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FORMULATION AND EVALUATION OF VALSARTAN NANO-EMULSION FOR ITS TRANSDERMAL EFFICACY

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Keywords:

Nanoemulsion, Antihypertensive, Transdermal flux, Stability study, Skin irritation study

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ABSTRACT: Valsartan is a selective angiotensin type I receptor agonist that has been used in the treatment of hypertension disease. The development of a thermodynamically stable and infinitely dilutable nanoemulsion (O/W) of valsartan with surfactant at a low concentration that can enhance its solubility and stability came about as a result of our investigation into the effectiveness of nanoemulsion as a carrier vehicle for Valsartan delivery in this study. Using the water titration methods, by adjusting the ratio of oil, surfactant, and co-surfactant for carrying out the nanoemulsion region, a pseudo-ternary phase diagram was constructed. For each phase, multiple concentrations of oil and surfactant were combined in variable amounts. After gradual titration with water phase utilizing a vortex mixer for each ratio of oil, surfactant, and co-surfactant (Smix), the translucent and transparent nano-emulsions were discovered. The optimization of surfactant and cosurfactant was done using a pseudo ternary phase diagram which were showing large covered area. According to the study, particle size, polydispersity index, zeta potential, physical stability, and shape were examined during the nanoemulsion evaluation. To examine the *in-vitro* permeability of valsartan nanoemulsion, a modified vertical Franz diffusion cell and a diffusion membrane were employed. In a nanoemulsion, valsartan was more stable. The penetration flow of valsartan from the hydrophilic matrix nanoemulsion was also greatly enhanced by nano emulsification.

INTRODUCTION:

Hypertension: Hypertension is among the most common causes of chronic disease in Europe, involving around 27% of the adult community. Hypertension is expected to impact up to 1 billion individuals around the world, resulting in 7.1 million deaths per year. It is estimated that 1% of high-risk patients will experience a high blood pressure at some point, and that hypertensive incidents report for 25% of all clinical visits to the clinical stage of an emergency unit (EU), with hypertensive incidents being identified in one-third of such cases [1].

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According to the National Health and Nutrition Examination Survey (NHANES), there are larger improvements in high blood pressure identification and prescription among younger adults, as well as a lower rate of blood pressure management amongst young adults, particularly among young men [2]. There are no clear guidelines for controlling blood pressure in women and men currently.

Valsartan: Valsartan, an angiotensin II receptor antagonist, is commonly used in clinical practice to the treatment of high blood pressure. Its poor water solubility results in low oral bioavailability. Because of its low water solubility, it has a low oral bioavailability. Valsartan has a limited oral bioavailability due to its solubility and dissolving restrictions. This drug has a pharmacokinetic property of about 23% and is easily absorbed after oral delivery. Blood vessels are relaxed by valsartan, which lowers blood pressure.

Blood and oxygen are given to the heart in greater quantities when blood pressure is reduced [3]. When taken alone or in combination with other drugs to treat high blood pressure, valsartan is effective and well tolerated. Valsartan is sold under the brand names Diovan, Entresto, Exforge and Byvalson. The most frequent adverse effects for those taking valsartan to treat hypertension, according to the pharmaceutical insert, are headache, dizziness, tiredness, flu symptoms, and stomach discomfort. Valsartan interacts with a variety of medications, including: Certain diuretics, Ritonavir, Rifampin [4].

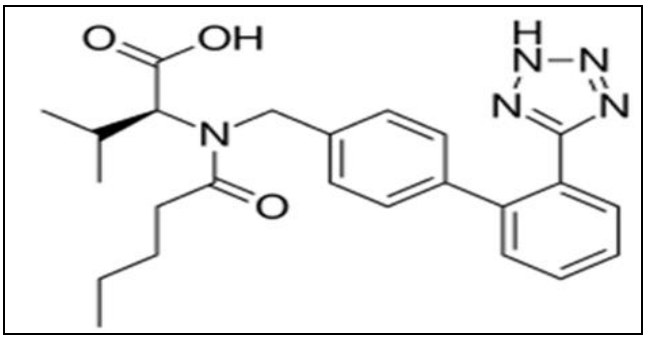


FIG. 1: CHEMICAL STRUCTURE OF VALSARTAN DRUG

Transdermal Drug Delivery System: Human civilizations have used many compounds on the skin for thousands of years as both aesthetic and therapeutic agents. However, the usage of drugs delivered through the skin did not begin until the 20th century.

A transdermal drug delivery system, sometimes called a transdermal patch or skin patch, is a medical treatment delivery method that applies a particular amount of medicine to the systemic circulation. When delivering medicinal chemicals for systemic effects through the human skin using an adhesive patch, the morphological, biophysical, and physicochemical properties of the skin must be considered [5]. Transdermal patch is the first transdermal patch that the FDA authorized in 1981.

Transdermal drug delivery systems (TDDS) are topically placed "patches" that transfer a medication over a patient's skin at a predetermined pace for a systemic impact. The stratum corneum, the skin's outermost layer that is relatively impermeable, is the main barrier to the administration of medications topically. Topical ways to provide local or systemic effects can be interestingly replaced by transdermal distribution in the form of a gel or patch. Additionally, it offers several advantages for valsartan, including avoiding first pass metabolism, reducing adverse effects, and permitting steady blood levels over extended periods of time. It can increase patient compliance [6].

Nanoemulsion: The liquid mixture of oil (O) and water (W) that is known as a nano-emulsion is clear or translucent, thermodynamically stable, and contains a substance called surfactant. The range of nanoparticle sizes is 10 to 1000 nm. Oil-in-water (O/W) and water-in-oil (W/O) nanoemulsion are the dispersion of two immiscible liquids stabilized with the help of a suitable surfactant. One of the most cutting-edge, contemporary, and effective methods for creating inorganic particles is the nano-emulsion approach or technology. When two structures are produced, each saturated with traces of a different substance, the oil and water phases are immiscible with it and cannot be distinguished from one another [7]. The dosage forms of nanoemulsion, such as liquids, creams, sprays, gels, aerosols, and foams, as well as the delivery methods, such as topical, oral, intravenous, intranasal, pulmonary, and ophthalmic, are all rather diverse. They are used in the cosmetic and pesticide industries as an aqueous foundation for organic deliverables because they have a better solubilization capacity than straightforward micellar dispersions and more kinetic stability than coarse emulsions. The nanoemulsion process or procedure is one of the newest and most efficient techniques to create inorganic particles [8].

MATERIALS AND METHOD:

Material: Valsartan was purchased in powder from jubilant generic Noida. Carbitol, Cween 80 and potassium phosphate monobasic from Sigma Aldrich Limited (New Delhi, India) and are of chemically pure grade. Castor oil (chemically pure grade) was provided by Labchem products. Acetonitrile and potassium dihydrogen phosphate buffers of HPLC grade were purchased from (RFCL limited, India).Water for HPLC was provided by RFCL limited, India. All other solvents and reagents used were of analytical grade.

Preformulation Studies: The following three important phases were included in preformulation research for the generation of nanoemulsion:

Selection of appropriate components, Investigations of intrinsic drug solubility and a study of drug-excipient interactions [9].

Solubility Analysis: Solubility analysis of Valsartan was determined in the Castor oil, IPM, Oleic acid, Olive oil, Glycerol, Tween 20, Span 20, Cween 80, Ethanol, Methanol, Benzene, DMSO, Toluene, Propanol, Butanol, Octanol and Carbitol [10].

Melting Point Determination: Valsartan was reduced in size to a very fine powder and dried at temperatures much below its melting point. It was then inserted into a glass capillary tube. A sufficient amount of dry medication powder was used to create a compact column with a height of 4-6 mm. This capillary tube, together with the thermometer, was then placed into the HICON melting point apparatus (Ningbo Hicon Industry Co. Ltd. Zhouxiang, China). The temperature at which the drug melted was recorded.

Screening of Excipients, Oils, Surfactants and Cosurfactants: Due to regulatory limits and pharmacological acceptability of excipients in terms of toxicity concerns, NE component selection is critical. As a result, significant care was taken to choose components that were both edible and GRAS (Generally Recognized as Safe).

In nanoemulsion formulation, the following important components are typically used: Oil phase, Primary surfactant and Secondary surfactant (co-surfactant). When screening components, it is crucial to consider the solubility of poorly soluble drugs in surfactants, co-surfactants, and oils. To test the solubility of Valsartan, a variety of oils, including olive oil, oleic acid, triacetin, castor oil, isopropyl myristate (IPM), jojoba oil, babchi oil, labrafac oil, and soybean oil, were used with cremophor EL.

In tiny vials, 2 ml of different oils were mixed with an excess of the medication, firmly sealed, and agitated regularly for 1 week at 37±0.5°C. The samples were then centrifuged for 10 minutes at 10,000 rpm. The supernatant was divided, filtered, and solubility was evaluated after suitable dilution with methanol. For detection of suitable surfactant and cosurfactant, the solubility of Valsartan was determined in several surfactants including carbitol, Tween 20, Tween 80, labrasol and combination of Cween 80: carbitol [11].

UV-Visible Spectroscopy of Valsartan: On a calibrated digital weighing scale, 10 mg of medication (Valsartan) was carefully weighed and transferred to a 100 ml volumetric flask. To dissolve the medication, a small amount of methanol was added. Using methanol to generate a 100g/ml stock solution, the volume was increased to 100 ml.1 ml of the above-mentioned solution was pipetted into a 10 mL volumetric flask, and the volume was increased to 10 ml with methanol. The absorbance maxima were found using a Shimadzu UV-1700 double beam UV-visible spectrophotometer [12].

HPLC (High Performance Liquid Chromatography):
HPLC Analysis of Valsartan:

Column: (5µm particle size, 10cm).

Mobile Phase: A mixture of 65% potassium dihydrogen phosphate buffer (0.025M, pH=6.0): 35% acetonitrile (an isocratic technique).

Flow Rate: 1.5 ml/min.

Detection: 249 nm.

Run time: 10 min.

Retention time: 3.566 min.

In Methanol: 10 mg of Valsartan was dissolved in 100 ml of methanol (100µg/ml) and different dilutions of 1-10 µg/ml was prepared after appropriate dilution with methanol. The samples were filtered using a 0.45µm filter and analyzed using HPLC at 249 nm.[13]

Fourier Transform Infrared spectroscopy (FTIR): The Valsartan sample was FTIR spectroscopically analyzed using the Potassium Bromide Pellet technique. A hydraulic press was used to transform the 5 mg of medication into a pellet after mixing it with 1:1 potassium bromide. The spectra were acquired by scanning the pellet at wavelengths of 4000 and 400cm^{-1} [14]. FTIR (Perkin Elmer) spectral data were obtained to detect any chemical interaction between Valsartan and Carbopol 934.

Development of Pseudo Ternary Phase Diagram: Oil phase Castor oil was chosen as the starting point for solubility investigations. Surfactant and cosurfactant were chosen as Cween 80 and carbitol, respectively. As an aqueous phase, distilled water was employed. Pseudo ternary phase diagrams were created using the water titration technique to determine the nanoemulsion phase (spontaneous emulsification method). Different weight ratios of surfactant and cosurfactant (Smix) were used (1:0, 1:1, 1:2, 1:3, and 1:4) these surfactant mixes were chosen in order of increasing cosurfactant content in relation to surfactant.

Oil and a particular surfactant combination were combined in varied ratios for each phase diagram. Fourteen distinct oil and Smix combinations (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 3:7, 1:2, 4:6, 5:5, 6:4, 8:2, and 9:1) were created to get the highest possible ratio.

For each weight ratio of oil and Smix, slow titration with aqueous phase was performed using a vortexer and eye inspection for translucent and clear nanoemulsion. A visual observation was performed and documented after each addition of the aqueous phase to the oil: Smix combination [15].

The pseudo ternary phase diagrams were created using the aqueous titration method. The aqueous phase was gradually titrated with each oil and Smix combination. Water concentrations ranged from 5-95 percent of volume at 5-percent intervals when the amount of water delivered was varied. The different percentages of oil, Smix, and water used in the aqueous titration process are provided for the purpose of constructing a phase diagram. A visual inspection was conducted and recorded after each 5% addition of water to the oil and Smix mixture. Visual observation was carried out using the following criteria:

1. It was classed as an o/w nanoemulsion (NE) if clear and readily flowable o/w nanoemulsion (NE) were formed during the addition (NE).
2. The translucent gel is referred to as NE gel.
3. If it had a milky or foggy appearance or phase separation, it is labeled as Emulsion.
4. Emulgel was the name given to milky gel.

Formulation Development:

Step 1: Screening of different oils on the basis of inherent solubilizing property.

Step 2: Screening Surfactant and Co-surfactant by miscibility and HLB value.

Step 3: Preparation of Smix (Surfactant and Cosurfactant) ratio 1:1, 1:2 and 2:1.

Step 4: Mixing oil with particular surfactant mixture ratio (Smix) in different ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 (oil: Smix).

Step 5: Titrating each (oil: Smix) with the aqueous phase from 5% to 95% of total volume, in 5% increments. Visual observation to be done on each addition.

Thermodynamic Stability Study:

Freeze thaw Cycle: Placebo formulations were carefully kept at -20^{0}C for 24 hours in a deep freezer. The NE was then removed out of the deep freezer and placed in a room temperature storage container. These formulas returned to their original condition within 5-8 minutes. 3-5 cycles were completed in total.

Centrifugation Research Analysis: Centrifugation tests were carried out after the thaw cycle method. They were spun for 15 minutes at 10000 rpm in a centrifuge in this study, and the formulations were determined to be stable and free of turbidity [16].

Characterization of Nanoemulsion:

Percentage Transmittance: A Shimadzu UV–vis spectrophotometer was used to determine the percent transmittance of the generated NE formulations. Before testing at 249 nm, the formulation was diluted 100 times in water.

Determination of Zeta Potential: Electrophoretic mobility was used to analyse the surface charge of the emulsions (EM). A mixture of 20 liters of sample and 40 liters of DDW was used for all measurements. The combination was then adjusted with a NaCl solution to a conductivity of 50 S/cm and evaluated using a zeta potential measuring equipment (Zetasizer-1000 HAS from Malvern Instruments in the UK: PCS) [17].

Viscosity Determination: The viscosity of the generated nanoemulsion compositions without dilution was evaluated using an R/S CPS plus Rheometer spindle # C 50-1 at 250.5°C. The programme that was used was RHEO3000. One cc of the formulation was used for the viscosity test. For a single run, the spindle speed was adjusted at 70 rpm and the temperature to 250.5°C. It took 50 minutes to perform the surgery. The spindle diameter was 50 mm, and the shear rate was 413 per minute [18]. The following parameters were set once the procedure had been improved:

TABLE 1: SPECIFICATION SET IN VISCOMETER FOR DETERMINING VISCOSITY

Sample	0.5 gm
Probe speed (rpm)	30 rpm
Data Interval (min)	60 second
Loop Start	60 second
Wait period	30 minute
Temperature	25±0.5 °C
Shear rate (1/sec)	60

Refractive Index: The refractive index of chosen formulations was measured using an Abbe refractometer. To standardize it, Castor oil was used.

Determination of Partition Coefficient: Excess valsartan was dissolved in a 10 mL combination of n-octanol and water (1:1) in a separating funnel to measure the partition coefficient. To establish equilibrium, the system was agitated intermittently for 30 minutes and then left alone overnight. The two phases were then separated and centrifuged for 15 minutes at 10000 rpm. After centrifugation, Using a UV-Visible spectrophotometer to measure absorbance, the concentration of valsartan in both phases was determined [19]. The partition coefficient is usually estimated using the shaking flask method using the following formula:

$$P_{(O/W)} = C_1(\text{oil}) / C_2(\text{aqueous})$$

Where, C_1= concentration of solute in organic phase

C_2= concentration of solute in aq. Phase

$P_{(O/W)}$= Partition coefficient

Log P = log $_{(O/W)}$

***In-vitro* Release Study:** The dialysis membrane employed in this study was cellulose membrane (Sigma, USA). It had a 60 mL/ft capacity, an average flat width of 2.5 mm, and a diameter of 16 mm. The molecular weight limit was set at 12000 g/mol.

The *in-vitro* release of valsartan from the valsartan solution was determined using the dialysis method. 4 mL of each formulation in a dialysis bag (MWCO 3500 Da) were dissolved in 40 mL of PBS with 0.1% Cween-80.

The dialysis bag was swirled at 100 rpm at 37 °C. At the predetermined intervals (0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, and 72 hours), 1 mL of the sample was taken out and the same volume of fresh medium was added. The concentration of curcumin was determined using the HPLC method.

***Ex-vivo* Permeation Study:** For the permeation studies, a vertical type Franz diffusion cell was manufactured by a local fabricator. It had a water jacket that kept the assembly temperature at 37 ± 0.5^{o}C. It was made up of two half cells, the donor compartment on top and the receiver compartment on the bottom (body). The diffusion area between 2 half cells was 3.0096 cm^2 (3 cm^2), and the receiver compartment had a capacity of 20 ml.

The entrance of the diffusion cell's receiver compartment was connected to a water bath, and the jacketed receiver compartment's output was placed in the sink. The flow of the water was adjusted to maintain a temperature of 37±0.5°C in the receiver chamber. The stratum layer corneum faced the giver partition and was wedged between the 2 compartments with the preparation [20].

% Drug release = (conc. (µg ml^{-1}) × Dilution factor × Vol. of release medium (ml)) / (Initial dose (µg)) × 100

***In-vitro* Kinetics Analyses of Nanoemulsion:** The rate constants for zero order and first order were calculated, along with their standard deviation (SD) and coefficient of variation (Cv), for *in-vitro* skin permeation studies after each time interval. The calculations were based on the cumulative percentage of drug that permeated from the skin permeation study.

Zero order rate constant, K_0

$$K_0 = X / t = (\text{Percentage drug permeated}) / (\text{Time (h)})$$

First order rate Constant, K_1

$$K_1 = 2.303 / T \times \log (Co/C)$$

Where, Co = Initial drug concentration.

C = Amount of drug remaining at time (t).

Skin Irritancy Test: The dermis irritancy test was carried out to verify the topical preparation efficacy. The mouse's left ear was treated with a single dose of nanoemulsion, and its right ear with control. The evolution of erythema, wheel or any allergic reaction was thoroughly investigated [21].

Solubility:

TABLE 2: SOLUBILITY OF VALSARTAN IN DIFFERENT OILS, SURFACTANT AND CO-SURFACTANT

S. no.	Oil	Solubility		
		24 hrs	48 hrs	72 hrs
1	Castor oil	In	Soluble	-
2	IPM	In	Slightly soluble	Slightly soluble
3	Oleic acid	In	Slightly soluble	Slightly soluble
4	Olive oil	In	Soluble	-
5	Glycerol	In	In-soluble	In-soluble

TABLE 3: SOLUBILITY OF VALSARTAN IN SURFACTANTS AND CO-SURFACTANTS

S. no.	Surfactant and Co-surfactant	Solubility		
		24 hrs	**48 hrs**	**72 hrs**
1	Tween 20	In-soluble	Soluble	-
2	Span 20	In-soluble	Soluble	-
3	Cween 80	In-soluble	Soluble	-
4	Ethanol	Soluble	-	-
5	Methanol	Soluble	-	-
6	Benzene	In-soluble	In-soluble	In-soluble
7	DMSO	Soluble	-	-
8	Toluene	In-soluble	In-soluble	In-soluble
9	Propanol	Soluble	-	-
10	Butanol	Soluble	-	-
11	Octanol	Soluble	-	-
12	Carbitol	Soluble	-	-

Preformulation tests were carried out to establish the drug's solubility and partition coefficient in order to determine its appropriateness for use in a topical system.

The drug's solubility in several oils was also investigated in order to choose the best oil phase for Nano emulsion formulations.

After evaluating the oils for Valsartan solubility, it was discovered that Castor oil had the highest solubility. As a result, castor oil was selected as the oil phase. Studies have demonstrated that Castor oil can enhance transdermal delivery by creating distinct domains that disrupt the continuity of the multilamellar stratum corneum, resulting in highly permeable pathways. This is achieved by increasing the fluidity of the intercellular lipid barriers in the stratum corneum.

Because there is no substantial variation in medication solubility among these surfactants and cosurfactants, screening surfactants and cosurfactants on the basis of solubility is challenging.

RESULTS:

Authentication of Valsartan: Valsartan was characterized using physicochemical characteristics such as color, smell, taste, and solubility in water and other organic solvents.

Melting point (DSC), UV-spectrophotometry, FTIR, and mass spectrum analysis (m/z value) were performed on the obtained sample and compared to those reported in the literature.

Melting point Determination:

TABLE 4: MELTING POINT OF VALSARTAN FOUND AS FOLLOWS

Reported melting point	117° C
Observed melting point	112° C

Vspectral Analysis:

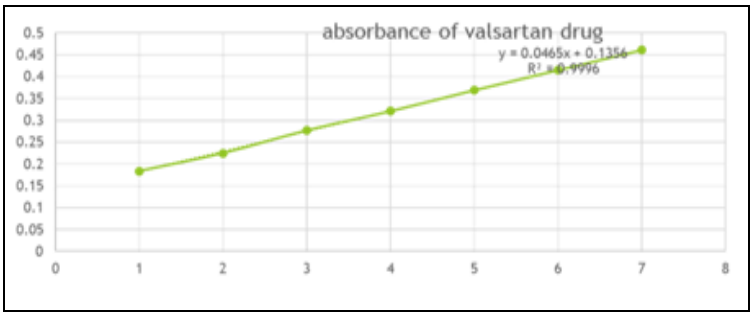


FIG. 2: VALSARTAN CALIBRATION PLOT IN METHANOL (UV)

Inference: The λ_{max} of Valsartan in methanol was 249 nm and was discovered to be the same as the reference spectrum. A Shimadzu Double Beam Spectrophotometer was used to scan the prepared sample between 200 and 400nm (UV 1601). The solution has absorption maxima at 249 nm. With the regression equation y= 0.0465x + 0.1356 and R^2 = 0.9996.

High Performance Liquid Chromatography Method:

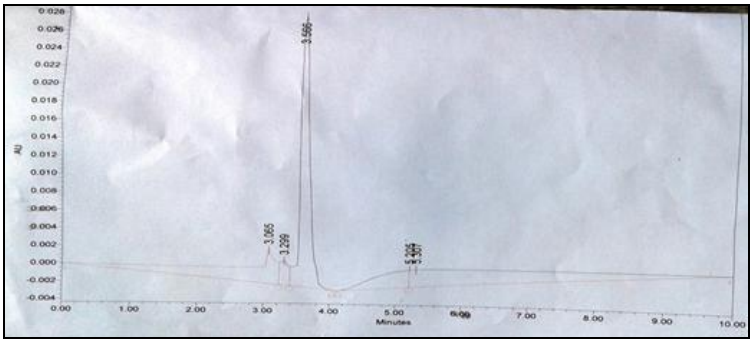


FIG. 3: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF VALSARTAN

TABLE 5: VALSARTAN CALIBRATION CURVE AT PH 7.4 IN PHOSPHATE

S. no.	Conc. (µg/ml)	Peak Area			Mean	SD	%CV=x100
1	2	65039.33	57198.45	61034.7	61091.43	3206.86	5.24
2	4	128979	114391.93	120571.7	121313.87	5985.10	4.92
3	6	172127.3	161582.72	169862.4	167857.47	4538.24	2.70
4	8	229093.3	238774.48	225691.8	231186.19	5548.61	2.39
5	10	284425.7	295966.6	289661.7	290017.66	4724.30	1.62
6	12	34218.2	363158.4	346554.7	352643.76	7471.92	2.11
7	14	417974.7	430351.3	414791.9	421038.96	6717.09	1.59
8	16	462416	477541.5	459871.7	466611.06	7805.86	1.67
9	18	519788	544734.8	517881.7	527468.16	12241.13	2.31
10	20	583773.7	599926.1	581482.4	588394.06	8213.85	1.39

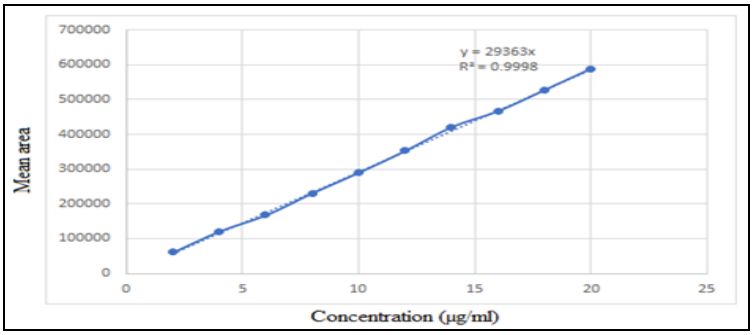


FIG. 4: VALSARTAN CALIBRATION USING THE RP-HPLC TECHNIQUE

Fourier Transformations Infrared Absorption Spectrum (FTIR):

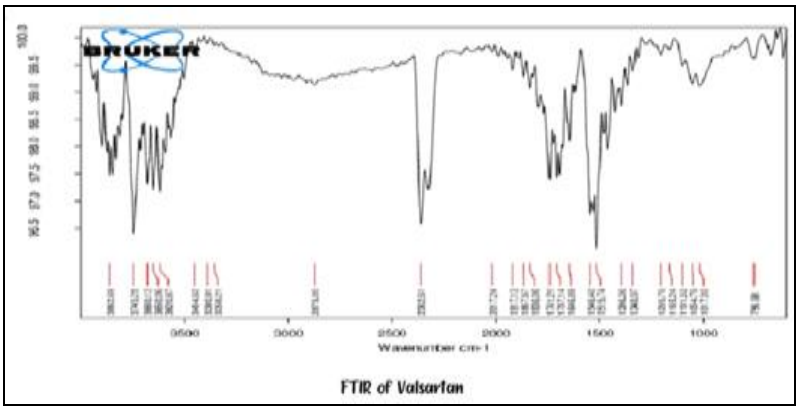
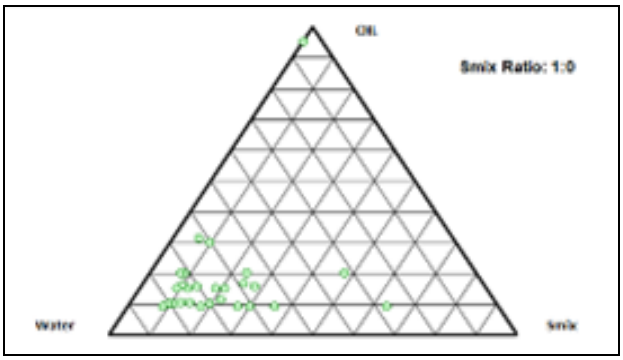
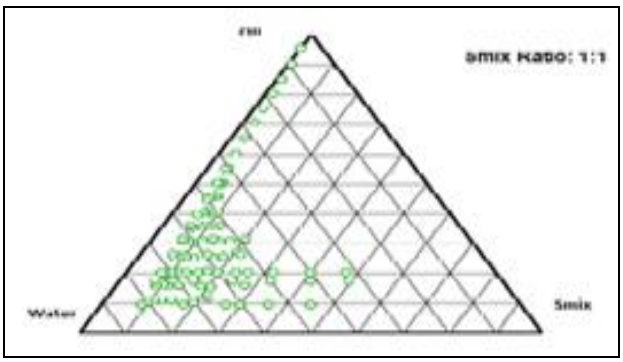


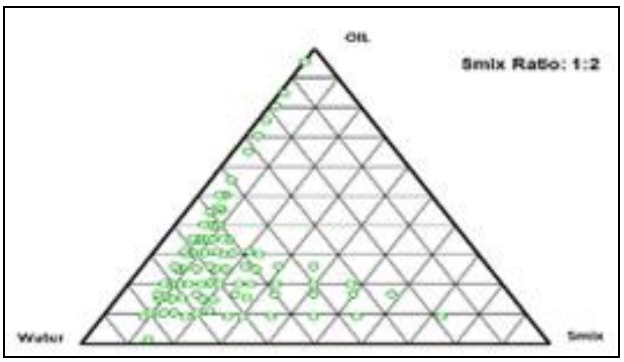
FIG. 5: VALSARTAN'S FTIR DISPLAYS THE DRUG'S DISTINCTIVE BAND AND STRETCHING

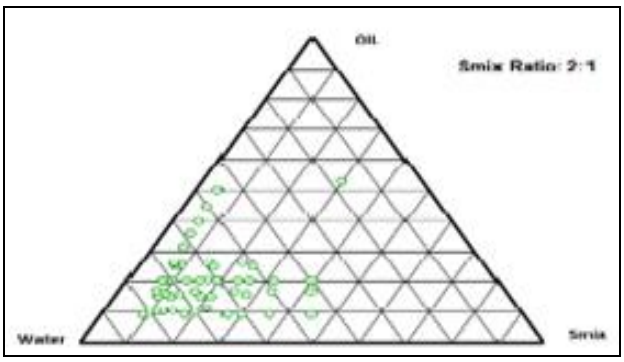
Fourier transformations infrared absorption Spectrum absorption spectrum of Valsartan was acquired utilizing the KBr pellet method and exhibited the characteristics (IR) peaks at approximately one or more of the positions about 3862, 3745, 3680, 3650, 3620, 3454, 3390, 3358, 2875, 2362, 2017, 1867, 1741, 1546, 1396, 1205, 1101, 1054, 1017 and 758 .

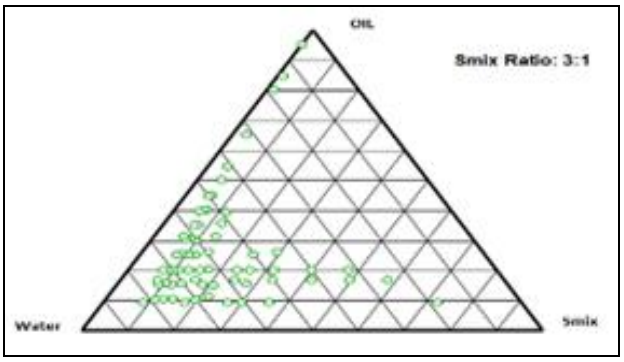
Development of Nanoemulsion by using Phase Tertiary Method:











Thermodynamic Stability of Nanoemulsion: All of the formulations had good physical stability, as demonstrated by stress tests that included centrifugation, freeze-thaw cycles, and heating-cooling cycles.

Valsartan was discovered to be stable after three months, with recovery rates of >97% for all the chosen formulations. Over the course of three months, there was no discernible change in the formulations' refractive indices' mean values (data not shown).

In light of this, it can be said that the Nanoemulsion formulations were both physically and chemically stable.

Characterization of Nanoemulsion:

Viscosity: The viscosity of the resulting nanoemulsion is measured by concentric cylinder Brookfield viscometer having a viscosity of 82 centipoise.

pH: pH was measured with a pH meter, and 6.95 was discovered.

Refractive Index: Determined using Abbe's refractometer at 25ºC and was found to be 1.463.

Particle size: Utilizing a quasi-dynamic light scattering device, the optimum nanoemulsion particle size was calculated and has been given in **Fig. 6** and **7.**

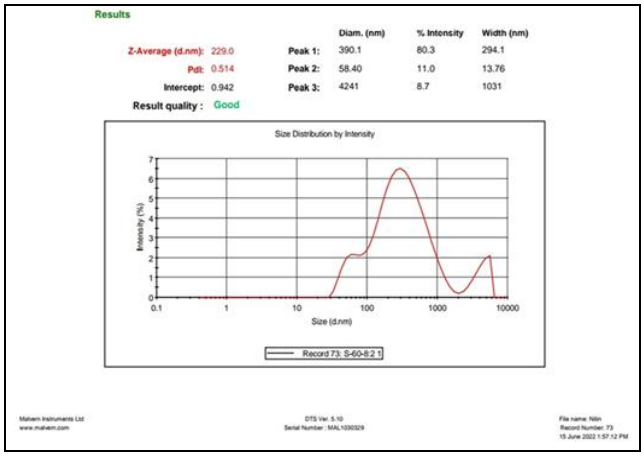


FIG. 6: ANALYSIS OF PARTICLE SIZE (NA)

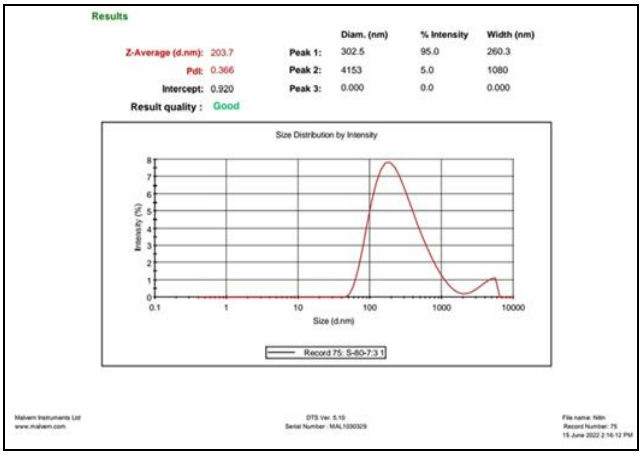


FIG. 7: ANALYSIS OF PARTICLE SIZE (NB)

Zeta Potential:

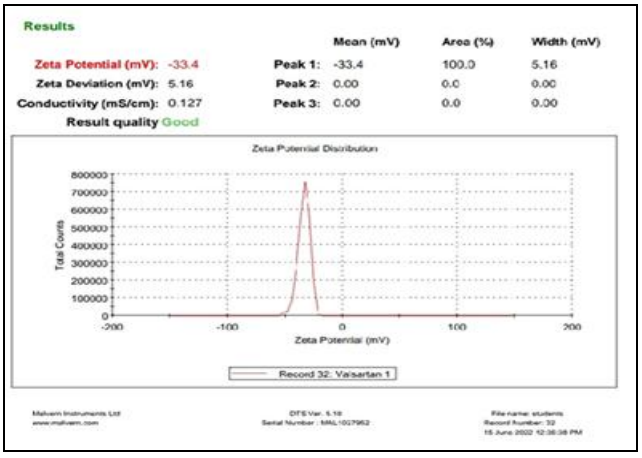


FIG. 8: ANALYSIS OF ZETA POTENTIAL

***In-vitro* Release Profile:**

TABLE 6: VALSARTAN DRUG RELEASE *IN-VITRO* AT PH 7.4 IN PHOSPHATE BUFFER

Time (hr.)	Mean area n=3 (±S.D)	The cumulative amount of drug release	Cumulative percentage of drug release
0	**0**	**0**	**0**
1	228130	644	3.22

2	387538	1094	5.47
3	685454	1935	9.67
4	900478	2542	12.71
5	1365595	3855	19.27
6	1637297	4622	23.11
7	1956821	5524	27.62
8	2251195	6355	31.77
10	2841713	8022	40.11
12	3384763	9555	47.77
24	4967153	14022	70.11

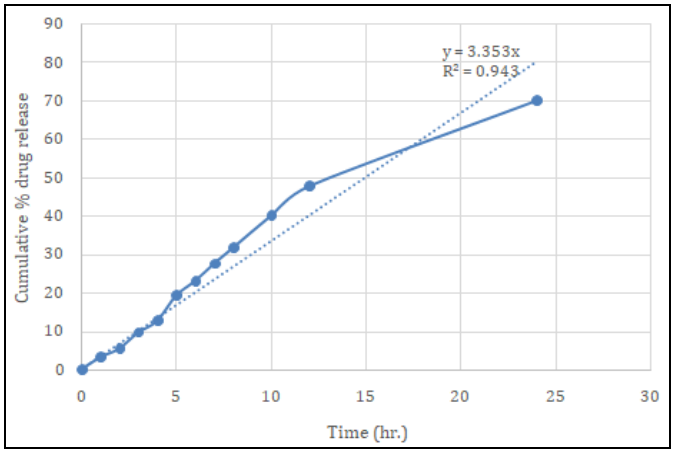


FIG. 9: VALSARTAN NANOEMULSION *IN-VITRO* RELEASE

***In-vitro* Skin Permeation Studies:** In order to evaluate the effect of Nanoemulsion vehicles on skin permeation, various Nanoemulsion formulas were selected from the phase diagrams.

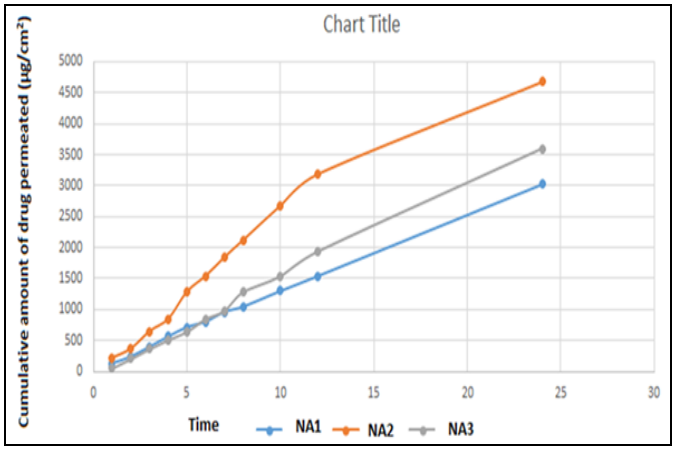


FIG. 10: VALSARTAN NANOEMULSION WITH A 1:1 S/COS RATIO *IN-VITRO* SKIN PERMEABILITY

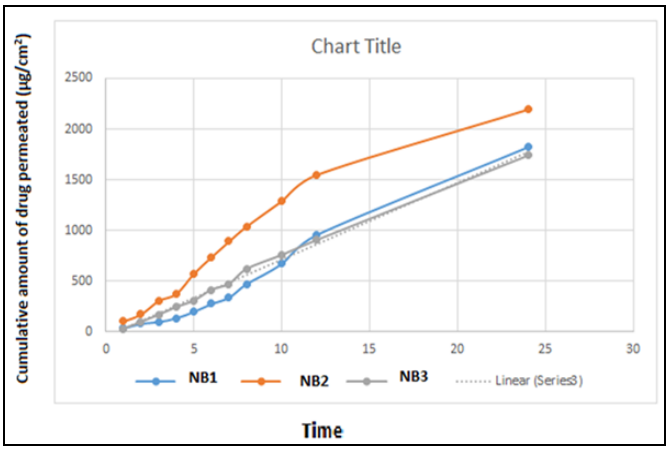


FIG. 11: VALSARTAN NANOEMULSION WITH A 1:2 S/COS RATIO *IN-VITRO* SKIN PERMEABILITY

Ex-vivo Permeation Study:

TABLE 7: *IN-VITRO* SKIN PERMEATION STUDIES

Formulation	Flux (µg/cm^2/h)	Permeability coefficient	Enhancement ratio
NA1	125.4	0.627	4
NA2	204.41	1.022	6.52
NA3	157	0.7859	5
NB1	82.055	0.410	2.61
NB2	97.10	0.485	3.09
NB3	75.94	0.3797	2.42
Control	31.353	0.156	-

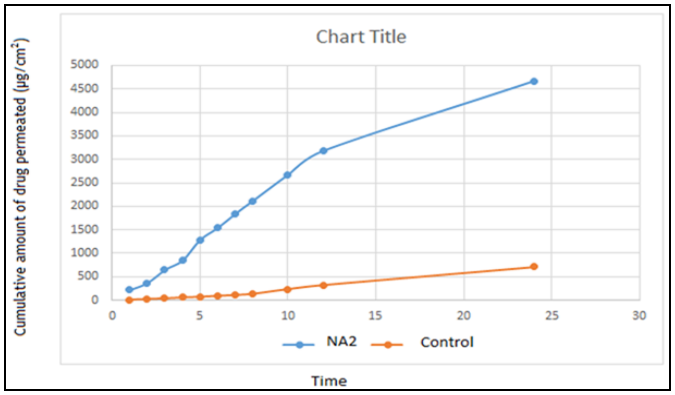


FIG. 12: *IN-VITRO* SKIN PERMEATION OF VALSARTAN FROM NANOEMULSION

In-vitro Release Kinetics Study:

TABLE 8: KINETICS ANALYSIS OF VALSARTAN LOADED NANOEMULSION

Time (min)	Square root of time	Log time	% drug release	Fraction drug release	Log % drug release	% drug remaining	Log % drug remaining
0	0	0	0	0	0	100	2
10	3.162	1	6.3	0.063	0.799	93.7	1.9717
20	4.472	1.301	17.1	0.171	1.232	82.9	1.9185
60	7.745	1.778	27.7	0.277	1.442	72.3	1.8591
120	10.954	2.079	38.1	0.381	1.580	61.9	1.7916
240	15.491	2.380	48.3	0.483	1.683	51.7	1.7134
480	21.908	2.681	67.1	0.671	1.826	32.9	1.5171
720	26.832	2.857	82.2	0.822	1.914	17.8	1.2504
1440	37.947	3.158	90.1	0.901	1.954	9.9	0.9956

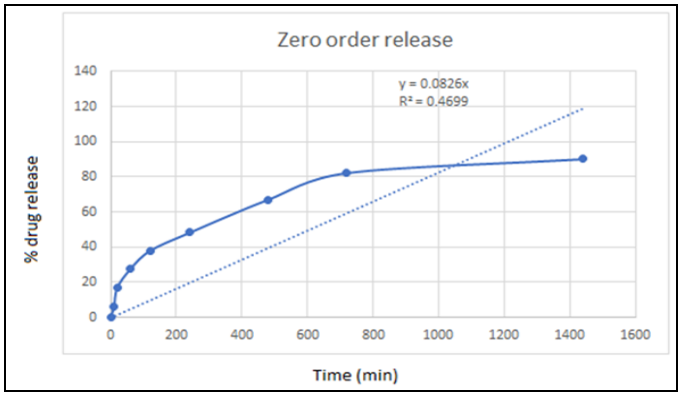


FIG. 13: ZERO ORDER RELEASE KINETICS OF OPTIMIZED NA2 FORMULATION

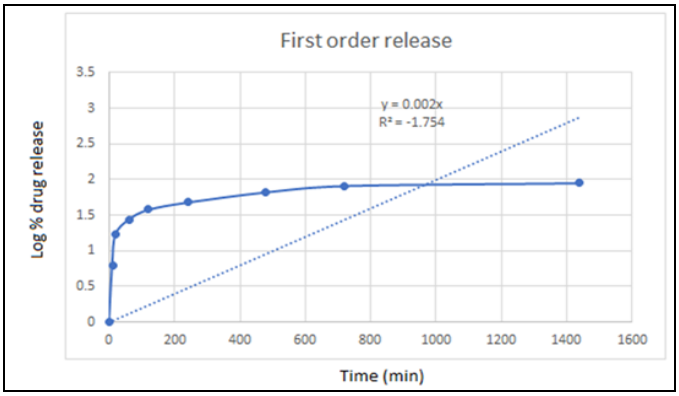


FIG. 14: FIRST ORDER KINETICS OF OPTIMIZED NA2 FORMULATION

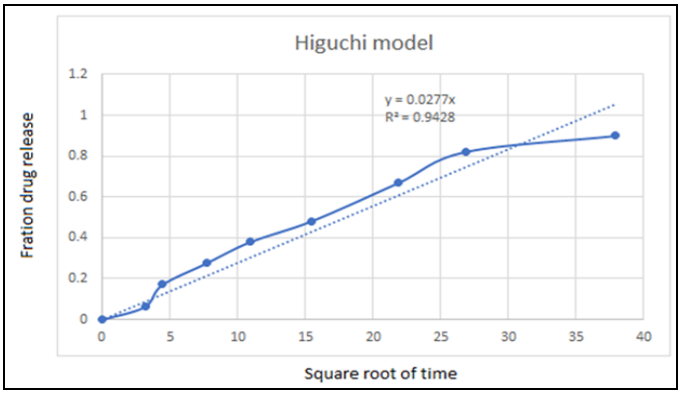


FIG. 15: HIGUCHI'S PLOT OF OPTIMIZED NA2 FORMULATION

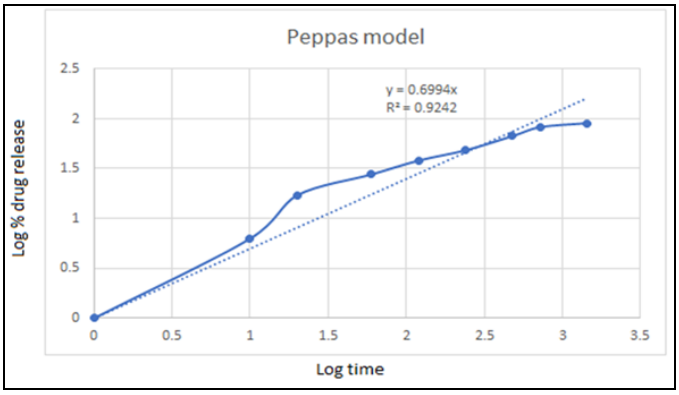


FIG. 16: KORSEMEYERPEPPAS PLOT OF OPTIMIZED NA2 FORMULATION

TABLE 9: MODEL FITTING OF THE DRUG RELEASE PROFILE OF FORMULATION NA2

Release model	R^2
Zero order model	0.4699
First order model	-1.754
Higuchi's square root of time plot	0.9428
Korsemeyer peppas plot	0.9242

Interpretation of Release Model (*In-vitro*): The formulation as stated uses first order release kinetics, and the drug is released by diffusion (first order release model, R^2= -1.754; Higuchi model, R^2=0.9428).

***In-vitro* Kinetics Analyses of Nanoemulsion:** The rate constants for zero-order and first-order were computed along with standard deviation (SD) and coefficient of variation (Cv) after each time interval during *in-vitro* skin permeation studies. The calculations were based on the cumulative percentage of drug that penetrated the skin.

Zero order rate constant, K_o

$$K_o = X/t = \text{(Percentage drug permeated)} / \text{(Time (h)}$$

First order rate Constant, K_1

$$K_1 = 2.303/T \log (Co/C)$$

Where, Co = Initial drug concentration. C = Amount of drug remaining at time t.

TABLE 10: KINETIC PROFILE OF *IN-VITRO* SKIN PERMEATION DATA OF NANOEMULSION

Time (h)	Cumulative % drug permeated	% of drug remaining	Zero order rate constant (K_o)	First order rate constant (K_1) (h^{-1})
1	3.22	96.78	3.22	0.032
2	5.47	94.53	2.73	0.027
3	9.67	90.33	3.223	0.033
4	12.71	87.29	3.177	0.0338
5	19.27	80.73	3.854	0.0426
6	23.11	76.89	3.851	0.0433
7	27.62	72.38	3.945	0.0460
8	31.77	68.23	3.971	0.049
10	40.11	59.89	4.011	0.0511
12	47.77	52.23	3.98	0.054
24	70.11	29.89	2.92	0.050
	Average		3.5347	0.04198
	SD		0.48325	0.009116

Cv was calculated using the following formulation:

$$Cv = (SD \times 100) / Average$$

Thus, Cv (K_o) = 13.671

Cv (K_1) = 21.7

TABLE 11: KINETIC PROFILE OF *IN-VITRO* SKIN PERMEATION DATA OF CONTROL

Time(h)	Cumulative % drug permeated	% of drug remaining	Zero order rate constant (K_o)	First order rate constant (K_1) (h^{-1})
1	.076	99.24	0.076	0.00755
2	2.6	97.4	1.3	0.02625
3	3.16	96.84	1.053	0.03208
4	3.8	96.2	0.95	0.03873
5	3.84	96.16	0.768	0.03912
6	4.16	95.84	0.693	0.04249
7	4.36	95.64	0.622	0.04449
8	4.52	95.48	0.565	0.04622
10	4.72	95.28	0.472	0.04831
12	5.04	94.96	0.42	0.05163
24	5.44	94.56	0.226	0.05591
	Average		0.649545	0.039343
	SD		0.359517	0.013509

$$Cv = (SD \times 100) / Average$$

Thus, Cv (K_o) = 13.671

Cv (K_1) = 21.7

Franz diffusion cells containing rat skin were used to conduct *in-vitro* skin permeation tests on the control and the nanoemulsions. According to the findings of the investigation, formulation NA2 produced good results since the total quantity of medicine penetrated. To determine penetration kinetics, the data from the nanoemulsion's skin permeation trials was further assessed. The permeation investigations yielded the rate constant, coefficient of variation (Cv), and deviation from the mean (SD) for zero and first order rate kinetics for each time period.

In the instance of nanoemulsion, lower Cv was recorded for zero order kinetics and higher Cv was seen for first order kinetics.

Skin Irritation Test Data:

TABLE 12: SKIN IRRITATION SCORES OF FORMULATION NA2

Scores for skin irritation caused by the formulations are measured using two types of scores: A for erythema formation and B for edema formation.

Rats	Intact skin				Abraded skin			
	24 hrs.		72 hrs.		24 hrs.		72 hrs.	
	A	B	A	B	A	B	A	B
1	0	0	1	0	0	1	0	1
2	0	1	0	1	0	1	1	0
3	1	1	0	1	0	2	1	1

The ultimate scores for skin irritation of the formulations (where * indicates the total of A and B from the section, and ** represents the average of all skin readings taken at 24 and 72 hours).

Rats	Intact skin		Abraded skin		Total Average
	24 hrs.	72 hrs.	24 hrs.	72 hrs.	(i)+(ii)
	(i)		(ii)		
1	1	1	1	1	1, 1.75, 1
2	2	1	2	2	
3	0	1	1	2	
					Combined avg.=1.25

The skin irritability test was carried out to verify the formulation's safety. The formulation used is considered safe for human skin and does not cause irritation as long as the skin irritancy score is between 0 and 2. The average score for skin irritancy of the formulation was determined to be 1.25. This number shows that every excipient employed in the formulation was suitable for the topical administration of drugs.

CONCLUSION: Preformulation tests were carried out to establish the drug's solubility and partition coefficient in order to determine its appropriateness for use in a topical system. The drug's solubility in different oils was also investigated in order to choose the best oil phase for nanoemulsion formulations. Valsartan HPLC was performed using Acetonitrile: Potassium Dihydrogen Phosphate Buffer with a flow rate of 1.5 ml/min. Particle size of the optimized nanoemulsion was found to be 229 nm. Zeta potential was found to be33.4 mV. In the Optimizenanoemulsion drug delivery system, the derived kinetic profile demonstrates the existence of two distinct release processes. *In-vitro* valsartan release was rapid at first, then continued steadily. Valsartan's release from the nanoemulsion surface may be responsible for the initial medication to be released quickly. A release order was established using a kinetic analysis of the *in-vitro* release profile of the Optimize nanoemulsion. For valsartan loaded nanoemulsion, the Higuchi model was best fitted. Valsartan nanoemulsion (NA2) was reported to have a maximal skin permeation flux of 204.41 µg/cm^2 /hr. and that of control is 31.353 µg/ cm^2/hr. The enhancement ratio of the optimize formulation was found to be 6.52 as compare to control. The optimize nanoemulsion was found to be non-irritating and safe for human skin with average skin irritancy score of 1.25. The develop nanoemulsiondrug delivery system will be proved to be a potential carrier for delivery of antihypertensive drug valsartan through

transdermal route with increased solubility with increased skin permeation flux and permeability coefficient, with increase enhancement ratio and better efficacy.

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REFERENCES:

1. Hughes AD, Eastwood SV, Tilin T and Chaturvedi N: Antihypertensive medication use and its effects on blood pressure and haemodynamics in a tri- ethics population cohort: Southall and Brent revisited. Frontier in Cardiovascular Medicine 2022; 8: 1-9.
2. Verma N, Rastogi S, Chia chin Y, Siddique S, Turana Y, Cheng H, Sogunuru GP, Tay JC, Teo BW, Wang TD, Fai KK and Kario K: Non- pharmacological Management of hypertension. Journal of clinical hypertension 2021; 1-9.
3. Andresen BT, Anderson SD, Yeon JK & Mireles R: Valsartan 2017; 1–8.
4. Ardiana F, Suciati & Indrayanto G: Valsartan. Profiles of Drug Substances, Excipients and Related Methodology 2015; 40: 431–493.
5. Nandgude T and Tekawade A: Transdermal drug delivery an emerging approach for antipsychotics. Int J Res Pharm Sci 2020; 11(4): 5615-25.
6. Singh NS, Mohammad VY and Khan RA: Non-invasive drug delivery technology: development and current status of transdermal drug delivery devices, techniques and biomedical applications. Biomed Eng 2020; 65(3): 243-72.
7. Garcia CR, Malik MH, Biswas S, Tam VH, Rumbaugh KP, Li W and Liu X: Nanoemulsion delivery systems for enhanced efficacy of antimicrobials and essential oils. Biomaterials Science 2022; 10(3): 633-53.
8. Naseema A, Kovooru L, Behera AK, Kumar KP & Srivastava P: A critical review of synthesis procedures, applications and future potential of nanoemulsions. Advances in Colloid and Interface Sci 2021; 287: 102318
9. Marzuki NHC, Wahab RA and Hamid MA: An overview of nanoemulsion: Concepts of development and cosmeceutical applications. Biotechnol Equip 2019; 33(1): 779-797.
10. Tamashiro FL, Yukuyama MN, Velasco MVR, De Araújo GLB and Bou-Chacra NA: Nanoemulsions containing plant oils: How do they influence hair treatment. Int J Cosmet Sci 2021; 43(2): 136-143.
11. Ashaolu TJ: Nanoemulsions for health, food and cosmetics: a review. Environ Chem Lett 2021; 19(4): 3381-3395.
12. Xu, Hong Y, Chang SL, Chuan LH, Li C, Yu RZ, Si HH, and Xiao YL: Nanoemulsion improves hypoglycemic efficacy of berberine by overcoming its gastrointestinal challenge. Colloids and Surfaces B: Biointerfaces 2019; 927-934
13. Garcia CR, Malik MH, Biswas S, Tam VH, Rumbaugh KP, Li W and Liu X: Nanoemulsion delivery systems for enhanced efficacy of antimicrobials and essential oils. Biomaterials Science 2022; 10(3): 633-53.
14. Majumder KK, Kumar M, Pahwa R, Lamba AK, Shankar R, Tiwari A and Tiwari V: Formulation and Characterization of Floating Tablet Dosage form of Dual Delivery of Drug Curcumin and Berberine Hydrochloride using Simultaneous Estimation by UV Spectroscopy. International J of Applied Pharmaceutics 2021; 306- 310.
15. Jaiswal M, Dudhe R & Sharma PK: Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech 2015; 5(2): 123–127.
16. Romes NB, Abdul WR, Abdul HM, Oyewusi HA, Huda N and Kobun R: Thermodynamic stability, *in-vitro* permeability, and *in-silico* molecular modeling of the optimal Elaeisguineensis leaves extract water-in-oil nanoemulsion. Scientific Reports 2020; 11(1): 1-19.
17. Ardiana F, Suciati & Indrayanto G: Valsartan. Profiles of Drug Substances, Excipients and Related Methodology 2015; 40: 431-493.
18. Naseema A, Kovooru L, Behera AK, Kumar KP & Srivastava P: A critical review of synthesis procedures, applications and future potential of nanoemulsions. Advances in Colloid and Interface Science 2021; 287: 102318.
19. Kumar N, Verma A and Mandal A: Formation, characteristics and oil industry applications of nanoemulsions: A review. JPSE 2021; 206; 109042.
20. Hashemnejad SM, Badruddoza AZM, Zarket B, Ricardo Castaneda C and Doyle PS: Thermoresponsive nanoemulsion-based gel synthesized through a low-energy process. Nat Commun 2019; 10(1): 1-10.
21. Kong M, Chen XG, Kweon DK and Park HJ: Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. CarbohydrPolym 2011; 86(2): 837-843.

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