Formulation and Evaluation of Etodolac Nanosuspension Loaded Topical Gel

Safiya Bee[1]*, Sakshi Aole[2], S.C. Mahajan[3], Vikas Jain[4]
[1,2,3,4]*Mahakal Institute of Pharmaceutical Studies, Ujjain, Behind Air strip, Datana, Dewas Road, Ujjain (M.P.) India-456664*

Abstract- This study aims to prepare a topical formulation of etodolac. Presently, a total of 5 formulations were formulated, for which 2 steps were followed. First the preparation of nanosuspension by using pearl milling technique, and the second one incorporates them into a gel formulation by adding a gelling agent carbopol 940. Nanosuspension was prepared to improve the poor solubility of the drug or to enhance the bioavailability of drug, and to improve the drug penetration into the skin. In this paper, a comparative study is illustrated between the etodolac nanosuspension-loaded gel and pure etodolac gel. In the formulation of nanosuspension different concentrations of poloxamer 407 and different speeds of stirrer were used. The formulation was evaluated for pH, clarity, spreadability, drug content, etc. The *in vitro* diffusion studies showed satisfactory results. On the basis of evaluation parameters, F3 and F4 showed a good release profile as well as also showed an ideal characteristic for gel formulation.

Keywords: Etodolac, Non-steroidal anti-inflammatory drugs, Arthritis, Pearl milling technique, Ultra violet spectroscopy.

INTRODUCTION

A Nanosuspension is the type of suspension in which drug particles in submicron size or nanosize are colloidally dispersed. The stability of nanosuspensions is usually achieved by the use of surfactants. Nanosuspension are colloidal in nature and generally stabilized by the addition of polymers and surfactants. Nanosuspension may be defined as a biphasic system which is consists of a drug particle that is pure and dispersed in an aqueous vehicle in which the suspended particle diameter is generally less than 1µm in size. Nowadays various methods are used for the treatment of drugs that are poorly soluble which includes solubilization using co-solvents, salt form, surfactant dispersions, techniques like precipitation, and oily solution. Other techniques involved are liposomal technique, emulsions technique, microemulsion technique, solid dispersion technique, and inclusion complexation which is usually uses cyclodextrins. The technique is always used for a drug that has no solubility in water and organic solvent. This results in solubility being higher, and also the flooding rate of the active pharmaceutical ingredient increases and reaches a maximum plasma level at a much faster rate. Nanosuspension may be used for both poor soluble drugs as well as poorly permeable drugs. They reduce particle size significantly so it is also useful for intravenous administration in order to prevent the blockage of blood capillary.

The gel may be defined as a semi-rigid system in which by an interacting 3- the dimensional network of particles the movement of dispersing medium is restricted.

As their origin indicates that there is the greatest idea of liquid setting to a solid-like material and it should elastic and also retains some liquid-like properties but should not flow like a liquid. The gel is known as a magma gel when in a 2 phase system the dispersed phase particle size is relatively large.

When we compare both gel and jelly with each other the gel to be found more rigid is due to it containing more covalent, cross-links, physical bond's higher density or its contain less amount of liquid. Some gel systems are clear like a liquid and some gel systems are not these is because the ingredients are not completely molecularly dispersed or dissolved.

The gelling agent plays an essential role in the formulation of gel the gelling agent concentration should be less than 10% or in a range between 0.5% - 2.0%.

MATERIAL AND METHODS

Etodolac was obtained from Angels Pharma India Pvt. Ltd. Paravada, Vishakhapatnam (A.P.) India, Glycerol (Cyano Pharma-Indore), Methylparaben, and

Propylparaben (JayshreeChemicals- Indore), Propylene glycol (Anmol Chemicals Group M.N. Globex Pvt. Indore. Ltd., Dimethyl sulfoxide (Merk Pvt. Ltd. Indore)

METHODS

The preformulation study of the drug carried out includes solubility, melting point, partition coefficient.

Solubility[5]

Solubility of drug was checked by adding small amount of drug in different solvents like distilled water, ethanol, methanol and dimethylsulfoxide, etc.

Meting point [6]

By the use of Melting point apparatus (Jindal Medical & Scientific Instrument P. Ltd., Delhi India) melting point of drug was analysed.

Partition coefficient [7]

A weighed amount of Etodolac was taken, then transferred into a conical flask followed by continuous shaking for 24 h, until the equilibrium was reached. 2 phases were separated with the help of separating funnel and through 0.2 µm filter paper, the aqueous phase was filtered. This was then suitably, diluted. It was in this aqueous phase, the amount of Etodolac was determined with the help of UV spectrophotometer by measuring the absorbance at required lambda max.

FORMULATION AND EVALUATION

Preparation of nanosuspension [8-9]

By utilizing the pearl milling technique, nanosuspension was prepared. Etodolac powder (1.0g) was dispersed in an aqueous solution containing glycerol (2.2%) and a different ratio of poloxamer 407.By utilizing zirconium oxide beads (milling technique) on a magnetic stirrer for 6 h. At different rotation speeds the stirring was continued. The resulting coarse pre dispersion was comminuted.

Formulation of Etodolac nanosuspension loaded topical gel or etodolac gel[10]

In the formulation of Etodolac gel as a gelling agent carbopol was used. Carbopol clear dispersion was prepared in water by agitation and soaked overnight to prepare a slurry of Etodolac nanoparticles. Under continuous stirring, propylene glycol mixture and water were added. The required quantity of Etodolac nanoparticles were slowly added with stirring to prepare carbopol slurry. Propylene glycol, Methylparaben, Propylparaben, and dimethyl sulfoxide were added to the homogeneous gel obtained, then the pH of the gel was adjusted with the help of Triethanolamine and finally, the volume was made up using distilled water. Similarly, the pure Etodolac gel was prepared.

Table 1: Composition of different Nanosuspension formulation

Ingredient	F1	F2	F3	F4	F5
Drug (gm)	1	1	1	1	1
Poloxamer 407(%)	1	1.5	2	2.5	3
Glycerol (%)	2.2	2.2	2.2	2.2	2.2
Rotation RPM	850	900	950	1000	1050
Time (h)	6.5	6.5	6.5	6.5	6.5

Table 2 Composition of different Nanosuspension loaded gel formulation and pure gel formulation.

	F1	F2	F3	F4	F5	pure etodolac gel
Etodolac nanoparticle (g)	1	1	1	1	1	-
Pure etodolac gel (g)	-	-	-	-	-	1
Carbopol 934 (%)	2	2	2	2	2	2
Methyl Paraben (g)	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (g)	0.05	0.05	0.05	0.06	0.07	0.07
propylene glycol (ml)	15	15	15	15	15	15
Dimethyl Sulfoxide (ml)	5	5	5	5	5	5
Triethanolamine	QS	QS	QS	QS	QS	QS
Distill Water Q.S.	Upto100 ml	Upto100 ml	Upto100 ml	Upto100 ml	Upto100 ml	Upto100 ml

Evaluation of optimized Etodolac nanosuspension loaded topical gel

Percentage entrapment efficiency [11]

The Percentage entrapment efficiency and loading capacity of prepared nanosuspension were determined by transferring about 1 ml of nanosuspension into a centrifugation tube. 5 ml volume was made up with phosphate buffer pH 7.4 and after that centrifuged at 600 RPM for about 1 hour to know the unentrapped drug. 1 ml supernatant fluid was withdrawn and Suitably diluted with the same solvent and the absorbance was measured at 254 nm. Finally the entrapped drug amount was determined by taking the pallet settled after the centrifugation process and the absorbance was measured at 254 nm.

It was calculated by following formula.

PEE = total drug – unentrapped drug / total drug x100

Zeta potential [12]

1 ml prepared formulation was taken and diluted (up to 100 ml,) with water, then subjected to zeta potential by using Malvern zeta size at 25°C.

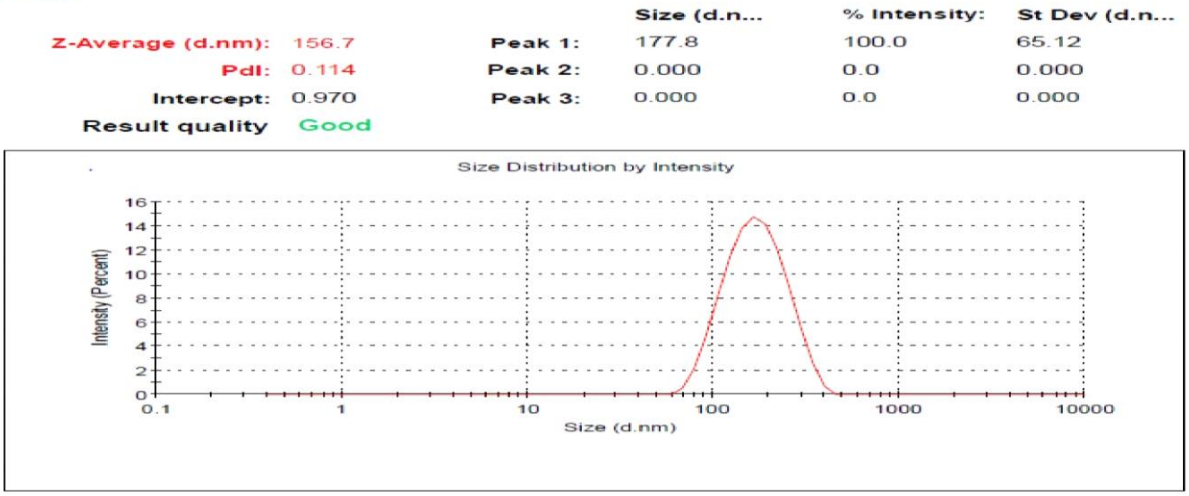

Figure 1: Zeta potential of optimized formulation

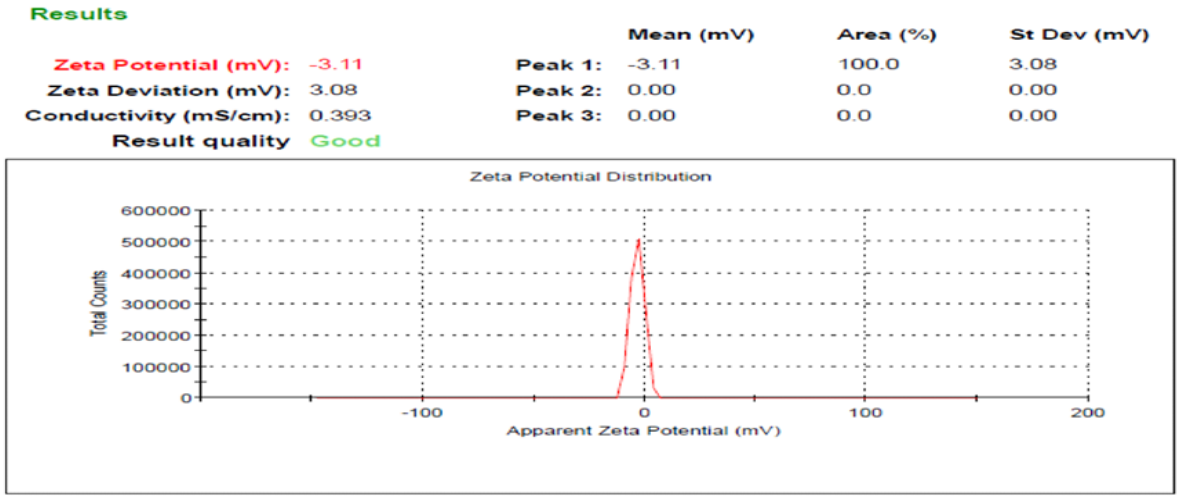

Figure 2: Optimized Formulation Particle Size Distribution.

Percentage yield of nanosuspension [13]

The percentage yield was determined by calculating raw material (initial weight) and the nanosuspension (final weight).

percentage yield = practical yield / theoretical yield x100

Clarity [14]

Prepared gel clarity was measured by visual examination under black and white background and it was graded as turbid (+), clear (+ +) and very clear (+ + +).

Homogeneity [15]

All the prepared gels were placed into the container, and then by visual inspection they were tested for homogeneity. The gel was tested for its appearance and for the presence of any kind of aggregates.

Spreadability[17]

To determine the spreadability of the prepared gel, circle of 2 cm diameter was marked on a slide, then the gel was transferred into the glass slide for uniform thickness . 100 gm of sample was placed for 5 min on a glass slide and the excess gel was scrapped out. The time, required to separate out 2 slides was considered as a measure of spreadability.

By the following formula Spreadability was calculated

S=M .L\T

Where,

S= spreadability

M= weight placed on upper slide,

L= glass slide length

T= time

Viscosity[18]

The viscosity measurement of prepared nanosuspension was done by a Brookfield Viscometer. In order to determine the viscosity of gel formulation the sample was transferred into the beaker then the spindle (no. 64) was attached to a Viscometer and 50 rpm was rotated. After each speed the respective reading was noted.

In vitro drug diffusion study [19]

By the use of Franz diffusion apparatus *in vitro* drug release of Etodolac Nanosuspension were studied. As a diffusion medium phosphate buffer, pH7.4 (freshly prepared) was used. For these specially designed glass cylinder were used. The Cellophane membrane which was previously soaked overnight in distilled water was tied on the end of a specially designed glass cylinder. Prepared nanosuspension 1 ml was transferred to this specially designed assembly. By using a magnetic receptor at 50 rpm the diffusion medium was stirred. Aliquots after that was withdrawn with 1 ml volume, then diluted and with the help of UV/visible spectrometer at 252 nm the absorbance was measured.

Table3: *In vitro* Drug diffusion of Etodolac Nanosuspension loaded topical gel and pure etodolac gel.

Time (hours)	% CDR					
	F1	F2	F3	F4	F5	Pure etodolac gel
0.5 hrs	1.96	3.96	7.13	6.27	5.56	0.78
1 hrs	3.72	5.72	9.62	8.13	8.74	1.31
1.5 hrs	6.29	8.42	14.52	10.23	10.66	4.58
2 hrs	11.77	13.77	25.45	23.56	20.84	7.82
2.5 hrs	20.95	22.95	28.36	29.02	24.20	10.16
3 hrs	29.51	32.13	39.23	47.24	33.97	18.53
3.5 hrs	37.59	41.59	47.25	61.09	42.36	26.25
4 hrs	43.35	49.35	59.52	69.13	55.36	36.59
4.5 hrs	52.38	57.69	71.31	80.26	66.21	43.32

Percentage Drug content of Etodolac nanosuspension laoded gel [20]

To determine the drug content of optimized formulation, 1 g gel was accurately weighed, then transferred into a graduated volumetric flask after that about 5 ml of ethanol was added and sonicated for 30 min. The volume was made up with phosphate buffer (pH 7.4) then serial dilutions were prepared with the same solvent then the solution absorbance was measured spectrometrically at 254 nm.

In vitro drug permeability study [21]

By the use of Franz diffusion apparatus *in vitro* drug permeability of Etodolac loaded nanosuspension were studied. For *in-vitro* release as a receptor medium phosphate buffer pH 6.8 was used. The Etodolac loaded nanosuspension gel and pure Etodolac gel was applied on the skin for albino mice skin and was fixed in Franz diffusion cell. Between the donor and receptor compartment of Franz diffusion cell 100 ml phosphate buffer (pH 6.8) contained in the receptor compartment diffusion medium temperature was

maintained thermostatically at 37±1^0 by surrounding water in jacket. By the use of magnetic stirrer at 500 RPM medium was stirred. Aliquots after the withdrawn 1 ml volume then diluted and with the help of UV visible spectrophotometer at 252 nm the absorbance was measured.

Table4: *In vitro* Drug permeability of Etodolac Nanosuspension loaded topical gel and pure etodolac gel.

Time (hours)	Cumulative amount of drug permeated					
	F1	F2	F3	F4	F5	Pure etodolac gel
0.5	675.71	702.03	732.6	767.77	710.04	382.07
1	711.64	738.21	775.32	809.75	749.26	439.59
1.5	767.47	803.05	852.53	838.03	829.03	527.28
2	789.69	856.16	916.88	916.21	883.13	571.5
2.5	844.91	928.16	993.32	1001.28	956.10	660.01
3	908.86	1030.09	1113.43	1050.22	1068.03	764.05
3.5	999.85	1123.07	1240.5	1189.29	1174.23	909.72
4	1049.25	1188.19	1269.27	1333.53	1242.20	909.88
4.5	1083.79	1191.17	1378.23	1402.5	1299.25	924.89

Table5: Evaluation parameters for etodolac nanosuspension loaded topical gel.

Formulation code	Evaluation parameters for etodolac nanosuspension loaded topical gel			
	Spreadability g.cm/sec	Viscosity (cps)	Drug content	pH
F1	48	8951	52.91	7.2
F2	46	8882	69.96	6.9
F3	45	9124	82.98	6.5
F4	44	9145	86.34	6.8
F5	45	8870	78.35	6.6

Table6: Evaluation parameters for pure etodolac gel

Evaluation parameters for pure etodolac gel					
Clarity	homogeneity	Spreadability g.cm/sec	Viscosity (cps)	Drug content	pH
Turbid (+)	Good	43	9118	44.24	6.6

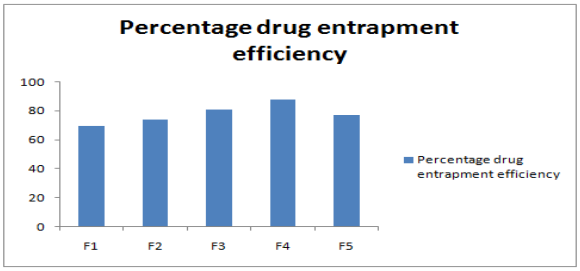

Figure3: Percentage drug entrapment efficiency of nanosuspension loaded gel F1 to F5 and pure etodolac gel

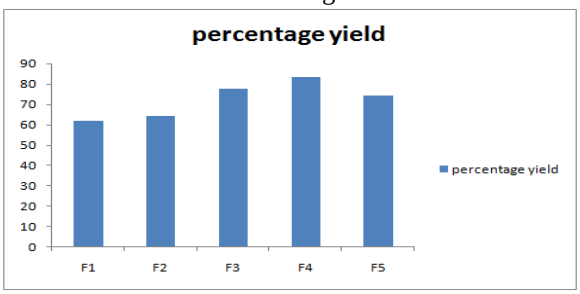

Figure 4: Percentage yield of nanosuspension

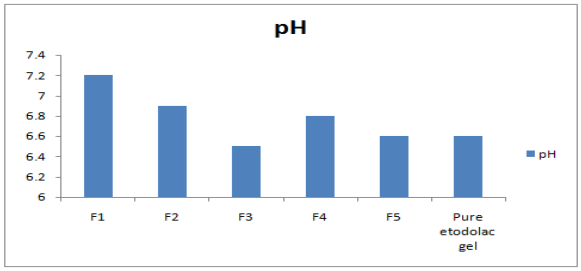

Figure5: pH of nanosuspension loaded gel F1 to F5 and pure etodolac gel

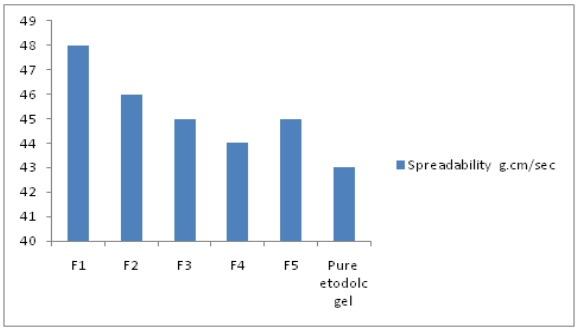

Figure 6: spreadability of nanosuspension loaded gel F1 to F5 and pure gel

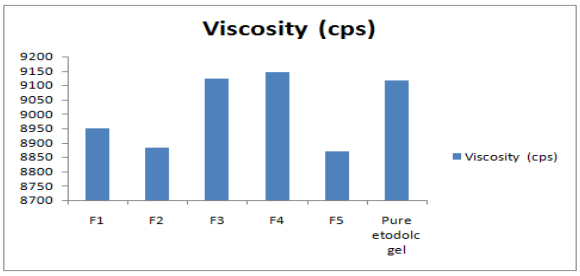

Figure 7: Viscosity of nanosuspension loaded gel F1 to F5 and pure gel

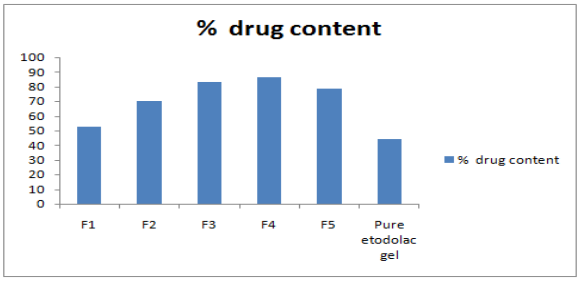

Figure 8: Percentage drug content of nanosuspension loaded gel F1 to F5 and pure gel

RESULTS AND DISCUSSION

In the formulation and evaluation of Etodolac loaded Nanosuspension gel different poloxamer 407 ratio were used and different rotation speed of RPM both independent variables and significantly affected the formulation hence the concentration of poloxamer 407 and rotation RPM play an essential role in the preparation of formulation.[8,9,10]

Percentage entraptment efficiency of nanosuspension were found to be 69.36, 73.69, 80.41, 87.21 and 76.81 respectively for F1 to F5.[22]

Percentage yield of prepared nanosuspension found to be 61.96 (F1), 64.32 (F2), 77.65 (F3), 83.45(F4), 74.17(F5).[23]

Clarity of prepared gel were found to be turbid (+), turbid (+), very clear(+++), very clear (+++) and clear (++) respectively for F1 to F5.While pure Etodolac gel was found to be turbid (+). Similarly prepared gel homogeneity were found to be poor, good, very good, very good and poor respectively for F1 to F5 while pure Etodolac gel was found to be good.[24]

Prepared gel formulation pH were found to be 7.2 (F1), 6.9 (F2), 6.5 (F3), 6.8 (F4), 6.6 (F5) while pure Etodolac gel pH was found to be 6.6.[25]

Prepared gel formulations spreadability were found to be 48 (F1), 46 (F2), 45 (F3), 44 (F4), 45 (F5) g.cm/sec while pure Etodolac gel spreadability was found to be 43.[26]

Viscosity of prepared gel were found to be 8951, 8882, 9124, 9145, 8870 (cps) while pure Etodolac viscosity was 9118 (cps).[26]

To determine the Etodolac release from the prepared formulation the graph was plotted between percent drug release v/s time and were found to be 52.38% (F1), 57.69% (F2), 71.31% (F3), 80.26% (F4), and 66.21% (F5) and drug release study of pure Etodolac gel was found to be 43.32%.

Drug content of prepared gel were found to be 52.91%, 69.96%, 82.98%, 86.34%, and 78.35% respectively for F1 toF5 formulation while drug content of pure gel was found to be 44.24%.[27]

The above results of clarity, homogeneity, pH, spreadability, viscosity, and drug content cleared that the F4 formulation shows all the characteristics results of the gel formulation, and the results of F4 formulation was also best than the results of pure etodolac gel thus, F4 formulation was better, on the other hand, drug content of nanosuspension loaded etodolac gel was also greater than the drug content of pure etodolac gel which cleared that the F4 formulation was best.

The permeability study was performed by plotting graph between cumulative amount of drug permeated v/s time the cumulative amount of drug permeation for prepared Etodolac loaded nanosuspension topical gel was found to be 1183.79 for F1 1219.17 for F2 1378.23 for F3 1402.5 for F4 and 1346.25 for F5 while 924.89 was found for pure Etodolac gel. On the basis of the above data, it was concluded that the permeability of Etodolac nanosuspension-loaded gel was much more as compared to pure Etodolac gel. [27]

The above data showed that the F4 was the best formulation and F4 was optimized for zeta potential and particle size distribution and zeta potential was found to be -3.11mv, as a stabilizer poloxamer-407 (non-ionic surfactant) was used which provide steric stabilization so negative zeta potential was attributed to drug nanocrystal. Usually, for nanosuspension stability ±17 mv zeta potential value was sufficient for the nanosuspension, stabilized by stearic stabilizer poloxamer 407 which assure that prepared nanosuspension would be stable and in the future prepared nanosuspension formulation would not suffer from instability problem. Polydispersity index was

found to be 0.114 and particle size was found to be 15.67nm.[12]

CONCLUSION

Etodolac nanosuspension-loaded gel was prepared with an aim to improve the poor solubility of etodolac and to enhance the poor bioavailability of the drug as well as to increase the penetration of the drug into the skin. As per the above studies, it was concluded that poloxamer 407 and speeds of the stirrer are independent variables and can greatly affect the percentage entrapment efficiency of drug and drug content also so, it is necessary to use poloxamer 407 in the proper concentration and also fix proper speed of the stirrer.

REFERENCE

[1] Chingunpituk J. Nanosuspension Technology for Drug Delivery Walailak J Sci & Tech. 2007; 4(2): 139-153.

[2] Geetha G, Poojitha U, Arshad Ahmed K. Various Techniques for Preparation of Nanosuspension. International Journal of Pharma Research and review, 2014; 3(9): 30-37.

[3] Kumari K. and Rao S. Nanosuspensions: A Review. Int J Pharm 2017; 7(2): 77-89.

[4] Arunkumar N, Deecaraman M, Rani C. Nanosuspension technology and its applications in drug delivery. Asian Journal of Pharmaceutics - July-September 2009.

[5] Banavath H, Sivarama RK, Ansari T, Ali S, Pattnaik G. Nanosuspension: an attempt to enhance bioavailability of poorly soluble drugs. International Journal of Pharmaceutical Sciences and Research 2010; 1(9):1-11.

[6] British Pharmacopoeia 2013 Published by British Pharmacopoeia Commission Office: Market Towers 1 Nine Elms Lane London.

[7] Lachman L, Herbert A. Lieberman, Joseph L. Kanig. The theory and practice of industrial pharmacy. Third edition. Varghese publishing house. Page no. 681-703.

[8] Nakarani M, Misra AK, Patel JK. Itraconazole nanosuspension for oral delivery: Formulation, characterization and in vitro comparison with marketed formulation. Pub Med.gov 2010;18 (2):84-90.

[9] Thakkar HP, Patel BV, Thakkar SP. Development and characterization of nanosuspensions of olmesartan medoxomil for bioavailability enhancement Journals of Pharmacy and Bioallied Sciences.2011 Jul;3(3):426-34.

[10] Parchuri DB, Kumar GSS, Goli DFormulation and evaluation of nanoparticulate drug delivery system of Acyclovir for topical drug delivery. *World* Journal of Pharmacy and Pharmaceutical Sciences. 2013; 2(6): 5602–17p.

[11] Bandr AE, Nair AB, Kumari R,Attimara M,Harsha S. Formulation And Evaluation of Nanoparticulate Drug Delivery System of Acyclovir for Topical Drug Delivery 2015 Dec 1;136:878-84.

[12] Mandal B, Alexander KS, Riga AT. Sulfacetamide loaded eudragit RL100 nanosuspension with potential for ocular delivery. J Pharm Pharmaceut Sci. 13(4):510–523.

[13] Hunter RJ, zeta potential in colloid science. London, England: academic press: 1981.

[14] Yadav V, Jadhav P, Dombe S , Bodhe A, Salunkhe P, Formulation and evaluation of microsponge gel for topical delivery of antifungal drug, International Journal of Applied Pharmaceutics ISSN- 0975-7058 Vol 9, Issue 4, 2017.

[15] Keck CM, Muller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homoginisation. Eur J Pharm Biopharm. 2006; 62:3-16

[16] Katkade M, Kalkotwar R, Jain N, Patil P, Gadakh R, Naikwade J. Ethyl cellulose based microsponge delivery system for antifungal vaginal gels of itraconazole. J Drug Delivery Ther 2013; 3:14-20.

[17] Mulani H, Bhise KS. QBD Approach in the Formulation and Evaluation of Miconazole Nitrate Loaded Ethosomal Cream-o-gel. Ind Res J of Pharm and Sci. 2017;8:001-037.

[18] Devi AS, Pinnika A, Divya P. Formulation and Evaluation of Candesartan Cilexetil Transdermal Proniosomal Gel. Journal of Drug Delivery Therapeutics. 2014;4(2):90-98

[19] Mulani H, Bhise KS. QbD Approach in the Formulation and Evaluation of Miconazole Nitrate Loaded Ethosomal Cream-o-gel. Ind Res J of Pharm and Sci 2017; 8: 001-037.

[20] Saiesh P , Shabaraya A.R, Shripathy D, Soman L. Formulation And Evaluation of Topical Gel Containing Econazole Nitrate.
[21] Bhanu P, Sahu, Maly K. Das nanosuspension for bioavailability app. Nanosci(2014) 4:189-197.
[22] Thadkala K, Nanam Pk, Rambabu B, Sailu C And Anukumar J. Preparation and characterization of amorphous ezetimibe nanosuspensions intended for enhancement of oral bioavailability International journal of pharmaceutical investigation 2014 Jul-Sep; 4(3): 131–137.
[23] Poonam Madhukar K, Kalyanisundarrao K. Dipti Ganesh PFormulation and Evaluation of Topical Antifungal Gel Containing itraconazole. Int J Curr Pharm Res. Vol 10, Issue 4, 71-7471
[24] Ankita M. and Abdul Wahid A.development and characterization of nanoemulsion gel for topical drug delivery of nabumetone. International journal of pharmacy and pharmaceutical science. October 2016 Vol.:7, Issue:3
[25] Kute SB, Saudagar RB. Emulsified gel A Novel approach for delivery of hydrophobic drugs: An overview. Journal of Advanced Pharmacy Education & Research. 2013; 3(4):368-373.
[26] Inayat BP. Rashmi D. Wahid A. 2Formulation and evaluation of ketoprofen loaded chitosan nanogel for pain management: Ex-vivo and In-vivo study. Ars Pharm. 2019; 60(2): 101-108.
[27] Basha BN, Prakasam K, Goli D. Formulation and evaluation of gel containing fluconazole-antifungal agent. Int J Drug Dev Res. 2011 Oct;3(4):119-27.