How to Cite:
Shetkar, M. A., Patil, S. S., Nagoba, S. N., & Panchal, C. V. (2022). Formulation and evaluation of nano-particulate topical gel containing Celecoxib. *International Journal of Health Sciences*, *6*(S6), 5942–5964. https://doi.org/10.53730/ijhs.v6nS6.11793

Formulation and evaluation of nano-particulate topical gel containing Celecoxib

Madhav A. Shetkar
School of Pharmacy SRTMU, Nanded, Maharashtra, India

Siddheshwar S. Patil
Maharashtra College of Pharmacy Nilanga, Maharashtra, India
*Corresponding author email: patil_ss21@rediffmail.com

Shivappa N. Nagoba
Channabasweshwer Pharmacy College, Latur, Maharashtra, India

Chandrawadan V. Panchal
Maharashtra College of Pharmacy Nilanga, Maharashtra, India

Abstract---The present research work describes formulation and evaluation of celecoxib containing gel using transdermal delivery. Transdermal potential of nanoparticulate gel was investigated using *in-vitro* and *in-vivo* study. In present study procured drug samples were investigated under preformulation studies. For development of nanparticles containing celecoxib, nanoprecipitation method is used. The formulated nanoparticles were evaluated for different parameters such as zeta potential, particle size, % entrapment efficiency, in-vitro drug release, SEM and stability studies. The formulated nanoparticles were formulated as gel using carbopol. Formulated carbopol gels were characterized for appearance, spreadability, pH, viscosity, drug content, consistency index and ex-vivo drug release, in-vitro drug release. Formulated gels were evaluated for in-vitro studies (skin irritation and skin permeation). Further evaluated for stability studies as per ICH guidelines, results of stability studies reveled that both the formulated gels were found to be stable at stuied temperatures.

Keywords---formulation development, celecoxib, nanoparticles.

Introduction

Since ancient times, medicinal plants remedies have been the primary source of medicine in India, and they are now gaining popularity in developed countries. They also play an essential role in the lives of tribal and rural people, particularly

in developing countries' remote areas. Clearly, these plants aid in the relief of human pain. These plants are being used as additives, beverages, and cosmetics in the food industry. During the last century, our country has seen a fast expansion of the allopathic medical system. However, these medicines are harmful to human health, and people are returning to nature in the hopes of finding safety and security. On the other hand, drugs derived from medicinal plants are safe, less expensive, and readily available, with no risk of adverse effects. [1-4]. In India, medicinal plants fueled demand for pharmacopoeial medications and their derivatives. Many of these medicine plants have been introduced to the general public in recent years. Agronomical procedures for growing a few medical plants have been devised, and these medicinal plants are currently commercially grown in many parts of our country. [5]

Many important herbal medications have been found as a result of learning that a particular plant was utilised by ancient folk healers to cure a particular condition. Furthermore, medicinal plant wealth is our national history, and it appears to be the first and primary line of defence for the treatment of many diseases, particularly in tribal and rural populations, and is a subject worthy of scientific investigation. [6-9]. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The drug is dissolved, entrapped, encapsulated or attached to the nanoparticles matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsulescan be obtained. Nanocapsules are delivery systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.

In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period of time and target to a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, nanoparticles help to increase the stability of drugs/proteins and possess useful controlled release properties. Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) used to treat pain, menstrual cramps, inflammatory diseases such as rheumatoid arthritis, gout and fever. It is taken orally. It is available in immediate and delayed release formulations. Onset of effects is within an hour and last for up to twelve hours. The present research aims to formulate Celecoxib nanoparticles. [10-15].

Materials and Methods

Preformulation Study [16-21]
Organoleptic properties

Organoleptic properties are the aspects of food, water or other substances that create an individual experience via the senses—including taste, sight, smell, and touch.

Procedure

About 1 g of drug samples was placed in watch glass and was observed for appearance, color, any peculiar odor and taste.

Melting point determination

Differential scanning calorimeter (DSC) is thermal analysis procedures which determine the temperature and heat flow associated with transitions in materials as a function of temperature and time. DSC is useful to determine qualitative and quantitative information about the physical and chemical changes, including in heat capacity or exothermic / endothermic processes. Glass transition temperature, melting factor, crystallization time and temperature, percentage crystallanity, purity, reaction kinetics, are derived from DSC graph. A differential scanning calorimeter (Mettler Toledo) was used for thermal evaluation of drug and excipients in separate and in mixture form. The samples of drug and excipients as well as physical mixtures were weighed instantly into the DSC aluminum crucible and scanned within the temperature variety of 50-400°C under a dry nitrogen atmosphere. The heating rate was 60-100ml / min and the thermograms received were determined for interactions. Melting point was determined by using Differential scanning Calorimetry (Mettler Toledo, United States).

Identification of Pure drug

Identification of pure drug was carried out by Fourier Transform Infrared Spectrophotometry (Shimadzu 8400s).

UV-Spectroscopy Study

The UV analysis was carried out to determine the UV spectrum of metal chelate and ligand co-relation. UV spectra were recorded in solvent DMSO for metal chelates on the Shimadzu instrument and UV scans of ligands were recorded in methanol solvents.

Determination of λmax Celecoxib
Selection of Solvent

The solubility of Celecoxib was checked in water, acetonitrile and methanol. It was found to befreely soluble in methanol, slightly soluble in water but insoluble in acetonitrile.

Preparation of standard stock solution

Standard stock solution of celecoxib was prepared by dissolving accurately weighed quantity of celecoxib 25mg in 25 ml of methanol and transferred it to 25 ml of volumetric flask. Volume was created to the mark with methanol for obtaining stock solution up to 1000µg/ml conc. Further dilution made to get the concentration of 100µg/ml.

Selection of Maximum Wavelength

Determination of Absorption Maximum: The standard solution of Celecoxib (10µg/ml) was scanned in the wavelength from 230 to 280 and absorption maximum was found to be 252nm.

Preparation of calibration curve

From the standard stock solution of Celecoxib, appropriate aliquots were pipette out into 25 ml of volumetric flask, 5%, 5ml acetyl chloride solution was added and dilutions were made with methanol to produce working standard solution of Celecoxib 10 to 70µg/ml concentrations. The absorbances of Celecoxib solutions were measured at 252nm. The calibration plot of the drug Celecoxib was plotted. The concentration range over which the drug followed linearity was chosen as an analytical concentration range i.e. 10 to 70µg/ml for Celecoxib.

Preparation and evaluation of nanoparticulate topical gel
Preparation of CNPs loaded Carbopol and HPMC gels

Gel forming polymer (Carbopol and HPMC) was soaked in water sepearatly for 24 hours and then dispersed by agitation to get a smooth dispersion. The dispersion was allowed to stand for 15 min to expel entrapped air. Simultaneously nanoparticles (CNPs), propylene glycol permeation enhancer was added to water and undergoes gentle stirring. This was added to carbopol mixture by stirring, triethanolamine is added to form gel.

Table 1
Preparation of CNPs loaded Carbopol and HPMC gels

	Concentartions							
Drug/Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Celecoxib NP's % (w/v)	10	10	10	10	10	10	10	10
Carbopol % (w/w)	0.5	1	1.5	2	-	-	-	-
HPMC % (w/w)	-	-	-	-	0.5	1	1.5	2
Propylene glycol (%w/v)	5	5	5	5	5	5	5	5
Triethanolamine (%w/v)	10	10	10	10	10	10	10	10

Evaluation of CNPs loaded Carbopol and HPMC gels
Measurement of pH

The pH measurements were carried out using a calibrated digital pH meter (Hanna instruments HI 9321, Michigan, USA).

Gelling capacity

Gelling capacity means to measure the speed as well as extent of gelation. The formulations which were undergone sol to gel transition are suitable. The formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time.

Drug entrapment efficiency

The percentage of drug entrapped into the developed formulation (CNPs,) gel was determined after removing the free drug by ultra dialysis against acetate buffer (pH 6.0) containing 30% PEG 400 (v/v) at 4°C for 4 hr(s) in a dialysis bag (Sigma, USA; MWCO 12000–14000). The dialyzed formulation was lysed using Triton-X 100 (0.1% v/v) and subsequently analyzed for drug content (A2) by UV spectrophotometry (Schimadzu, 1800 spectrophotometer) at 252nm and 331nm. The percent of drug entrapment efficiency (% E.E) was computed by using Equation,

$$E.E\ (\%) = \frac{A_2}{A_1} \times 100$$

Where, A1 is the total amount of drug added in the formulation.

Transmission electron microscopy (TEM) analysis

The morphology of the developed formulation (CNPs) gel was examined by transmission electron microscopy (TEM) (Morgagni 268D, FEI, Holland). CNPs loaded Carbopol and HPMC gels was diluted with distilled water in a ratio of 1:10. One drop of the diluted formulation was subsequently taken and placed onto a carbon-coated copper grid. The excess liquid was removed with filter paper and allowed to stand for 10 m. The grid was then stained with 1% phosphotungstic acid (PTA) and allowed to air dry for 5 m. The sample was then viewed under transmission electron microscope (TEM) and photomicrographs were taken.

Thermal behaviour

Differential scanning calorimetry (DSC) analyses were performed in a Mettler DSC 821e differential scanning calorimeter (Mettler Toledo, Gieβen, Switzerland) to study the thermal characteristics of the drug containing lipid matrix and CNPs loaded Carbopol and HPMC gels. Weighed amounts of the samples (approximately 1-3 mg based on lipid content) were scanned in 40 µl aluminium pans at heating rate of 5 K/m over a temperature range of 25-85°C. An empty standard aluminum pan was used as a reference. The DSC parameters were evaluated using STARe software (Mettler Toledo, 821e, Switzerland).

Occlusion factor

The occlusive property of the gel formulations was evaluated by in-vitro occlusion test. Briefly, beakers (100 ml) with a diameter of 4.9 cm were filled with 50 ml of water and covered with a filter paper (Whatman number 6, cutoff size: 3 µm, USA) on the upper surface of which 250 mg of the test formulation was evenly distributed. The beakers were subsequently stored at 32±0.5°C for 48 hr(s) in order to mimic the temperature of the skin surface. The beaker covered with the filter paper without sample was used as a control. The evaporation of water through the filter paper was measured and the occlusion factor “F” was calculated at 6, 24, and 48 hr(s) following Equation,

$$F = \frac{A-B}{A} \times 100 .$$

Where:
A stands for the water flux through the filter paper without sample (control) and
B is the water flux through the filter paper with sample. An F value of 0 means no occlusive effect compared to the reference, while an F value of 100 means maximum occlusiveness.

Rheogram

The rheogram of the formulation was obtained at 25±1°C with a Brookfield Viscometer (Model RVT, Brookfield Engineering Laboratories, Inc., USA) at rotational speed of 0.5, 1.0, 2.0, 2.5, 4, 5, 10, 20, 50 and 100 rpm using an appropriate spindle (spindle ≠2). The shear rate was increased from 0.5 to 100 rpm (up curve) and then decreased from 100 to 0 rpm (down curve), and the resulting shear stress (τ) was noted. The position of the up and down curves in the rheogram was analyzed to indicate the type of flow behavior.

Viscoelasticity

The viscoelastic analysis of the formulations was performed at 25±0.2°C using a rheometer Rheo Stress RS 6000 (Haake Instrument, Germany) equipped with a cone-and-plate test geometry (plate diameter 35 mm, cone angle 2°). The linear viscoelastic region (LVR) was determined through stress sweep test by measuring the complex modulus (G*) and phase angle (δ) as a function of stress ranging between 0 Pa and 10 Pa at a constant frequency (1Hz in our experiment). Oscillation frequency sweep test was then performed by measuring the elastic modulus (G′), viscous modulus (G″), and complex viscosity (η*) as a function of frequency ranging between 0 Hz to 10 Hz at a constant stress amplitude selected from the observed LVR.

Spreadability

For determining the spreadability (www.floratech.com) of the CNPs loaded Carbopol and HPMC gels, the cellulose acetate filter paper was weighed (W1) and kept in the center of the aluminum foil sheet. Twenty drops (1ml) of the formulation was then pressed out of the syringe (BD syringe, Becton Dickinson & Co. USA) on the marked area in the center of the filter paper and allowed to stand for 10 m. Afterwards, unsaturated portion of the filter paper was cut away and weighed out precisely (W2). % Spread by weight was calculated by using the Equation.

$$\% \text{ Spread by Weight} = \frac{W_1 - W_2}{W_1} \times 100$$

where W1 is the initial weight of cellulose acetate filter paper and W2 is the weight of unsaturated portion of the paper after application of formulation.

In-vitro release

In-vitro release of CNPs loaded Carbopol and HPMC gels was evaluated by using a dialysis membrane (MWCO 12–14,000). The membrane was mounted between the donor and receptor compartments of a locally fabricated Franz diffusion cells (diffusion area of 2.26 cm2; receptor volume of 22.5 ml). Phosphate buffer of pH 7.4 was selected as receptor medium and maintained at 32±0.5°C in the receptor compartment. Unentrapped drug was removed from the formulation by ultra dialysis against Phosphate buffer of pH 7.4 containing 30% PEG 400 (v/v) at 4°C for 4 hr(s) using dialysis bag (Sigma, USA; MWCO 12–14,000). 250mg of the dialyzed formulation was then applied evenly on the surface of the membrane in the donor compartment. The aliquots from the receptor compartment were withdrawn at predetermined time interval and replenished immediately with a similar volume of fresh medium. The samples were centrifuged, and analyzed for drug content by HPLC assay. The experiment was performed in triplicate. The cumulative percentage of drug released at different time intervals (Qt) was calculated and the data obtained was fitted to Zero order (Qt vs. t), Higuchi (Qt vs. t1/2), and First order kinetic model (log Qt vs. t) to find out the mechanism of CNPs loaded Carbopol and HPMC gels release from the developed formulations.

Ex-vivo skin permeation and skin deposition studies
Animals

All procedures were conducted as per guidelines of the committee for the purpose of control and supervision of experimental animals (CPCSEA). The study protocol was approved by the Institutional Animal Ethical Committee of SGRS College of Pharmacy, Saswad, Pune, India. The skin irritation study was carried out using male albino rabbits (weighing 2.0-2.5 kg) as per the OECD (Organisation for Economic Co-operation and Development) guidelines 404 (OECD 2002) for the testing of acute dermal irritation/corrosion. Ex-vivo skin permeation and skin deposition studies were performed as per Organization for Economic Cooperation and Development (OECD) guidance notes on dermal absorption (OECD GD156 2011) following the protocol approved by the Institutional Animal Ethical Committee of SGRS College of Pharmacy, Saswad, Pune, Maharashtra, India. The study was performed in a locally fabricated Franz diffusion cell with a diffusion area of 2.26 cm2 and receptor volume of 22.5 ml using abdominal rat skin. Abdominal rat skin was excised and washed with isotonic NaCl.

The excised skin was then mounted between the donor and receptor chambers of the Franz diffusion cell with the dermal side in contact with the receptor medium and the SC side facing upwards into the donor compartment. Then, 250 mg of the dialyzed formulations of (CNPs) loaded carbopol gel F1, (CNPs) loaded carbopol gel F2 was evenly applied on the surface of the rat skin in the donor compartment. The receptor compartment was filled with Phosphate buffer of pH 7.4 containing 30% PEG 400 (v/v) and stirred continuously. The temperature in the receptor compartment was maintained at 32±0.5°C to simulate the skin temperature. At predetermined time intervals [1, 2, 3, 5, 7, 9, 12, and 24 hr(s)], aliquots from the receptor compartment were withdrawn and replenished immediately with a similar volume of fresh medium equilibrated at 32±0.5°C to maintain sink condition. All the collected samples were centrifuged and analyzed for celecoxib

content by HPLC. For evaluation of drug deposition into rat skin, the skin sample was removed from the Franz diffusion cell at the end of the skin permeation experiment [24 hr(s)]. The formulations remaining on the skin surface was removed by gentle washing with PBS (pH 7.4). SC was removed by stripping the skin surface with hypoallergic, transparent adhesive tape (Transpore 3M surgical tape, 3M India Ltd, India). The epidermis was separated from the dermis with a surgical sterile scalpel. Tape strips, epidermis, and dermis were placed each in methanol and sonicated for 20 m to extract the drug. All the samples were then centrifuged; the supernatants collected were analyzed for drug content by HPLC.

Stability study

The globalization of manufacturing operation, the final product should be stable in various climatic conditions. The climatic condition include tropical, sub tropical and temperate. Stability studies were performed as per ICH guidelines. The stability studies of CNPs) loaded carbopol gel F1 were stored at 25^0C, and 40^0C for 3 months. The gel was analyzed for psychochemical parameter like color change, drug content, pH, and viscosity.

Results and Discussion

Preformulation Study
Identification of drugs by FT-IR

Samples of Celecoxib and physical mixture of both drugs and polymers were subjected to FT-IR spectroscopy.

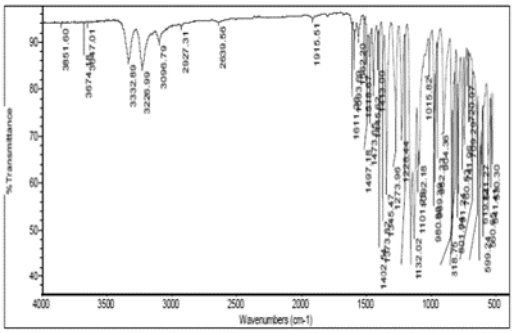


Figure 1. FTIR Spectra of Celecoxib

Wave Number (cm-1)	Interpretation
3332.89	NH_2
1345.47	S = O
1373.82	CN
1273.96	CF

Major functional groups like NH2 stretching, H stretching, Aromatic CH stretching, and S=O stretching (Sulfonamide group) were present in Celecoxib showed characteristic peaks in FTIR spectrum. The major peaks were identical to functional group of Celecoxib. Hence, the sample was confirmed as Celecoxib.

Melting point

Melting point values of Celecoxib was found to be in range of 160°C - 164°C which was represented in below table.

Table 2
Melting point of Celecoxib

Drug name	Melting point (°C)
Celecoxib	162

Organoleptic properties

Table 3
Organoleptic properties of Celecoxib

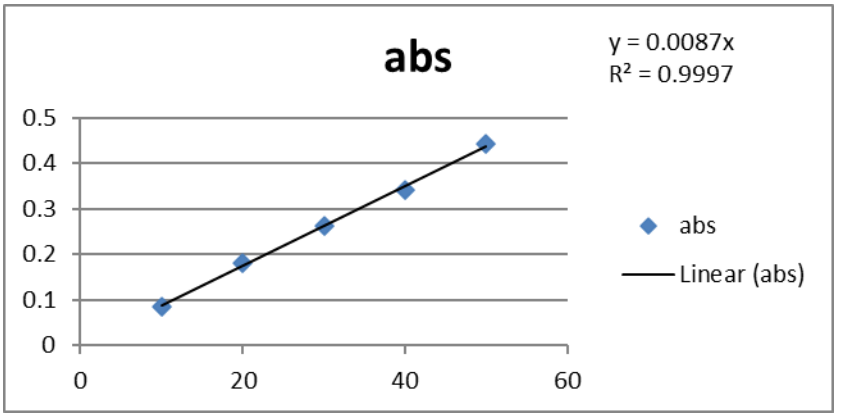
Properties	Specification	Observation
Color	White or almost white	White color
Nature	Amorphous or crystalline powder	Amorphous powder
Odour	NA	Odourless

UV-Spectroscopy Study
Determination of λmax Celecoxib

The absorption maximum for Celecoxib in methanol was found to be 252nm.

Calibration curve of Celecoxib

The experiments were performed in triplicate. The calibration curve was obtained in the range of 10-70µg/ mL. The regression equation obtained was y=0.0087x + 0.9 and the correlation coefficient (R2) was 0.999 shown in figure below.



Graph 2. Calibration curve for Celecoxib

Evaluation of nanoparticulate topical gel

Evaluation of CNPs loaded Carbopol and HPMC gels
Measurement of pH

Table 4
pH of CNPs loaded Carbopol and HPMC gels

Sr. No.	Formulation	pH
1	F1	7.4
2	F2	7.4
3	F3	7.2
4	F4	7.3
5	F5	7.5
6	F6	7.3
7	F7	7.2
8	F8	7.1

Gelling capacity

Gelling capacity means to measure the speed as well as extent of gelation. The formulations which were undergone sol to gel transition are suitable. The formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time.

Table 5
Gelling capacity of CNPs loaded Carbopol and HPMC gels

Sr. No.	Formulation	Gelling Capacity
1	F1	+++
2	F2	+++
3	F3	++
4	F4	++
5	F5	+++
6	F6	+++
7	F7	++
8	F8	++

([a]values are mean ± SD [b] + gels slowly and dissolves ++ gelation immediate and remains for few hours +++ gelation immediate and remains for an extended period.)

Drug entrapment efficiency

The entrapment efficiency of CNPs loaded Carbopol gels F1 formulation and CNPs loaded Carbopol gels F2 formulation were observed in the range of 90.10 percent and 87.34 percent respectively indicating higher drug entrapment efficiency.

Table 6
% Entrapment efficiency of CNPs loaded Carbopol and HPMC gel

Formulations	Entrapment efficiency (%)
F1	90.10
F2	87.34
F3	84.20
F4	81.22
F5	87.12
F6	84.55
F7	83.00
F8	80.11

Transmission electron microscopy

Below is a TEM micrograph of CNPs loaded Carbopol gels F1 formulation and CNPs loaded Carbopol gels F2 formulation The particle size ranges from 30 to 90 nanometers, with a 60 nanometer average. The particles have a spherical form.

The same sample was also subjected to electron diffraction examination to ensure that no other forms of metal oxide were present.

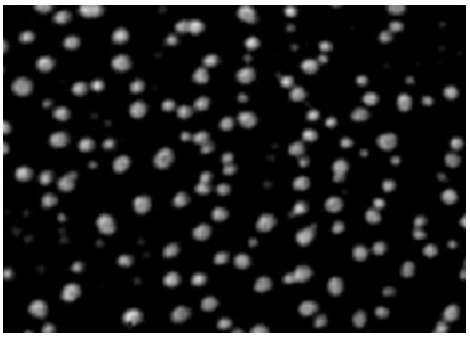


Fig 3. TEM of CNPs loaded carbopol gel F1

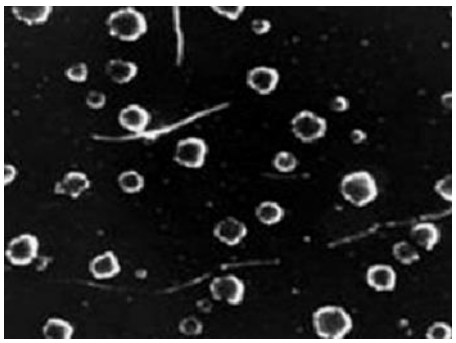


Fig 4. TEM of CNPs loaded carbopol gel F2

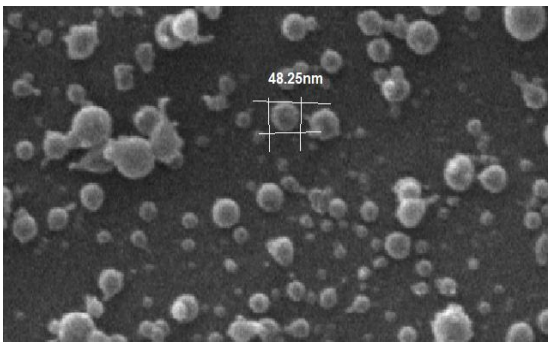


Fig 5. TEM of CNPs loaded carbopol gel F3

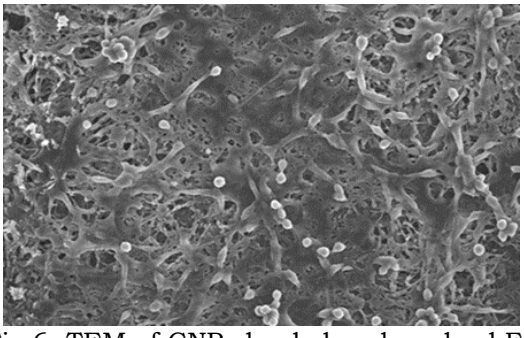


Fig 6. TEM of CNPs loaded carbopol gel F4

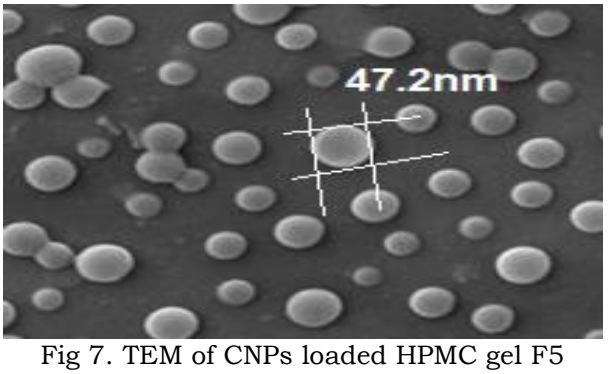


Fig 7. TEM of CNPs loaded HPMC gel F5

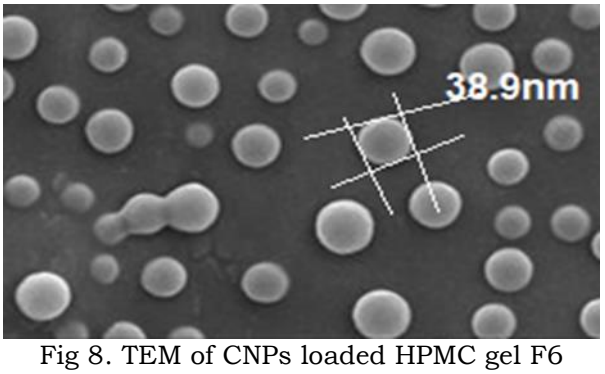


Fig 8. TEM of CNPs loaded HPMC gel F6

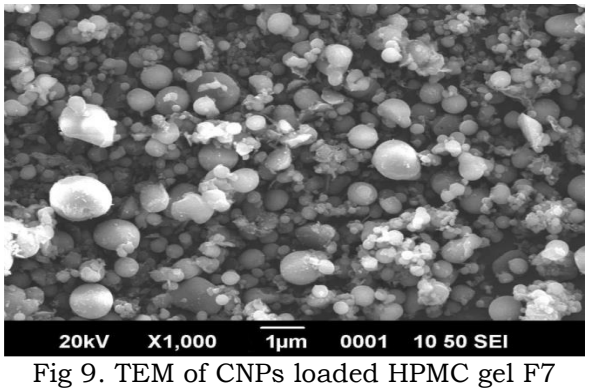


Fig 9. TEM of CNPs loaded HPMC gel F7

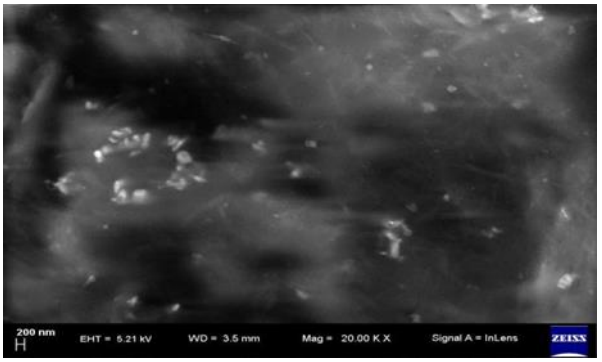


Fig 10. TEM of CNPs loaded HPMC gel F8

Thermal behaviour

DSC thermogram of CNPs loaded Carbopol gels F1 formulation and CNPs loaded Carbopol gels F2 formulation showed the temperature as well as their heat flow values as stated in DSC thermograms. Thermograms shows characteristic energy absorbing peak corresponding to drug/s melting was perceived at 159.24°C for celecoxib. This can be credited to nearness of medication in incomplete crystalline state.

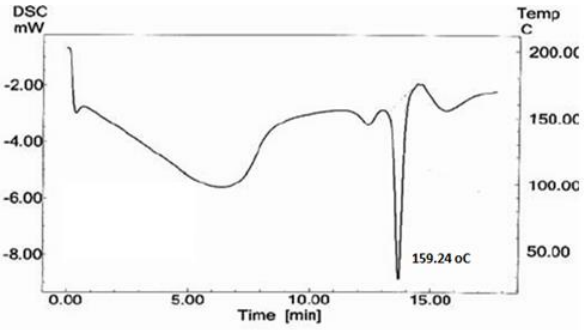


Figure 11. DSC thermogram of CNPs loaded carbopol gel F1

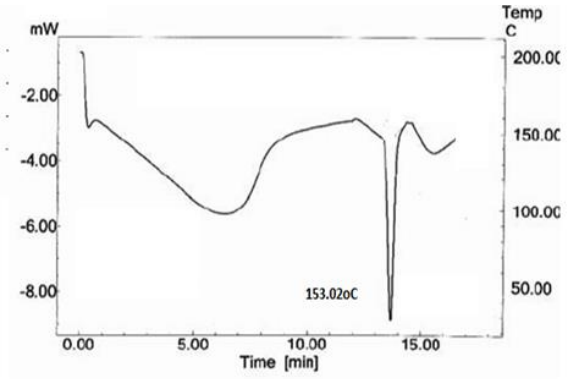


Figure 12. DSC thermogram of CNPs loaded carbopol gel F2

Rheogram

Rheological characterizations of gels were carried out using fixed proportion of carbopol corresponding to rotation speed from 0-100 and 100-0 in downward and upward.

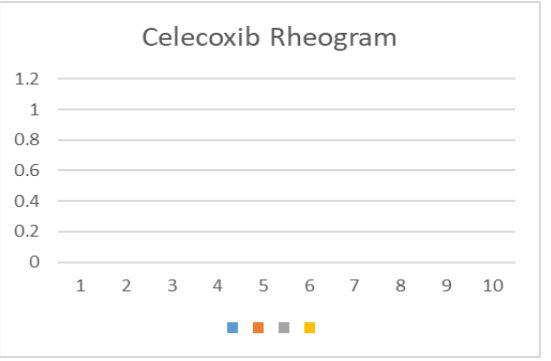


Fig. 13. Rheological behavior of CNPs loaded carbopol gel F1

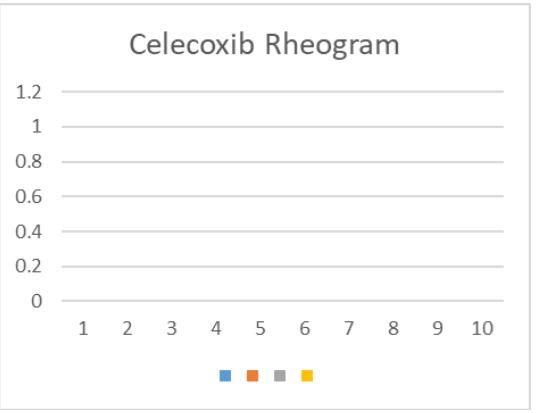


Fig 14. Rheological behavior of CNPs loaded carbopol gel F2.

Occlusion factor

The water flux through the filter paper (simulating skin) providing the measure of the occlusion factor was tested.The results indicating that there is significant prevention of water loss at the end of 48hrs.

Table 7
Occlusion factor of CNPs loaded carbopol gel

Formulation	Occlusion Factor		
	6 hrs	24 hrs	47 hrs
CNPs loaded carbopol gel F1	77.46±0.11	77.11±0.25	77.21±0.30
CNPs loaded carbopol gel F2	76.10±0.54	76.31±0.11	76.89±0.31

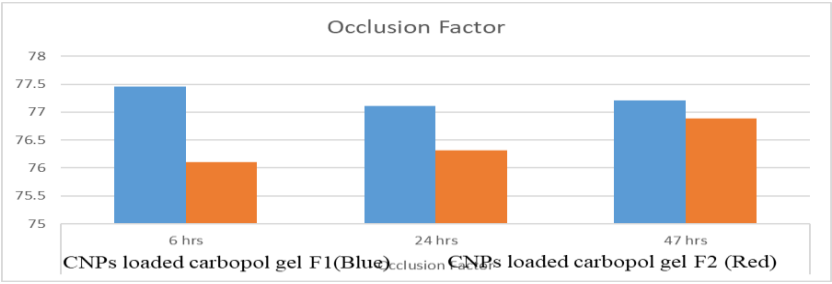


Figure 15. Occlusion factor of CNPs loaded carbopol gel F1 and F2

Spreadability

In the below mentioned table the % of spreadability is given which indication the good spreadability for both gel formulations of Celecoxib.

Table 8
Spreadability of formulation CNPs loaded carbopol gel

Formulations	% Spread by weight
F1	46.23±0.25
F2	47.12±0.30
F3	45.23±0.25
F4	44.15±0.31
F5	45.23±0.27
F6	40.12±0.34
F7	40.23±0.22
F8	41.12±0.31

In-vitro release

CNPs loaded gels formulations were studied in vitro for drug release. For CNPs loaded carbopol gel F1 the maximal drug release was found to be about 92.42 percent respectively.

Table 9
% Drug release of CNPs Loaded Gel

Time Interval (in Hrs.)	Drug Release (%)							
	CNPs Gel F1	CNPs Gel F2	CNPs Gel F3	CNPs Gel F4	CNPs Gel F5	CNPs Gel F6	CNPs Gel F7	CNPs Gel F8
1	9.12	8.11	7.02	6.01	7.12	6.02	6.02	5.01
2	17.45	16.44	15.42	14.41	16.45	15.42	14.40	12.41

3	27.45	26.44	25.16	22.15	26.45	25.16	22.14	20.15
4	32.16	31.15	30.12	28.11	31.16	30.12	28.11	24.11
5	46.58	45.57	43.96	41.95	45.58	43.96	41.95	40.95
6	52.98	51.96	50.14	49.13	51.98	50.14	49.13	44.13
7	60.21	59.22	58.23	56.22	59.21	57.23	54.21	51.21
8	64.26	63.25	62.21	60.22	64.26	61.21	59.20	58.20
9	71.45	70.46	69.15	68.14	71.45	68.15	67.14	68.14
10	81.57	79.53	78.14	77.13	80.57	77.14	76.11	75.12
11	85.66	83.68	82.23	81.21	84.66	81.23	80.22	79.21
12	92.42	89.42	88.21	86.25	89.41	87.21	85.22	83.25

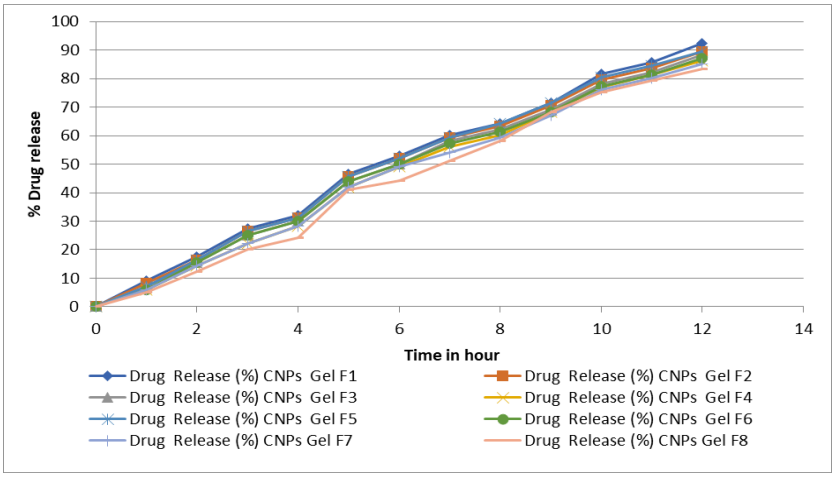


Figure 16. % Cumulative drug release (CNPs) Loaded Gel

Ex-vivo skin permeation and skin deposition studies
Ex-Vivo Skin Permeation Study of DH Containing Batches F1 to F8

From this plot, permeation kinetic parameters such as permeation flux, permeability coefficient and enhancement ratio were calculated. The results revealed that gel containing celecoxib 90% released in 12 hrs.

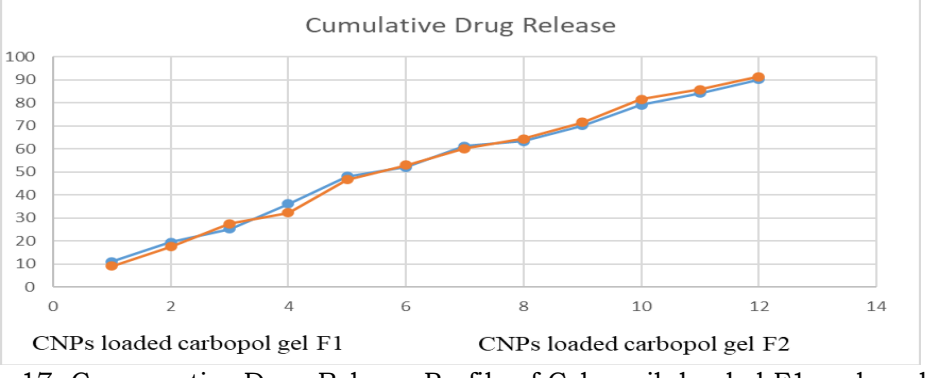


Figure 17. Comparative Drug Release Profile of Celecoxib loaded F1 carbopol gel and Celecoxib loaded F2 carbopol gel

Table 10
Permeation Kinetic parameters of Celecoxib loaded F1 carbopol gel and Celecoxib loaded F2 carbopol gel

Formulation	Transdermal Flux Jss (µg/cm2/hr)	Lag time (h)	Permeability Coefficient (Kp)	Diffusion Coefficient (D) (cm/h×10-8)	Enhancement Ratio
CNPs loaded carbopol gel F1	172.25 ± 0.98	1.31 ± 0.24	1.711×10-5 ± 2.8	0.0284 ±0.02	1.352 ± 0.09
CNPs loaded carbopol gel F2	170.50 ± 0.94	1.29 ± 0.15	1.774×10-5 ± 2.32	0.031 ± 0.20	1.226 ± 0.23

R^2 values of Different Kinetic Models

Table 11
Kinetic Models and Regression coefficient

Sr. No.	Equation	Regression coefficient (r)
1	Zero order	0.991
2	First order	0.705
3	Higuchi	0.978
4	Korsmeyer-Peppas	0.942
5	Hixson Crowell	0.849

In ex-vivo drug release data of gels were subjected to different kinetic models to study the mechanism of drug release from the patch and through the skin. Regression coefficient also suggested that drug release from the patch follow zero order and from the patch drug was release continuously in a controlled manner up to 12 hrs. The correlation coefficient (R2) of Higuchi's model was found to be 0.978 that indicate diffusion occurred. Thus, the selected CNPs loaded carbopol gel F1 followed zero order.

Stability of CNPs loaded carbopol gel F1

CNPs loaded carbopol gel F1 was subjected for stability studies. It was done for 3 month at two temperatures. The temperature was 25 ± 2^{0}C and 40 ± 2^{0}C respectively. In this relative humidity was 75%. A sample of this gel was withdrawn after complete of every 1 month. The physiochemical parameter was studied for this sample. The result of this study showed that gels are pretty stable at both of the temperature used for stability.

Storage condition at 40^{0}C ± 2^{0}C / 75% RH ± 5%

The findings of Accelerated stability study demonstrated that the values of pH and viscosity at different intervals were similar.

Description

Table 12
Color changes in selected formulation at accelerated condition

Gel	Observation	Inference
CNPs loaded carbopol gel F1	No changed of color	Complies with the stability condition

Table 13
Viscosity and pH of CNPs loaded carbopol gel F1 at accelerated temperature

Parameters	**Initial (0 days)**	**30 days**	**60 days**	**90 days**
Viscosity(cp)	29542 ± 4.19	30214 ± 3.28	30178 ± 2.62	29857 ± 3.47
pH	6.7 ± 0.12	6.9 ± 0.63	6.7 ± 0.42	6.8 ± 0.53

Values are mean ± S.D

Room Temperature (25^{0}C ± 2^{0}C / 60% RH ± 5%)

The findings of stability study at room temperature of CNPs loaded carbopol gel F1 demonstrated that the values of pH and viscosity at different intervals were similar.

Description

Table 14
Color changes in selected formulation at room temperature

Gel	Observation	Inference
CNPs loaded carbopol gel F1	No changed of color	Complies with the stability condition

Table 15
Viscosity and pH of CNPs loaded carbopol gel F1 at RT te0mperature

Parameters	Initial (0 days)	30 days	60 days	90 days
Viscosity(cp)	30215 ± 4.19	29684 ± 3.28	30214 ± 2.62	29015 ± 3.47
pH	6.8 ± 0.12	6.7 ± 0.63	6.9 ± 0.42	7.1 ± 0.53

Values are mean ± S.D

Summary and Conclusion

Topical delivery systems comprising non-steroidal anti-inflammatory drugs like Celecoxib are being developed and evaluated in the current study. According to BCS Class 2, both medications have the potential to increase solubility by considering present formulation, which might circumvent the potential pharmacokinetic and dynamic limitations associated with conventional drug administration. Zeta potential and percent yiled were used to establish an optimised recipe for the formulation of Celecoxib nanoparticles. After analysing

the data, it's clear that the nanoparticles in Celecoxib trial Batch 3 are more stable than those in the other trial batches, at least when looking at the percent yield figures. It is therefore regarded as the best trial batch for Celecoxib. The nanoprecipitation method was used to make the nanoparticles, and they were then characterised using the following techniques: Zeta potential analysis, Zeta sizer particle size determination, scanning electron microscopy, drug entrapment efficiency, nanoparticle production yield, in-vitro drug release analysis, transmission electron microscopy, and nanoparticle stability.

The particle size of the nanoparticles was found to be between 1 and 100 nanometers. Nanoparticles were mostly round in shape, according to the results (CNPs). Celecoxib nanoparticles measured 46.21 nm in size. There were results showing that the nanoparticles encapsulating Celecoxib had an entrapment effectiveness of 88.12% for CNPs respectively. Polymer content was found to be inversely proportional to yield. As a result, the solution was difficult to pour and clung to the beaker's wall because to an increase in polymeric concentration. A dialysis bag approach was used to assess in-vitro release. All formulations of nanoparticles containing Celecoxib was tested for drug release in vitro. The maximum drug release for CNPs was determined to be 92.25 %. The particle size ranges from 30 to 60 nanometers, with an average of 45 nanometers, according to a TEM research. It is clear that the particles are round. In order to rule out the presence of any other types of metal oxide, the same sample was examined using electron diffraction. The nanoparticle stability was assessed by storing the best formulation (CNPs) at 4°C in a stability chamber for three months. Absorption spectroscopy was used to determine the stability of the CNPs nanoparticles after 8 weeks. The nanoparticles did not cluster, suggesting that they were more stable, over the storage time.

The carbopol and HPMC gels coated with nanoparticles were developed and tested for. pH, Gelling capacity, drug entrapment efficiency, thermal behaviour, occlusion factor, rheogram, and spreadability may all be measured in the lab. Tests on rabbits for ex-vivo skin penetration and skin deposition investigations was carried out with in vitro release. The formulation with a basic pH produces gel, indicating that the pH Value of the formulations was judged to be acceptable. Measurement of the rate and amount of gelation is referred to as "gelling capability." The gel-to-gel transition formulas are suited. Over time, the gel should not dissolve or erode because of its integrity has been preserved. 87.34 percent for Celecoxib-loaded carbopol gel were reported for CNPs respectively, demonstrating a greater efficacy of drug entrapment Both CNPs loaded carbopol gels were shown to have the same temperature and heat flow values in their DSC thermograms. Celecoxib melting points were found to be 159.24°C respectively, in thermograms. That's because of a drug that's still in an unfinished crystalline state being so close to where you are.

In vitro occlusion tests were used to evaluate the gel compositions' occlusive properties. Gels were characterised rheologically using carbopol proportions set to rotation speeds ranging from 0-100 to 100-0 in both directions. Using filter paper (simulating skin), the occlusion factor was measured in terms of water flow through the filter paper. At the end of 48 hours, there was a considerable reduction in water loss. From the perspective of the patient, spreadability is a

crucial consideration. It's a sign of how simple it is to use for Celecoxib spreadability percentage was determined to be high enough. A dialysis bag approach was used to assess in-vitro release. Drug release data from gels was analysed in ex-vivo using several kinetic models in order to better understand how the patch and skin release drugs. There is evidence from the regression coefficient that the patch's medication release follows zero order and continues for up to 12 hours in a regulated way. Correlation coefficient (R2) was determined to be 0.978, which indicates diffusion. As a result, there was no order to the CNPs gel batches that were picked. All gel formulations must be able to withstand a wide range of temperature and environmental conditions over the course of their life span. They found that both Optimized CNPs loaded carbopol gels F1 and F2 were quite stable at both temperatures employed in this investigation.

Conclusion

In present study drug samples were investigated under preformulation studies. For development of nanoparticles containing celecoxib by nanoprecipitation method is used. The formulated nanoparticles were evaluated for different parameters such as zeta potential, particle size, % entrapment efficiency, in-vitro drug release, SEM and stability studies. The formulated nanoparticles were formulated as gel using carbopol and HPMC. Formulated carbopol and HPMC gels were characterized for appearance, spreadability, pH, viscosity, drug content, consistency index and ex-vivo drug release, in-vitro drug release. Formulated gels were evaluated for in-vitro studies (skin irritation and skin permeation). Further evaluated for stability studies as per ICH guidelines, results of stability studies reveled that the CNPs loaded Carbopol Gel F1 was found to be stable.

References

1. Deore BV, Mahajan HS, Deore UV. Development and characterization of sustained release microspheres by quasi emulsion solvent diffusion method. Inter. J. of ChemTech Res.2009; 1(3): 634-642.
2. Eliana Maria, Barbosa Souto. SLN and NLC as Drug Carriers of Clotrimazole for Hydrogel Topical Formulations. Faculdade de Farmácia. 2003.
3. Elisabetta Esposito, Paolo Mariani, Laura Ravani, Catia Contado, Mattia Volta, Simone Bido, Markus Drechsler, Serena Mazzoni, Enea Menegatti, Michele Morari, Rita Cortesi. Nanoparticulate lipid dispersions for bromocriptine delivery: Characterization. Eur Journ. Pharm Biopharm. 2012; 80: 306-314.
4. European Medicines Agency. Evaluation of Medicines for Human Use. London, 20 November 2008. Doc.Ref.:EMEA/387246/2009.
5. Frank HJL, Pardridge WM. A direct in vitro demonstration of insulin binding to isolated brain microvessels. Diabetes. 1981;30:757-761.
6. Garner C, Brown PD. Two types of chloride channel in the apical membrane of rat choroid plexus epithelial cells. Brain Res. 1992; 591:137-145.
7. Gattefosse. Technical literature: Compritol 888 ATO., 2000.
8. Gattefosse. Technical literature: Labrasol., 2004.
9. Gattefosse. Technical literature: Precirol ATO 5, 2004.
10. Gowda D. V. and Karamsetty V. M. et al. 2016, Nano structured lipid carrier based drug delivery system. J. Chem. Pharm. Res., 8(2):627-643.

11. Hajare Ashok A., Mali Sachin S. Lipid nanoparticles: A modern Formulation Approach in Topical drug Delivery Systems. JADD, January– February 2014. 1(1): 30-37.
12. Hanin I, Pepeu G. eds. Phospholipids: Biochemical, Pharmaceutical and Analytical Considerations. New York. Plenum Press, 1990.
13. Hawkins BT, Thomas PD. The Blood brain barrier / neurovascular unit in health and disease. Pharmacol Rev. 2005; 57: 173-185.
14. Hend Mohamed Abdel Bar et al, 2016. Targeted and synthetic nanotubies brain compounds conducted by members of the systems active nervous system system Clonazepam. Journ. Pharm Pharm Sci; 16(3) 456 – 469.
15. Hu P et al. Acta Pharmacol Sin. 2000. 214:306-308.
16. Jin Ki Kim, Jeong Sook Park, Chong Kook Kim. The development of Bipolar Nanoparties through the Isreconozole control and control of the injection. Int Journ. Pharm 2010. 383: 209-215.
17. Joseph Schwarz, Michael Weisspapir. Pharmaceutical composition for parenteral administration of idebenone. 2010. U. S. Patent No. 20100130619A1.
18. Joseph Schwarz, Michael Weisspapir. Pharmaceutical composition for parenteral administration of Idebenone. 2010. U. S. Patent No. 20100130619A1.
19. Khandadi P, Syed MA, Goparaboina S, Veerabrahma K. Provide direct emulsions on the intenavir submicron fat tip. Europ. Journ. Pharm Sci. 2011; 42:423-432.
20. Kotla Niranjan G. et al. 2016, A new analysis of drug release tests from the nanosailleval polysaccharide delivery system, the sharing method of other colonists. Int. J. of Nanomedicine; 11 1089–1095.
21. KV Voronkova, MN Meleshkov. Newbine (IDBN) is used to cure mental illness and a weak memory without depression. Neuroscience and Behavioral Physiology. 2009. 39(5): 501-506.
22. Liang Yan et al. 2017. The combined turbine pressure for the joint care of adipose skeletons of biological capacities to integrate polyclinic arms with pure silk. Int. J. of Nanomedicine:12 1699–1715.
23. Lookatillino is Info content /notes sesquicentennial history Woodstock 2002.; page-188.
24. M Cirri, M Bragagni, N Mennini, P Mura. Developing a new drug system that includes cyclodoxygen drugs on nanostructured fat carrier that is sent by poisonous carotrophones. Europ. J. Pharm Biopharm. 2012; 80: 46-53.
25. Mansoor S. Sheik et al. 2012, The problem of polynalinean nanostructured (water), through the use of cheetahsons, through chemicals, between interfaces. Der Chemica Sinica, 3(3):683-688.
26. Michael Bodmer, Pierre Vankan, Manfred Dreier, Klaus W Kutz, Jurgen Drewe. Pharmacokinetics and Liped Meabolism in mens affairs. Europ. J. Clin. Pharmacol 2009; 1-9.
27. Kardina, I., Ramadany, S., Sanusi B, Y., Made, S., Stang, S., & Syarif, S. (2020). Children's midwifery learning media application about android-based rough motor development in improving midwifery student skills. International Journal of Health & Medical Sciences, 3(1), 146-152. https://doi.org/10.31295/ijhms.v3n1.300

28. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, *6*(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949