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New Product Development, Evaluation, With Optimization Diclofenac Transport For Gout

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ABSTRACT

Background: Gout is a common form of inflammatory arthritis. When this happens to one or more joints, it can cause inflammation, redness, and discomfort. Developing solid lipid nanoparticles (SLNs) containing diclofenac (DCE) was the primary objective of this research. Because of their reputation as the most effective lipid-based colloidal carriers, SLNs have become the standard approach for improving the oral bioavailability of drugs that have low water solubility.

Methodology: The microemulsion method was used to create diclofenac-loaded SLNs that were coated with chitosan (CS) for targeted drug delivery. Next, a battery of tests was run on them, including in vitro drug release in phosphate buffer, polydispersity index, and particle size.

Results: The formulation parameters of Diclofenac-SLN were optimized using the Box-Behnken design of response surface methodology. Particle size was 94.39 nm, PDI was 0.239, and entrapment efficacy was around 0.89 for the improved formulation. An 8.75 mV zeta potential is associated with diclofenac-SLN. TEM examination showed that Diclofenac-SLNs ranged in size from 100 nm and had a spherical form. Endothermic transitions occurred at 243°C in the DCE DSC curve. Over the course of a day, 85% of the medicine was released from the SLNs containing diclofenac, demonstrating a regulated release of the medication from the lipid matrix. When compared to pure drug solutions, optimized diclofenac-SLNs demonstrated sustained drug release for up to 24 hours in the drug release trials.

Conclusion: First, FTIR and physiological testing identified and purified the drug and polymer. The design strategy was followed to synthesize DCE and DCE-coupled SLNs using micro-emulsion technology. In-situ coating of SLNs with chitosan ligand improved oral bioavailability and maybe tailored drug distribution at the site of action. Lipid phase affinity for negatively charged Diclofenac was increased by cationic lipid stearic acid. The increased batch had a 300-nm diameter and 0.324 PDI, as proven by TEM and particle size analysis.

KEYWORDS: Solid lipid nanoparticles, Diclofenac, Optimized Gout.

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INTRODUCTION

Many different areas of medicine have been profoundly affected by nanoparticle-based drug delivery systems. These include general medicine (immunology, cardiology, pulmonology, etc.), specialized medicine (gene delivery, brain targeting, tumor targeting, etc.), and even oral vaccine formulations. Because of their high drug loading capacity and facile permeability, solid lipid nanoparticles (SLN) are essential nanotechnology tools for the administration of arthritis treatment [1-3]. A gout attack often subsides within a week or two. Flares of gout may begin in the lower thigh or big toe. Excess urate crystals can build up over time and cause gout, which is marked by the presence of needle-shaped stones in and around the affected joint. Inflammation and arthritis manifest in the joints. When urine production is either excessive or insufficient, the urate level rises. Non-steroidal anti-inflammatory drugs (NSAIDs) have a lengthy history of usage in the treatment of gout. Medications for gout have historically come in many forms, including transdermal patches, oral liquids, parenteral liquids, tablets, capsules, and formulations for children and the elderly. In addition to non-steroidal anti-inflammatory pharmaceuticals (NSAIDs), including aspirin, celecoxib, diclofenac, and aceclofenac, anti-rheumatic medications such as gold, aceclofenac, penicillamine, leflunomide, sulfasalazine, and hydroxychloroquine can be utilized for the treatment of arthritis. In the past, nonsteroidal anti-inflammatory drugs (NSAIDs) were used to treat gout [5]. Tablets, capsules, transdermal patches, topical solutions, parenteral solutions, oral solutions, pediatric solutions, and geriatric solutions have all been used to treat gout. Doctors can use a combination of anti-rheumatic medications, including gold, penicillamine, leflunomide, sulfasalazine, and hydroxychloroquine, along with non-steroidal anti-inflammatory medicines like aspirin, celecoxib, aceclofenac, and diclofenac to treat arthritis[4]. That is why there is a need for non-toxic, low-cost gout treatments. For pharmaceutical distribution over an extended period of time, novel dosage forms, including solid lipid nanoparticles, are under development. The half-life, bioavailability, and solubility of medications, as well as their potential toxicity, could be improved. Special lipid nanocarrier systems (SLNs) are biocompatible nanocarriers with a diameter of 10–1000 nm that are mostly made of lipids or modified lipid nanostructures, such as triglycerides, fatty acids, or waxes. Because of this, the current research is focused on developing and refining SLNs to effectively administer diclofenac while also studying drug release properties, particle size, and entrapment capabilities.

MATERIALS AND METHODS

Diclofenac, stearic acid, Tween 80, hydrochloric acid, sodium hydroxide, and methanol were extracted from the Maharna Pratap College of Pharmacy in Kanpur's Department of Pharmaceutics.

Drug Diclofenac's Physiochemical Characterization

DCE was identified using FTIR spectroscopic data for drugs, melting point and partition coefficient determination, absorption maxima (λmax) computation, and medicine excipient interaction studies.

Drug Solubility Study in Various Lipids

To determine which lipid had the highest potential for Diclofenac, the drug's solubility in various lipids was evaluated. A glass vial containing a predetermined weight (100 mg) of fat was melted. The amount of medication in the vial was gradually raised. The previously mentioned mixture was heated above the melting point of the lipid. The drug's dissolution into the melting lipid is indicated by a translucent solution, which serves as the experiment's endpoint.

FTIR for a compatibility study

FTIR analysis was done to confirm any potential chemical interactions between the drug DCE and polymers such stearic acid and chitosan. FTIR was used to examine the medication by first combining it with dried KBr and then using an FT/IR 4100-TypeA to run spectra between 4000 cm-1 and 400 cm-1. When notable peaks linked to the important functional groups were found, subsequent samples of the same medication were compared to the initial running.

Diclofenac standard calibration curves in PBS pH 7.4

Diclofenac's maximum absolute wavelength was found to be 276 nm. In triplicate, drug sample dilutions ranging from 10 to 50 µg/mL were prepared. In a regression analysis, the calibration equation and correlation coefficient are obtained using absorbance (y) and concentration (x).

Creation of Diclofenac SLNs Using a Micro-Emulsion Approach:

This technique involved magnetically stirring a warm microemulsion dissolved in cold water to create solid lipid nanoparticles (SLNs). This technology differs from conventional ones in several ways, including its low cost, simplicity of usage, possibly biocompatible components, reliable and well-defined solid nanoparticle manufacturing, and remarkably high drug entrapment efficiencies within SLNs. SLNs were made with stearic acid, a lipid, soy lecithin, Tween 80, sodium taurodeoxycholate, ethanol, and distilled water. SLNs were made by melting stearic acid at 70°C above its melting point (65–70°C). After adding 300 mg of Diclofenac, which had dissolved in ethanol, the mixture was agitated for five minutes and sonicated for sixty seconds at 120 W. Tween 80 and soy lecithin, surfactants, were added and mixed for two minutes. In-situ chitosan mixing was done. The melted lipid phase received 50 mg of co-surfactant sodium taurodeoxycholate after heating an aqueous phase to 80°C. This liquid was mechanically stirred for 20 minutes at various rpms. The heated microemulsion was mechanically agitated and dissolved in distilled water. Next, the emulsion was cleaned three times with distilled water [6].

Optimization of Diclofenac-SLN Formulation Variables

SLN formulations (RSM) were improved using the surface response technique. An efficient model uses an RSM Box–Behnken experimental design (BBD) to assess the independent variables' responses and interactions. Lipid content, surfactants, and homogenization speed determined Diclofenac-loaded chitosan nanoparticle (DCE-SLNs) particle size, PDI, drug entrapment effectiveness, and drug release percentage. Table 1 details the design. A three-factor, three-level statistical experimental design optimized formulation variables, and 17 experiments were needed to execute the response surface approach.

Table-1 Factors displaying coded values with predetermined objectives for answers - Diclofenac -SLNs using BBD

Independent Variables	Symbol	Unit	Coded levels			Response (Y1)	Response (Y2)	Response (Y3)
			-1	0	+1			
Surfactant Concentration	X1	%w/v	0.5	1	1.5	Particle Size(nm)	PDI	% EE
Homogenization Speed	X2	rpm	12k	15k	18k			
Lipid content	X3	mg	200	300	400			

To assess the extended influence of formulation variables X1 (surfactant concentration), X2 (homogenization speed), and X3 (lipid content) on responses, the Box–Behnken design of response surface approach was used to record Y1 (particle size), Y2 (PDI), and Y3 (%EE).

Evaluation and description of solid lipid nanoparticles of diclofenac:

Particle size and distribution measurement

Photon correlation spectroscopy evaluated the SLNs' z-average and PDI. UK-based Malvern Instruments Ltd. Zetasizer ZS 90 measuring devices were used. Dilute 1 millilitre of SLN dispersion with 10 ml HPLC-grade water before measuring. We tested and improved the average particle size of the SLN formulation using various drug and fat ratios to find the best particle size formulation. The particle z-average diameter and polydispersity index (PDI) were determined for each triplicate experiment.

Shape and morphology of particles

Transmission electron microscopy investigated the created SLNs' shape and morphology. Transmission electron microscopy utilizes magnetic lenses to concentrate the structural picture after electrons pass through nanoparticles. Field emission scanning electron microscopy examined nanoparticle surface properties and form. After negative staining with a 1% w/v aqueous phosphotungstic acid solution, the samples were air-dried at room temperature before being examined under a microscope at the appropriate magnifications [7].

Zeta potential measurement of SLNs
Zeta potential can be used to enhance formulation and forecast long-term stability. Zetasizer Ver. 7.0 was used to evaluate the zeta potential after 1 ml of SLN dispersion was diluted with 10 ml of HPLC-grade water. Three attempts were made at each measurement.

Drug content determination:
Drug content was estimated using UV spectrophotometry at 286 nm.

$$\%Drug\ loading = \frac{Amount\ of\ drug\ in\ SLNS}{Total\ weight\ of\ SLNS} \times 100$$

Here, the type equation experiment was conducted. conducted three times. The following formula was used to determine the drug loading of SLNs:

Entrapment efficiency calculation:The method below was used to evaluate entrapment efficiency after evaluating the supernatant for unentrapped drugs (Eq 2).

$$E.E\ (\%) = \frac{\text{Amount of drug added in the formulation } - \text{Amount of drug in supernatant}}{Amount\ of\ drug\ add\ in\ the\ formulation} \times 100$$

SLN in vitro drug release studies:
A water bath incubator shaker was employed for in vitro release tests. The dialysis membrane's molecular weight threshold for 12,000–14,000 Da pore diameters was employed. At 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hours, a 5 ml aliquot was taken. The volume was taken regularly and refilled with fresh medium at the same temperature to maintain a consistent volume. Samples were analysed using 276 nm UV spectrophotometry. Concentrations and cumulative release % were calculated using the calibration curve. To understand drug release, numerous release kinetic models were fitted to release data[10].

Testing drug release kinetics:
For the purpose of determining the best drug-loaded nanoparticle compositions, the release data was examined using mathematical models such as the Zero order kinetic, first-order kinetic, Higuchi kinetic, Hixson-Crowell model, and Korsmeyer-Peppas model.

Studying Storage Stability
Storage stability of the enhanced nanoparticle formulation was tested at various temperatures and relative humidity. The Diclofenac nanoparticle formulation was stored at 4°C in the fridge, 25°C in a stability chamber with 60% humidity, and 40°C with 75% humidity for six months following ICH recommendations. At 0, 2, 4, and 4-month intervals, particle size and EE were measured[8-9].

RESULTS AND DISCUSSION

Diclofenac's physiochemical characteristics:
It was discovered that every physiochemical property was within the specified range. The drug's melting point and partition coefficient values matched those found in the standard monograph.

Drug Solubility Study in Various Lipids
According to a solubility study, Diclofenac is poorly soluble in water and is soluble in acetone, ethanol, and chloroform but is only weakly soluble in ethanol and alcohol. According to the solubility investigation, Diclofenac was a hydrophobic medication.

Initial FT-IR Compatibility Analysis

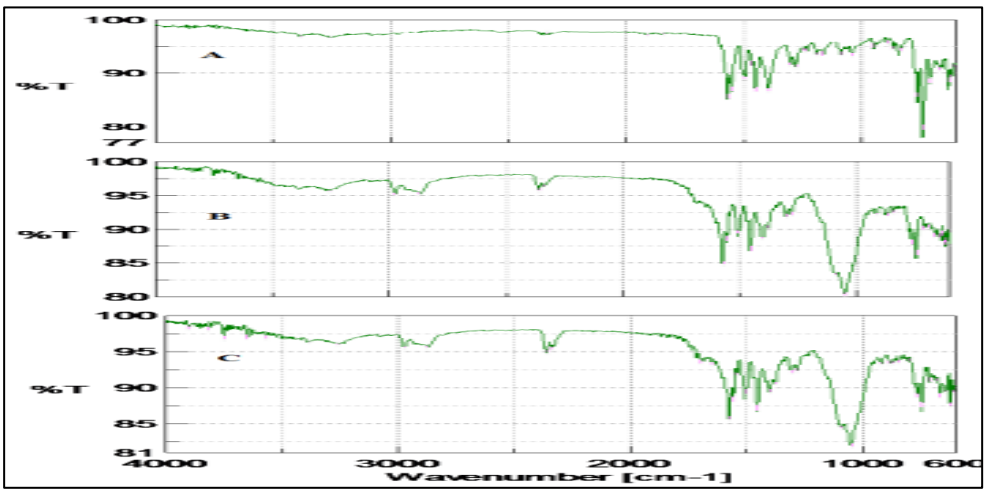

Fig. 1 FT-IR Diclofenac and polymer spectrum of a physical mixture FTIR spectra of (a) Diclofenac, (b) Chitosan, and (c) Stearic Acid

Diclofenac's identity was confirmed when the FTIR peak of the test sample matched that of the standard spectra, particularly in the fingerprint area.

Wavenumbers 3675.8 cm^{-1} (O-H stretch groups), 1645.6 cm^{-1}, 1592 cm^{-1}, 1545 cm^{-1} (skeletal C-C), and 2954.2 & 3078.1 cm^{-1} (skeletal C-H vibrations) were the locations of the DCE functional group peaks. When the FTIR peak of the test sample, especially in the fingerprint region, matched the reference spectra, the validity of diclofenac was verified. Through physical mixing, compatibility between DCE and lipid and chitosan were demonstrated.

Table 1 Interpretation of Diclofenac's FTIR spectra

Stretching type	Spectra Range Standard cm-1	Observed peak cm-1 Drug sample
N-H stretching	3500-3300	3359
O-H stretching	3100-2550	2875.2
C=O stretching	1850–1550	1710.5
C-C stretching for NH	3000-2800	2910.5
O-H in plane bending	1450-1350	1314.8
C-Cl	850–550	715.5

Table 2: FT-IR interpretation of a diclofenac combination with polymers

Stretching type	Spectra Range Standard cm-1	Observed peak cm-1 Drug sample
O-H Stretching	2970-2535	2870
N-H Stretching	3450-3210	3340
C-H Stretching (aliphatic)	3000-2850	2980
C-C and C-N Stretching	1370-1160	1203
C=O stretching	1540–1870	1710

Diclofenac Calibration Curve in PBS pH 7.4

The standard plot for diclofenac was created in triplicate using a range of 10–50 Microgram/milliliter. Data Available in table-3

Table 3 Calibration curve in Phosphate Buffer pH 6.8

S. No	Concentration (Microgram/milliliter)	Avg. Absorbance PBS pH 6.8 (276nm)
1	0	0
2	10	0.098
3	20	0.225
4	30	0.31
5	40	0.405
6	50	0.49

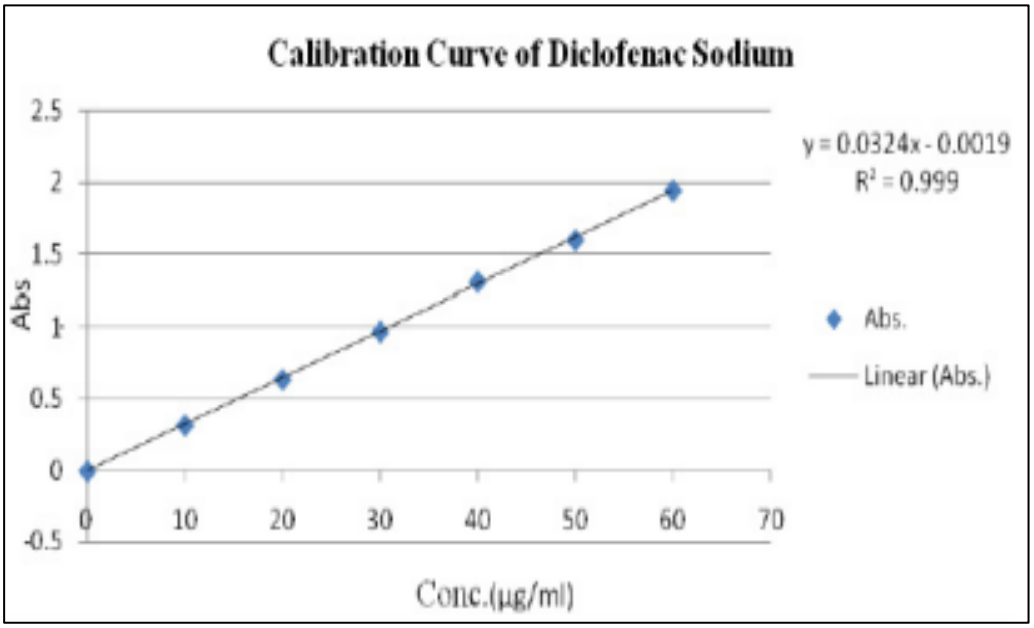

Fig 2 Diclofenac's standard calibration curve in phosphate buffer

Optimization of CS-DCE-SLN Formulation Variables

A linear model was found to be appropriate for particle size and PDI since no influence of interactions between the factors was noticed on these attributes, but the quadratic model was found to be appropriate for %EE since interactions between the components were shown to affect the attribute.

Table 4 Runs and Design Matrix produced by Design-Expert® software with recorded run answers for Diclofenac-SLN optimization.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A: Surfactant	B: Speed	C: Lipid Content	Particle Size (Y1)	PDI	Percentage EE
		Mg/ml	rpm	Mg	nm		Percentage
16	1	1	15000	300	336	0.385	70.12
10	2	1	18000	200	372	0.425	75.65
15	3	1	15000	300	186	0.315	71.85
1	4	0.5	12000	300	212	0.275	62.74
17	5	1	15000	300	342	0.312	80.55
4	6	1.5	18000	300	196	0.254	79.35
5	7	0.5	15000	200	232	0.345	74.35
7	8	0.5	15000	400	270	0.295	74.9
2	9	1.5	12000	300	306	0.386	78.12
13	10	1	15000	300	254	0.310	74.25
14	11	1	15000	300	310	0.365	77.12
11	12	1	12000	400	254	0.285	68.25
12	13	1	18000	400	312	0.405	62.35
3	14	0.5	18000	300	325	0.316	76.86
9	15	1	12000	200	275	0.325	71.38
6	16	1.5	15000	200	245	0.267	76.4
8	17	1.5	15000	400	198	0.312	79.42

The improved formulation's particle size, %EE, and drug release were assessed. Entrapment efficiency (77.9±1.45 nm), particle size (268.2±1.8 nm), and PDI (0.324±0.02) matched projected values for the improved formulation. The absolute error for response parameters is 2.72 ± 0.5%, with prediction errors of 1.45, 2.4, and 2.1%. Table 5 compares the response variable's actual and predicted values. These numbers show how well the models functioned and how well the predictions matched the measured data.

Table 5: Comparison between the experimentally observed and expected responses for the optimized Diclofenac-SLNs

Process	X1	X2	X3	Predicted	Experimental (n=3)	Error (%)
Particle size (Y1)	200.04	0.52	15330	275.83	268.2±1.8	2.54
PDI (Y2)	200.04	0.52	15330	0.332	0.324±0.02	2.4
EE % (Y3)	200.04	0.52	15330	80.60	77.9±1.45	3.22

Table 6 The Diclofenac-SLNs criterion for numerical optimization

Parameter	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Surfactant	is in range	0.5	1.5	1	1	3
B:Speed	is in range	12000	18000	1	1	3
C:Lipid	is in range	200	400	1	1	3
Particle Size	is in range	186	372	1	1	3
PDI	is in range	0.254	0.425	1	1	3
EE	maximize	62.35	80.55	1	1	3

Solution

	Lipid	Surfactant	Speed	Particle Size	PDI	Percentage EE	Desirability	
	200.04	0.52	15330	275.83	0.332	80.60	1.00	Selected

Diclofenac Solid Lipid Nanoparticle: Evaluation and Characterization

Measurement of particle size and distribution

The diameter of each formulation was determined by the particle size analyser in accordance with the design, and the findings indicated that the range in figure-3

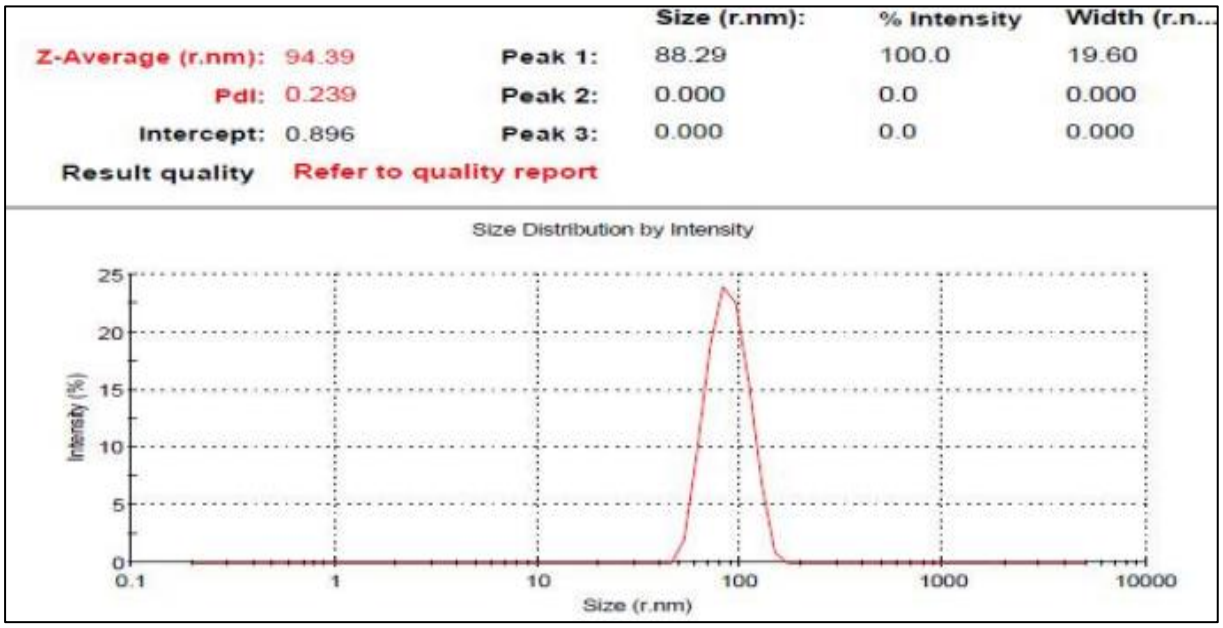

Fig: 3 Distribution of particle sizes in an improved batch of Diclofenac-SLNs

Zeta potential measurement of SLNs

The zeta potentials of nearly neutral nanoparticles range from 14.7 to 14.7 mV. The 3.76 mV zeta potentials in Figure 4 demonstrate the stability of the formulation. Lipid and Tween 80 surface coatings on NPs have the potential to reduce electrostatic repulsion and sterically stabilize them.

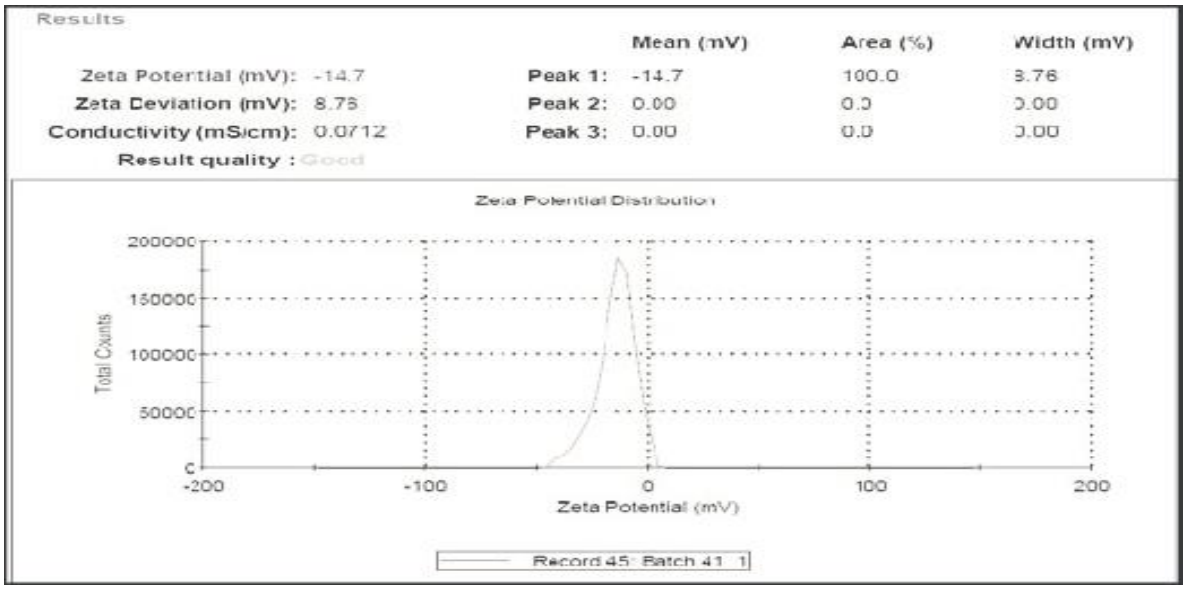

Fig: 4 Zeta potential of the Diclofenac-SLNs optimized batch

Entrapment efficiency:

With Diclofenac-SLNs, the entrapment efficiency was shown to be 0.896. In the SLNs, DCE demonstrated an excellent EE, according to the findings. Due to its low water solubility, DCE is more likely to partition out of the lipid once it has hardened into the crystalline matrix. Some drugs have poor solubility in both water and fat; therefore, cationic SLNs were created to enhance the EE of these drugs. Stearic acid's ability to increase DCE affinity for the lipid is what allows the SLNs to have such a high EE of the medicine.

Shape and morphology of particles

Particles of the right size and narrower size distributions, as determined by TEM examination, can be produced using this method. The diclofenac-SLNs varied in size from 100 nm and had a spherical form, as shown in Figure 5. To achieve this, we tweaked the ideal parameters for Diclofenac-SLN synthesis. The creation of perfectly spherical polymeric nanoparticles is aided by chemically polyelectrolytic compounds that are formed when varying concentrations of CS, tween, and

stearic acid are combined.

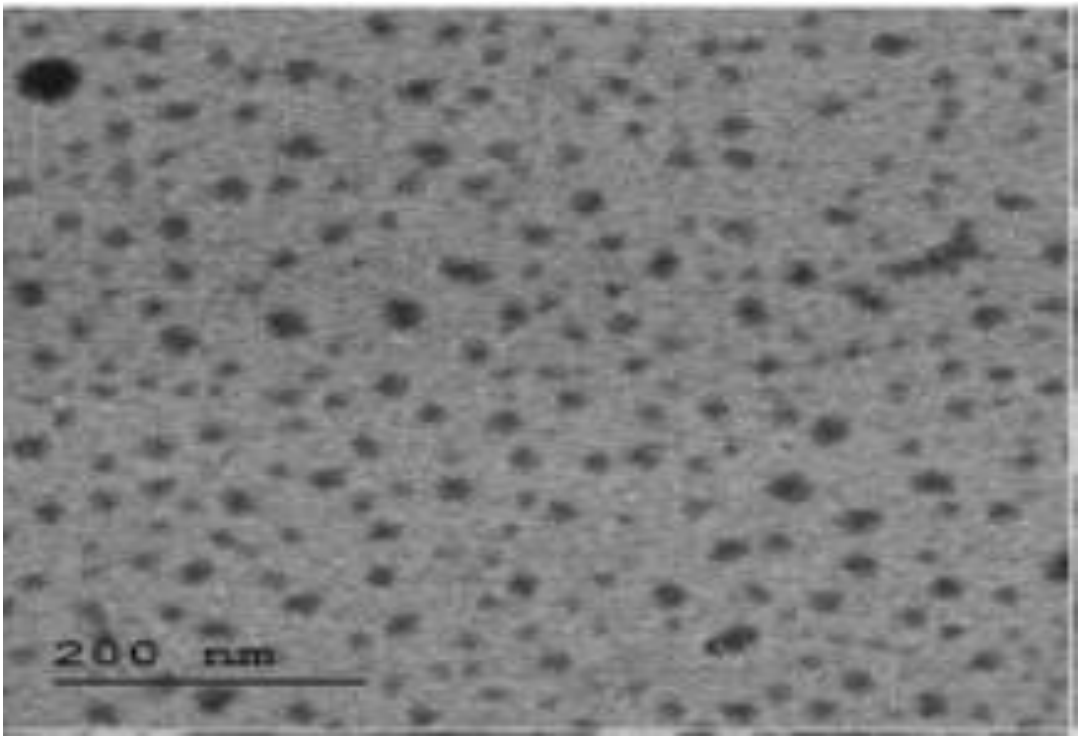

Fig: 5 TEM picture of the Diclofenac-SLNs optimized batch

Drug release studies from SLNs in vitro:

Table 11 and Figure 7 show that optimum Diclofenac-SLNs were released at a rate of 89.6±1.75 in 7.4 pH phosphate buffers. The findings show that although pure DCE had a greater drug release (91.1±2.1), the controlled release profile was not displayed since the drug was released entirely in less than 16 hours. On the other hand, the regulated release from nanoparticles lasted more than 24 hours, and the medication release was good.

Both an initial burst release and a prolonged release were included in the optimized formulation. A brief burst release of the drug from the Diclofenac-SLNs occurred in the first few hours, as shown in Fig. 6. This was followed by a prolonged release of the drug (86.6%) that lasted for up to 24 hours. One crucial feature of a delivery method that is frequently linked to better pharmacokinetics and efficacy is the controlled, extended release of a medicine.

Table 7: Investigations into the dissolution of optimized Diclofenac-SLNs in different fluids

Time Hr	Percentages Cumulative Drug Release in PBS pH 7.4)	
	Diclofenac -SLNs	Plain Diclofenac
0.5	19.5±0.8	17.6±0.78
1	27.65±1.22	31.5±1.3
2	37.8±1.5	45.25±1.6
4	46.3±0.95	59.6±1.1
8	59.4±1.3	70.1±0.8
12	68.1±2.1	80.8±1.8
16	74.2±1.5	91.1±2.1
20	82.1±2.25	
24	89.6±1.75	

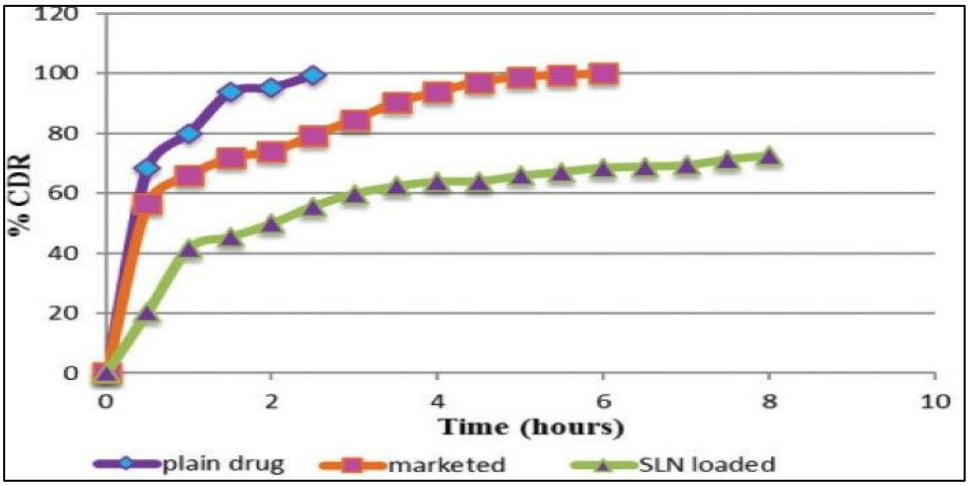

Fig: 6 Comparison of in vitro drug release experiments using plain DCE and commercially available optimized DCE-SLNs in PBS

Assessment of the kinetics of drug release

Based on the slope of the pertinent plots, the regression coefficient (R2) and release constant were calculated; Table 8 displays the outcomes.

Table 8: Kinetic models adapted to data for Diclofenac-SLN optimization

	0 Order (R2)	Ist Order (R2)	Higuchi (R2)	Korsmeyer- Peppas	
				(R2)	(n)
Diclofenac -SLNs	0.991	0.961	0.981	0.968	0.45

Storage Stability Study:

At low temperatures, the average particle size and entrapment efficacy of the nanoparticles were 265 nm and 79.25%, at 40°±2°C and 75%±5% relative humidity, they were 267 nm and 78.9%, and at ambient temperature, they were 263 nm and 78.53%. These measurements were taken after four months. These results demonstrated that Diclofenac -SLNs provided the highest level of stability. The stability was good at room temperature, 4°±1°C in the fridge, and 40°±2°C/75%±5% relative humidity over the four-month period.

CONCLUSION

First, FTIR and physiological testing identified and purified the drug and polymer. The design approach was followed to synthesize DCE and DCE-coupled SLNs using microemulsion technology. In-situ coating of SLNs with chitosan ligand improved oral bioavailability and maybe tailored drug distribution at the site of action. Lipid phase affinity for negatively charged Diclofenac was increased by cationic lipid stearic acid. The increased batch had a 300-nm diameter and 0.324 PDI, as proven by TEM and particle size analysis. DCE EE was 80–85%. Drug release experiments demonstrated that Diclofenac-SLNs followed the zero-order model with Fickian diffusion and prolonged drug release compared to pure drug solutions.

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REFERENCES:

1. Manzari MT, Shamay Y, Kiguchi H, Rosen N, Scaltriti M, Heller DA. Targeted drug delivery strategies for precision medicines. Nat Rev Mater, 6, 351–70 (2021) https://doi.org/10.1038/s41578-020-00269-6.
2. Mishra V, Bansal KK, Verma A, Yadav N, Thakur S, Sudhakar K, Rosenholm JM. Solid Lipid Nanoparticles: Emerging Colloidal Nano Drug Delivery Systems. Pharmaceutics, 10, 191 (2018) https://doi.org/10.3390/pharmaceutics10040191.
3. Asghari KM, Zahmatyar M, Seyedi F, Motamedi A, Zolfi M, Alamdary SJ, Fazlollahi A, Shamekh A, Mousavi SE, Nejadghaderi SA, Mohammadinasab R, Ghazi-Sha'rbaf J, Karamzad N, Sullman MJM, Kolahi A-A, Safiri S. Gout: global epidemiology, risk factors, comorbidities and complications: a narrative review. BMC Musculoskelet Disord, 25, 1047 (2024) https://doi.org/10.1186/s12891-024-08180-9.
4. Dubey A, Dash SL, Mukherjee C, Mishra T, Kumari M, Shahi S. In Vitro Anti-arthritis Activity of Asparagus Racemosus Linn Seed Extract Against Albumin Denaturation. JNR, 1677–81 (2025) https://doi.org/10.18311/jnr/2025/49172.
5. Dubey A, Tiwari M, Singh Y, Kumar N, Srivastava K.Investigation of Anti-pyretic Activity of Vinpocetine in Wistar Rat. IJPR, 12, (2020) https://doi.org/10.31838/ijpr/2020.12.02.254.
6. Khatra DS, Harikumar SL, Nirmala N. Nanoparticles: An Overview. J. Drug Delivery Ther., 3, (2013) https://doi.org/10.22270/jddt.v3i2.407.
7. Wan C, Muya L, Kansara V, Ciulla TA. Suprachoroidal Delivery of Small Molecules, Nanoparticles, Gene and Cell Therapies for Ocular Diseases. Pharmaceutics, 13, 288 (2021) https://doi.org/10.3390/pharmaceutics13020288.
8. Friedman B, Cronstein B. Methotrexate mechanism in treatment of rheumatoid arthritis. Joint Bone Spine, 86, 301–7 (2019) https://doi.org/10.1016/j.jbspin.2018.07.004.

9. Freitas C, Lucks JS, Müller RH. P238 effect of storage conditions on long-term stability of "solid lipid nanoparticles" (SLN) in aqueous dispersion. European Journal of Pharmaceutical Sciences, 2, 178 (1994) https://doi.org/10.1016/0928-0987(94)90411-1.
10. Mukherjee S, Ray S, Thakur R. Solid lipid nanoparticles: A modern formulation approach in drug delivery system. Indian J Pharm Sci, 71, 349 (2009) https://doi.org/10.4103/0250-474X.57282.