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Develop Suitable Ra Animal Model For The In Vivo Evaluation Of Developed Nano-Emulsion Gel

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Abstract

This study details the design, optimization, and assessment of a nanoemulsion (NE)-based gel loaded with Quercetin (QCT) for the efficient treatment of rheumatoid arthritis (RA). QCT- NE was formulated utilizing the Box- Behnken experimental design, which relies on spontaneous emulsification procedures. Two different cell lines, HIG-82 and RAW 264.7, were used to test the cytotoxicity and determine the impact on TNF- production. QCT- NE was shown to suppress LPS-induced TNF- generation while having no harmful effects on synoviocytes. Drug penetration is enhanced in QCT- NE gel, and its rheological behavior is verified to be satisfactory. For RA sufferers as a whole, the QCT-NE gel formulation is a very efficient type of localized treatment.

Keywords: Quercetin Nanoemulsion Rheumatoid arthritis Gel.

INTRODUCTION

Among the most prevalent illnesses, arthritis is a leading reason for adult joint dysfunction and disability. However, these medications come with a slew of undesirable consequences, such as gastrointestinal distress, loss of protein, toxicity, failure to hit the intended target, and suppression of the immune system. Hence, plant-based treatments linked to the fewest adverse effects are now being used to treat rheumatoid arthritis, diminishing the significance of different anti-inflammatory pharmaceuticals.

Flavonoids' many biological features, including antioxidant, anti-proliferative, and anti-inflammatory effects, have garnered a lot of attention in recent years. QCT is one of the most powerful antioxidants found in plants and is a prominent flavonol found in many different types of food plants. Antiviral, anti-diabetic, anti-inflammatory, neuroprotective, and anticancer properties are only some of the therapeutic benefits of this common and safe dietary flavonoid. TNF-, nitric oxide, interleukin (IL)-6, interleukin (IL)-1, chemokines, and other pro-inflammatory cytokines are all inhibited by QCT. As a result, it aids in the control of conditions including diabetes, chronic inflammation, and arthritis that have been linked to oxidative stress. QCT also has limited GI retention, poor skin penetration, weak water solubility, and fast elimination. As a result, QCT is not suitable for oral administration, and its clinical applications are inefficient. Not only does it break down quickly in alkaline circumstances, but it also breaks down quickly when exposed to heat during manufacturing and storage. Because of these challenges, a new formulation is needed to stabilize QCT and take advantage of its therapeutic potential.

For decades, researchers have examined the pharmacotherapeutic potential of herbs for use in complementary and alternative medicine, including inflammatory immune-related illnesses, with the goal of restoring normal bodily processes. There are obstacles and hurdles to treating RA since the present pharmacotherapeutics supplied in standard dose forms only offer therapeutic advantages to a suboptimal level. To achieve this goal, innovative

drug delivery techniques must be designed to provide efficient, precise, and safe drug delivery systems with enhanced therapeutic performance. Nanomedicines have been extensively reported in recent decades to serve as drug delivery carriers for the efficient management of rheumatoid arthritis (RA). Topical nanomedicines have advantages over other routes of drug administration for RA because they are better able to stay on the skin, have a more focused impact, reduce medication doses, have less side effects, are more widely accepted, and increase patient compliance. There are a number of positive aspects to these therapeutics, but they face significant barriers to widespread use due to factors such as higher production costs, negative effects on long-term stability, insufficient clinical data demonstrating safety and effectiveness, and challenging regulatory approval. Benefits and cons of currently available medications are also discussed, and a basic pathophysiology of RA is provided.

LITERATURE REVIEW

Anwer, M.K. et al. (2019), Because of the complexity and fragility of the wound-healing process, chronic wounds that never heal might form. Seeking medical assistance quickly may alleviate this problem by preventing further problems and hastening the skin wound's recovery. Functionalized artificial biomaterials have been shown to be a viable option for initiating cutaneous wound treatment. Further testing and incorporation of these DSM-loaded NEs (F1) into a 1% carbopol 940 gel yielded positive results. The drug content, spreadability, in vitro release, wound healing, and anti-inflammatory investigations of F1-loaded gel were then described. DSM gel was shown to have considerably higher anti-inflammatory and wound-healing action (p 0.05).

R.R. Lala and N.G. Awari (2014), We have looked at the possibility of etoricoxib as a model medicine for topical delivery of COX-2 inhibitors by employing a nanoemulsion. The process of spontaneous emulsification was used to create a wide range of oil-in-water nanoemulsions. Pseudo-ternary phase diagrams were used to locate the nanoemulsion region. The thermodynamic stability of the produced nanoemulsions was evaluated. Those who made it through were further analyzed using differential scanning calorimetry, droplet size analysis, and viscosity measurements. The Franz diffusion cell was used to determine the topical penetration of etoricoxib into porcine belly skin. Optimized formulations' skin penetration profiles were compared to those of standard etoricoxib gel ex vivo. Our findings point toward the viability of nanoemulsions as vehicles for enhancing the transdermal administration of anti-inflammatory drugs like etoricoxib.

B. B. Panigrahi, S. B. Bhanja, and Vikram V.B.K. Mishra (2019), The thermodynamic stability of the produced Nanoemulsions was evaluated. The zeta-potential, scanning electron microscopy (SEM), and droplet size were all studied. There was a notable improvement in the enhancement ratio (Er), the permeability ratio (Kp), and the flux (Jss). When comparing the anti-inflammatory effects of Valdecoxib gel with formulation NG2, the anti-inflammatory impact of the latter was shown to be significantly higher (72% inhibitory effect in 24hrs) on Carrgeenan-induced paw edema in rats. Nanoemulsion gels showed promise as carriers for enhanced transdermal administration of valdecoxib.

Gajanan Mogal, Vaibhav Changadiya (2018), The goal of this research was to improve the efficacy, stability, and bioavailability of mefenamic acid, a medication used to treat rheumatoid arthritis. The manufactured nanoemulsions were stored in sealed glass vials at room temperature. These drug-loaded formulations were put through a battery of physical stability tests, including centrifugation (5000 rpm for 30 minutes), heating and cooling (0 and 45 degrees Celsius for eight cycles), and freeze-thawing (-21 degrees Celsius for 48 hours). In conclusion, The results of the mefenamic acid nanoemulsion's physical stability tests, appearance, % transmittance, refractive index, electrical conductivity, and reduced surfactant content all met or surpassed expectations. Mefenamic acid nanoemulsion has been demonstrated to be equally effective as the more traditional forms of the drug in the current investigation of rheumatoid arthritis.

S.B. Bhanja, B.B. Panigrah, and Vikram V.B.K. Mishra (2019), Ketoprofen, a medicine used to treat rheumatoid arthritis, is only marginally soluble in water, therefore a nanoemulsion gel was created to increase its solubility and permeability. The oils, surfactants, and co-surfactants that are most soluble in ketoprofen are CAPTEX 200, tween 80, and PEG 400, respectively. Using a constructed pseudo ternary phase-diagram, the optimal concentration was identified. Thermodynamic stability tests were performed on the manufactured nanoemulsions, analyzed for zetapotential, pH, viscosity, and diffusion, and seen under a scanning electron microscope (SEM). Nanoemusion H-2's optimal formulation has a zeta potential of -16.3mV, a particle size of less than 100 nm, a pH of 7.2, and diffusion experiments that demonstrate a drug release efficiency of 90.84% after 24 hours. Nanoemulgel was measured to have a viscosity of 2050 mPaS, a pH of 7.5, and no potential for causing localized skin irritation. When optimized formulation H2 was compared to commercially available ketoprofen gel and nanoemulsion gel. The enhancement ratio (Er), flux (Jss), and permeability ratio (Kp) all increased significantly. The findings indicated that nanoemulsion gels may be useful carriers for enhancing ketoprofen transdermal distribution.

Materials and methods

Materials

BASF India Ltd. was able to provide us with Cremophor RH 40. (Mumbai, India). Gattefosse was the source for the capryol 90. (France). Noveon India Ltd. was contacted for supplying the Carbopol 940. (Mumbai, India). Novartis Pharmaceuticals Ltd. generously provided us with a sample of diclofenac sodium for review purposes (Mumbai, India). All of the analytical-grade, GRAS solvents used in the experiments came from Merck Ltd. (Mumbai, India).

The substance's solubility has been evaluated to establish which oil phase is optimal for its formulation. Oleic acid, coconut oil, castor oil, olive oil, arachis oil, sesame oil, sunflower oil, soy oil, and idebenone polyunsaturated methyl ester were all tried to see whether they would dissolve QCT. The solubility was also estimated using oil mixtures. At 37 1.0 °C for 72 hours, the mixtures were shaken orbitally before being subjected to further vortex mixing. Centrifuged at 3000 rpm, the equilibrated samples' supernatants were filtered, and the resulting filtrates were diluted with the appropriate solvent. Validated UV spectrophotometric analysis of 258 nm measured QCT solubility (UV 1700, Shimadzu, Japan). Triangulation was used for all calculations.

Formulation of nanoemulsion

The process of spontaneous emulsification was used in the creation of nanoemulsion. In short, 10 mg of QCT was carefully measured and put to the oil phase. After that, we added some surfactant and co-surfactant (Smix), and gave the whole thing a good 15 minutes of vortexing at 300 revolutions per minute. Rapid formation of nanoemulsion. After that, the whole thing was thrown into a vortex mixer for 20 minutes.

After 2 hours of equilibration, the resultant nanoemulsion was evaluated for particle size, PDI, and zeta potential and found to be transparent and easily flowable.

Characterization of QCT loaded nanoemulsion

Using a Malvern Zetasizer and a photon correlation spectrometer (PCS), we determined the average globule size, polydispersity index, and zeta potential. The samples were diluted with double-distilled water and vigorously shaken to achieve the desired scattering intensity before being measured. All of the readings were taken at 25 degrees Celsius and a 90 degree angle.

In order to examine the nanoemulsion's morphology, a transmission electron microscope was used. A carbon-coated copper grid was applied with a drop of nanoemulsion that had been properly diluted with distilled water. 2% phosphotungstic acid was used to create the negative stain, and it was left on for 30 seconds. Transmission electron microscopy at an accelerating voltage of 60-80 kV was used to examine the grid.

Degree of transparency, drug content, % EE

Percentage transmission (% T) was used to evaluate the level of optical transparency in the QCT- NE formulation. Spectrophotometric analysis at 258 nm was performed after diluting the formulation to 1 mL with alcohol.

The HPLC analytical technique was used to estimate the amount of QCT in the formulation. The basic minimum is dissolving 5 mL of nanoemulsion in 10 mL of methanol, centrifuging at 3500 rpm for 15 minutes, then evaluating the supernatant using HPLC. An HPLC system was coupled with Autochro 3000 software and a UV-visible dual-wavelength detector. We triple checked all measures for precision.

$$\%EE = \left(\frac{Amount\ of\ drug\ content}{Total\ amount\ of\ drug}\right) \times 100$$

The nanoemulsion (F16) was studied in vitro using a 10-mL Franz diffusion cell. The diffusion membrane was a dialysis membrane pre-soaked for 24 hours in phosphate buffer saline (PBS) with a pH of 5.8 (Himedia, India). The diffusion cell was assembled with a dialysis membrane and then filled with phosphate-buffered saline (PBS) at a pH of 5.8. The same method was used to a QCT suspension that was given out for free. Carbopol 940 (1.0% w/v) was used to create a gel matrix. After homogenizing the gel to the correct consistency, the pH was adjusted to 5-6 using triethanolamine.

In order to avoid adverse reactions, the topical gel should be hypoallergenic and suitable for use on all skin types. Thus, it is crucial to monitor the gel formulation's pH. The gel's pH was checked using a pH meter that had been previously calibrated with buffers of known pH values. The Brookfield Viscometer was used for the rheological analysis. Spindle 7 was used to measure the QCT-NE gel's viscosity at 5, 10, 20, 30, 60, and 100 rpm.

Gel's mechanical qualities including consistency, firmness, cohesiveness, work of adhesion, gumminess, deformation at hardness, and springiness may be estimated using texture profile analysis. A Brookfield CT3 Texture analyzer was used to get the measurements. A sample cone was used to hold the measured amount of gel. The TA3/100 probe was set to move downward into the sample for 20 mm at a rate of 0.5 mm/s and then upward for another 20 mm to return to its starting position. Target mode distance was 10 mm, trigger load was 3 g, trigger type was auto, and data rate was 20 points per second with the TPA configured as follows. The point of maximal force created by the probe as it began to rise out of the gel was determined. We tested its adhesiveness, cohesion, hardness, gumminess, and springiness. All of the readings were taken three times for accuracy. The computations were done using the instrument's included software, and results were reported as the mean standard deviation.

Evaluation of the severity of Arthritis

Rats from each group had their ankle joints evaluated using the arthritis index (AI). Butler et al. described an evaluation scale that was used to quantify joint stiffness, and this scale was followed in order to arrive at a final score. On a scale from 0 to 10, 2 indicates a limitation in the subject's ability to bend and extend their ankles fully.

A score of 1 indicates that neither the bending nor the extension of the ankle was completely free. Limitation factor = 0.

The blood indicators help with medication assessment for rheumatoid arthritis. As a result, there were tests for erythrocyte sedimentation rate, hemoglobin, C-reactive protein, rheumatoid factor, and white blood cell count. The anticoagulant was added to the test tube after the venipuncture, and the red blood cell count, white blood cell count, hemoglobin level, and erythrocyte sedimentation rate were measured. The cells were counted using an automated hematology analyzer. Latex agglutination was used to measure CRP and RF levels in accordance with the manufacturer's instructions. The findings were summarized as the mean standard deviation from three replicates of each experiment (n = 3). Student's t-test and one-way analysis of variance were used to analyze the data statistically. A p-value less than 0.05 was judged significant in every situation.

RESULTS AND DISCUSSION

Traditionally, oils were chosen for topical applications because they provided the highest solubility for QCT. In this investigation, we chose to estimate QCT's solubility in a number of different oils. Arachis oil had the highest solubility of any of the oils tested for QCT, so it was chosen as the oil phase. Second, oleic acid, with its high solubility, was selected to improve QCT's oil-phase solubility and penetration. The surfactant and co-surfactant were chosen based on their solubility efficiency and HLB value. The HLB value of Tween 20 was 16.7, and its solubility in QCT was 9.28 mg/mL. As a co-surfactant, PEG400 was chosen due to its HLB value of 11.4, as well as its QCT solubility of 13.59 mg/mL. Morphological analyses (Fig. 1) revealed a regular distribution and size distribution of globules. They were non-aggregated and spherical, demonstrating their resistance to Oswald ripening caused by globular collapse.

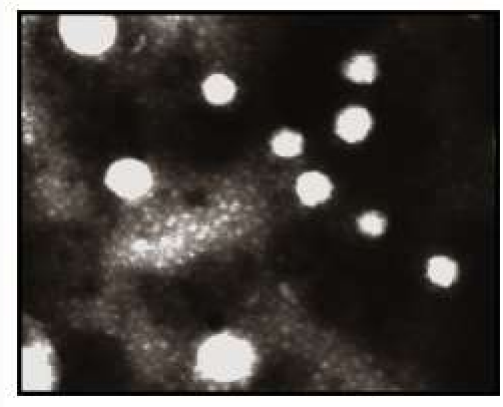


Fig. 1. Viewing the morphology of QCT-loaded nanoemulsion globules at a TEM voltage of 60-80 kV

The results showed that nanoemulsion had a transmittance of 97.68 0.05%. Formulation clarity and transparency are best represented by a value closer to 100%. This batch of nanoemulsion was chosen for the development of a gel formulation because its drug content was 95.65 0.14%. The optimized nanoemulsion was found to have an entrapment efficiency of 94.65 0.14%.

Because of the nanoemulsion's tiny globule size, it is a useful method for increasing the solubility of hydrophobic medications and, therefore, the drug's bioavailability. To maximize medication release and therapeutic effect, the drug was disseminated throughout the nanoemulsion globules. After 4 hours, free QCT suspension only released 28.23 1.72% of the drug, but QCT- NE released 84.12 0.51% of the drug, a significant difference (p = 0.002). Smaller

globule size in nanoemulsions means more available surface area for diffusion, leading to greater release. As QCT-NE is lipoidal and has a tiny globule size, it is safe to say that it increased QCT's solubility and diffusion rate.

Characterization of QCT-NE gel

The QCT-NE gel pH was measured to be 5.81 0.03. The pH is somewhat acidic, so it wouldn't irritate the skin since it's already at that level. QCT-NE gel was measured to have a viscosity of 71,210 1.12 cP at 5 rpm. It was also revealed that the viscosity of QCTNE gel reduced as the rotational speed increased. In reality, when the rpm was raised, the contact sites inside the gels started to break, causing internal structural disruption. The uniformity of the particles diminished with increasing rotational speed, having been aligned until they began to flow. Pseudoplastic flow was seen in the gel. Unfortunately, thixotropy was not detected. The gel formulation's rheological features were analyzed using the power law model, which allowed us to calculate the flow index. Having a flow behavior index of 0.415, it was established that QCT- NE gel exhibited shear thinning behavior.

Texture profile analysis

Mechanical properties such as stickiness, rigidity, hardness, and ease of application are important for topical formulations. Analysis of texture profiles was used to learn about QCT-NE gel's mechanical characteristics. Texture PRO CT 1.4 build 17 was used to create the texture profile analysis results.

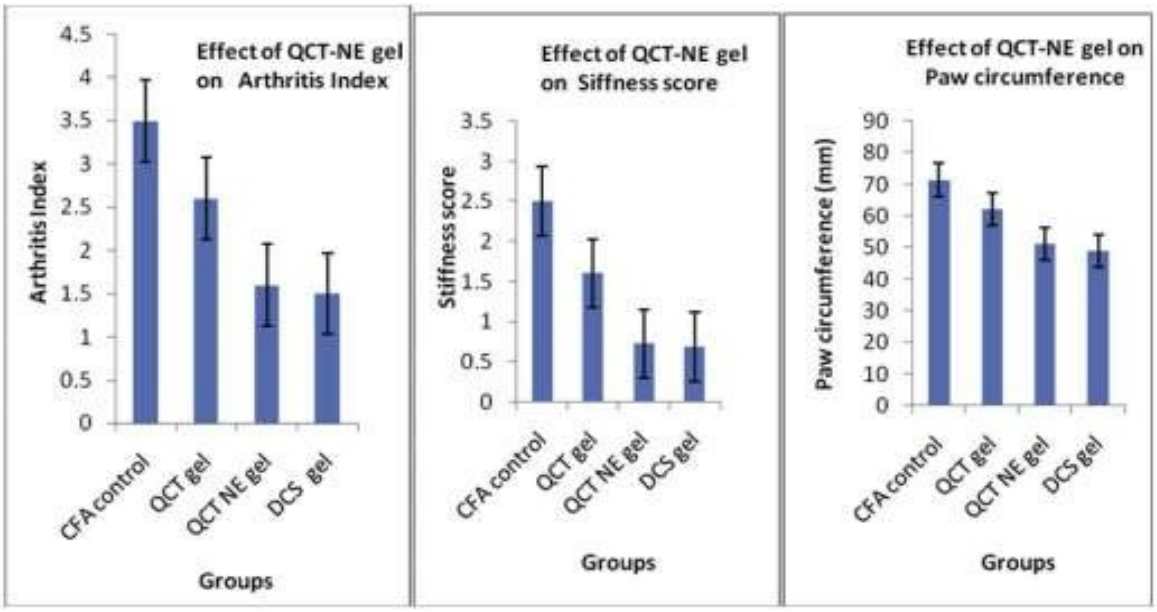
QCT- NE gel was measured to have an adhesiveness of 0.3 0.61 mJ. QCT- NE gel's value close to zero indicates increased adhesion and a smoother feel. The cohesiveness (consistency) of a gel sample reveals both the stability of its internal linkages and its plasticity prior to application. Maximum negative force is a good indicator of how cohesive a sample is. The group's cohesiveness was measured at 0.79 1.98.

Hardness and cohesiveness combine to create gumminess. Because of its low hardness and high cohesiveness, it is a crucial characteristic in gel systems. The hardness of a crushed gel is equivalent to the maximum force applied during the first compression cycle. This is the pressure required to apply the gel system to the skin, and it corresponds to the sense of a light touch. A stronger gel structure corresponds to a greater hardness value.

Antiarthritic activity

Measurements of paw volume. Rats developed rheumatoid arthritis after being given CFA for two weeks. Joint stiffness, difficulty moving, and an increase in paw circumference were all noticed. On days 7, 14, 21, and 28 after CFA injection, the volume of the paw was measured using a digital plethysmometer. The QCT-NE gel reduced the paw circumference from 71.210.33 mm in the CFA control group to 51.131.35 mm. QCT-NE gel significantly reduced paw volume compared to the CFA-control group.

Arthritis index and stiffness score. After CFA administration, arthritis indicators were measured on days 7, 14, 21, and 28. After treatment with QCT-NE gel, When compared to the CFA-control group, both the arthritic index and the stiffness score dropped from 3.71 to 1.61 to 1.53 to 0.73. Synovial membrane inflammation, hyperplasia, and macrophage activity define days 4 through 14 of the proliferative phases. Results for the arthritic index, stiffness score, and paw circumference from groups I–IV are shown in Figure 2.



(a) (b) (c)

Fig. 2. Effect of QCT-NE gel on rats following CFA induced arthritis

Measurement of hematological parameters. One of the clinical symptoms of RA was a reduction in the amount of RBCs and Hb in the CFA-control rats. When compared to the CFA control group and the QCT-NE gel formulation treatment group, the RBC and Hb levels in the CFA control group were considerably lower.

After QCT- NE gel therapy, the fast sedimentation rate (an indicator of inflammation in the ESR test) was reduced from 15 0.5 mm/h to 9.9 1.13 mm/h. One of the hallmarks of arthritis is a high white blood cell count. The QCT-NE gel formulation successfully lowered the WBC count from 12 0.9 103 /mm3 to 5.6 1.5 103 /mm3 after therapy.

C-reactive protein (CRP) has emerged as a powerful indicator for RA diagnosis in recent years. The liver produces CRP, a pentraxin protein, in reaction to inflammation. Bone deterioration is aided by this protein, which lines the synovium of rheumatoid arthritis patients. Eighty percent of arthritis cases are inflammatory, and RF is often regarded as the most important serological marker for this kind of arthritis. The Fc region of IgG antibodies is the target of RF, an autoantibody. Immune complexes composed of RF and IgG have been linked to the development of RA [46]. Compared to the CFA-control group, the QCT-NE gel dramatically decreased CRP and RF levels ($p = 0.032$ and 0.004, respectively), indicating a decrease in systemic inflammation and antibody formation against the injected adjuvant. Table 1 displays the aggregated data.

Table 1 Rats with CFA-induced arthritis show changes in hematological markers, CRP, and RF.

Group	RBC ($\times 10^6/mm^3$)	WBC ($\times 10^3/mm^3$)	ESR (mm/h)	Hb (mg%)	CRP (mg/dL)	RF (IU/mL)
Group I-CFA control	7.4 ± 0.5	12 ± 0.9	15 ± 0.5	11.5 ± 1.2	8.7 ± 0.2	71 ± 1.3
Group II-QCT- gel	8.2 ± 0.6	7.9 ± 0.5	10.5 ± 0.5	13.7 ± 0.2	3.6 ± 0.8	34 ± 1.9
Group III-QCT-NE gel	8.8 ± 0.4	5.6 ± 1.5	9.9 ± 1.13	15.0 ± 0.1	2.1 ± 0.3	23 ± 0.2
Group IV- Std. DCS gel	8.9 ± 1.7	5.7 ± 0.13	9.2 ± 1.4	14.3 ± 0.5	1.9 ± 0.9	22 ± 1.6

CONCLUSION

In conclusion, the focus of the current body of work is on improving upon the limitations of QCT administration by creating a unique formulation that may be applied topically. As QCT has an effect comparable to that of NSAIDs, it has found widespread application as a phytoconstituent. Nevertheless, poor skin permeability restricts its use through the topical approach. A carrier system for QCT was achieved with the incorporation of oleic acid: arachis oil, tween 20, and PEG-400 (15:6:6). The improved physicochemical stability, tolerable mechanical characteristics, and enhanced skin permeability of the modified formulation led to a significant uptick in its anti-arthritic effectiveness. QCT-NE gel has been proven in studies, when used topically, may be an effective treatment for rheumatic problems.

REFERENCES

1) Vikram V.B.K.Mishra, S.B. Bhanja , B.B Panigrah (2019), Development and Evaluation of Nanoemulsion gel for Transdermal Delivery of Ketoprofen, Int. J. Pharm. Sci. Rev. Res., 56(2), May - June 2019; Article No. 03, Pages: 10-21
2) Gajanan Mogal, Vaibhav Changadiya (2018), Formulation and Evaluation of Nanoemulsion for Non-Steriodal Anti-inflammatory Drug, Volume 9 Issue 2, DOI: 10.21275/ART20204616
3) Vikram V.B.K. Mishra, S. B. Bhanja, B. B Panigrahi. (2019), Development and Evaluation of Nanoemulsion gel for transdermal delivery of Valdecoxib, Research J. Pharm. and Tech; 12(2):600-610. doi: 10.5958/0974-360X.2019.00107.0
4) Lala, R.R., Awari, N.G. (2014), Nanoemulsion-based gel formulations of COX-2 inhibitors for enhanced efficacy in inflammatory conditions. Appl Nanosci 4, 143–151. https://doi.org/10.1007/s13204-012-0177-6
5) Anwer, M.K.; Aldawsari, M.F.; Iqbal, M.; Almutairy, B.K; Soliman, G.A.; Aboudzadeh, M.A. (2019), Diosmin-Loaded Nanoemulsion-Based Gel Formulation: Development, Optimization, Wound Healing and Anti-Inflammatory Studies. Gels, 9, 95. https://doi.org/ 10.3390/gels9020095
6) Abd-Allah F, Dawaba HM, Mansour A, Ahmed AMS. Evaluation of the anti-inflammatory and analgesic effects of piroxicam-loaded microemulsion in topical formulation. Int J Pharm Pharm Sci ,2011; 3(2), pp. 66- 70.
7) Panwar AS, Upadhyay N, Bairagi M, Gujar S, Darwhekar GN, Jain DK. Emulgel: A Review. Asian Journal of Pharmacy and Life Science .2011;1(3):335.
8) Aboalnaja KOet al.Utilization ofnanoemulsions to enhance bioactivityof pharmaceuticals, supplements, andnutraceuticals: nanoemulsion deliverysystems and nanoemulsion excipientsystems.Expert Opin Drug Deliv2016;21: 1–10
9) M€uller RHet al.Development ofindustrially feasible concentrated 30%and 40% nanoemulsions for intra-venous drug delivery.Drug Dev IndPharm2012; 38: 420–430

10) Sharipova AAet al.Polymer-surfactantcomplexes for microencapsulation ofvitamin E and its release.Colloids SurfB Biointerfaces2016; 137: 152–157
11) Liu Tet al.Effect of drug physico-chemical properties on the efficiencyof top-down process and characterization of nanosuspension.Expert OpinDrug Deliv2015; 12: 1741–1754.
12) Kumar S, Talegaonkar S and Lalit M. Design and development of Ciclopirox topical Nanoemulsion gel for the treatment of subungual onchomycosis. Ind J Pharma Edu Res. 46(4); 2012: 303-311.
13) Chopade VV and Chaudhari PD. Development and evaluation of self emulsifying drug delivery system for Valdecoxib, Int J Res Dev Pharma Life Sci. 2;2013: 531-537
14) Debnath S, Satayanarayana K and Kumar GV.. 'Nanoemulsion-a method to improve the solubility of lipophilic drugs', PHARMANEST - An Int J of Advances In Pharma Sci.2(3); 2011:72-83.
15) Sharma N, Mishra S ,Sharma S and Deshpande R. Preparation and Optimization of Nanoemulsions for targeting Drug Delivery. Int J of Pharma Professional Res 5(4); 2013:37-48.