e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2025; 17(3); 03-10

Original Research Article

Formulation and Evaluation of Nanostructured Lipid Carriers for management of Rheumatoid Arthritis

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Received: 25-05-2025 / Revised: 23-06-2025 / Accepted: 26-07-2025
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Conflict of interest: Nil

Abstract:

Nanostructured Lipid Carriers (NLCs) are very potential formulations for topical delivery of anti-inflammatory and anti-arthritic drugs. In the present study NLCs containing methotrexate and acelofenac were formulated for treatment efficiency in arthritis. The evaluation of different formulation parameters based on variation of solid lipid - liquid lipid ratio (SL: LL), Drug - lipid ratio (D:L), type of liquid lipid (LL), surfactant concentration was carried out. The NLCs were prepared by microemulsion technique method with fixed amount of methotrexate (100mg) using cetyl Alcohol, compritol 888 ATO and clyceryl monostearate. Optimization of process variables was carried out using Taguchi design. The particle size of the optimized batch for methotrexate (NLCMop) and aceclofenac (NLCAop) was found to be 136.2 nm and 145.3 nm, zeta potential -25.0 and -24.4 with drug entrapment of 89.47 ± 6.8 % and 77.46 ±0.76% respectively. Permeation rate and controlled release property of drugs loaded NLCs was studied through egg membrane and was found to be 89.038 ± 2.63% and 82.7±1.34 after 24 hrs. Stability study of the optimized formulation (NLCMop) and (NLCAop) showed that the formulation was more stable at 5 ± 1^0C than room temperature. These results suggested that NLC based formulations can be potential for topical delivery of methotrexate and aceclofenac for the management of arthritis.

Keywords: Nanostructured Lipid Carriers, Aceclofenac, Methotrexate, Rheumatoid Arthritis, Topical.

Introduction

Inflammation is a normal protective respective to tissue injury caused by physical trauma noxious chemicals or microbiologic agents. Inflammation is the body's effort to inactivate or destroy invading organism remove irritants and set the stage of tissue repair. Inflammation is sometimes inappropriate triggered by an innocuous agent such as pollen or by an autoimmune response as in asthma or rheumatoid arthritis [1].

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by a distinctive pattern of bone and joint destruction. RA is also a systemic disease, and several patient subsets can be distinguished based on the presence of extra- articular manifestations [2]. It is a systemic disorder where inflammatory changes not only affect synovial joints but also many other sites including the heart, blood vessels and skin. Rheumatoid arthritis (RA) is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage. Immune complexes composed of IgM activate complement and release cytokines (mainly TNF and IL-1) which are chemotactic for neutrophils. These inflammatory cells secrete lysosomal enzymes which damage cartilage and erode bone, while PGs produced in the process cause vasodilatation and pain. RA is a chronic progressive, crippling disorder with a waxing and waning course. NSAIDs are the first line drugs and afford symptomatic relief in pain swelling, morning stiffness, irnmobility, but not arrest the disease process [3]. Methotrexate (MTX) is an immunosuppressant class of DMARDs and this dihydrofolate reductase inhibitor has prominent immunosuppressant and anti-inflammatory property thus used for the treatment of rheumatoid arthritis. The systemic use of this drug (Trexall, Rheumatrex etc.) causes numerous side effects, the most important being the hepatic toxicity. Topical delivery of MTX would be beneficial to reduce its side effects. But a disappointing result has been reported which was mainly due to insufficient percutaneous absorption of MTX [4].

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) used in the first line treatment of rheumatoid arthritis, osteo arthritis and ankylosing spondylitis. Aceclofenac is partially water insoluble drug which undergoes first pass metabolism when taken orally also it produces some GI problems.

Hence better alternate route of administration of aceclofenac is transdermal route. Encapsulation of aceclofenac in to nano structured lipid carrier with the objective of prolonging its action and avoiding its most side effect [5]. Nanostructure lipid carriers (NLC) are the novel colloidal carriers in the new generation of lipid nanoparticles which have been gaining attention in recent trends. Solid lipid Nano particles have been administrated through several routes such as parenteral, oral and topical routes by controlled and sustained release formulations. It has to be explored for its delivery in commercial market [1]. Some of the advantages of this drug delivery includes controlled release profile, drug targeting, high stability, good entrapment efficiency, incorporation of both lipophilic and hydrophilic drug, non biotoxicity of the carrier, avoidance of organic solvents, good scalability and also this system is attractive for their potential to improve performance of pharmaceuticals, nutraceuticals and other materials. Topical drug application are in use from past many years to treat various infections on skin surface, epidermis, and dermis.

A lot of research is going on topical application of NLC. The major advantage of NLCs is that they are composed of physiological and biodegradable lipids that show low toxicity. Their small size furthermore provides a close contact to the stratum corneum which in turn enhances the amount of drug penetrated into the skin [6].

The aim of the present work was to formulate and characterize NLCs containing Methotrexate and aceclofenac that could achieve enhanced skin targeting effect enhanced skin bioavailability, good stability and sustained and prolonged drug release.

Materials

Aceclofenac was received as gift sample from Akums Drugs & Pharmaceuticals Ltd., Haridwar, Uttarkhand. Methotrexate was received as gift sample from Kwality Pharmaceutical Pvt. Ltd. (Amritsar, India). Compritol 888 ATO was a gift from Gattefosse, India. Glyceryl Monostearate Cetyl alcohol, propylene glycol, and Tween 80 were purchased from S. D. Fine Chem Ltd (Mumbai, India). All other solvents and reagents were of analytical grade.

Methodology

Preparation of NLCs containing methotrexate and aceclofenac by microemulsion technique: The lipids were melted at 80°C and drug was added followed by stirring for 5 minutes. Subsequently sonication was carried out at 120W for 60 seconds. To this mixture, tween 80 was added and stirring was carried out for two minutes. The aqueous phase containing co-surfactant was heated to 80°C and added to the lipid phase. The microemulsion (o/w) was formed under mechanical stirring at 80°C for 15 minutes at 150 rpm.

The resulted microemulsion was sonicated for 5 minute followed by stirring to cool down (40°C). The warm microemulsion was dispersed in cold (2-4°C) mixture of propylene glycol and distilled water (2:8) at a fixed ratio of 1:5 (microemulsion: dispersing medium) under mechanical stirring for 3 hours. The resultant suspension was centrifuged at 8000 rpm for 30 min to separate free drug and microparticles from NLCs suspension. The NLCs were stored in cool place till further evaluation [7].

Design of Experiment: In order to find an optimum formulation condition, a large number of experiments are required to prepare test specimens and also to carry out lengthy tests. To reduce the number of experiments, several experimental designs have been used [8]. Taguchi method is one which is a frequently applied experimental design method [9].

Table 1: Code for four variables at three different levels (methotrexate)

S.No	Variables	Levels		
		Low(1)	Medium(2)	High(3)
1.	Solid lipid - liquid lipid ratio (SL:LL)	65:35	70:30	75:25
2.	Drug - lipid ratio (D:L)	1:5	1:7	1:10
3.	Type of liquid lipid (LL)	almond	castor	canola
4.	Surfactant concentration	8	10	12

Dependent variables	Constraints
Particle size (nm)	Minimizes
Entrapment Efficiency (%)	Maximizes

Table 2: Code for four variables at three different levels (aceclofenac)

S.No	Independent Variables	Levels		
		Low (1)	Medium (2)	High (3)
1.	(A) Solid lipid - liquid lipid ratio (SL:LL)	60:40	70:30	80:20
2.	(B) Drug - lipid ratio (D:L)	1:5	1:7	1:10
3.	(C) Mechanical Stirring time (hours)	1	1.5	2
4.	(D) Surfactant concentration	0.5	1	1.5

Dependent variables	Constraints
Particle size (nm)	Minimizes
Entrapment Efficiency (%)	Maximizes

Characterization of methotrexate and aceclofenac loaded nanostructured lipid carriers

Shape and Surface Morphology: The shape and surface morphology of NLCs suspension was studied by transmission electron microscopy [Phillips EM416LS]. Room temp. And 80 kV potential was used, at 20000 magnifications. The sample was prepared by placing a drop of NLCs that was previously diluted 50-folds with double-distilled water onto a 400-mesh copper grid coated with carbon film and followed by negative staining with 1% phosphotungstic acid.

Particle Size and Size Distribution: Average particle size and polydispersity index were measured by Malvern ZetaSizer Ver. 2.0.

Zeta Potential Measurement: The surface charge of NLCs is denoted as zeta potential, and it was determined by the electrophoretic mobility of NLCs in U type tube at 25^0C, using a Zetasizer (Malvern).

Drug Entrapment Efficiency: A volume of NLCs suspension equivalent to 10 mg of drug was pipetted out (10 ml, Borosil), and centrifuged at 12000 rpm for 10 min at 10^0C using refrigerated centrifuge (SIGMA 3-18K, Sartorious). The supernatant was removed and concentration of drug in the supernatant was determined spectrophotometrically at 259.5nm using calibration curve. The percent drug entrapment was calculated by the following equation:

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Amount of drug in supernatant} \times 100}{\text{Amount of drug added}}$$

In-*vitro* Drug Release Study: *In vitro* drug release study was carried out using Keshary-Chien (K-C) cell of 25 ml capacity using egg membrane, in phosphate buffer saline (PBS) pH 6.8. The receptor compartment was filled with phosphate buffer saline pH 6.8 while a 2ml volume of formulation was taken in the donor compartment. The temperature of the receptor compartment was maintained at 37 ± 0.5^0C with the help of a circulating water bath. Samples (1 ml) were withdrawn at regular interval and replaced with equal volume of PBS pH 6.8 to maintain the sink conditions. Samples were filtered through Whatman filter, diluted with 0.1M NaOH and analyzed spectrophotometrically against reagent blank.

Mechanism of drug release: The mechanism of drug release was determined by fitting the release data into various kinetic models such as zero-order (% drug release vs time), first-order (log % drug retained vs time), Higuchi (% drug release vs square root of time) and Korsmeyer-Peppas.

Stability studies of optimized formulation NLCMopt and NLCAopt: The optimized formulation NLCMopt and NLCAopt were stored in screw capped amber color glass bottles at 5 ± 1^0C (Refrigerator) and room temperature for a period of 45 days. Samples were analyzed for residual drug content by plotting a graph between drug content vs time and log % drug content vs time in order to evaluate shelf-life of the formulation.

Shelf-life was calculated by the equation: $T_{10\%} = 0.104/K$

Half-life was calculated by equation: $T_{10\%} = 0.152 \times t_{1/2}$

Results and Discussion

The drug loaded NLCs optimized using Taguchi design were formulated by the microemulsion technique as shown in Table 1 & 2. Out of nine formulations for both methotrexate (NLC1-9) and aceclofenac (NLCA1-9) loaded NLCs the particle size of the optimized batch for methotrexate (NLCMop) and aceclofenac (NLCAop) was found to be 136.2 nm and 145.3 nm, zeta potential of -25.0 and -24.4 with drug entrapment of 89.47 ± 6.8 % and 77.46 ±0.76% respectively.

Permeation rate and controlled release property of drugs loaded NLCs was studied through egg membrane and was found to be 89.038 ± 2.63% and 82.7±1.34 after 24 hrs.

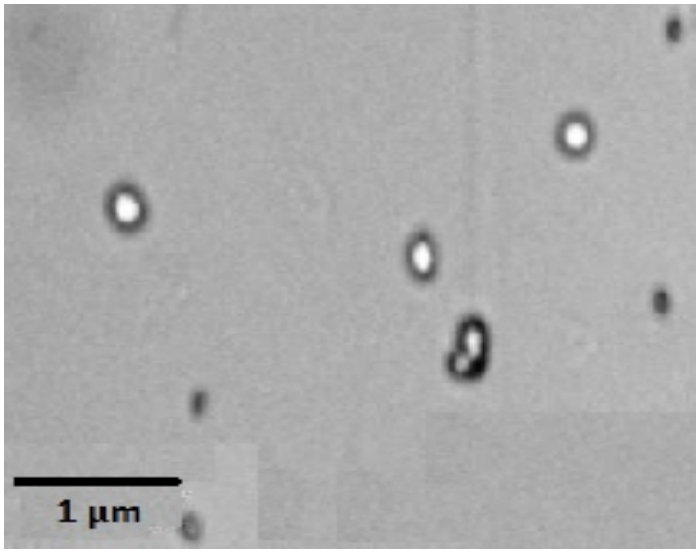

Figure 1: TEM of optimized formulation containing methotrexate NLCMop

Table 3: Optimization of process variables of methotrexate loaded NLCs

Batches	Parameters				Zeta Potential (mV)	Particle size (nm)	Drug entrapment (%)	*In-vitro* drug release (%) after 24 hours
	A	B	C	D				
NLC1	3	3	2	1	-15.6	195.3±1.4	72.83±3.5	77.37±2.5
NLC2	3	2	1	3	-21.3	205.9±4.6	81.97±8.5	80.26±2.1
NLC3	1	2	2	2	-22.98	201.6±4.6	85.87±5.7	78.38±2.1
NLC4	1	1	1	1	-28.70	133.5±5.8	79.60±7.6	84.17±2.6
NLC5	1	3	3	3	-27.50	137.8±8.6	80.13±3.2	86.19±1.4
NLC6	2	3	1	2	-27.10	176.8±3.7	88.23±6.2	87.63±3.8
NLC7	3	1	3	2	-26.80	185.9±6.4	85.13±6.6	66.86±1.7
NLC8	2	2	3	1	-23.60	152.7±5.3	88.23±8.5	71.42±1.6
NLC9	2	1	2	3	-25.0	133.2±6.7	91.47±7.9	90.03±2.8

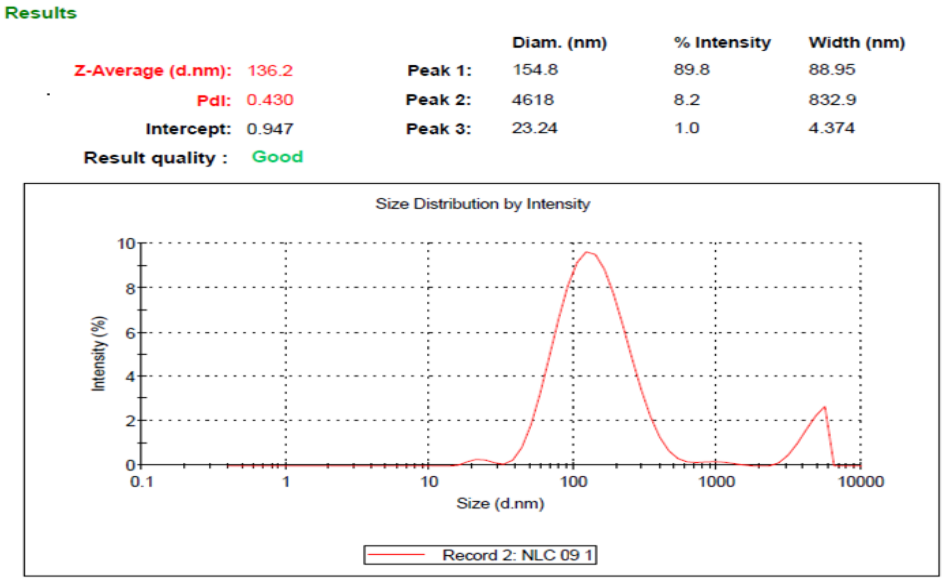

Figure 2: Particle Size of optimized formulation containing methotrexate NLCMop

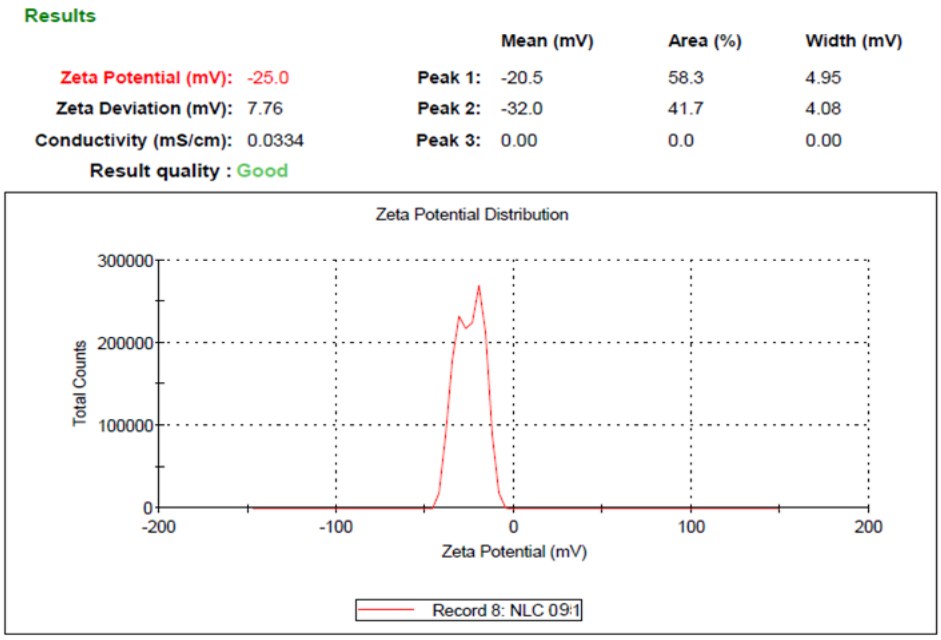

Figure 3: Zeta Potential of optimized formulation containing methotrexate NLCMop

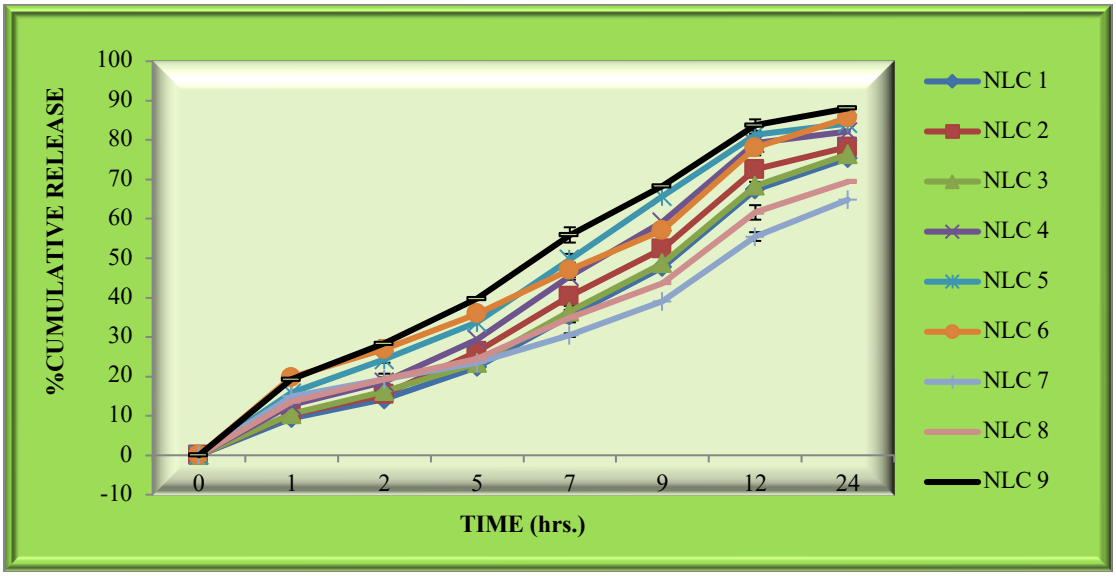

Figure 4: *In-vitro* drug release profile of Methotrexate loaded NLC batches

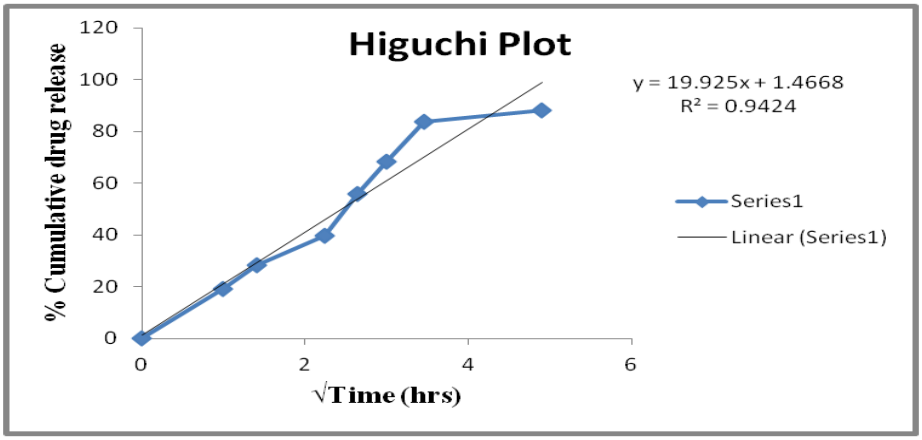

Figure 5: Higuchi Plot of optimized formulation containing methotrexate NLCMop

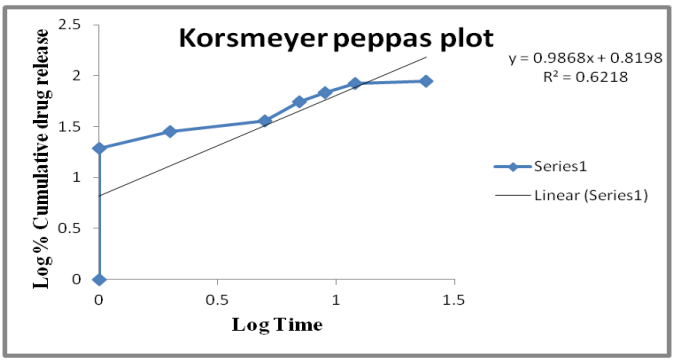

Figure 6: Korsmeyer and Peppas plot of optimized formulation containing methotrexate NLCMop

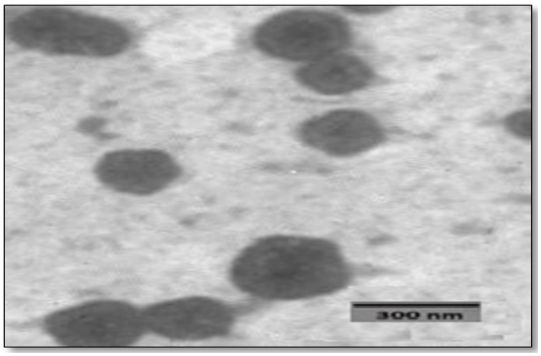

Figure 7: TEM of optimized formulation containing aceclofenac NLCAop

Table 4: Optimization of process variables of aceclofenac loaded NLCs

Formulations	Parameters				Particle Size (nm)	Zeta Potential (mV)	Drug entrapment (%)	*In-vitro* drug release (%) after 24 hour
	A	B	C	D				
NLC_A1	1	2	2	2	223	-7.80	59.35±1.12	76.1±0.8
NLC_A2	1	3	3	3	275	-13.90	68.77±1.25	74.3±1.23
NLC_A3	2	3	2	1	187	-26.3	72.57±1.0	74.5±1.8
NLC_A4	3	1	2	3	295	-23.4	61.06±1.65	70.6±1.65
NLC_A5	2	1	3	2	186.6	-19.6	65.31±1.4	72.5±1.23
NLC_A6	3	2	3	1	268	-22.13	72.43±1.03	77.6±1.22
NLC_A7	3	2	1	2	145.3	-24.4	77.27±1.1	82.7±1.34
NLC_A8	2	2	1	3	167	-22.65	73.13±1.69	78.4±0.5
NLC_A9	1	1	1	1	205.5	-22.80	69.24±1.36	76.2±0.2

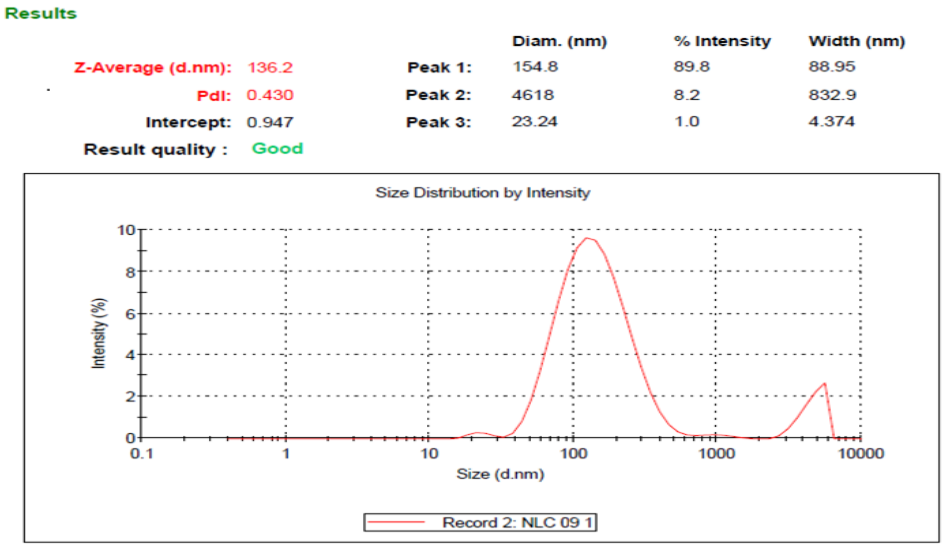

Figure 8: Particle Size of optimized formulation containing aceclofenac NLCAop

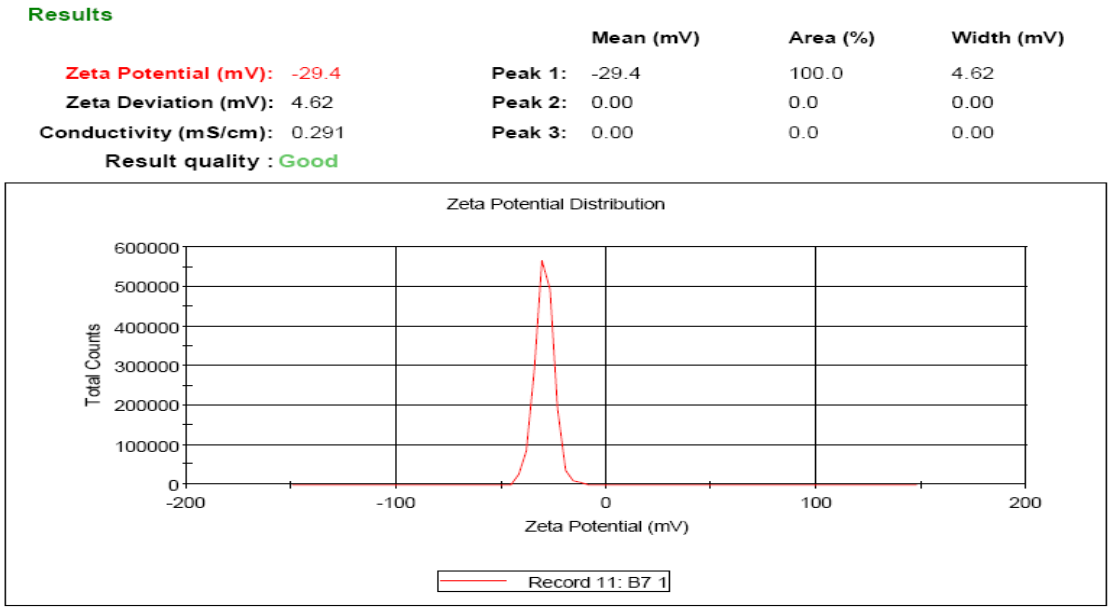

Figure 9: Zeta Potential of optimized formulation containing aceclofenac NLCAop

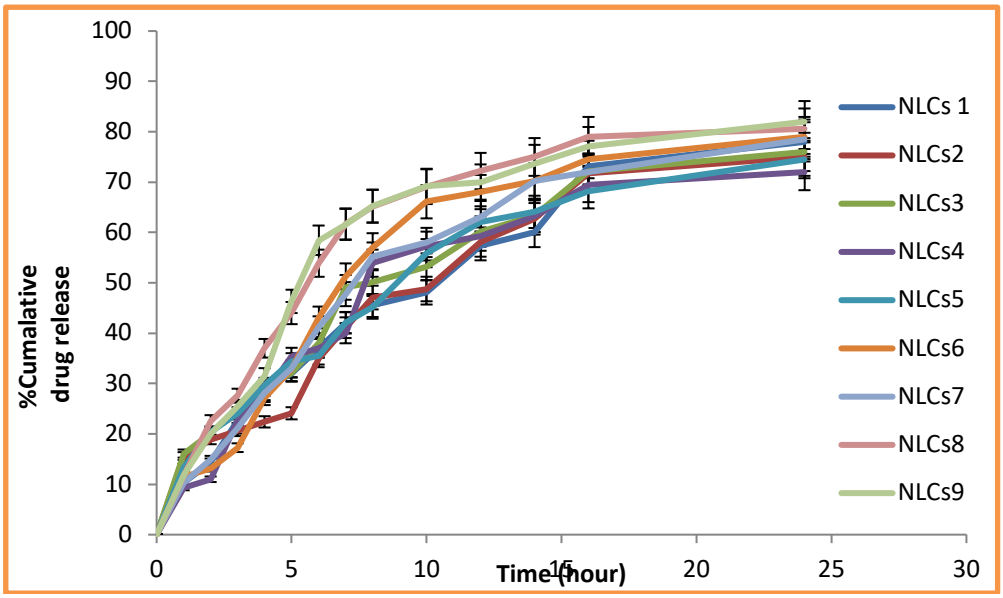

Figure 10: *In-vitro* drug release profile of Aceclofenac loaded NLC batches

Table 5: Kinetic study and 'n'valve for NLCAopt

Release kinetics of Optimized NLC_A7	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	k	R^2	k	R^2	k	R^2	n	R^2
	3.67	0.954	0.0061	0.878	0.866	0.9743	0.676	0.930

In vitro release data of optimized NLCs (NLCMopt and NLCAopt) was then subjected to various release kinetic models. The kinetic models used were zero-order, first order, Higuchi and Korsmeyer-Peppas model. The interpretation of data was based on the value of the resulting regression coefficients. The release kinetics of Methotrexate and Aceclofenac followed higuchi as highest linearity was displayed for this curve. The Korsmeyer-Peppas release exponent n for the optimized formulation NLCMopt and NLCAopt indicated the anomalous (non-fickian) diffusion indicating that drug release is controlled by more than one process i.e. superposition of both phenomenon, the diffusion controlled as well as swelling controlled release. The drug release from the optimized batches NLCMop and aceclofenac NLCAop displayed a biphasic drug release pattern with burst release at the initial stage followed by sustained release as shown in the Figure 4&10. These results indicated that the NLCs is a suitable carrier of methotrexate and aceclofenac with improved drug loading capacity and sustained drug release properties following Higuchi kinetics.

Stability study of the optimized formulations (NLCMop) and (NLCAop) showed that the formulation was more stable at 5 ± 1^0C than room temperature.

In conclusion, the optimized batch of methotrexate and aceclofenac loaded nanostructured lipid carrier having low particle size and prolonged release was successfully prepared using microemulsion technique. Taguchi design was found to be a promising tool for obtaining the optimum condition and also reducing the sets of experiments. The optimised NLCs formulations has the potential to be useful for site-specific delivery of drugs through skin.

References

1. Lucinda Hampton, https://www.physio-pedia.com/Inflammation_Acute_and_Chronic
2. Tobón G.J., Youinou P., Saraux A., The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis. Journal of Autoimmunity. 2010 Aug; 35(1):10-4. doi: 10.101 6/j.jaut.2009.12.009. Epub 2010 Jan 18. PMID: 20080387.
3. K.D.Tripathi, Essentials of Medical Pharmacology, 7th Edition, Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, 2013.
4. Javadzadeh, Y. and Hamishehkar, H., Enhancing Percutaneous Delivery of Methotrexate Using Different Types of Surfactants. Colloids and Surfaces B: Biointerfaces, 2011; Vol. 82, pp. 422–426.
5. Anand Kumar K., Parameswara Rao V., Priyanka K., Sanjay Kumar S., Sanjay S., Development and evaluation of solid lipid nanoparticles of raloxifene hydrochloride for enhanced bioavailability. BioMed Research International, Volume 2013, Article ID 584549, pp.1-9.
6. Rahul N., Vishnu Priya K., Arun Kumar K.S., Badivaddin T.M., Sevukarajan M., Formulation and evaluation of solid lipid nanoparticles of water soluble drug: Isoniazid. Journal of Pharmaceutical Sciences and Research. 2011; 3(5):1256-64.
7. Misra, A., Kalariya, M., Padhi, B.K. and Chougule, M., Methotrexate-loaded solid lipid nanoparticles for topical treatment of psoriasis: formulation & clinical implications. Drug Discovery and Development. [Online] http://www.drugdeliverytech.com/ME2/dirmod.asp?sid=&nm=&type=Publishing&mod=Publications%3A%3AArticle&mid=8F3A702 7421841978F18BE895F87F791&tier=4&id=53FFF1CD14794962B2A80E7BDF475ECD,(accessed on 20 Aug, 2025)
8. Suresh, S., Narendra, C., Maheshwari, D. and Kundawala, A.J., Formulation and evaluation of liposomes containing antitubercular drugs by taguchi's orthogonal array design. Acta Pha rmaceutica Sciencia, 2010; Vol. 52, pp. 79-88.
9. Senthilkumar, R., Vaidyanathan, S. and Sivaraman, B., Thermal analysis of heat pipe using taguchi method. International Journal of Engineering and Science Technology, 2010; Vol. 2, Issue 4, pp. 564-569.
10. Dubey A., Prabhu P., Kamath J.V., Nano Structured lipid carriers: A Novel Topical drug delivery system. Internaional Journal of Pharm Tech Research, 2012; 4(2):705-14.
11. Patel D., Dasgupta S., Dey S., Roja Ramani Y., Ray S., Mazumder B., Nanostructured lipid carriers (NLC) - based gel for the topial delivery of aceclofenac: preparation, characterization and in-vitro evaluation. Scientia Pharmaceutica., 2012; 80(3):749-64.
12. Uner L. and Yener G., Importance of Solid Lipid Nanoparticles (SLN) in various admin-istration routes and future perspectives, Inter-national Journal of Nanomedicine, 2007; 2(3):289-300.