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Formulation and Characterization of Nimodipine Nanoemulsion

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Abstract: ***Nimodipine is a dihydropyridine calcium channel blocker which is used in the treatment of hypertension. It is a highly lipophilic, poorly water soluble API. When we take nimodipine in oral dosage form, it shows bioavailability of only around 13% due to its extensive first-pass metabolism. Topical transdermal option of this potential API has been reported. Chemical penetration enhancers in many such products may cause skin irritation on prolonged therapy. As nanoemulsions do not need the chemical enhancers, they are advantageous over the conventional transdermal drug delivery systems. The aim of the present study was to develop nimodipine nanoemulsion formulation for topical administration. The nanoemulsion formulation consists of eucalyptus oil: tween 20/propanol-2 (1: 1, 1: 2, 1: 3, 3: 1, 2: 1) as an internal oil phase in external aqueous phase, Tween 80 as surfactant and propanol-2 as cosurfactant. Pseudoternary phase diagram was developed to determine the effect of the surfactant to cosurfactant mass ratio on the nanoemulsion formation, a transparent region. Optimized nanoemulsion was prepared by Sonication method and characterized by droplet size, SEM, viscosity and refractive index.*** **In-vitro** ***skin permeation of nimodipine through rat abdominal skin was determined by the Franz diffusion cell. Optimized nanoemulsion was also subjected to the thermodynamic stability studies. All the selected formulations were found to be stable. Novel nimodipine nanoemulsion formulation could be designed and projected to be suitable for transdermal application.***

Keyword: Nanoemulsion, Surfactant, Co-Surfactant, Transdermal.

1. Introduction

Nimodipine is an effective dihydroperidine calcium channel blocker used in the treatment of hypertension. It is a lipophilic poorly-water soluble drug and undergoes extensive first-pass metabolism after oral dosing. This results in its low bioavailability. The elimination half-life is also very short i. e. about 1-2 hours. Transdermal drug delivery system provides a novel platform for lipophilic drugs, where through proper selection of the formulation not only the bio availability might be improved, the side effects of oral administration would also be avoided. [1]

Nanoemulsions are a new class of emulsion which can be defined as an emulsion with uniform and extremely small droplet sizes, typically in the range of 20–200 nm. The physical appearance of nanoemulsion is transparent or translucent because of their small droplets size. Their small droplets size makes it kinetically stable against sedimentation or creaming for a long period of time. [2] The use of nanoemulsions in oral dosage forms, achieve promising results in increasing the effectiveness of the drug at the target site, as well as can increase drug bioavailability, enhanced permeability and therapeutic functions. [3]
One of the important properties of nanoemulsion is that they improve therapeutic efficacy of the drug and reduce the volume of the drug delivery system, which in turns minimizes the toxic side effects. [4] The key for the successful formulation of such desirable transdermal option lies in proper selection of oil, surfactant and cosurfactant. No such work has been reported for nimodipine. The objective of this study was to provide a logistic screening approach for transdermal drug delivery of nimodipine. [5, 6]

2. Materials and Methods

Components:
Nimodipne was a purchased from Yarrow Chem. Products Distributor.7 Potassium chloride, 2-propanal, Ethanol were purchased from SD Fine Chemical Ltd (Mumbai). Tween 20, n-octanol, Ethyl acetate, Potassium dihydrogen phosphate, Sodium chloride, Disodium hydrogen phosphate were purchased from Central Drug House (P) Ltd. (New Delhi). Eucalyptus oil was purchased from Shri Krishna Enterprises, Delhi. Excised mice skin was taken from Pharmacology Lab P. S. I. T., Kanpur.

Screening of components: [7, 8]
Selection of oil, solvents, and surfactants were done on the basis of their solubilizing capacity for nimodipine. Best criteria for the selection of component is that all the excipient should be comes under Generally regarded as safe category and compatible with each other and pharmaceutically acceptable for topical use.

Screening of Oils, Surfactants, and Co-surfactants: Screening of oils, surfactants, cosurfactants was done to optimized best formulation. Components for nanoemulsion formulation were selected on the basis of their solubilizing capacity for nimodipine and their HLB value. This study was performed on different oils such as castor, corn, equcalyptus, clove etc. surfactants such as tween 20, tween 80, span 20 etc. co-surfactants such as propanol, peg 200, butanol etc. For this study, following method was applied: **[9]**

5ml of different oils, surfactants, co-surfactants are taken in a volumetric flask separately then, added the excess amount of drug in it separately. shaken the flask for few hours on vortex mixture if the solution become clear then again added

some amount of drug and drug was added until saturation phase becomes. The solution was centrifuged for 10min. at 3000rpm and allowedto stand for 24 hrs. After 24 hrs, filtered the solution through membrane and solubility was determined by U. V. Spectrophotometer. [**10]**

Finally propanol-2 as co-surfactant, eucalyptus oil, and tween 20 were selected for the development of formulation.

Formulation of nanoemulsion: [11, 12]

Formulation Development: Nanoemulsion was prepared by using Sonication method. Components used in the formulations are as follows: Surfactant – tween 20 Co-surfactant-propanol-2 Oil-eucalyptus oil.

Procedure: Firstly, mixture of tween 20 and propanol-2 was prepared in different ratio after that stock solution of tween 20 and propanol-2 in the ratio 1: 1, 1: 2, 1: 3, 3: 1, 2: 1 were prepared in sufficient quantity by proper mixing. Then, oil was added to the mixture of tween 20 and propanol-2. In this final mixture, Drug was added in required quantity. This mixture was sonicated for 12 hours at 25^{o}C. this process was repeated for different compositions of formulations in the ratio of 1: 9, 2: 8, 3: 7, 4: 6, 5: 5, 6: 4, 7: 3, 8: 2, 9: 1 (eucalyptus oil: tween 20/propanol-2) for each ratio of tween 20 and propanol-2 (1: 1, 1: 2, 1: 3, 3: 1, 2: 1). Finally, formulations were prepared.

Formulation Control (C)

Table 1: Formulation Control (C)

Formulations	Oil: Surfactant w/w	Oil (ml)	Surfactant With Cosurfactant (ml)	Water (ml)	Drug (mg)
C1	1: 9	0.2	1.8	2.8	20
C2	2: 8	0.4	1.6	2.0	20
C3	3: 7	0.6	1.4	1.2	20
C4	4: 6	0.8	1.2	0.9	20
C5	5: 5	1.0	1.0	0.2	20
C6	6: 4	1.2	0.8	0.1	20
C7	7: 3	1.4	0.6	00	20
C8	8: 2	1.6	0.4	00	20
C9	9: 1	1.8	0.2	00	20

Formulation F2: 1

Table 2: Formulation F2: 1

Formulations	Oil: Surfactant (2: 1) w/w	Oil (ml)	Surfactant With Cosurfactant (ml)	Water (ml)	Drug (mg)
F21 (1: 9)	1: 9	0.2	1.8	2.8	20
F21 (2: 8)	2: 8	0.4	1.6	1.4	20
F21 (3: 7)	3: 7	0.6	1.4	0.7	20
F21 (4: 6)	4: 6	0.8	1.2	0.2	20
F21 (5: 5)	5: 5	1.0	1.0	0.1	20
F21 (6: 4)	6: 4	1.2	0.8	00	20
F21 (7: 3)	7: 3	1.4	0.6	00	20
F21 (8: 2)	8: 2	1.6	0.4	00	20
F21 (9: 1)	9: 1	1.8	0.2	00	20

Formulation F3: 1

Table 3: Formulation F3: 1

Formulations	Oil: Surfactant (3: 1) w/w	Oil (ml)	Surfactant With Cosurfactant (ml)	Water (ml)	Drug (mg)
F31 (1: 9)	1: 9	0.2	1.8	2.4	20
F31 (2: 8)	2: 8	0.4	1.6	1.5	20
F31 (3: 7)	3: 7	0.6	1.4	0.8	20
F31 (4: 6)	4: 6	0.8	1.2	0.3	20
F31 (5: 5)	5: 5	1.0	1.0	0.1	20
F31 (6: 4)	6: 4	1.2	0.8	00	20
F31 (7: 3)	7: 3	1.4	0.6	00	20
F31 (8: 2)	8: 2	1.6	0.4	00	20
F31 (9: 1)	9: 1	1.8	0.2	00	20

Formulation F1: 1

Table 4: Formulation F1: 1

Formulations	Oil: Surfactant (1: 1) w/w	Oil (ml)	Surfactant With Cosurfactant (ml)	Water (ml)	Drug (mg)
F11 (1: 9)	1: 9	0.2	1.8	1.5	20
F11 (2: 8)	2: 8	0.4	1.6	1.2	20
F11 (3: 7)	3: 7	0.6	1.4	0.5	20
F11 (4: 6)	4: 6	0.8	1.2	0.1	20
F11 (5: 5)	5: 5	1.0	1.0	00	20
F11 (6: 4)	6: 4	1.2	0.8	00	20
F11 (7: 3)	7: 3	1.4	0.6	00	20
F11 (8: 2)	8: 2	1.6	0.4	00	20
F11 (9: 1)	9: 1	1.8	0.2	00	20

Formulation F1: 2

Table 5: Formulation F1: 2

Formulations	Oil: Surfactant (1: 2) w/w	Oil (ml)	Surfactant With Cosurfactant (ml)	Water (ml)	Drug (mg)
F12 (1: 9)	1: 9	0.2	1.8	1.4	20
F12 (2: 8)	2: 8	0.4	1.6	0.8	20
F12 (3: 7)	3: 7	0.6	1.4	0.6	20
F12 (4: 6)	4: 6	0.8	1.2	0.1	20
F12 (5: 5)	5: 5	1.0	1.0	00	20
F12 (6: 4)	6: 4	1.2	0.8	00	20
F12 (7: 3)	7: 3	1.4	0.6	00	20
F12 (8: 2)	8: 2	1.6	0.4	00	20
F12 (9: 1)	9: 1	1.8	0.2	00	20

Formulation F1: 3

Table 6: Formulation F1: 3

Formulations	Oil: Surfactant (1: 3) w/w	Oil (ml)	Surfactant With Cosurfactant (ml)	Water (ml)	Drug (mg)
F13 (1: 9)	1: 9	0.2	1.8	1.2	20
F13 (2: 8)	2: 8	0.4	1.6	0.7	20
F13 (3: 7)	3: 7	0.6	1.4	0.5	20
F13 (4: 6)	4: 6	0.8	1.2	0.1	20
F13 (5: 5)	5: 5	1.0	1.0	00	20
F13 (6: 4)	6: 4	1.2	0.8	00	20
F13 (7: 3)	7: 3	1.4	0.6	00	20
F13 (8: 2)	8: 2	1.6	0.4	00	20
F13 (9: 1)	9: 1	1.8	0.2	00	20

Selection of nanoemulsion formulations for in vitro studies: Following formulations were selected for In vitro studies. F21 (1: 9), F21 (2: 8), F21 (3: 7) F31 (1: 9), F31 (2: 8), F31 (3: 7) Control (C).1 In vitro skin permeation study Mice skin was used for permeation study. Skin of albino mice obtained from animal house of PSIT, Kanpur which is approved by Institutional Animal Ethical Committee (CPCSEA/1273) in 2009. Franz diffusion cell was used to determine the permeation of drug through skin. Skin was clamped between donor and receptor compartment of diffusion cell.20ml (7.4) phosphate buffer: ethanol (60: 40) was filled in receptor compartment of diffusion cell and temperature was maintained at 37oC and stirred at 400 rpm throughout the experiment. Place the formulation in the donor compartmemt of the cell on the mice skin.1ml sample withdrawn at definite time intervals such as 0.5, 1, 2, 3, 4, 5, 6, 7, 8 hours and replaced immediately with equal volume of fresh solution. Absorbance of all sample were determined by U. V. spectroscopy at 237nm. **[13, 14]**

Characterization of nanoemulsion: [15, 16, 17]

1) **Determination of pH:** pH of the formulation was determined by the electronic pH meter. Firstly pH meter was calibrated by using the standard buffer solution of pH 4 and pH 7. pH of the formulations were detected.
2) **Viscosity measurement:** Viscosity of the formulations were determined by using Oswald viscometer. In this method, formulation filled in a viscometer upto A point then started stopwatch and allowed the formulation to reach the point B. Time taken by the formulation toreach the point B from point A were noted. Viscosity were determined by using the following formula,
   $n_1 = d_1t_1 / d_2t_2 * n_2$
   where, n_2= viscosity of water, d_1 = density of formulation, t_1= time taken by formulation and d_2= density of water, t_2= time taken by water.
3) **Refractive index measurements:** Refractive index of formulations were determined by using ABBE Refractometer. Refractive index of substances determined to check the composition or purity.
4) **Conductivity measurements:** Conductivity of formulations was determined by using digital conductometer and it was firstly calibrated by distilled water then, conductivity of formulations were determined. It was determined to find out the formulation is oil continuous or water continuous.
5) **Stability studies: [18, 19, 20]**
   a) **Centrifuge stress test:** This test was mainly done to check the phase separation of formulation in this, formulations were centrifuged at 3500 rpm for 30 minutes. The formulation which was passed the test was applied for next test.
   b) **Heating and cooling cycle:** In this, the formulations which were passed the centrifuge test go for 6 cycles between refrigerator temperature (4°C) and 45°C with storage not less than 48 hrs. Stable formulations were subjected to next test that is freeze thaw cycle.
   c) **Freeze-thaw cycle:** Finally the formulations which are selected were subjected to 3 freeze thaw cycles between-21°C and 25°C atleast for 24 hrs. The formulations which were passed all the three tests are thermodynamically stable and passed the phase separation test.6.14 Short term stability study: Short term stability study of formulations was carried out for 2 months at 25+2 o and 75% RH. Checked the formulation at definite time interval such as 1week, 2 weeks, 3 weeks so on by visual observation and checked the degradation of formulation by UV spectrophotometer.

3. Result and Discussion

1) **Screening of component for nanoemulsion:** Various oils, surfactants, co-surfactants were analyzed and checked the solubility for the selection of formulation development. Among various component eucalyptus oil was selected for formulation development because it has better solubilizing capacity for nimodipine and does not show any dermal reaction means it is safe for topically use. Tween 20 was selected as a surfactant because nimodipine show better solubility in tween 20 compare to other surfactants. In place of co-surfactant propanol-2 was used because it has good ability to form nanoemulsion with tween 20 and eucalyptus oil and it is topically safe, act as a penetration enhancer.
2) **Stability studies**
   a) **Centrifuge stress test:** All the selected formulations were passed this test and found to be stable means no phase separation.
   b) **Heating and cooling cycle:** In this test, also all the selected formulations were passed and shows no phase separation.
   c) **Freeze-thaw cycle test:** All the selected formulations were found to be stable and does not show phase separation. All the selected formulations were passed the stability test.
3) **Short term stability test:** This study for nanoemulsion was done for one month at 25+2 C and 45% RH. In this time period, the selected formulation passed all the tests such as phase separation, pH, viscosity. F31 (1: 9) shows highest % drug content compare to other selected formulations.

Table 7: Stability test result

Formulation	Storage condition (temperature/ Relative humadity)	pH	Centrifugation	Drug transport profile	% drug content
F21 (2: 8)	25+2 ^{O}C/ 45% RH	6.65	No phase separation	Similar	97.3%
F31 (1: 9)	25+2 ^{O}C/ 45% RH	6.47	No phase separation	Similar	98.3%
CONTROL (C)	25+2 ^{O}C/ 45% RH	6.83	No phase separation	Similar	85.0%

4) **Determination of pH:** pH of the all selected formulations were found to be within a range or according to pH of skin means formulations were safe for topical use.

Table 8: pH of formulations

Formulations	pH without drug	pH with drug
F21 (2: 8)	6.58	6.65
F31 (1: 9)	6.30	6.47
Control (C)	6.54	6.83

5) **Refractive index:** All the selected formulations were shows the value of refractive index in a range of 1.34-1.37.

Table 9: Refractive index of formulations

Formulations	Refractive index without drug	Refractive index with drug
F21 (2: 8)	1.348	1.354
F31 (1: 9)	1.352	1.359
Control (C)	1.360	1.374

6) **Determination of viscosity:** Viscosity of selected formulations found to be satisfactory. Viscosity of selected formulation was within a range so it is easy to apply and also gives satisfactory penetration of drug into skin.

Table 10: Viscosity of formulations

Formulations	Viscosity (CPs)
F21 (2: 8)	72.18
F31 (1: 9)	54.14
Control (C)	33.27

7) **Conductivity measurement:** Conductivity of selected formulations was done. Conductivity of all selected formulation within a 0.54-0.55Ms/cm range and F21 (2: 8) shows high conductivity.

Table 11: Conductivity measurement of formulations

Formulations	Conductivity (mS/cm)
F21 (2: 8)	0550
F31 (1: 9)	0.547
Control (C)	0.546

8) **In vitro study:** In vitro drug release of formulations were determined and results were found to be satisfactory.

Table 12: Comparative release study of different formulations % Cummulative Drug Release
% Cummulative Drug Release

Time (hrs.)	F21 (1: 9)	F21 (2: 8)	F21 (3: 7)	F31 (1: 9)	F31 (2: 8)	F31 (3: 7)	C
0.5	00	00	00	00	00	00	00
1	2.65	3.19	2.19	3.22	2.67	3.17	2.08
2	6.28	6.28	5.17	7.58	5.46	7.25	3.92
3	9.05	10.62	9.28	10.37	8.16	11.03	6.37
4	13.25	15.75	14.25	15.98	13.42	16.23	9.58
5	16.70	21.35	18.77	23.21	20.33	21.71	12.39
6	22.62	32.98	23.43	36.18	27.63	26.20	15.03
7	28.71	38.22	30.43	54.69	37.78	32.35	19.20
8	33.01	43.15	35.85	67.24	44.08	37.11	21.84

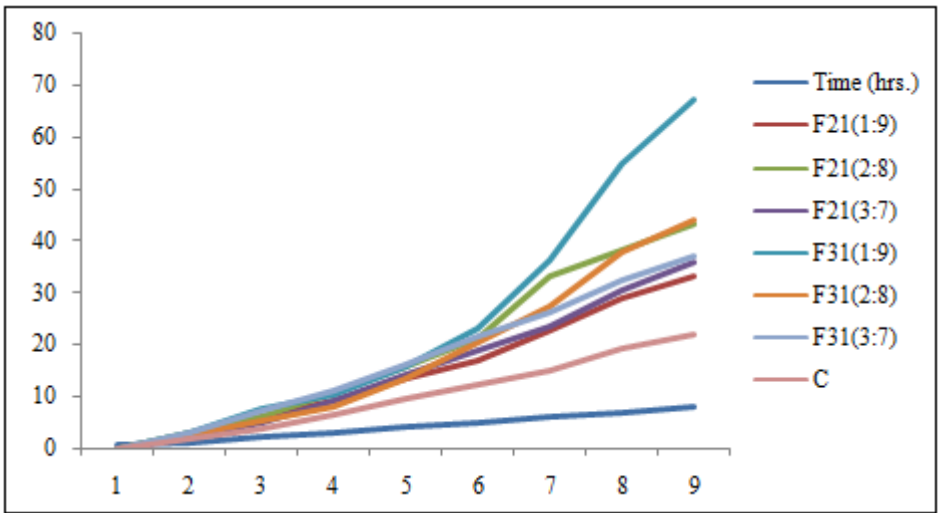

Figure: Comparative release study of different formulations

4. Conclusion

Recently, Nanoemulsions are receiving great attention as drug carrier for improving the delivery of neutron capture therapy agents, various anticancer drugs and pharmaceutical ingredients. Now-a – days, many studies have focused on using nanoemulsion for transdermal drug delivery. The Nanoemulsion formulations which are formulated is found to be transparent because its particle size in nm and these formulations contains oil, surfactants, and co-surfactants. In formulations drug Nimodipine was used which is hydrophobic and photosensitive in nature. The concentration of components which are used in nanoemulsion formulations were determined or screened by using the construction of pseudo ternary phase diagrams. Formulated nanoemulsions contain eucalyptus oil as oil, propanol-2 as co-surfactant, and tween 20 as surfactants. Nimodipine nanoemulsion was formulated for transdermal or topical application. Following studies were done for nimodipine nanoemulsion such as drug release, pH, viscosity, refractive index, phase separation study, stability test, conductivity, SEM etc. Selected formulations were showed increase in bioavailability, proper stability, least dose with more effect, no irritant effect, patient compliance etc. In invitro release, all the formulations showed sustained release drug permeation on excised mice skin and zero order model fit for these formulations with non-fickian diffusion over a period of 8 hours. Formulations F21 (2: 8), F31 (1: 9), and C were showed better drug release on excised mice skin. But F31 (1: 9) showed the highest drug release in all formulations. All three selected formulations [F21 (2: 8), F31 (1: 9), C] showed the satisfactory results of all studies that is pH, viscosity, refractive index, conductivity, stability etc. Stability studies of formulations were done at ambient

temperature (25^0C) and humidity (75%). All the evaluations of developed formulations showed result within a range or satisfactory and passed the stability test for 2 months. Hence it can be conclude that the selected formulation F31 (1: 9), C (CONTROL), F21 (2: 8) showed that they passed all evaluation studies such as pH, viscosity, conductivity, stability etc. so that they could be applicable for transdermal or topical delivery.

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