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DEVELOPMENT AND EVALUATION OF HERBAL MICROEMULSION OF *FICUS RELIGIOSA* FOR TOPICAL DELIVERY

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Abstract- For local dermatological activity, topical drug administration is usually preferable. Drug solubility, residence duration, lipophilicity, and permeability are all factors that restrict the effectiveness of topical medications. Creams, ointments, and other traditional dosage forms have problems such as lack of stability, stickiness, poor absorption, and penetration, especially when dealing with giant molecules. To combat this, microemulsion was created, which is a drug delivery system that focuses on hydrophobic medicines. This study aims to make an herbal microemulsion and test it for improved solubility, permeability, and superior anti-inflammatory activity. Herbal Microemulsion made from linseed oil, Tween 20, and Span 20 can be a topical anti-inflammatory medication.

Keywords: Ficus Religiosa, Tween 20, Span 20, Anti-inflammatory, Microemulsion, Topical drug delivery.

1. Introduction

Inflammation is the body's rapid response to pathogens, unpleasant stressors such as toxins, or muscular trauma that causes tissue and cell deterioration. The individual's defensive endeavor is to eliminate the harmful stimuli and start the recovery mechanism[1]. Cytokines, chemokines, PGs, platelet stimulating factor, NO, and histamine are some arbitors involved in inflammation. Among all these, PGs are often regarded as powerful pro-inflammatory mediators[2]. Furthermore, statistics indicate that the production of reactive oxygen species (ROS) significantly increased through inflammation. Mast cell degranulation has been reported to have a role in inflammation by releasing various messengers such as Histamine associated with inflammation and allergies[3].

Ficus religiosa (FR) L. (Moraceae), also known as 'Peepal,' is an Indian fig species and sacred tree[4]. Leaves decoction has been utilized for the medication of asthma, cough, sexual disorders, diarrhea, hematuria, earache and toothache, migraine, eye troubles, gastric problems, and scabies; also used as an analgesic for toothache[5]. Ficus religiosa has been discovered to have

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anti-inflammatory and analgesic properties. The suppression of PG production is the principle behind the activity[6]. The inhibitory function of Ficus religiosa leaf extract has been discovered due to the inhibition of histamine, serotonin (5HT), Kinins, and PG's release[7]. Mast cell degranulation causes inflammation, and Ficus religiosa extract suppresses the proportion of degranulation generated by propranolol or carbachol substantially[5].

1.1. Microemulsions

Micro-emulsified materials were found in 1943 by Hoar and Schulman, who noted that an opalescent emulsion treated by a surfactant turned apparent after the inclusion of medium-chain alcohol. Micro emulsified components are regarded as dispersions of oil, surfactant, aqueous phase, and, sometimes, co-surfactants[8]. They are excellent diverse transporter with several notable qualities, including increased bioavailability of poorly soluble medications, high absorption, and penetration due to low surface tension and small droplet size, with a premium methodology[9]. It has been easier to develop diverse microstructures by varying the quantities of ingredients, which may be categorized into three types based on their physicochemical features, respectively[10]

- Oil-in-water microemulsions,
- Bi-continuous microemulsions,
- Water-in-oil microemulsions[8].

Microemulsions are defined as emulsions with droplet sizes of less than 0.1 m. Despite their tiny size (far smaller than the wavelength of visible light (400–800 nm), such droplets are unlikely to reflect light and are therefore undetectable across an optical microscope, rendering the Microemulsion technology translucent[11].

As a result, microemulsions have received much attention as a viable delivery mechanism for hydrophilic, lipophilic, and ampiphilic bioactive chemicals for pharmaceutical, neutraceutical, and aesthetic purposes[12].

2. Materials and Methods

2.1. Collection and Authentication of Plant

The plant's crude drug (fresh leaves) was collected from the nearby garden in New Delhi and authenticated by Sci. A.S. Upadhyay. The aerial roots were washed under tap water, dried in sunlight for 1-2 months, and then finely powdered in a grinder. Vidya S. Kukde et al. (2021) studied the physiochemical parameters of powdered material.

2.2. Extraction[13]

The extraction process was done with the help of the three solvents. The solvents were selected based on the % yield and other extractive values. 200 g of the leaves powder was taken in the Soxhlet apparatus and heated at 80°C for 8 hours for each solvent. The collected extract was air dried and stored in an air-tight container. The extract was then subjected to phytochemical screening and extractive values. The final extract was selected and used to formulate the micro elusion based on the percentage yield. (Table-2&3)

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Fig.1: Leaves of *Ficus religiosa*[14]

2.3. Chemicals

Carbopol 940, Triethanolamine, Petroleum ether, ethanol, propylene glycol, Tween 20, Span 20 PEG 400 were purchased from Loba, CDH, and Isochem. All were laboratory grade and extra pure.

2.4. Preparation of Microemulsion[15]

For the preparation of Microemulsion Span 20 concentration utilized in (F1= 1%, F2= 0.75%, F3= 0.5%) and 500mg of the drug content (*Ficus religiosa*) has been dispersed in linseed oil (5%), which provided the oil phase. Tween 20 and PEG-400 was dissolved in purified water at the following concentrations: F1= 0.25%, F2 = 0.5%, 0.75%. The oil and surfactant concentrations have all been considered depending on the HLB value. Both parts were held in a water bath and heated to 70-80°C independently[15]. At last, both phases were mixed and cooled to room temperature after 15 minutes of continuous stirring at 5 rpm.

Table-1: Formulae of Microemulsion

Ingredients	F1	F2	F3
Extract	500mg	500mg	500mg
Tween 20 (%)	0.25	0.5	0.75
Span 20 (%)	1	0.75	0.50
Linseed Oil (%)	5	5	5
Water (ml)	q.s	q.s	q.s

2.5. Evaluation Parameters[16]

The formed microemulsions were finally identified and characterized based on their physical attributes that can be used to interpret and adjust the system's functional features. Microdroplets can readily distinguish from macro droplets by their optical characteristics, potential gravitational behavior, and rheological studies.

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2.6. Assessment of Globule size[17]

A dynamic light dispersion particle size analyzer (Nanotrac A-150) was utilized to determine the globule size of emulsions. It can capture particles as small as 0.08 nm and as large as 6.8 m. The dilution was accomplished by diluting 1 ml of emulsion to 20 ml with Millipore water and obtaining values.

2.7. Translucency[18]

Because Microemulsion droplets are more minor than visible light wavelengths, white light can flow through the dispersed system, rendering it transparent or translucent. Optical transparency and homogeneity of the Microemulsion solutions were checked using standard assessment under bright light. Insoluble drugs or other solid ingredients were also examined in the processes.

2.8. The integrity of the thermodynamic system

The preparation was centrifuged at 3500 rpm for 30 minutes to maintain physical integrity.

2.9. Measurement of pH[19,20]

The pH of Microemulsion compositions was determined utilizing a digital pH meter that had been calibrated before use with buffered solutions at pH 4 and pH 9.2. A certain quantity of the formulation was weighed out and diluted with calibrated distilled water before thoroughly blended. The pH meter's electrode was immersed in the developed formulation for pH evaluation. Approx. 2gm of prepared Microemulsion has been dispersed in 20ml of distilled water with the help of a pH meter.

2.10. Viscosity[21]

With the help of a digital viscometer (IGenelab) viscosity of the Microemulsion was obtained. The internal friction that occurs when a fluid film is compelled to flow for another layer is measured by viscosity. The spindle no. 64 was rotated at 50 revolutions per minute. Microemulsion specimens were expected to rest at room temperature for 30 minutes before observations were conducted.

2.11. Analysis of Mechanical Stress[22]

Mechanical stress testing was used to assess the chemical and physical integrity of a prepared Microemulsion. The various Microemulsions preparations (F-1 to F-3) were centrifuged at 2000 rpm for various time intervals (10, 30, and 60 minutes), and the proportion of phase separation of the formulation was recorded.

2.12. Drug Content Analysis[23]

1 mL of Microemulsion Formulations was put into a beaker with 10 mL of methanol. After stirring for 30 minutes, the material of the beaker was retained for 24 hours. The materials of the beaker were subsequently transferred to a centrifuge tube after 24 hours and centrifuged for 10 minutes at 3000 rpm. The supernatant was filtered and collected. Then, 0.1 mL of the supernatant was diluted with Phosphate Buffer Saline (PBS) pH 7.4, and drug concentration was determined spectrophotometrically.

2.13. Optical Birefringence[24]

Light transmittance was measured by sandwiching Microemulsion between two polarizing plates in a series. Following that, one of the plates was rotated 90 degrees about the other

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(crossed polarizers) and evaluated.

2.14. Measurement of electrical conductivity[25]

The conductivity measurements indicate whether the resulting Microemulsion system is oil- or water-continuous. By evaluating the electrical conductivity (□), the solubilization of the water phase in the proposed oily mixture was systematically assessed. A conductivity meter (Digital Conductivity meter from Cell Constant) was used to determine the conductivity (□□ of the formed samples.

3. Results

3.1. Droplet Size Determination

The globules were spherical with an average size range of 4.14 - 5.12 m throughout the investigation duration and under all experimental circumstances, as per the microscopic analysis. The morphology of the globules did not alter significantly throughout the investigation.

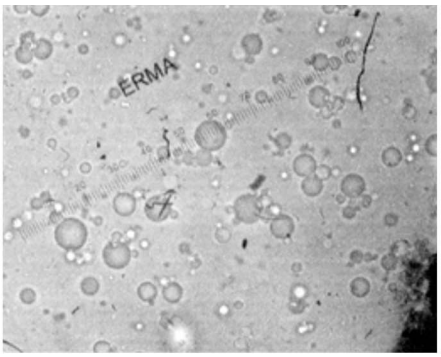
The photomicrographs show a slight rise in globule size as a function of time (Fig.2).

3.2. Translucency

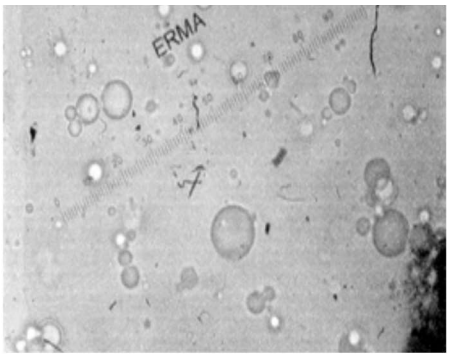
All the microemulsions produced were transparent and seemed to be a homogeneous single-phase liquid when viewed under bright light. There was no evidence of undissolved medication or solid substance in each sample.

3.3. Centrifugation:

On centrifugation at 3500 rpm for 30 minutes, none of the Microemulsion solutions exhibited symptoms of phase separation. The system was quickly and wholly identified as microemulsion only due to this performance.



F1



F2

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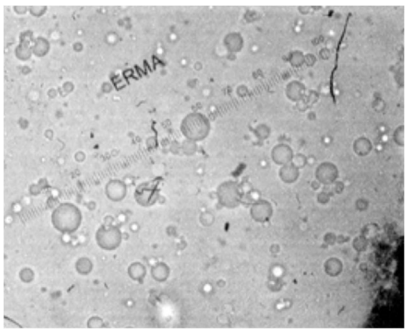

F3

Fig.2- Globule Size Determination

3.4. pH

The pH change in this investigation did not deviate significantly from the average human skin pH, indicating that the composition remained consistent and acceptable for topical administration (table-5).

3.5. Optical Birefringence

The specimens were evaluated by eye inspection in a cross polarizer for sample homogeneity and birefringence. When examined with a cross polarizer, the microemulsions seemed dark. According to the findings, all of the microemulsions seemed to be optically isotropic colloidal dispersions.

3.6. Conductivity Test

The conductivity of the optimized microemulsions (F1) was found to be 0.244 as calculated by the conductivity meter. According to the electro conductivity investigation, the system is of the o/w type (table-10).

3.7. Staining Test

Methylene blue solution, a water-soluble dye, was introduced to the optimized Microemulsion (F1). Because the dye dissolves consistently across the solution just because the continuous phase was water. As a result, the improved formulation F1 was discovered to be an o/w Microemulsion.

3.8. Viscosity Studies

The Microemulsion solutions' rheological behavior suggested that they were non-Newtonian, with viscosity decreasing as shear rates increased (table-6).

Table-2: % Yield of Microemulsion

Sn.	% Yield	Result
1	Ethanol	11.1%
2	Methanol	9.02%
3	Water	10.05%

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Table-3: Extractive Values of Microemulsion

Sn.	Extractive Values	Results
1	Water Soluble Extractive	1.90%
2	Alcohol Soluble Extractive	2.01%
3	Ash Value	1.3%
4	Acid Insoluble Ash	2.8%
5	Water soluble Ash	2%

Table-4: Phytochemical screening of Microemulsion

Sn.	Test	Solvents		
		Methanol	Ethanol	Water
1	Alkaloids	+	+	+++
2	Carbohydrates	++	-	++
3	Saponins	+	+	++
4	Glycosides	-	-	-
5	Steroids	+	+	+
6	Phenolic	+	+	+
7	Flavonoids	+	+	+

Table-5: pH of Microemulsion

Formulations	pH
F1	6.1±0.2
F2	5.9±0.2
F3	6.2±0.1

Table-6: Viscosity of Microemulsion

Formulations	Viscosity (cps)
F1	387.7±2.3
F2	415.7±4.0
F3	399.3±10.5

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Table-7: Mechanical stress of Microemulsion

S.No.	Centrifugation time (min)	% Phase Separation		
		F1	F2	F3
1	10	-	2	6
2	30	5	-	4
3	60	3	6	-

Table-8: Particle Size of Microemulsion

Formulations	Particle Size
F1	108.3±3.1
F2	95.7±4.9
F3	100.0±1.0

Table-9: Drug Content of Microemulsion

Formulations	% Drug Content
F1	91.3±0.6
F2	90.3±1.5
F3	91.0±1.0

Table-10: Conductivity of Microemulsion

Sn.	Microemulsion	Conductivity (Mean±SD)
1	F1	0.244±0.005
2	F2	0.212±0.003
3	F3	0.208±0.003

4. DISCUSSION

The physical appearance, viscosity, pH, and stability characteristics of Microemulsion systems used in cosmetics are significantly responsible for their quality and desirability under all storage circumstances. The ionization of water and other additional compounds in a sample has been facilitated by electrical current, which is demonstrated in the emulsion's consistency[19].

Microemulsion systems with oil as the external component are poor conductors of electricity, whereas systems with water as the external component are strong conductors. Because it did not facilitate electricity, the created emulsion was stable and W/O type. An identical outcome

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has been mentioned in prior research for identifying Microemulsion types using conductivity and dilution tests in a corresponding approach[21].

The viscosity of the produced system has a significant impact on flow characteristics. In context, viscosity can determine the quality and stability of microemulsions. Creaming happens in this sort of Microemulsion due to the settling of water globules, resulting in the formation of two distinct layers. According to Stroke's law, the exterior phase's viscosity influences the interior phase's sedimentation (water globules). As a result, as the temperature raised, the viscosity of the preparation reduced, causing the Microemulsion to liquefy[23].

A Microemulsion is a biphasic, thermodynamically volatile formulation in which the density imbalance between the dispersed and continuous components can generate instability, such as creaming or sedimentation of the dispersed phase under gravity, leading to phase segregation[26]. Centrifugation is a quick method for determining the physical stability of novel preparations. Under the influence of centrifugal force, both components are isolated on the grounds of their densities. Centrifugation causes the globules to collide and agglomerate, resulting in emulsion de-stabilization. The modest phase separation seen in the last week of the study could be attributable to the sedimentation or creaming of dispersed globules under accelerated circumstances due to the steady loss in surfactant action with time. Induced rapid phase separation and instability in produced W/O microemulsions have been documented in prior investigations using the centrifugation method[26].

Human skin has pH values varying from 4.5 – 6, and a pH of 5.5 is typically regarded as an average human skin pH. Consequently, preparations intended for topical administration should have a pH value nearer to the skins. The pH change in this investigation did not deviate significantly from the average human skin pH, indicating that the formulation was stable and acceptable for topical administration. Previous studies have also assessed topical microemulsions' pH, with results comparable to those found in this study[24].

Microscopy can examine globules' morphology, size, and distribution in the continuous phase. It can also be used to learn more about emulsion destabilization sources. Particles of uniform size should be evenly distributed throughout the external phase of a stable emulsion[25].

Physical stability, flow properties, structural features, and deformation of semisolid compositions under stress are investigated using rheological investigations. The flow index values in this investigation stayed within the range of 0 - 1, indicating pseudo plastic behavior (non-Newtonian flow), which is an essential property for topical medication application[22].

5. CONCLUSION

The results of this study reveal that the optimized water-in-oil (W/O) emulsion is highly stable under test conditions, with no changes in physicochemical parameters over time. As a result, the proposed formulation could be a sound Microemulsion system for topical distribution of diverse plant extracts applications.

6. REFERENCES

1. Charde R. M., Dhongade H. J., Charde M. S and Kasture A. V. Evaluation of antioxidant, wound healing and anti-inflammatory activity of ethanolic extract of leaves

DOI: 10.5281/zenodo.6945690

of Ficus religiosa. International Journal of Pharma Sciences and Research. 2010; 1: 73-82.

2. Prakash PR, Rao NGR, Chowdary S. Formulation, evaluation and anti-inflammatory Activity of topical Etoricoxib gel. Asian journal of pharmaceutical and clinical research, 2010; 3:126- 128.
3. Biswajit Biswal, Nabin Karna, Jyotiranjan Nayak, Vivek Joshi, Formulation and Evaluation of Microemulsion Based Topical Hydrogel Containing Lornoxicam. Journal of Applied Pharmaceutical Science Vol. 4 (12), 2014, pp. 077-084
4. Kumar, A.; Sandeep, D.; Tomer, V.; Gat, Y.; Kumar, V. Ficus religiosa: A wholesome medicinal tree. J. Pharmacogn. Phytochem. **2018**, 7, 32–37.
5. Rathod, V.D.; Digambar, N.W.; Pillai, S.; Bhangale, J.O.; Bhangale, P.J. Antiarthritic activity of ethanolic extract of Ficus religiosa leaves in FCA induced arthritis in rats. World J. Pharm. Res. **2018**, 7, 778–789.
6. Jayant, K.K.; Vijayakumar, B.S. In-Vitro anti-oxidant and anti-diabetic potential of endophytic fungi associated with Ficus religiosa. Ital. J. Mycol. **2021**, 50, 10–20.
7. Bhalerao SA, Sharma AS. Ethenomedicinal, phytochemical and pharmacological profile of Ficus religiosa Roxb. Int J Curr Microbiol App Sci 2014; 3(11): 528-538.
8. Kaur, G.; Mehta, S.K. Developments of Polysorbate (Tween) based microemulsions: Preclinical drug delivery, toxicity and antimicrobial applications. Int. J. Pharm. **2017**, 529, 134–160.
9. Mouri, A.; Legrand, P.; El Ghzaoui, A.; Dorandeu, C.; Maurel, J.C.; Devoisselle, J.M. Formulation, physicochemical characterization and stability study of lithium-loaded microemulsion system. Int. J. Pharm. **2016**, 502, 117–124.
10. Mouri, A.; Diat, O.; Lerner, D.A.; El Ghzaoui, A.; Ajovalasit, A.; Dorandeu, C.; Maurel, J.C.; Devoisselle, J.M.; Legrand, P.Water solubilization capacity of pharmaceutical microemulsions based on Peceol®, lecithin and ethanol. Int. J. Pharm. **2014**, 475, 324–334.
11. C. Nastiti, T. Ponto, E. Abd, J.E. Grice, H.A.E. Benson, M.S. Roberts Topical nano and microemulsions for skin delivery Pharmaceutics, 9 (4) (2017).
12. L. Chiappisi, L. Noirez, M. Gradzielski A journey through the phase diagram of a pharmaceutically relevant microemulsion system J. Colloid Interface Sci., 473 (2016), pp. 52-59.
13. N. Aggarwal, S. Goindi, R. Khurana Formulation, characterization and evaluation of an optimized microemulsion formulation of griseofulvin for topical application, Colloids and surfaces B, Biointerfaces, 105 (2013), pp. 158-166
14. U.A. Shinde, S.H. Modani, K.H. Singh Design and development of repaglinide microemulsion gel for transdermal delivery AAPS PharmSciTech, 19 (1) (2018), pp. 315-325.
15. S. Sunitha, W. Jitendra, D. Sujatha, M. Santhosh Kumar Design and evaluation of hydrogel-thickened microemulsion for topical delivery of minoxidil Iran. J. Pharm. Sci., 9 (4) (2013), pp. 1-14

DOI: 10.5281/zenodo.6945690

16. Kansagra H, Mallick S. Microemulsion-based antifungal gel of luliconazole for dermatophyte infections: Formulation, characterization and efficacy studies. Journal of Pharmaceutical Investigation. 2015;2(1):2-12.
17. Scher RK, Nakamura N, Tavakkol A. Luliconazole: A review of a new antifungal agent for the topical treatment of onychomycosis. Mycosis Journal of Diagnosis, Therapy and Prophylaxis of Fungal Diseases. 2013;1(1):390-3
18. F.R. Ali, M.H. Shoaib, Design, Development, and Optimization of Dexibuprofen Microemulsion Based Transdermal Reservoir Patches for Controlled Drug Delivery, 2017 (2017) 4654958.
19. Khanum R, Thevanayagam H. Lipid peroxidation: its effects on the formulation and use of pharmaceutical emulsions. Asian J Pharm Sci 2017; 12(5): 401-411.
20. Mohsin S, Akhtar N. Formulation and stability evaluation of Bauhinia variegata extract topical emulsion. Acta Pol Pharm 2017; 74(3): 945-954.
21. Arshad AI, Khan HM, Akhtar N, Mustafa R, Aslam I, Mohammad IS. Stability assessment of polysiloxane polyalkyl polyether copolymer based cosmetic emulsion loaded with Ananas comosus extract. Lat Am J Pharm 2014; 33(8): 1363-1370.
22. Anindya Hana Iradhati, Mahdi Jufri. Formulation and physical stability test of griseofulvin microemulsion gel. Int J Appl Pharm 2017; 9:23-6.
23. Vishal Yadav, Prakash Jadhav, Shailaja Dombe, Anjali Bodhe, Pranali Salunkhe. Formulation and evaluation of microsponge gel for topical delivery of the antifungal drug. Int J Appl Pharm 2017; 9:30-7.
24. R. Mishra, K.S. Prabhavalkar, L.K. Bhatt Preparation, optimization, and evaluation of Zaltoprofen-loaded microemulsion and microemulsion-based gel for transdermal delivery J. Liposome Res., 26 (4) (2016), pp. 297-306.
25. M. Cao, L. Ren, G. Chen Formulation optimization and ex vivo and in vivo evaluation of celecoxib microemulsion-based gel for transdermal delivery AAPS PharmSciTech, 18 (6) (2017), pp. 1960-1971
26. Pang B, Liu H, Liu P, Peng X, Zhang K. Water-in-oil Pickering emulsions stabilized by stearoylated microcrystalline cellulose. J Colloid Interface Sci 2018; 513: 629-637.
27. Tanriverdi ST, Yapar EA. Preparation and characterization of herbal emulsion formulations. Marmara Pharm J 2017; 21(4): 756-761.
28. G. Sharma, G. Dhankar, K. Thakur, K. Raza, O.P. Katare Benzyl benzoate-loaded microemulsion for topical applications: enhanced dermatokinetic profile and better delivery promises AAPS PharmSciTech, 17 (5) (2015), pp. 1221-1231
29. M. Naeem, N. Ur Rahman, G.D. Tavares, S.F. Barbosa, N.B. Chacra, R. Lobenberg, M.K. Sarfraz Physicochemical, in vitro and in vivo evaluation of flurbiprofen microemulsion Anais da Academia Brasileira de Ciencias, 87 (3) (2015), pp. 1823-1831
30. T. Wan, T. Xu, J. Pan, M. Qin, W. Pan, G. Zhang, Z. Wu, C. Wu, Y. Xu
31. Microemulsion based gel for topical dermal delivery of pseudolaric acid B: in vitro and in vivo evaluation Int. J. Pharm., 493 (1-2) (2015), pp. 111-120