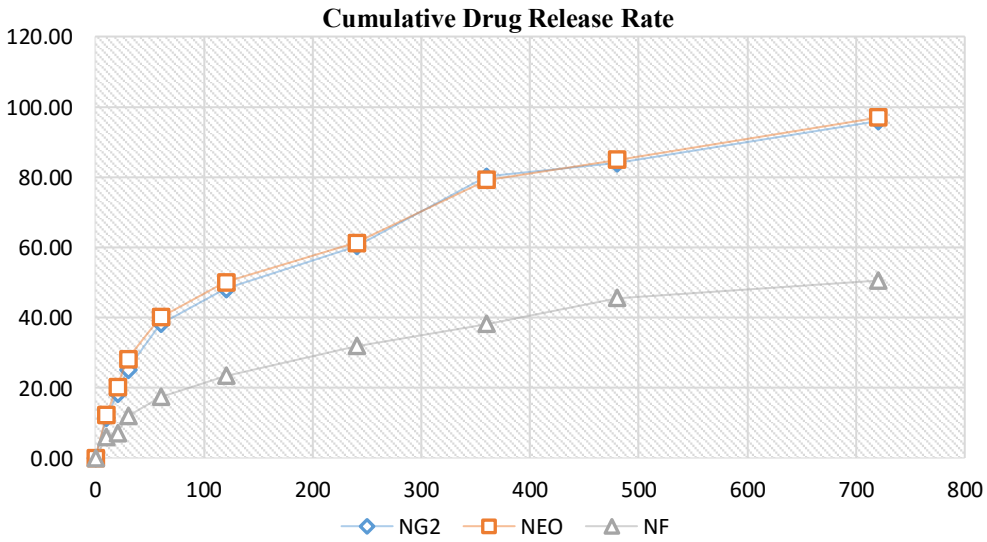

Figure 4: Graph showing the comparative drug release form gel formulations

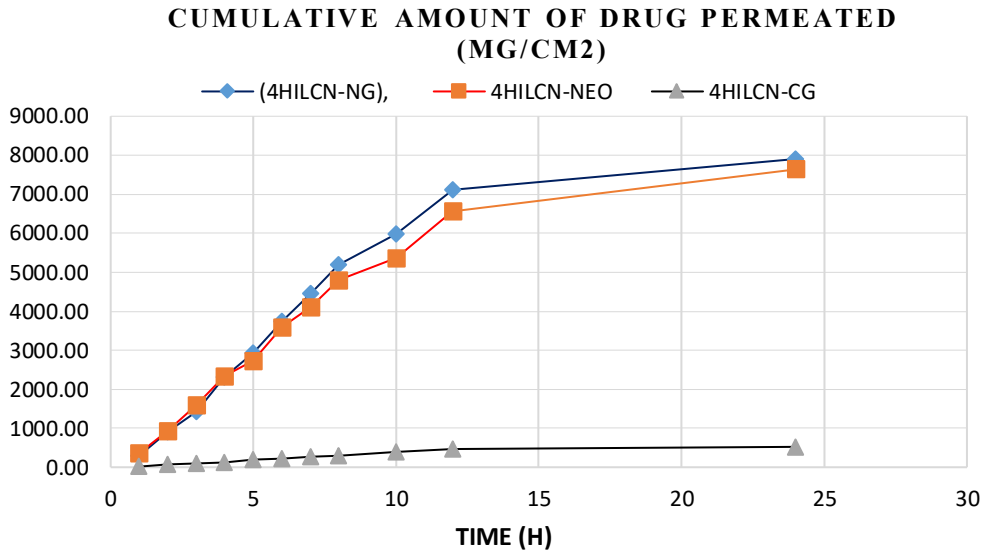

Figure 5: Plot-In-comparison: Permeation Profile of NE4C, 4HILCN-NG & NF through rat skin

efficiency.

Nanogel Formulation

Preparation of Nano Gel

The NEO was converted into a hydrogel to impart the necessary application marks. NEO exhibits low viscosity, so NG was formed for facilitating application on the skin. The optimized nanoemulsion 4HILCN-NEO was converted into a nanogel. These materials were utilized due to their biocompatibility, biodegradability, bioadhesive properties, non-irritating nature, and lack of absorption into the body.[19] Selection was done on the basis of required characteristics of NG.

To make a gel base of different concentrations, a suitable amount of distilled water was added to a precisely measured amount of polymer (1% to 3% w/v). Once the gel was created, the pH was reduced to 5.5 with the addition of Triethanolamine. The ideal formulation of 4HILCN-NEO added to each gel base at a 1:1 ratio to produce 4HILCN-NG, which was then retained for further characterization. A comprehensive mixing procedure that includes ongoing homogenization came next. Using the same methods, a simple gel containing free 4HILCN solution (4HILCN-NFc) may also be created.

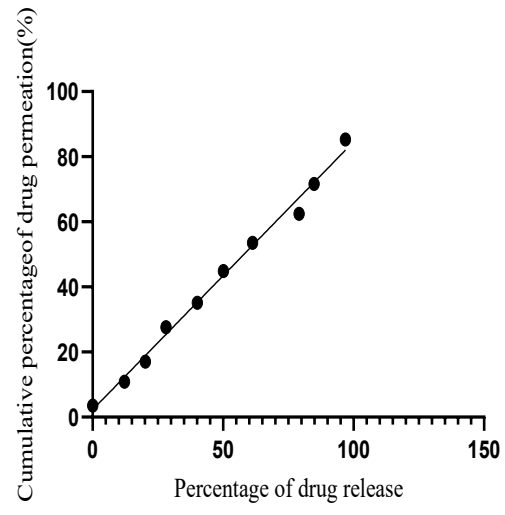

Figure 6: Plot-In-comparison

Screening of the Optimised NG

Homogeneity

The study found that formulations NG1, NG2, and NG3 was consistent for homogeneity that was observed and reported after transferring the gel into flint-colour glass containers. It was concluded that concentration of the gelling ingredient did not affect the homogeneity.

PH Measurement

When it comes to the value of cosmeceutical, however, the pH range of 5.5–7.2 is regarded to be optimum since it corresponds to the usual pH of the skin and it was found to be in range of it (Table: 3).

Size of Globules

The frequency distribution of droplets concerning size was assessed using Photon Correlation Spectroscopy (P.C.S.). It examines the variations in light scattering anticipated as a result of the Brownian motion of the particles. The Zetasizer 1000 HS, manufactured by Malvern Instruments in Worcestershire, UK, is utilized for this purpose, with light scattering monitored at a 90-degree angle.

Spreadability

The Spreadability of topical nanogel formulations NG1, NG2, and NG3 was assessed, yielding values of 3.2 cm, 2.7 cm, and 2.3 cm, respectively (Table: 3). The concentration of Carbopol 934 diminished the spreading ability of these formulations. The optimised batch, NG2, was selected for its excellent consistency and Dispersibility.

Rheological Studies

The viscosities of the topical nanogel formulations NG1, NG2, and NG3 were measured at 207.4±12.5, 460.2±10.6, 904.5±12. Respectively (Table: 3.). the data indicate that an increase in the concentration of the gelling agent correlates with an increase in viscosity (Fig.3). The NG2 batch was selected as the optimized batch due to its desired viscosity and consistency.

Nanogel demonstrate shear-thinning behaviour, characterized by a reduction in viscosity with increasing shear rate. This phenomenon is commonly ascribed to the disruption of particle-particle interactions or the reorganization of the nanogel structure during flow.

Evaluation of NG

Stability Studies

Based on the findings, there was no discernible change in the color, odor, homogeneity, pH, viscosity, or net content of the 4HILCN-NG formulation when it was applied in stressed conditions of 0, 1, 2, 3, and 6 months. The findings demonstrate a very high degree of consistency.[27]

Kinetics of Release of NanoGel

The *in vitro* drug release profile from 4HILCN-NEO, 4HILCN-NG, and 4HILCN-NF was examined over 12 hours, with findings shown in Fig. 3. The drug release percentage after 12 hours was 96.9% for 4HILCN-NEO and 95.9% for 4HILCN-NG, both surpassing the release of 4HILCN-NF at 50.5%. The significant increase in drug release from 4HILCN-NEO and 4HILCN-NG compared to 4HILCN-NF. It is ascribed to be due to improvements in the solubility of poorly soluble constituents encapsulated in the NE. these has been shown to boost solubility and dissolution due to its tiny size, viscosity, and the presence of Smix, which are acknowledged variables that facilitate drug release.[28]

Drug Permeation from Nanogel

A series of comparative tests were conducted to examine the differences and similarities between drug loaded nanogel (4HILCN-NG), nano-emulsion chosen (4HILCN-NEO), and drug loaded neat formulation (NF) (mean ± standard deviation, n=6)* (Table: 6; Fig. 5). At 24 hours, the penetration profile of Nano-Gel was superior to that of NF gel (5.48%) and also to that of 4HILCN-NEO (90.76%). The cumulative percentage of drug-CPP that was able to pass through Nano-Gel was 93.56%. The formulation known as 4HILCN-NG was able to obtain the highest possible flux and permeability of 4HILCN when compared to other formulations.

The Karl Pearson coefficient of correlation, which has a value of ±1 for best relation ship, was used in order to investigate the relationship between the cumulative percentage of permeability and the cumulative percentage of drug release from nanogel.

It was very near to 1 (r = +0.9088, mean ± SD, n=3) * (Fig. 6).

CONCLUSION

The suggested nanogel method seeks to enhance the solubility and transdermal flux of a weakly water-soluble Antidiabetic medication, ensuring prolonged activity via systemic circulation after absorption into the bloodstream. The successful creation of a nanogel from an optimized nanoemulsion formulation containing 4-hydroxyisoleucine indicates that a transdermal delivery method for diabetes therapy is a viable technique. This method circumvents first-pass metabolism, enhances therapeutic effectiveness, Dispersibility, and storage stability, while decreasing the therapeutic dosage and dosing frequency.

The formulation exhibited exceptional physicochemical stability, enhanced skin penetration, and prolonged drug release. The nanogel may boost the bioavailability of 4-hydroxyisoleucine as a result of these observations. This would provide a viable alternative to traditional oral administration methods and facilitate further *in vivo* research and practical applications in diabetes therapy.

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REFERENCES

1. IDF Diabetes Atlas Group. IDF Diabetes Atlas Fourth Edition [Internet]. Idf Diabetes Atlas. 2009. 1–527 p. Available from: http://www.diabetesatlas.org/resources/2015-atlas.html%5Cnhttps://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf%5Cnwww.ecuadorencifras.gob.ec
2. World Health Organization. Global Report on Diabetes. Isbn [Internet]. 2016;978:88. Available from: http://www.who.int/about/licensing/%5Cnhttp://apps.who.int/iris/bitstream/10665/204871/1/9789241565257_eng.pdf
3. Gothai S, Ganesan P, Park SY, Fakurazi S, Choi DK, Arulselvan P. Natural phyto-bioactive compounds for the treatment of type 2 diabetes: Inflammation as a target. Nutrients. 2016;8(8).
4. Singh S, Mazumder A, Chakaraborthy GS. Potential herbs against diabetes mellitus - An update. Int J Pharm Sci Res [Internet]. 2019;10(8):3619–26. Available from: https://www.embase.com/search/results?subaction=viewrecord&id=L629274509&from=export%0Ahttp://dx.doi.org/10.13040/IJPSR.0975-8232.10(8).3619-26
5. Santhiveeran J, Ph D, Kim M, Ph D, Ranney M, Ph D. A QUANTITATIVE STUDY ON THE DISEASE MANAGEMENT OF DIABETES AND THE EFFECTS ON MINORITY POPULATIONS Presented to the School of Social Work California State University , Long Beach In Partial Fulfillment of the Requirements for the Degree Master of Social W. 2019;(May).
6. Anwar S, Desai S, Eidi M, Eidi A. Antidiabetic Activities of Fenugreek (Trigonella foenum-graecum) Seeds. Nuts Seeds Heal Dis Prev. 2011 Jan 1;469–78.
7. Upadhyay TK, Das S, Mathur M, Alam M, Bhardwaj R, Joshi N, et al. Medicinal plants and their bioactive components with antidiabetic potentials. Antidiabetic Med Plants Appl Oppor. 2023 Jan 1;327–64.
8. Geberemeskel GA, Debebe YG, Nguse NA. Antidiabetic Effect of Fenugreek Seed Powder Solution (Trigonella foenum-graecum L.) on Hyperlipidemia in Diabetic Patients. J Diabetes Res [Internet]. 2019 Jan 1 [cited 2025 Jan 26];2019(1):8507453. Available from: https://onlinelibrary.wiley.com/doi/full/10.1155/2019/8

507453
9. Khursheed R, Singh SK, Wadhwa S, Kapoor B, Gulati M, Kumar R, et al. Treatment strategies against diabetes: Success so far and challenges ahead [Internet]. Vol. 862, European Journal of Pharmacology. Elsevier B.V.; 2019. 172625 p. Available from: https://doi.org/10.1016/j.ejphar.2019.172625
10. Alam S, Iqbal Z, Ali A, Khar RK, Ahmad FJ, Akhter S, et al. Microemulsion as a potential transdermal carrier for poorly water soluble antifungal drug itraconazole. J Dispers Sci Technol. 2010 Jan;31(1):84–94.
11. Ganesan P, Arulselvan P, Choi DK. Phytobioactive compound-based nanodelivery systems for the treatment of type 2 diabetes mellitus - Current status. Int J Nanomedicine. 2017;12:1097–111.
12. Harwansh RK, Mukherjee PK, Bahadur S, Biswas R. Enhanced permeability of ferulic acid loaded nanoemulsion based gel through skin against UVA mediated oxidative stress. Life Sci [Internet]. 2015;141:202–11. Available from: http://dx.doi.org/10.1016/j.lfs.2015.10.001
13. Azeem A, Talegaonkar S, Negi LM, Ahmad FJ, Khar RK, Iqbal Z. Oil based nanocarrier system for transdermal delivery of ropinirole: A mechanistic, pharmacokinetic and biochemical investigation. Int J Pharm [Internet]. 2012;422(1–2):436–44. Available from: http://dx.doi.org/10.1016/j.ijpharm.2011.10.039
14. Rashid M, Sindhu RK, Ahmad FJ. Development and evaluation of optimised nanogel loaded with herbal bioactive antidiabetic agent. Eur Chem Bull. 2023;12(8):435–63.
15. Amjad Pasha M, Amjad M, Ehtheshamuddin M, Chand S, Sabreesh M, Asia R, et al. FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES OF ATENOLOL. Adv Res Pharm Biol. 2011;1(2).
16. Sturm S, Stuppner H. Analysis of cucurbitacins in medicinal plants by high-pressure liquid chromatography-mass spectrometry. Phytochem Anal. 2000;11(2):121–7.
17. Aslan C, Çelebi N, Değim IT, Atak A, Özer Ç. Development of Interleukin-2 Loaded Chitosan-Based Nanogels Using Artificial Neural Networks and Investigating the Effects on Wound Healing in Rats. AAPS PharmSciTech. 2017;18(4):1019–30.
18. Sengel-Turk CT, Ozmen N, Bakar-Ates F. Design, characterization and evaluation of cucurbitacin B-loaded core–shell-type hybrid nano-sized particles using DoE approach. Polym Bull [Internet]. 2021;78(6):3327–51. Available from: https://doi.org/10.1007/s00289-020-03256-7
19. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Brazilian J Pharm Sci. 2016;52(3):493–507.
20. Khan MA, Pandit J, Sultana Y, Sultana S, Ali A, Aqil M, et al. Drug Delivery Novel carbopol-based transfersomal gel of 5-fluorouracil for skin cancer treatment: *in vitro* characterization and *in vivo* study Novel carbopol-based transfersomal gel of 5-fluorouracil for skin cancer treatment: *in vitro* characterization and *in vivo* study. Drug Deliv [Internet]. 2015 [cited 2023 May 26];22(6):795–802. Available from: https://www.tandfonline.com/action/journalInformation?journalCode=idrd20http://informahealthcare.com/
21. Abdhesh, Gupta SK. Formulation and evaluation of nanoemulsion based nanoemulgel of aceclofenac. J Pharm Sci Res. 2020;12(4):524–32.
22. Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharm J. 2012 Jan 1;20(1):63–7.
23. Sultana S, Talegaonkar S, Bhatnagar A, Ahmad FJ, Mittal G. Optimization of nifedipine loaded gastroretentive microcapsules for biliary colic. Asian J Pharm. 2012;6(4):295–306.
24. Mutalik S, Udupa N. Transdermal delivery of glibenclamide and glipizide: *in vitro* permeation studies through mouse skin. Pharmazie [Internet]. 2002 Dec 1 [cited 2023 Sep 7];57(12):838–41. Available from: https://europepmc.org/article/med/12561248
25. Tian Q, Quan P, Fang L, Xu H, Liu C. A molecular mechanism investigation of the transdermal/topical absorption classification system on the basis of drug skin permeation and skin retention. Int J Pharm. 2021 Oct 25;608:121082.
26. Attar UA, Ghane SG. Optimized extraction of anti-cancer compound – cucurbitacin I and LC–MS identification of major metabolites from wild Bottle gourd (Lagenaria siceraria (Molina) Standl.). South African J Bot [Internet]. 2018;119:181–7. Available from: https://doi.org/10.1016/j.sajb.2018.09.006
27. Rai VK, Mishra N, Yadav KS, Yadav NP. Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications [Internet]. Vol. 270, Journal of Controlled Release. Elsevier B.V; 2018. 203–225 p. Available from: http://dx.doi.org/10.1016/j.jconrel.2017.11.049
28. Mauri E, Cappella F, Masi M, Rossi F. PEGylation influences drug delivery from nanogels. J Drug Deliv Sci Technol [Internet]. 2018;46(January):87–92. Available from: https://doi.org/10.1016/j.jddst.2018.05.003