A

Report on Progress of Sanctioned Ongoing Research Project

ON

Development of Transdermal Drug Delivery Systems of Iron Using Natural Skin Permeation Enhancers

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Submitted by-

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Marathwada Mitra Mandal's College of Pharmacy Thergaon, Pune-33 Estd. 2006

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Introduction

The therapeutic efficacy of Active Pharmaceutical Ingredients (API) is determined by various processes that takes place in body such as absorption, distribution, metabolism, and excretion. The rate and extent of absorption of drugs from the dosage forms determines its bioavailability and onset of action. Similarly, distribution, metabolism and elimination of drug determines the duration of action. The bioavailability of drugs having smaller particles is more due to more specific surface area and thereby enhanced absorption. Thus, the nano-particulate drug delivery system may increase the bioavailability. Numerous nanoparticulate drug delivery systems have been explored so far for their various advantages. One of such nanoparticulate drug delivery system is solid lipid nanoparticles which have numerous benefits and few drawbacks when compared to other colloidal carrier systems. Because of their biodegradable and biocompatible characteristics, as well as low toxicity, solid lipid nanoparticles (SLN) have gained popularity as carriers for the production of a wide range of poorly water-soluble medicines. SLNs are made up of a solid lipid core that is biodegradable and biocompatible, and an exterior shell that is made up of a non-hazardous surfactant/co-surfactant. Particle size (PS) and polydispersity index (PDI) are important features and factors in the stability and manufacturing of SLNs. These properties are mostly determined by the particle composition and manufacturing processes used. The major goal of this study is to make Iron oxide-loaded solid lipid nanoparticles with lecithin and cholesterol as lipid matrix, tween 80 as a stabilizer and Eugenol as permeation enhancer with the goal of increasing the dissolution rate of Iron oxide and its permeation through skin and therefore may replenish the iron loss from the body.

Iron is an essential mineral nutrient which has a key physiological role and is required for numerous functions such as oxygen transport, ATP production, and DNA replication. When the body's iron requirements are not met by dietary iron, deficiency of iron arises. Reduced iron delivery to target sites such as the liver parenchyma, bone marrow and muscle myoglobin results in an impairment of iron-dependent functions such as erythropoiesis. A decrease in the number of red blood cells may also be characterized by a smaller mean cell size (microcytic anemia). The net outcome is decrease oxygen carrying capacity and consequent tissue hypoxia. Iron depletion and deficiency in its mildest form is not particularly detrimental. However, progression to iron deficiency anemia (IDA) or sideropenicanaemia can have severe physiological consequences. Anemia affects one-fourth of the world's population, accounting for 8.8% of the

total global burden of disease. Iron deficiency is the predominant cause of anemia across countries and in both sexes, with women more commonly afflicted, around 30% of women in high-resourced countries, and increasing to over 50% of women in low-resourced countries. Thus, there is clear evidence to support prompt treatment in all patients with iron deficiency anemia especially in adolescent girls and women from underdeveloped and developing countries. It is known that the treatment improves quality of life and physical condition as well as alleviates fatigue and cognitive deficits.

Thus, in this study attempt has been made to formulate patient friendly Iron oxide loaded solid lipid nanoparticulate transdermal films using natural permeation enhancer with the goal of increasing its permeation through skin to replenish the iron loss from the body.

Materials and methods

Iron oxide was obtained as gift sample from ASTRRA chemicals (Tamil Nadu), India. Tween 80, soya lecithin, and cholesterol were obtained from Loba Chemie (India). HPMC, Xanthum Gum were supplied by Sigma-Aldrich. All the other chemicals used such as Poly vinyl alcohol (PVA); polyvinyl pyrrolidone (PVP); propylene glycol (PG) were of analytical grade (Sisco Research laboratories Pvt. Ltd, Maharashtra, India).

Standard calibration curve

Standard Calibration Curve of Iron oxide in 1% HNO_3

Absorbance of five known concentrations of Iron oxide (5, 10, 15, 20 and 25µg/mL) in 1% HNO_3 were measured at λmax 480 nm in Atomic Absorption Spectrophotometer (Pinnacle 500, Perkin Elmer, USA). Then Concentration vs Absorbance curve was plotted and the absorbance is given in table 1 and the calibration curve in figure 1.

Table 1. Concentration vs absorbance data for iron oxide

Concentration (µg/ml)	Absorbance
5	0.1789
10	0.2518
15	0.3569
20	0.4015
25	0.4768

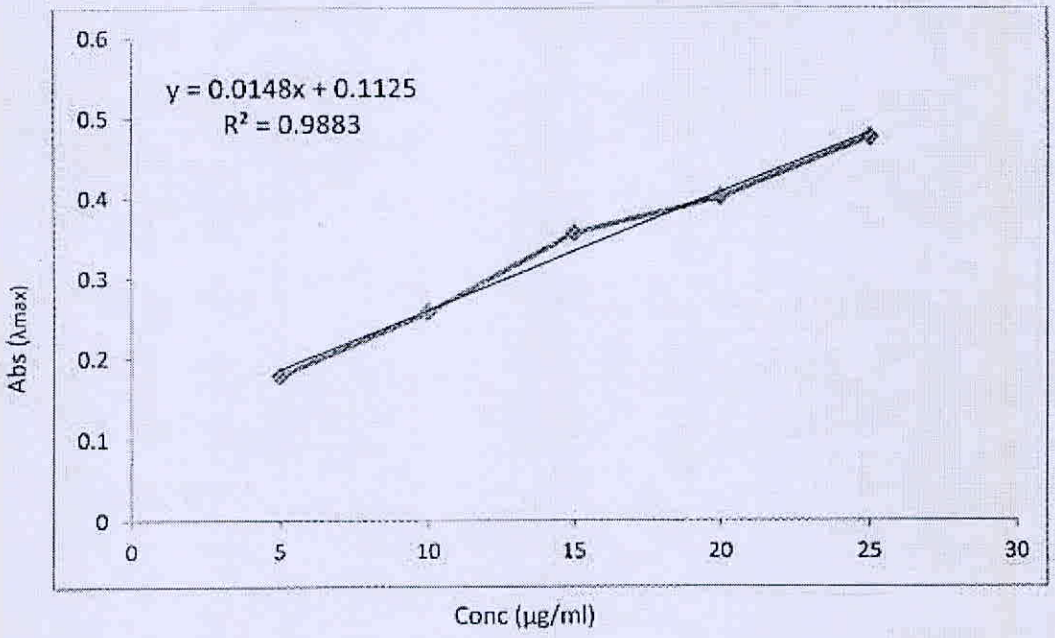

Figure 1. Calibration curve of iron oxide

Preparation of solid lipid nanoparticles loaded with Iron oxide

The modified high pressure homogenization and probe-sonication method has been used for preparation of seventeen formulations of Iron oxide-SLNs. Lipids (Soya lecithin and cholesterol) were heated by 50^0C above their melting points. Then 50mg iron oxide was added to the lipid matrix to obtain a drug-lipid mixture. While the aqueous phase was obtained by dissolving the surfactant (Polyvinyl alcohol) in Distilled water and heated up to the temperature of the melted lipid phase. After that, the melted lipid phase was poured onto the warm aqueous phase and subjected to sonication for 10 minutes using probe sonicator (VCX 750, Sonics Vibracell, India) followed by homogenization at pressure 800 bar for 15 min using high pressure homogenizer (Panda 2K Plus, Niro Savy, Italy).

Experimental design

The optimization technique is very important for the developer as it assist in the development of the best possible formulation design under a given set of conditions with minimum experimentation, saving considerable time and effort. The response surface methodology technique was selected to get in depth understanding as well as ability to seek ranges of polymers, formulation excipients and processing parameters. The concentration ranges of the

lipids and Polyvinyl alcohol for the preparation of Iron oxide SLNs by above mentioned method were optimized by trial-and-error basis.

A box Behnken design was used to examine the combined influence of two–level, three factor variables on the formulation of Iron oxide SLNs. In this design, three factors were evaluated, each at two level and experimental trial were performed at all 17 possible combinations. The concentration of lipids (Soya lecithin & cholesterol), concentration of surfactant and pressure of high pressure homogenizer were selected as independent variables

Entrapment Efficiency (Y_1), Particle size (Y_2) and Drug content (Y_3) were selected as dependent variables. These three factors might affect the designed characteristics of nanoparticles formulations were varied over two levels and arranged according to box Behnken design. Then the formulations generated by this design of experiment using Design Expert Version 11 were used to study the effect of three independent factors on the dependent variable response.

Table 2. Independent and dependent variables with their levels and goals

Independent Variables	**Level**		
	Low (-1)	**Middle (0)**	**High (1)**
(X_1) = lipid Conc. (%)	5	7.5	10
(X_2) = concentration of surfactant (mg)	2	5	8
(X_3) = pressure of high pressure homogenizer (bar)	500	650	800
Dependent Variables		**Goals**	
(Y_1)= Entrapment Efficiency		Maximize	
(Y_2) = Particle size		Minimum	
(Y_3) = Drug content		Maximize	

Table 3. Formulation Composition and Process Parameters

Formulation No.	Drug (mg)	Lipid (%)	Surfactant (mg)	Pressure (bar)	Water (ml)
1	50	5	2	500	100
2	50	10	8	500	100
3	50	5	8	650	100
4	50	5	5	650	100
5	50	7.5	5	800	100
6	50	10	2	500	100
7	50	7.5	5	650	100
8	50	7.5	5	650	100
9	50	7.5	2	500	100
10	50	7.5	5	500	100
11	50	10	5	650	100
12	50	7.5	5	650	100
13	50	5	5	800	100
14	50	7.5	2	800	100
15	50	10	5	800	100
16	50	7.5	8	650	100
17	50	7.5	8	800	100

Characterization of Iron oxide-SLN

Particle size determination:

The particle size of nanoparticles was measured using a particle size analyzer (LA-960, HORIBA Scientific, Japan) at 25^{0}C after appropriate dilution with double distilled water to avoid multi-scattering phenomena and placed inside the sample holder of the instrument. Once the intensity of the sample was within the range recommended by the instrument, analysis was performed to obtain the particle size and is shown in table 4.

Determination of Entrapment Efficiency:

Nanoparticle formulations were centrifuged at 60,000 rpm for 45 min at 37°C using a Cooling Ultra Centrifuge to separate nanoparticles from non-entrapped drug. Concentration of the free drug in the supernatant was determined spectrophotometrically at λ max 480 nm with UV spectrophotometer (1800, Shimadzu, Japan). The percentage of drug entrapment in nanoparticles was calculated using equation and is shown in table 4.

$$\% \text{ Entrapment Efficiency (\%EE)} = \frac{W\text{total} - W\text{ free}}{W\text{ total}} \times 100 \quad \text{.....(1)}$$

Determination of Drug Content:

Nanoparticle formulations were ultracentrifuged at 60,000 rpm for 45 min at 37°C to form a pellet. Then the obtained pellet was digested with 0.1 N HCl overnight and sonicated for 15 min to obtain a clear solution. The drug content in the nanoparticles was determined using a UV spectrophotometer at λ max 480 nm against dummy nanoparticles as a reagent blank, which had also been prepared and treated similarly to the drug-loaded particles. The % drug content was calculated using equation 2 and drug content is given in in table 4.

$$\% \text{ Drug Content} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100 \quad \text{--------} \ (2)$$

Table 4. Evaluation of Solid-lipid Nanoparticles of Iron oxide

Formulation Code	Entrapment Efficiency (%)	Drug content (%)	Particle size (nm)
F1	73.41	70.59	1140
F2	62.52	60.34	674
F3	73.43	66.49	1187
F4	79.83	82.57	372.8
F5	74.11	60.83	674
F6	69.11	67.32	505.2
F7	82.88	79.19	505.6
F8	72.75	67.05	649
F9	70.26	69.48	1063
F10	64.22	47.33	806
F11	73.01	79.79	378.2

F12	**81.81**	**95.64**	**310.5**
F13	73	60.06	729
F14	74.01	42.83	833
F15	63.17	32.21	703
F16	80.90	64.71	370.8
F17	68.90	67.85	505.5

Formulation of transdermal film

Transdermal films loaded with iron oxide SLNs were prepared by the solvent evaporation/Solvent casting method. Transdermal formulations were prepared using HPMC, Xanthum Gum, PVA, and eugenol as natural permeation enhancer. Weighed quantity of polymer was dispersed in 10 ml of distilled water and stirred well using magnetic stirrer at 1000 rpm until it gets a uniform translucent thickened dispersion. Then the required volume of permeation enhancers and plasticizer polyethylene glycol (PEG) was added to it with continuous stirring until the above solution becomes viscous. In this solution, suspension of SLN equivalent to 50 mg of the drug was added followed by stirring until it gets a uniformly dispersed. The above dispersion was then poured into Petri plates uniformly with utmost care to avoid air entrapment or avoid bubble formation. The solvent in the film was allowed to evaporate at room temperature until it dried. The dried films were then removed from the petri plates and stored in a desiccator until further evaluation.

Experimental design

A Box Behnken design was used to examine the combined influence of two–level, three factor variables on the formulation of Iron oxide Transdermal film. In this design three factors were evaluated, each at two level and experimental trial were performed at all 17 possible combinations. The Polymer ratio (HPMC: Xanthum Gum), Penetration Enhancer and Concentration of surfactant were selected as independent variables.

WVTR (Y1), Drug Release (Y2) and Drug content (Y3) were selected as dependent variables. These three factors might affect the designed characteristics of nanoparticles formulations were varied over two levels and arranged according to Box Behnken design. Then the formulations generated by this design of experiment using Design Expert Version 11 were used to study the effect of three independent factors on the dependent variable responses.

Table 5. Independent and dependent variables with their levels and goals

Independent Variables	Level		
	Low(-1)	Middle (0)	High (1)
(X_1) = polymer ratio (HPME: Xanthum gum) (mg)	1:1	1:2	2:1
(X_2) = concentration of surfactant	50	100	150
(X3) = Penetration Enhancer (ml)	1	2	3
Dependent Variables		**Goals**	
(Y_1)= WVTR		Minimum	
(Y_2) = Drug Release		Maximum	
(Y_3) = Drug content		Maximize	

Table 6. Formulation batches with their respective composition as per Box Behnken Design

Formulation Code	NP Suspension (ml)	Polymer Ratio (mg)	Pressure HPH (bar)	Penetration Enhancer (ml)
F1	5	66.6:133.4	50	2
F2	5	133.4:66.6	50	2
F3	5	66.6:133.4	100	2
F4	5	133.4:66.6	100	2
F5	5	66.6:133.4	150	1
F6	5	133.4:66.6	100	1
F7	5	100:100	100	2
F8	5	133.4:66.6	50	3

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F9	5	100:100	100	1
F10	5	100:100	150	1
F11	5	100:100	150	3
F12	5	100:100	100	3
F13	5	100:100	100	2
F14	5	100:100	50	2
F15	5	100:100	50	2
F16	5	100:100	100	2
F17	5	100:100	150	2

Evaluation of transdermal films

Physico-Chemical Evaluation

Physical appearance

All the transdermal films were visually inspected for color, clarity, elasticity and softness (Figure 2).

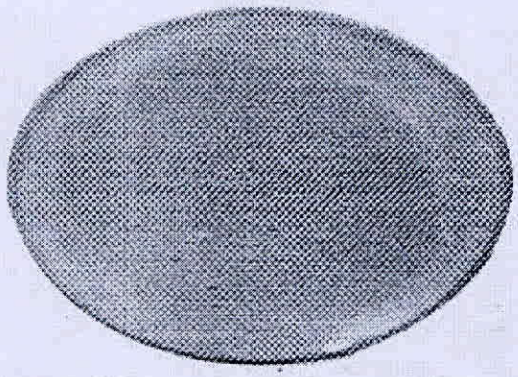

Figure 2. Dried film of iron oxide.

Thickness of the film

The thickness of the drug loaded film was measured at different points by using a digital Degimatic Caliper and determined the average thickness and standard deviation for the same to ensure the thickness of the prepared patch. The results are shown in table 10.

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Surface pH:

The Transdermal film requires strict conformity to pH requirement. The preparations having pH 7.0 – 7.4 is tolerable to human skin. The pH of the film was determined by using the calibrated digital pH meter (EQ-610, Equip-tronics, India)

Weight uniformity

The prepared films were dried at 60°C for 4h before testing. A specified area (1x1 cm2) of film was cut from different parts of the film and weighed on digital balance. The average weight and standard deviation values were calculated from the individual weights.

Folding endurance

A strip of film (3 × 3 cm) was cut evenly and repeatedly folded at the same place till it breaks. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance. The results are shown in table 10.

Drug content

A specified area of film was dissolved in a suitable solvent in specific volume. Then the solution was filtered through a filter medium and analyze for the drug content using Atomic Absorption Spectrophotometer (Pinnacle 500, Perkin Elmer, USA).

Percentage moisture content

The prepared films were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature for 24 h. After 24h the films were reweighed percentage moisture content was determined using following formula.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad \text{----------(3)}$$

Percentage Moisture uptake

The weighed films were kept in desiccators at room temperature for 24h containing saturated solution of potassium chloride in order to maintain 84% RH. After 24h the films were reweighed and percentage moisture uptake was determined using following formula.

$$\text{Percentage moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Final weight}} \times 100 \quad \text{----------(4)}$$

The films were removed and weighed at various time intervals like 3, 6, 12, 18 and 24h to note down the weight gain. The results are shown in table 10.

Flatness Test

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For the calculation of percent flatness longitudinal strips were cut from the prepared patches and thickness of each strip was measured and the variation in the thickness (vertical length) due to non-uniformity of flatness was measured. Flatness was detrmined by measuring constriction (unevenness) of strips and a zero percent constriction was considered to be equal to 100 percent flatness. The results are shown in table 10.

$$\% \text{ Constriction} = \frac{(\text{average thickness}) - (\text{thickness at sampling point})}{(\text{Average thickness})} \times 100$$

In vitro drug release studies

The paddle over disc method (USP apparatus V) was employed for studying release of the drug from the prepared films. Dry films of known thickness were cut into definite shape, weighed, and fixed over metal disc. The metal disc was then placed in a dissolution vessel followed by addition of 900 mL of the phosphate buffer (pH 7.4). The paddle speed was kept at 50 rpm. Samples (5 mL) were withdrawn at different time intervals up to 6 h and analyzed by Atomic absorption Spectrophotometer. The results are shown in table 7, 8 and 9.

Table 7. *In vitro* percent drug release F1- F5

Time (h)	F1	F2	F3	F4	F5
0.5	2.67	2.52	2.41	1.96	2.74
1	7.13	7.26	5.66	6.26	7.41
2	13.17	13.2	11.44	11.75	13.38
3	20.98	20.41	39.58	49.57	40.77
4	48.5	48.6	49.33	75.95	65.45
5	61.5	50.55	68.24	80.17	69.47
6	72.4	63.4	72.8	86.31	70.5

Table 8. *In vitro* percent drug release F6- F10

Time (h)	F6	F7	F8	F9	F10
0.5	2.47	2.1	2.01	2.68	2.19
1	7.31	6.35	5.85	7.45	3.43
2	20.5	35.5	11.4	13.6	25.5
3	45.6	45.6	30.5	35.5	30.5
4	50.6	65.5	40.8	55.6	40.5
5	60.8	75.8	68.5	60.5	50.4
6	69.4	81.6	75.3	68.6	55.6

Table 9. *In vitro* percent drug release F11- F17

Time (h)	F11	F12	F13	F14	F15	F16	F17
0.5	15.8	8.74	6.33	5.62	6.54	9.9	9.77
1	22.56	28.56	17.37	13.09	13.43	27.56	14.03
2	34.9	33.85	28.67	23.75	25.69	34.8	24.31
3	41.17	46	35.5	29.1	34.57	45.7	32.89
4	54.58	55.55	47.21	42.64	46.91	54.95	42.52
5	66.35	66.7	58.46	52.53	53.66	64.17	53.36
6	78.3	92.8	78.3	56.8	55.2	86.9	75.8

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Table 10. Evaluation parameters of Iron oxide transdermal films

Formulation	Thickness (mm)	Weight variation (mg)	Folding Endurance	Percent Moisture content	Flatness %	Swellability	% moisture uptake	Surface pH	Drug Release
F1	0.25±0.013	40±1.25	46	10.52	72	25±0.18	7.91	7.2	72.5
F2	0.24±0.014	42±2.25	68	11.21	88	27±0.15	6.85	7.4	63.4
F3	0.25±0.020	35±2.22	72	10.61	85	25±0.18	7.92	7.2	72.8
F4	0.26±0.022	41±1.25	45	12.51	75	27±0.17	6.65	7.0	86.3
F5	0.23±0.014	45±2.25	47	13.23	82	28±0.15	7.21	7.0	70.5
F6	0.23±0.015	32±2.22	46	12.51	71	25±0.16	6.23	7.4	69.4
F7	0.24±0.015	42±2.26	68	12.32	70	25±0.18	5.52	7.4	81.6
F8	0.27±0.017	50±2.25	70	11.62	85	27±0.18	7.21	7.2	75.3
F9	0.29±0.018	47±2.23	70	11.9	90	28±0.15	7.21	7.0	68.6
F10	0.35±0.019	45±2.26	65	10.61	92	30±0.16	6.52	7.4	55.6
F11	0.41±0.022	35±2.25	46	11.21	86	30±0.15	7.72	7.4	78.3
F12	0.51±0.023	40±3.35	70	12.53	72	27±0.16	6.65	7.2	92.8
F13	0.61±0.018	35±2.25	72	12.52	86	26±0.15	7.91	7.2	78.3
F14	0.35±0.017	42±2.25	46	13.2	72	30±0.15	7.52	7.0	56.8
F15	0.39±0.018	41±2.23	65	11.51	75	30±0.15	6.65	7.2	55.2
F16	0.42±0.017	32±2.25	65	10.52	86	35±0.18	6.65	7.4	86.9
F17	0.53±0.019	50±2.25	60	12.61	85	37±0.18	7.21	7.4	75.8

Remaining work/Work to be done:

- Drug diffusion study from prepared films.
- Preparation and evaluation of transdermal microneedle patches for drug release and drug diffusion.
- Skin irritation study and in vivo pharmacokinetic study of optimized transdermal film/patch formulation in rabbits.
- Stability studies as per ICH guidelines.
- Report writing and filing of patent/paper communication.

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Annual Progress Report for AICTE funded project under
Modernisation and Removal of Obsolescence (MODROBS)

1. **Principal Investigator** : Dr. Manohar J. Patil

   a. (Name & Address) : Principal & Professor of Pharmacognosy
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2. **Project Title** : Upgradation of Pharmacognosy Laboratory to Develop Inventive TSM's Formulations with Contemporary Technology

3. **Objectives of the Project:**

Though knowledge about traditional formulations exists in literature as well as in practices, is still faces a challenge of analytical standardization as per regulatory guidelines. There was little effort to develop creative formulations inspired by TSM. Thus the objective of the current project is to develop inventive TSM's Formulations with contemporary technology. It is also planned to study the scope for futuristic development in the area of standardization of TSM's formulation and their biomedical applications using the tools of modern techniques.

- ❖ To explore the traditional process of drug manufacturing in a view of modern techniques.
- ❖ To achieve the modernization of lab to meet the research and academic needs of the department.
- ❖ To Develop and nurture the research workforce.

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4. Salient Research Achievements:

Expected outcome/ Achievements

- The history of development of pharmaceutical dosage forms can be traced back to presence in systematic documentation of Traditional Systems of medicines.
- Plan of the project involves a multidisciplinary approach i.e develop inventive TSM's Formulations with contemporary technology, which would elaborate the technological details of these formulations.
- It is also planned to study the scope for futuristic development in the area of standardization of TSM's formulation and their biomedical applications using the tools of modern techniques.
- These activities would help in directing student's interest in research involving traditional medicines.
- Thus, the outcome of project is to fulfill the commitments of academic requirement, to establish research activities of high standards and at the same time great help to society.
- The outcome is aimed for the development of commercial value to the products which will be of help to the society and economy of the country.
- The Department will support traditional system of medicines and develop their importance through this project.
- The Department value the social responsibilities and wishes to fulfill the commitment through competence with technological excellence.

Improvements in the Scope of old experiment conducted

- TSM's comprises of various types of medicines. These are regarded as valuable therapeutics due to their efficacy and desirable features.
- The department works on Preparation and Evaluation of TSM's formulations by using traditional process as per literature.
- For a long time Traditional medicines were not considered for development as novel formulations owing to lack of scientific justification and processing difficulties, such as standardization, extraction and identification of individual drug components in complex systems.

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Improvements over conventional processes/experiments like

- The project explores the advantages of traditional formulations.
- The drugs of TSM's origin can be utilized in a better form with enhanced efficacy by incorporating in modern dosage forms.
- The project offers a glimpse on the traditional medicines to be incorporated in novel drug delivery system using a scientific approach to overcome non-compliance, and also help to increase the therapeutic value by reducing toxicity and increasing the bioavailability and so on.
- The current project focuses on the possible avenues of further investigations using the tolls of modern science for the characterization, validation and improvement of traditional processes.
- The outcomes of this project would provide information that can support to the ability to make sound and safe decisions and give the consumer a better product in terms of safety and efficacy.

Summary of Project Report:

- Completed working on TSM formulations preparation and their evaluation through conventional mode.
- Conversion of TSM formulations into novel formulation and their evaluation by modern techniques with the use of sophisticated equipment's like Rotary Vacuum evaporator and Spectrofluorometer which are purchased from the MODROB funding.
- The equipments purchased form the funding mentioned above are also utilized for the spectroscopic characterization of novel compounds and preparation of novel drug delivery system based formulations.

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Progress Report

For

ASPIRE Research Mentorship Grant

Savitribai Phule Pune University, Pune

Sponsored Research Project

On

"Nano emulsified in situ gel as a potential ophthalmic delivery system for non-steroidal anti-inflammatory drug"

By

Mr. Sachin K. Jagdale

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Sanctioned Year - 2019-21

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Title of research proposal

Nano emulsified *in situ gel* as a potential ophthalmic delivery system for non-steroidal anti-inflammatory drug

1. **Introduction of research proposal**

In ophthalmology, topical application is the preferred route of drug administration due to the relative low risk of systemic effects. But the most interesting and challenging accomplishments faced by scientist for Ocular drug delivery is poor permeation and bioavailability of drugs. Main reasons for low bioavailability of drugs from orthodox formulations are quick drainage of drugs from the precorneal area along with prompt elimination of drugs by defense mechanisms of the eye like reflex blinking and increased tear flow [1]. Because of this, numerous instillations of concentrated eye drops are needed for achieving the anticipated therapeutic effects, which may results in poor patient compliance and sometimes causing side effects due to nonproductive absorption of drugs in stomach through the nasolacrimal duct. Many attempts have been made to maximize ocular bioavailability and therefore, its therapeutic effect and patient compliance. Most of the evaluated dosage forms, for example, oily solutions, ointments, aqueous gels and suspensions. They have been shown to increase the residence times of instilled dose and increase ocular bioavailability. However, they have not been universally accepted and used widely, due to some shortcomings like hazy vision from semisolid dosage forms, limited efficacy and poor patient compliance from inserts [2]. Consequently, superior ophthalmic bioavailability of a drug following topical delivery to the eye remains a challenge that is yet to be solved acceptably.

The major pros of the ocular nanoemulsions (NE) compared to conventional eye formulations are it provides sustained release of the drug, better penetration and higher ocular bioavability. It can be easily sterilized, comparatively simple, do not require any complex requirement of equipment's for production and economical [3]. The therapeutic window of the drug is achieved with less amount of dose which may reduce systemic and ocular side effects. The viscosity of NE is comparable with conventional ophthalmic formulations, so it is a challenge for formulation scientist to increase its residence time in the eye, incorporating the NE into the in-situ gel is an alternative method to improve the therapeutic performance of the drug into the eye by increasing its residence time. Recent trend is to develop cationic oil in water nanoemulsions for ophthalmic delivery to increase ocular drug bioavailability. The reason is ocular surface being negatively

charged, which forms electrostatic interactions with cationic nanoemulsion and thus increases precorneal residence time of formulations [4]. The in-situ gels are simple transparent polymeric solutions which are liquids at storage conditions, but converted into viscoelastic gel once inserted into eye because of phase transition properties of polymers. The reported advantages of gel are increase in ocular residence time and bioavailability; permit delivery of exact reproducible doses and improve patient compliance [5]. This conversion occurs because polymers incorporated in the system may undergo to modifications due to the change in temperature, pH or electrolyte composition of the lacrimal fluids. Various polymeric combinations have been successfully used for desired formulation and tailor-made release profile.

An immune mediated inflammatory disease affects all ages and remains a significant cause of visual loss. Their management requires the use of anti-inflammatory agents. Treatment with topical steroids is associated with serious ocular side effects. In order to overcome the potentially blinding complications of topical steroids; non-steroidal anti-inflammatory drugs (NSAIDs) are being used more frequently. Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin, flurbiprofen, ketorolac, aceclofenac and diclofenac have been found to be viable alternatives to corticosteroids in the management of ocular inflammation.

The major hindrance in developing ocular formulation of NSAIDs; is its poorly soluble nature in water and gets hydrolyzed in aqueous environment after long term storage. The excellent stability and better dissolution profile of NSAIDs were observed when they were blended with oily vehicles which lead in improving the shelf life and efficacy of NSAIDs for relief from ocular inflammatory diseases. Therefore, several attempts have been made to develop non irritative and more comfortable formulations. But they have some shortcomings like hazy vision from semisolid dosage forms, limited efficacy and poor patient compliance. Therefore, we have proposed formulation development of NSAIDs loaded nano emulsified *in situ* ophthalmic gel for sustained delivery and enhanced ocular bioavailability. The first advantages of NE gels are nanosize of globules, which may increase the solubility of the drug, permeation into cornea and aqueous humor. The second advantage is higher residence time into eye due to its viscous nature, which allows modifying the frequency of the drug release such that circumvents numerous instillations of the formulations and leads to enhance the ocular drug bioavailability.

In the light of the above facts, the current study is proposed to develop in-situ ocular nano emulsion gel of NSAIDs which will be capable of upgrading the performance of drug.

2. Review of research

Nadia Morsi *et. al*

They formulated an ion induced nanoemulsion based in situ gel for ocular delivery aiming a sustained drug release and an improved therapeutic efficacy. Acetazolamide loaded nanoemulsion formulations were prepared using peanut oil, tween 80 and/or cremophor EL as surfactant in addition to transcutol P or propylene glycol as cosurfactant. It is then incorporated into ion induced in situ gelling systems composed of gellan gum. They found higher therapeutic efficacy and more prolonged intraocular pressure lowering effect relative to that of commercial eye drops (Azopt®) and oral tablet (Cidamex®) (Nadia Morsi *et. al*., 2017)[6].

Hanan M. El-Laithy *et. al*

Two different chitosan (CS) nanocarriers namely nanoparticles and nanoemulsion were developed to prolong Indomethacin (IM) precorneal residence time and to improve its ocular bioavailability the main limitations in its management of post-operative inflammation and intraocular irritation after cataract extraction. CS-nanoparticles were developed by modified ionic gelation of CS with tripolyphosphate while nanoemulsion was prepared by spontaneous emulsification technique. In vivo studies and histopathological examination revealed that eyes of rabbits treated with nanoemulsion showed clearer healing of corneal chemical ulcer with moderate effective inhibition of polymorph nuclear leuckocytic infiltration (PMNLs) compared with nanoparticles preparation. Moreover, following topical instillation of CS-nanoemulsion to rabbits, it was possible to achieve therapeutic concentration of IM in the cornea through out the duration of the study and fairly high IM level in inner ocular structure, aqueous humor (Hanan M. El-Laithy *et. al*., 2008)[7].

Vaidehi Garg *et. al*

Proposed that tacrolimus nanoemulsion administered topically is a promising therapeutic approach to treat uveitis. Based on previous evidences, they hypothesized that nanoemulsion formulation of tacrolimus can improve efficacy and safety profile (Vaidehi Garg *et. al., 2013)*[8].

Pathak M K *et. al.*

They developed a novel pH triggered nanoemulsified in - situ gel for ophthalmic delivery of fluconazole to enhance the permeation and residence time of the formulation, by overcoming the limitations associated with protective ocular barriers. Pseudoternary phase diagrams were constructed using capmul MCM (oil phase), tween 80 (surfactant) and transcutol P (cosurfactant)

to identify the NE region. Nanoemulsions were prepared by spontaneous emulsification method and evaluated for various pharmacotechnical characteristics. They concluded that nanoemulsified in-situ gel may offer a more intensive treatment of ocular fungal infections due to higher permeation, prolonged precorneal residence time and sustained drug release along with higher in-vitro efficacy, safety and greater patient compliance (Pathak M K et. al., 2012)[9].

3. Objectives

To develop, characterize NSAID loaded nano emulsion.

To develop and characterize a novel ophthalmic nanoemulsion based in situ gelling system to exploit the benefits of nanoemulsion and gelling system in enhancing drug solubilization and permeation across corneal membrane in addition to prolonging the contact time of the formulation to the corneal surface.

To perform *ex vivo* and *in vivo* studies - pharmacokinetics studies.

4. Plan of work for first year

1. Detail Literature survey
2. Procurement of Material,
3. Development and validation of analytical method
4. Preformulation study of selected components
5. Drug Polymer Compatibility Studies

I. *Fourier Transform Infrared Spectroscopy (FTIR)*

II. *Differential Scanning Calorimetric (DSC)*

6. Formulation of Nano emulsion
   - I. Screening of oil
   - II. Screening of surfactants
   - III. Screening of co-surfactant
7. Construction of pseudoternary phase diagram
8. Physicochemical Characterization of Nanoemulsions
   - I. Particle Size Analysis and zeta potential analysis
   - II. Rheological Measurements
   - III. Refractive Index
   - IV. Surface Tension
   - V. pH and Osmotic Pressure

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5. Methodology

Preformulation Study

Preformulation testing was the first step in rational development of dosage forms of drug substance.

Characterization of Drug

Organoleptic properties like Colour, Odour, and Appearance of the aceclofenac (ACE) were determined. Colour of drug was visually observed and compared with standard and reported. Odour of drug were taken by smelling carefully and compared with standard and reported. Appearance of drug was observed under microscope with care and compared with standard and reported.

Melting point

Melting point of drug sample was determined by using melting point apparatus. A few quantity of drug sample was taken and placed in a thin walled capillary tube; the tube was approximately 10-12 cm in length with 1mm in diameter and closed at one end. The capillary which contain sample was placed in melting point apparatus and heated and when drug sample was melted the melting point of sample powder was noted.

Determination of absorption maxima of aceclofenac (λmax) and calibration curve

10 mg of aceclofenac was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved in 30 ml Methanol volume was made up with phosphate buffer 7.2 pH to obtain a stock solution of 100 µg/ml. From this solution 0.5 ml, 1ml, 1.5ml, 2ml, 2.5 ml up to 5ml was taken and diluted up to 10ml using Methanol: pH 7.2 phosphate buffer solution (in the ratio 3:7) to obtain a working standard solution of 5- 50 µg/ml. This solution was scanned between 400 nm to 200 nm with a double beam UV Visible spectrophotometer (Shimadzu 1800) having two matched quartz cells with 1 cm light path[10].

Validation of analytical method

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.

Infrared Spectroscopy

FTIR study of drug sample and identification study was performed by FTIR (Shimadzu Model 8400). The spectrum of ACE and the corresponding reference standard was recorded in the region of 4000 to 500 cm^{-1}. The FTIR absorption spectrum of aceclofenac exhibits maxima.

DSC Thermogram

Differential Scanning Calorimetry (DSC) was performed to determine the thermal behaviour of aceclofenac. Accurately weighed sample (5.0mg) was transferred to aluminium pans and sealed. The sample was run at a heating rate of 10^0C/min over a temperature range 40 to 300^0C using Mettler Tolledo DSC-1 Thermal Analyzer.

Identification of drug

10mg of drug is dissolved in 10 ml of ethanol. 1ml was then added in 0.2ml mixture of 0.6% of potassium ferricynide and 0.9% ferric chloride. This solution was protected from light for 5 minutes. Then 3ml of 1% HCL was added. They allowed to stand it for 15 minute.

Screening of Oil, Surfactant and Co-surfactant for nanoemulsion

The solubility of aceclofenac in several Oils / Surfactants / Co - surfactants was identified by adding an excessive amount of aceclofenac in 2mL of the Oils/Surfactant/Co-surfactant individually in 5mL stopper vials and mixed with vortex mixer for 10min[11]. The vials were then stored at 25±1°C for 3 days to attain equilibrium. The samples were removed and centrifuged at 3000rpm for half an hour. Supernant liquid was collected and diluted suitably. Absorbance was taken by using UV-spectrophotometer at 272nm and solubility was determined.

Construction of pseudo ternary phase diagram

The aqueous water titration method was used to make pseudo ternary system consisting of oil (Peppermint oil), surfactant (Triacetin), and a co-surfactant (Ethanol) [12]. The equivalent psedoternary phase diagrams were created with Tri-plot software. Surfactant and co-surfactant (S/CoS or S_{mix}) in altered weight proportions (1:1, 1:2, 1:3, 1:4, 1:5) were selected and their concentration proportions were identified from available range of nanoemulsions for the study of phase diagrams. For every phase diagram, altered weight ratios of oil and S_{mix}, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 were prepared in vials. Aqueous phase was added drop wise to the each weight ratios of oil and *S*mix continuously till one phase or clear solution was observed. The solution was inspected for the appearance and flow ability after each addition, and the end point of titration were considered when solution converts turbid.

Evaluation of ternary phase diagram

Phase separation time

The prepared nanoemulsion with different weight proportions of oil- surfactant-Cosurfactant mixture evaluate for phase separation time by visual observation.

Refractive Index

Refractive index of the formulations was done by using the Abbey's refractometer (Rolex India).

% Transmittance

Percent transmittance was measured by diluting nanoemulsion 1000 times in distilled water and transmittance was measured by spectrophotometrically at 650nm.

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6. Result and Discussion

Preformulation Study

Preliminary Evaluation and Characterization of drug-

The preliminary characterization of drug i.e physical properties of drug were evaluated (Table 1). All the observed characteristics matches with standard results.

Table 1: Preliminary Evaluation and Characterization

Sr.no	Characteristics	Standard	Observation	Inferences
1	Color	White	White	Complies
2	Odour	Odourless	Odourless	Complies
3	Appearance	Amorphous powder	Amorphous powder	Amorphous

Melting Point:

The average melting point of aceclofenac was determined by capillary method and was found to be 151°C. This is in good agreement with reported melting point.

Determination of absorption maxima of aceclofenac (λmax) and calibration curve:

UV spectrum for aceclofenac in methanol and buffer pH 7.2 is shown in Figure (1). It shows absorbance maxima at 272 nm and it matches with the reported value.

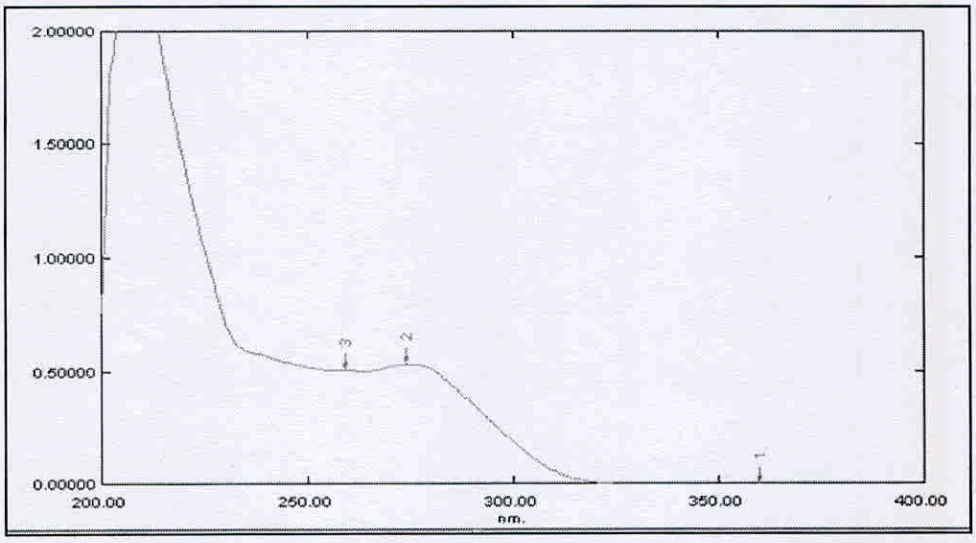

Figure 1: UV Spectrum of aceclofenac at 272 nm

Standard Calibration Curve of aceclofenac:

Its general method for determining the concentration of the substance in an unknown sample by comparing the unknown to a set of standard samples for known concentration. Calibration Curve of aceclofenac in Methanol and buffer pH 7.2 at 272nm.

Table 2: Absorbance of aceclofenac

Concentration	Absorbance
5ug/ml	0.08577
10ug/ml	0.16432
15ug/ml	0.2364
20ug/ml	0.30677
25ug/ml	0.38016
30ug/ml	0.45325
35ug/ml	0.52959
40ug/ml	0.5932
45ug/ml	0.67537
50ug/ml	0.7549

Construction of calibration curve:

The calibration curve of aceclofenac was plotted in 7.2 pH buffer solution at 272 nm. The equation obtained was y = 0.0735x + 0.0138 .The correlation coefficient was found to be 0.9997 as shown in figure 4.

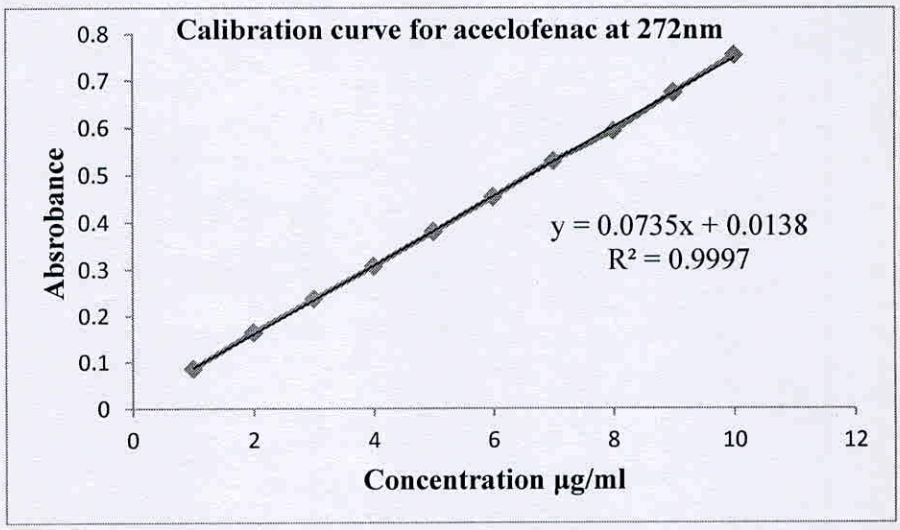

Figure 2: Construction of calibration curve

Table 3: Standard Calibration Curve Statistics

Sr. No.	Parameter	Observation
1	λmax (nm)	272
2	Beer's law limit (µgm/ml)	5-50
3	Slope (b)	0.0735
4	Intercept (a)	0.0138
5	Correlation Coefficient	0.9997

Validation of analytical method

Validation of an analytical method is the process to establish the performance characteristics of the developed method to meet the requirements of the intended analytical application. The UV method was validated in terms of precision, LOD, LOQ, linearity and sensitivity.

Table 4: Validation of method Statistics

Validation Parameter	Aceclofenac
Absorption Maxima	272nm
Linearity Range	5-50µg/ml
Std Regression Equation	Y = 0.0735x + 0.0138
Correlation Coefficient	0.9997
Molar Absorptivity	248597.9599
A % 1 Cm	352.3014
Precision	Intraday = 105.0045 %, Inter-day = 100.1766 %
LOD	0.4815 ug/ml
LOQ	1.4593 ug/ml
Sensitivity	0.029259

Infrared Spectroscopy

IR spectrum was taken by FTIR and graph is shown in Figure (3) and Table shows peaks and it gives conformity of structure of drug.

An IR spectrum was showing characteristics peaks of C-N, C=O, N-H groups. In aceclofenac spectrum, it has characteristics peaks at 1139 cm^{-1} and that corresponds to C-N stretching. The peak at 1714 cm^{-1} indicates the presence of C=O stretching functional group.

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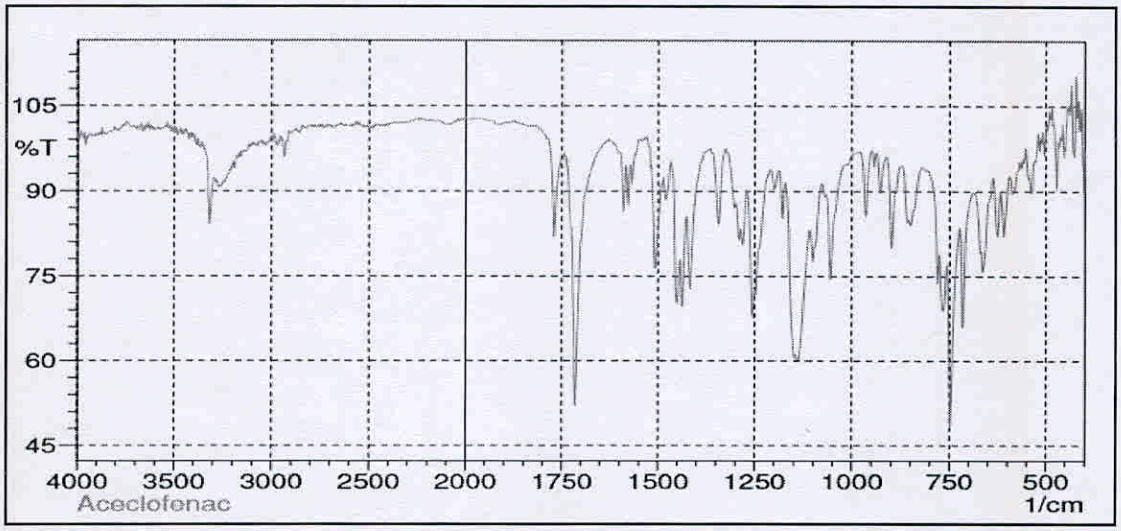

Figure 3: IR spectra of Aceclofenac

Spectrum also indicates the presence of N-H group. The spectrum reveals the characteristic peak in the typical range at 1506 cm^{-1} confirms the presence of amide group in the aceclofenac. These are quite identical with the reported wave numbers. This gives confirmation for drug.

Table 5: IR peaks of Aceclofenac

Group	Observed (cm^{-1})	Standard (cm^{-1})
C-N	1139	1350-1000
C=O	1714	1725-1700
C-O	1247	1300-1000
N-H	1506	1640-1550

DSC Thermogram:

DSC study was conducted to check the identity and purity of the aceclofenac. DSC Spectrum of aceclofenac is shown in Figure (4).

The thermogram of drug was characterized by melting endotherm at 152.90°C. Thus the DSC thermogram of the drug was found to be in agreement with specifications (Table 6).

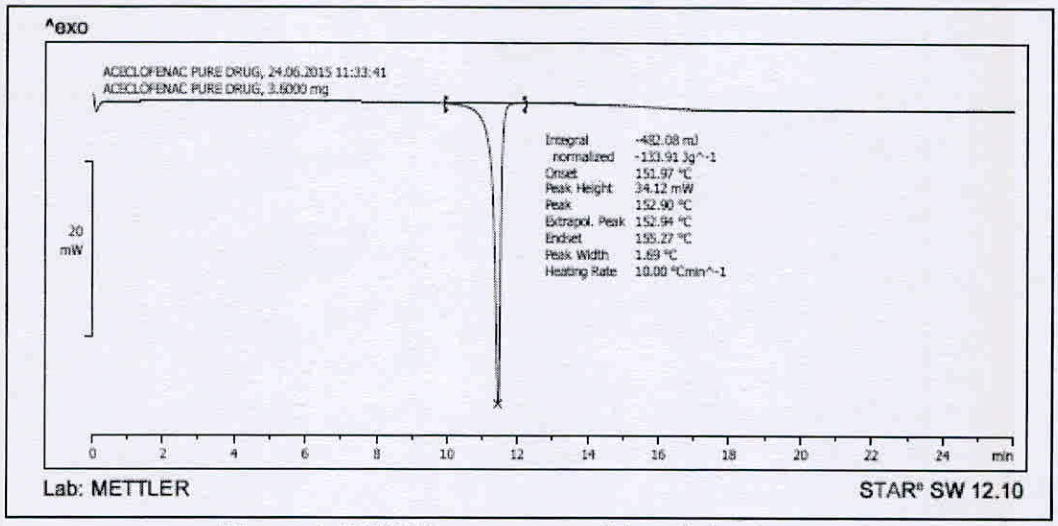

Figure 4: DSC Thermogram of Aceclofenac

Table 6: Observed value of DSC Thermogram of Aceclofenac

Parameters	Observation
Onset	151.97 °C
Normalized	-133.91Jg^-1
Peak Height	34.12mW
Peak	152.90 °C
End set	155.27 °C
Heating Rate	10.00 °Cmin^-1

Identification of drug

After allowing the test solution for 15 minutes the blue colour precipitation was observed. That means the aceclofenac drug was confirms.

Screening of Oil, Surfactant and Co-surfactant

Drug loading in the formulation is precarious step for the development of nanoemulsion system because drug solubility is dependent on formulation component for less soluble API's. Solubility of the drug in oil phase is one of the significant benchmarks for the selection of oils. The solubility of aceclofenac was found to be highest in Peppermint oil (26.63mg/ml), Triacetin (14.88mg/ml) and Ethanol (73.06mg/ml) as related to other oils, surfactant and co-surfactant. Peppermint oil was selected as the oil phase for the development of nanoemulsion formulation. Based upon the solubility of aceclofenac; surfactant Triacetin was selected for further study. The objective was to recognize the surfactant that has the maximum solubilization capability for the selected oil. A preliminary sign on the possibility of nanoemulsion formation with this system

should be given from surfactant-oil miscibility. Other advantage of the Triacetin is, it is a nonionic surfactant with less toxicity. A supplementary significant benchmark for the selection of the surfactant is its HLB value, which must be greater than 10 to form nanoemulsion and the Triacetin also satisfying this requirement. HLB After selection of oil phase and surfactant, the objective was identifying the co-surfactant. As the drug shows higher solubility in the Ethanol i.e (73.06mg/ml), it was chosen as the co-surfactant for the nanoemulsion development. Based upon the solubility of aceclofenac in co-surfactant Ethanol was selected for further study. Co-surfactant was incorporated to acquire nanoemulsion system, it decreases the interfacial tension. All the co-surfactant studied are pharmaceutically accepted components.

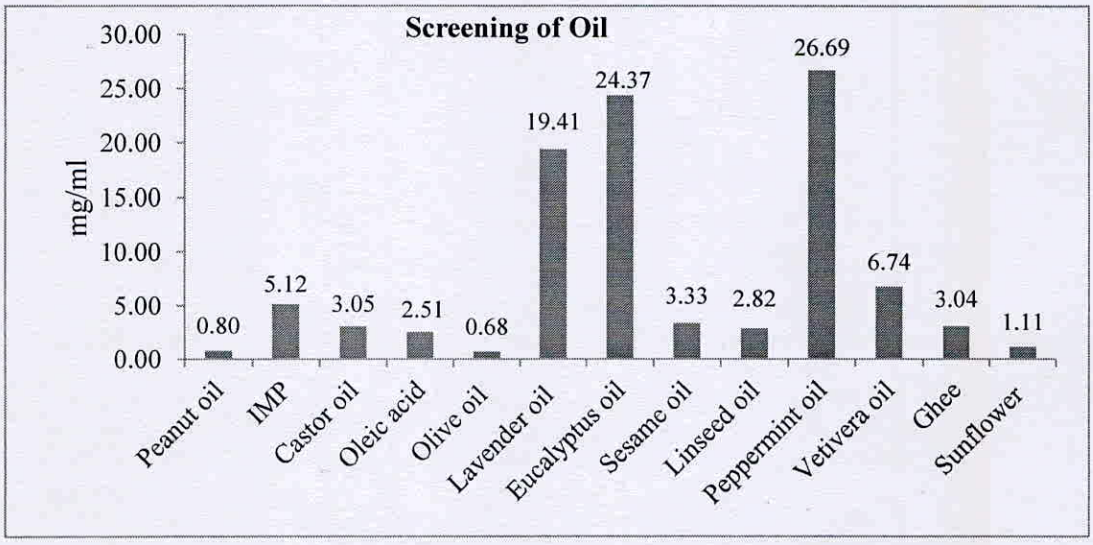

Figure 5: Screening of Oil

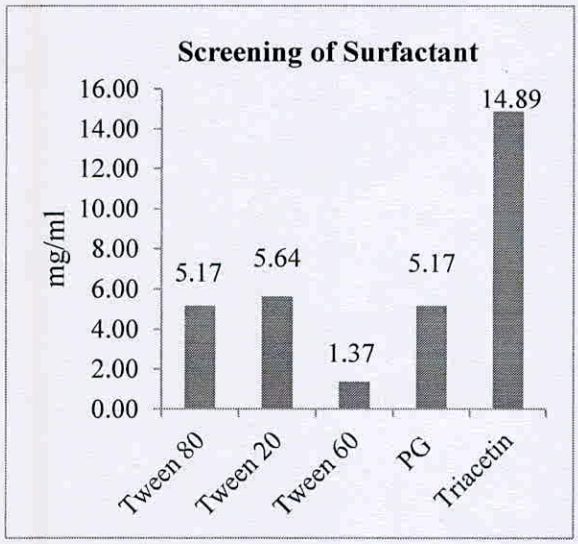

Figure 6: Screening of surfactant

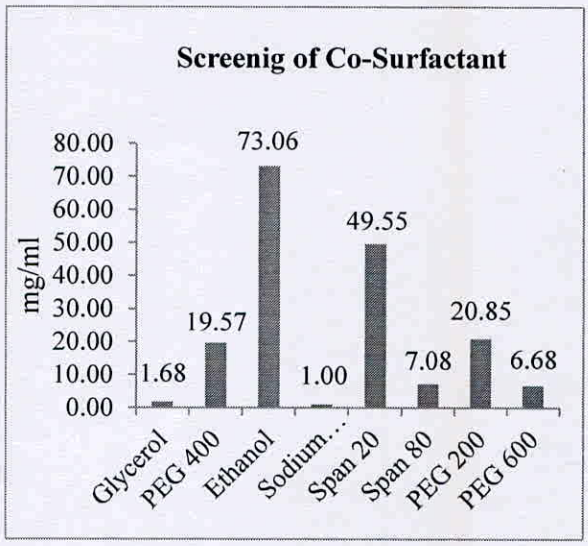

Figure 7: Screening of Cosurfactant

Construction of pseudo ternary phase diagram

The pseudoternary phase diagram determines concentration range of the components for the existence of nanoemulsion in the absence of aceclofenac. Figure represents the pseudoternary phase diagrams by varying the *S*mix (Surfactant: Co-surfactant) ratio at 1:1, 2:1, 3:1, 4:1 and 5:1. The dark area of the diagram represents the NE region which is selected for the study, while remaining portions exhibits emulsion region which is not selected for further study. The area of NE region became smaller as the *S*mix ratio increased from 1:1 to 5:1. The smaller region designated better nanoemulsifying efficacy of the prepared NE formulation and improve the interaction between the components. The surfactant ad co-surfactant mass ratio and type and concentration of oil employed had been found to be an important point for the phase properties. *S*mix ratio is inversely proportional to the microemulsion area, as the Smix ratio increases the microemulsion region get decreases. Depending upon the region of Microemulsion area (Figure 8) the ratio of *S*mix 1:1 was selected.

Evaluation of ternary phase diagram:

After selecting the Smix ratio based upon the NE region the concentrations i.e (1:9, 2:8, 3:7, 4:6, 5:5, 7:3, 8:2, 9:1) of Oil and Smix ratio was further evaluated for the Phase separation time, Refractive index and % Transmittance

Phase separation time

Phase separation of NE was determined visually. It was determined for the stability of nanoemulsion.

Refractive Index

Refractive index measurements were done by using Abbey's Refractometer. RI measurements identify likely impairment of vision or comfort to the patient after administration of formulation. Refractive index of tear fluid 1.340-1.360 and it is recommended that eye formulation should have refractive index value not greater than 1.476. Aceclofenac loaded nanoemulsion had refractive index values ranging from 1.36 to 1.458, which are within the recommended values.

% Transmittance

The % transmittance was found to be in the range of 68.75–94.55%. Based upon this parameter 0.8:1.2 ratio of oil and *S*mix was selected for the further study.

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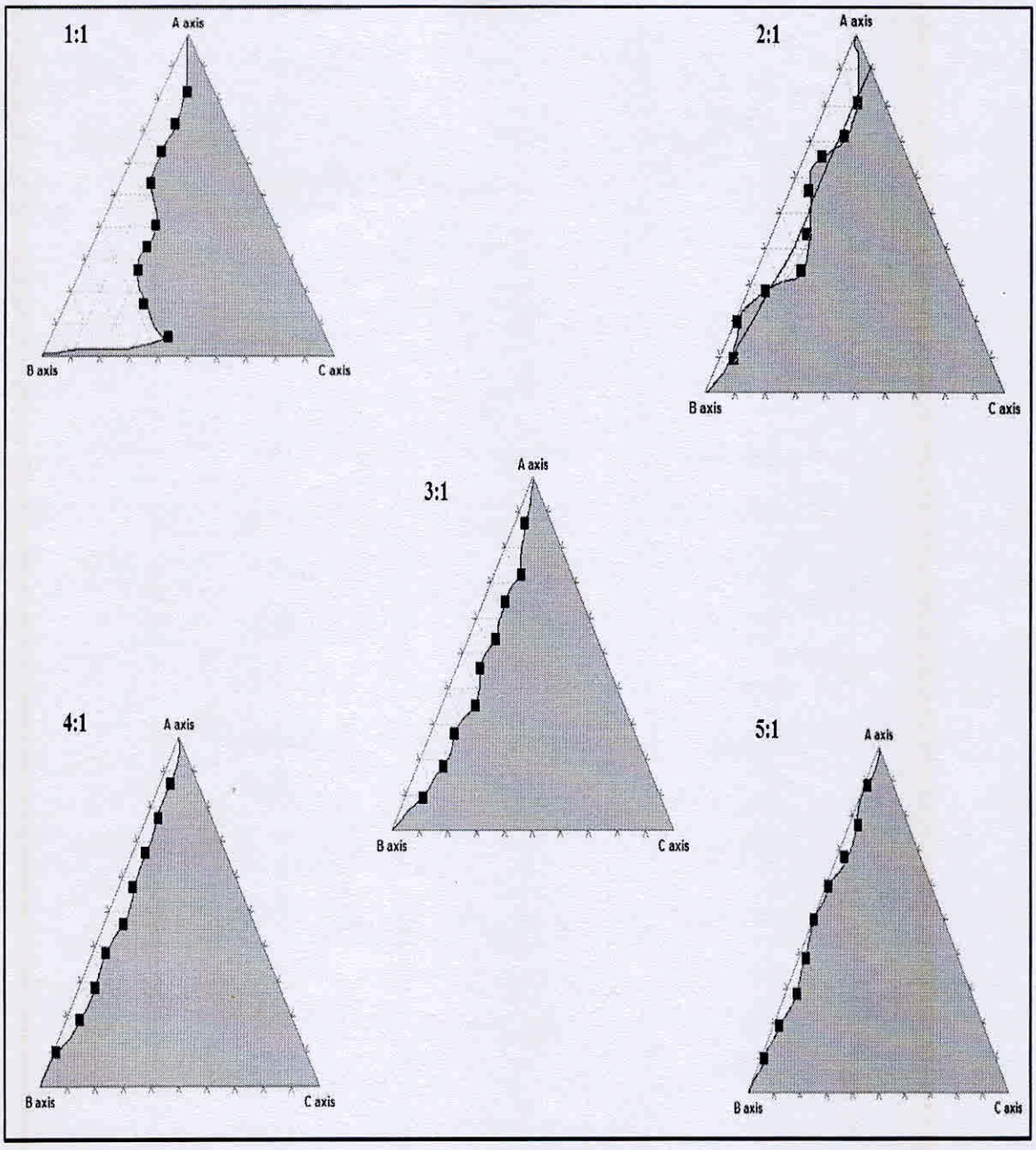

Figure 8: Ternary phase diagram A axis- Oil, B axis- Smix, C axis- Water

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- Certificate –

This is to certify that the work incorporated in the report entitled "Design & Development of Nasal Drug Delivery System for Brain Targetting" is an original contribution submitted to the Savitribai Phule Pune University, Pune by Mr. Shailendra S. Salvankar, based on the research work carried out by him during 2016-2018.

Such material has not been submitted to any other University / Institute for any financial support. The literature related to the problem investigated has been appropriately cited and duly acknowledged wherever facilities and suggestions have been availed of.

Date: 11/12/2019
Place: Pune

Mr. Shailendra S. Salvankar

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This is to certify that the work incorporated in the report entitled "Phytoalexins: A Novel Remedy" is an original contribution submitted to the Savitribai Phule Pune University, Pune by Mrs. Kavita N. Yadav, based on the research work carried out by him during 2016-2018.

Such material has not been submitted to any other University / Institute for any financial support. The literature related to the problem investigated has been appropriately cited and duly acknowledged wherever facilities and suggestions have been availed of.

Date: 11/12/2019
Place: Pune

Mrs. Kavita N. Yadav

Forwarded through:

Principal/ Director

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Thergaon (Kalewadi), Pune-411 033

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This is to certify that the work incorporated in the report entitled "Studies on CYP450 based Drug Metabolism using RP-HPLC" is an original contribution submitted to the Savitribai Phule Pune University, Pune by Dr.(Mrs.) Sampada D.Dalvi, based on the research work carried out by him during 2016-2018.

Such material has not been submitted to any other University / Institute for any financial support. The literature related to the problem investigated has been appropriately cited and duly acknowledged wherever facilities and suggestions have been availed of.

Date: 11/12/2019

Place: Pune

Dr.(Mrs.) Sampada D. Dalvi

Forwarded through:

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This is to certify that the work incorporated in the report entitled "Neuroprotective Activity of Green Tea Flavonoids in Parkinson's Disease Models of Zebrafish" is an original contribution submitted to the Savitribai Phule Pune University, Pune by Dr. Digambar B. Ambikar, based on the research work carried out by him during 2016-2018.

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Date: 11/12/2019
Place: Pune

Dr. Digambar B. Ambikar

Forwarded through:

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Exec. President

Shri. Kishor H. Mungale
Secretary

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This is to certify that the work incorporated in the report entitled "Implementation of Design of Experiments for Optimization of Forced Degradation Conditions and Studying the Degradation Mechanism of an Anticancer Drug" is an original contribution submitted to the Savitribai Phule Pune University, Pune by Mrs. Babita A. Agarwal, based on the research work carried out by him during 2016-2018.

Such material has not been submitted to any other University / Institute for any financial support. The literature related to the problem investigated has been appropriately cited and duly acknowledged wherever facilities and suggestions have been availed of.

Date: 11/12/2019
Place: Pune

Mrs. Babita A. Agarwal

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This is to certify that the work incorporated in the report entitled "Ecochemicals: An Evolution of Ecological Phytochemistry" is an original contribution submitted to the Savitribai Phule Pune University,Pune by Dr. Manohar J. Patil, based on the research work carried out by him during 2016-2018.

Such material has not been submitted to any other University / Institute for any financial support. The literature related to the problem investigated has been appropriately cited and duly acknowledged wherever facilities and suggestions have been availed of.

Date: 11/12/2019

Place: Pune

Dr.Manohar J. Patil

Forwarded through:

Principal/ Director

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This is to certify that the work incorporated in the report entitled "Design & Evaluation of Targeted Delivery of Gefitinib Using Polyethyleneglycolated Multiwall Carbon Nanotube" is an original contribution submitted to the Savitribai Phule Pune University, Pune by Mr. Mukesh P. Ratnaparkhi, based on the research work carried out by him during 2014-2016.

Such material has not been submitted to any other University / Institute for any financial support. The literature related to the problem investigated has been appropriately cited and duly acknowledged wherever facilities and suggestions have been availed of.

Date: 17/4/2017
Place: Pune

Mr. Mukesh P. Ratnaparkhi

Forwarded through:

Principal/ Director

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This is to certify that the work incorporated in the report entitled "Design, Synthesis And Biological Evaluation of some Novel Triazolo-oxadlazole Derivatives" is an original contribution submitted to the Savitribai Phule Pune University, Pune by Dr. Rahul H. Khiste, based on the research work carried out by him during 2014-2016.

Such material has not been submitted to any other University / Institute for any financial support.The literature related to the problem investigated has been appropriately cited and duly acknowledged wherever facilities and suggestions have been availed of.

Date:

Place: Pune 17/4/2017

Dr. Rahul H. Khiste

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Principal/ Director

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