Research Article

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Design, Development and Evaluation of Etodolac Loaded Chitosan based Nanogel for Topical Drug Delivery

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ABSTRACT

Nanogels, also known as nanosized hydrogels, are small, swelling particles that range in size from 100 to 400 nm. They are composed of hydrophilic or amphiphilic polymer networks that are malleable and can be cross-linked mechanically or chemically.Drugs that are poorly soluble in water can be made more soluble by employing a variety of technologies.Using the spontaneous emulsification method, oleic acid was used as the oil phase while Tween 80 and PEG 400 served as the surfactant and co-surfactant, respectively, to form etodolac nanoemulsion (ETD NE). It was discovered that the ideal zeta potential, polydispersity index (PDI), and ETD NE particle size were, respectively, -36.2 mV, 0.363, and 220.0 nm. Particle size, PDI, and zeta potential of the ETD loaded chitosan-based nanogel were found to be 230.4 nm, 0.376, and -37.1 mV, respectively. There were no interactions between the drug and excipients, according to the Fourier Transform Infrared Spectroscopy (FTIR) spectra. The transmission electron microscope pictures of the NE and the ETD nanogel show that the oil droplets have a spherical shape. For eight hours, it was discovered that the *in-vitro* drug release of NE and nanogel was 80.47% and 74.61%, respectively.

Keywords: Etodolac, Nanoemulsion, Nanogel, Design of experiments, Topical drug delivery

1. Introduction

Applying a pharmaceutical dosage form topically to treat a cutaneous condition or a cutaneous symptom of a general disease is known as topical drug administration. The goal is to limit the drug's pharmacological or other activity to the skin's surface [1]. Topical drug administration illustrates the most basic and straightforward form of localized drug delivery, which occurs throughout the body via the skin, rectum, vagina, eyes, ears, and nose. Because the skin is the most easily accessible organ on the human body for topical administration, it is the primary route of the topical drug delivery system. Most topically applied pharmaceutical preparations are likely to have some localized effects and are designed to offer sustained local contact with little systemic effects [2]. The main advantage of topical drug delivery techniques is that they do not necessitate intravenous therapy, which carries risks and difficulties in addition to various absorption conditions such pH changes, the presence of enzymes, and gastric emptying time [3]. The novel drug delivery technique aids in reducing first pass metabolism, increasing drug transdermal permeability, and improving drug molecule bioavailability.An innovative drug delivery method can breathe new life into an already-existing medicinal molecule [4]. The largest organ in the body, the skin makes up about 15% of the adult body weight [5]. The most visible and vulnerable organ that interacts with its environment is the skin [5]. The outermost few microns of the skin known as the stratum corneum, aid in maintaining the skin's barrier properties. Composed of a lamination of compressed keratin-filled corneocytes linked in a lipophilic matrix, this is the skin's most impermeable layer [6].

Research is being done on submicron-sized emulsions, also known as nanoemulsions, as drug carriers to improve the delivery of therapeutic medicines. These are isotropic systems that are thermodynamically stable, in which two immiscible liquids are mixed into a single phase using an appropriate cosurfactant and surfactant. Nanoemulsion droplet sizes typically have a narrow size distribution and fall between 100 and 400 nm [7]. These carriers have a negatively charged, amorphous, lipophilic surface. They are solid spheres. The location specificity of magnetic nanoparticles can be enhanced. They reduce adverse reactions and side effects while increasing the medication's therapeutic efficacy [8]. Miniemulsions, submicron emulsions, and ultrafine emulsions are other names for nanoemulsions. The classification of nanoemulsions is based on the composition of the water and oil components. a) Oil-in-water (O/W) nanoemulsions: these consist of dispersed oil droplets in a continuous aqueous phase. Water in oil (W/O) nanoemulsions, as described in [9-12], include the dispersion of water droplets inside a continuous oil phase. There are many prospects for intelligent drug delivery and drug manufacture, or nanomedicine, in the relatively new subject of nanotechnology. This covers the creation, synthesis, and characterization of substances, compounds, and apparatuses with practical nanoscale uses. This approach focuses mostly on significant advancements in modern therapy and diagnostic techniques. The development of innovative nanoparticle drug delivery systems (DDS) has a major impact on illness prevention, diagnosis, and treatment, according to research from academic labs and pharmaceutical businesses worldwide [13,14].

In addition to increasing the solubility and stability of poorly soluble medications, nanogels have also improved the drugs' potential for cellular absorption over free drug, providing a novel means of drug administration. They are considered as promising carriers for protein, peptide, and other biological compound delivery and cellular uptake because of their remarkable stability, inertness in internal fluids and systemic circulation, reasonable affinity for aqueous solutions, and suitability for molecular incorporation in bulk [15]. In dermatology and cosmetics, nanogels have been used to treat contact dermatitis brought on by allergies and psoriatic plaque, as well as to deliver nonsteroidal anti-inflammatory medications (NSAIDs) topically [4]. In order to improve etodolac's solubility, permeability, and bioavailability, a nanogel for topical administration is currently being designed, produced, and evaluated.

2. Materials and Methods

2.1. Materials

We bought etodolac from Aarti Chemicals in Mumbai. We bought Tween 80, Tween 20, Olive Oil, Soybean Oil, Isopropyl Myristate, and Castor Oil from Research-Lab Fine Chem Industries. Mumbai. From Loba Chemicals in Mumbai, we acquired ethanol, chitosan, glycerin, oleic acid, polyethylene glycol

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400, and propylene glycol. We purchased Span 20 and Span 80 from MolychemPvt. Ltd. in Mumbai. Every chemical and solvent utilized in this investigation was of the grade of an analytical reagent.

2.2. Solubility studies and Pseudoternary phase diagram

The solubility of etodolac in various oils (Olive oil, Castor oil, Isopropyl myristate, Oleic acid, Soybean oil), various surfactants (Span 80, Tween 80, Tween 20, Span 20) and various cosurfactants (Glycerin, PEG 400, Propylene Glycol, Ethanol) was evaluated by dissolving an excess quantity of the drug in 5 mL of selected oils, surfactants and cosurfactants in 15 mL capacity stoppered vials and thoroughly mixing them. To achieve equilibrium, the vials containing the mixtures were held at 37±1.0°C in a rotating shaker for 72 hours. After centrifuging the sample for 15 minutes at 3000 rpm in a rotary shaker. The supernatant was filtered, and the etodolac content was measured using a UV spectrophotometer at 270 nm [16-18].

The water titration method was utilized to create pseudo-ternary diagrams that depicted the boundaries and characteristics of the NE region. Different volume ratios (Km) of the surfactant and cosurfactant (Smix) were combined, including 1:1, 1:2, 1:3, 2:1, and 3:1. For every phase diagram, i.e., 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, the oil to Smix ratio was changed. Water was added drop-by-drop, with magnetic stirring, at room temperature to each oil:Smix mixture. The appearance of each addition was monitored. The titration's end was reached when the solution became opaque or turbid. It was noted how much water was needed to get the mixture opaque or turbid. The CHEMIX School 4.00 program was used to create pseudoternary diagrams. Plotting the component percentages on the pseudoternary phase diagram was done. A line was drawn connecting the spots to demarcate the area that is clear and turbid [16, 19].

2.3. Preparation and optimization of ETD NE

ETD NE formulation was prepared using a low-energy emulsification (spontaneous) method with varied oil and Smix ratios determined by a pseudoternary phase diagram. To make sure the drug was completely dissolved; two grams of ETD were added to the oil and mixed. Then, combinations of surfactants and co-surfactants in certain ratios were added while stirring continuously. Drop by drop, aqueous phase was added to the formulation mixture with continuous vortex mixing for 15 minutes at room temperature to generate a clear, transparent, and uniform NE formulation [20].

The nanoemulsion component proportion was improved using Design expert software Version 12.0. Thermodynamic stability data from actual batches of ETD were used to enhance the generated o/w nanoemulsion formulation. Based on early findings, a 3^2-complete factorial design was adopted in this investigation, in which two components were studied independently at three levels and up to nine combinations were created [21-23]. The statistical design produced numerous theoretical runs with three levels with low (-1), intermediate (0), and high (+1) values in order to optimize the nanoemulsion formulation. The factorial design used particle size (Y_1) and zeta potential (Y_2) as dependent variables, with the quantity of oil concentration (X_1) and Smix concentration (X_2) as independent variables (Tables 1 and 2).

2.4. Thermodynamic stability testing of ETD NE formulation

Thermodynamic stability testing of ETD nanoformulation was carried out to detect the presence of metastable formulation batches from pseudoternary phase diagrams and to identify stable formulation that will remain stable for a longer period of time during thermodynamic changes.

Heating-cooling cycles were applied to batches of ETD nanoemulsion formulation in order to investigate the impact of temperature variations on the stability of the formulation. The formulations that were stable at these temperatures passed to centrifuge testing [24].

The formulations that passed were centrifuged for 30 minutes at 3500 rpm. Diluted to 1:10, 1:50, and 1:100.Freeze-thaw stress testing was carried out on formulations that showed no signs of creaming, cracking, or phase separation.

The formulations were stored at each temperature for a minimum of 48 hours over three freeze-thaw cycles in arrange from -21°C and +25°C. For further research, a stable and homogeneous ETD formulation was chosen.

Table 1. Independent variables

Coded values Level	Independent variables	
	X_1, Oil (%v/v)	X_2, S_{mix}(%v/v)
-1	10	35
0	15	45
+1	20	55

Table 2. Formulation of ETD Nanoemulsion

Batches	ETD (gm)	Oil (Oleic acid) (%v/v)	S_{mix} (Tween 80: PEG 400) (2:1)(%v/v)
F1	2	15	45
F2	2	10	45
F3	2	10	55
F4	2	20	45
F5	2	15	55
F6	2	15	35
F7	2	10	35
F8	2	20	55
F9	2	20	35

2.5. Determination of NE type

Water-soluble dye distribution indicates the kind of NE if it distributes in the exterior phase, indicating that the NE is O/W and vice versa. Methylene blue drops were added to the NE formulations (in a tiny glass dish) for the dye test [25].

Fill a beaker halfway with pure water and a small amount of NE. After that, blend and examine the concoction. If it separates into two layers, the emulsion is W/O; if it does not separate into two layers, the emulsion is O/W.

2.6. Preparation of ETD loaded chitosan based Nanogel

The optimized ETD NE formulation was then modified to develop an ETD loaded chitosan-based nanogel. Chitosan (1% w/w) was used, which was first dissolved in 0.5% v/v acetic acid solution. To get a completely uniform mixture10 mL of the ideal formulation was gradually added to the viscous chitosan dispersion (1% w/w) while swirling constantly with a magnetic stirrer. Triethanolamine was used to adjust the pH. The completed nanogel formulation was stored in a firmly sealed container [19].

2.7. Evaluation of optimized formulation of ETD NE and chitosan based nanogel formulation

2.7.1. Particle size, polydispersity index (PDI) and zeta potential

The particle size analyzer was used to examine the mean particle size and degree of particle size distribution of the improved ETD NE and ETD loaded chitosan based nanogel formulation after appropriate dilution with distilled water. All measurements were performed at a temperature of 25±1^{0}C and a scattering angle of 90^0 [26-34].

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A zeta sizer is used to determine the zeta potential of nanoparticles. A frequency spectrum is generated using zeta sizer software, and the electrophoretic mobility and zeta potential are computed from this spectrum [35-36].

2.7.2. Transmission Electron Microscope (TEM)

TEM was used to examine the surface shape of distributed oil globules in an optimized ETD formulation. Both the ETD NE and the ETD loaded chitosan-based nanogel were diluted with distilled water and a drop of each was placed on a 300 mesh carbon-coated grid to dry. The grid was then put under a light microscope, and pictures were taken using a TEM at 100 kv [37-38].

2.7.3. Fourier Transform Infrared Spectroscopy (FTIR)

A test was performed to identify and validate the functional groups of drugs used in the preparation of chitosan-based nanogels. The sample was placed on a sample holder, and the spectrum was scanned from 4000$cm^{-1}$to 400cm^{-1} [39].

2.7.4. Measurement of pH, viscosity and spreadability

The pH of optimized NE and ETD loaded chitosan-based nanogels was determined using a calibrated digital pH meter at 25 ± 2˚C. Two standard buffers (pH 4.00 and 9.00) were used to calibrate the glass electrode.

The viscosity of the NE formulation and the ETD loaded chitosan-based nanogel formulation was determined using a Brookfield Viscometer. The apparent viscosity was determined by rotating the RV spindle 2 at 5 rpm at 30^0C [40].

Spreadability of ETD NE and ETD loaded chitosan based nanogel was determined by using spreadability apparatus by using "slip and drag" method. In this case, spreadability is determined by the formula:

S = mass x length/time …. Eq. (1)

2.7.5. Extrudability

The extrudability of a closed, collapsible aluminum tube containing nanogel was tested by applying force to its crimped end. The nanogel extruded until the pressure was released after the cap was removed. It was determined how much weight was required to extrude a 0.5 cm ribbon of nanogel in 10 seconds. The average extrusion pressure was recorded in grams. This test was useful only for nanogel formulation [41,42].

Extrudability = Applied Weight to extrude gel from tube (gm)/Area (cm^2)…. Eq. (2)

2.7.6. Percentage transmittance

The percentage transmittance of 2 millilitres NE and chitosan based nanogel of ETD was checked against distilled water using UV-Vis spectrophotometer at 270 nm.

2.7.7. In-vitro drug release

The dialysis bag used in the in vitro release experiment was MW 12,000–14,000 Da. To sum up, the dialysis bag spent an entire night submerged in a buffer solution. The dialysis bag was filled with the ETD NE and ETD loaded chitosan-based nanogel (2 mL), which was fastened at both ends of the tube. The device was continuously stirred at 100 revolutions per minute and maintained at 37 ± 0.5°C. The study took eight hours to complete. The 1 mL sample was obtained at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours, and it was replaced with the same freshly prepared buffer solution. The spectrophotometric characteristics of chitosan-based nanogels loaded with ETD and ETD NE were investigated. By contrasting different release kinetic models for ETD NE and ETD loaded chitosan-based nanogel, the drug's release mechanism was investigated. The optimal mechanism for drug release kinetics was determined to be a high correlation coefficient (R^2) value [43-45].

2.8. Short term stability studies

For two months, ETD NE and ETD loaded chitosan-based nanogel was maintained at ambient temperature and in the refrigerator (2-8°C). The formulation was visually inspected for any signs of instability, as well as particle size, zeta potential, and PDI [46].

3. Results and Discussion

3.1. Solubility studies

3.1.1. Solubility studies in various oils

A study was conducted to assess oils for the development of an ETD nanoemulsion. When compared to other oils, ETD has the highest solubility in Oleic

acid (46.5±2 mg.mL^{-1}) as shown in Figure 1. Oleic acid demonstrated excellent emulsification and stability. As a result, oleic acid was chosen as the oil phase for the formation of a NE.

3.1.2. Solubility studies in various surfactants

The highest solubility of ETD in surfactant was used to select the surfactant for the development of nanoemulsion. Tween 80 (59.18±2.26 mg.mL^{-1}) surfactant has the highest solubility of ETD (Figure 2). As a result, Tween 80 was chosen as a surfactant.

3.1.3. Solubility studies in various co-surfactants

The maximal solubility of ETD in co-surfactant was used to select a co-surfactant for the construction of a nanoemulsion. As a co-surfactant, ETD has the highest solubility in PEG 400 (42.56±2 mg.mL^{-1}) (Figure 3). As a result, PEG 400 was chosen as a co-surfactant.

3.2. Construction of pseudo-ternary phase diagrams

The NE area was determined using pseudoternary phase diagrams with various surfactants and co-surfactants ratios (1:1, 1:2, 1:3, 2:1, and 3:1). The ternary phase diagrams were created with the CHEMIX SCHOOL 4.0 program. Figure 4 displays the shift in the NE region as the concentrations of Smix and oil vary. Increasing the ratio of poly ethylene glycol 400 in the formulation to Tween 80 (e.g., Smix ratio from 1:2 to 1:3) led to a decrease in the NE, as Fig. 4 demonstrates clearly.This may be attributed to the reality that oleic acid solubility reduces when Tween 80 level falls and PEG 400 content increases. With a Smix ratio of 2:1, the NE area rose as the amount of Tween 80 in the solution increased as compared to PEG 400.This demonstrates how adding Tween 80, a surfactant, improves emulsification and expands the pseudoternary phase diagram's size. The NE area was seen to be smaller in the Smix ratio of 3:1 as opposed to the Smix ratio of 2:1, which could be attributed to the insufficient presence of PEG 400, the cosurfactant that further liquefies the Tween 80 interfacial layer. Consequently, a Smix ratio of 2:1 was selected for further formulation development research and optimization.

3.3. Preparation and optimization of the ETD NEby central composite design

Low-energy emulsification, or spontaneous emulsification, was used to create ETD NE formulations. In order to determine the impact of independent variables like the percentage of oil and Smix on dependent variables like particle size and zeta potential, nine experimental batches were examined in the current statistical design (Table 3).

3.3.1. Impact of independent factors on particle size

If the medicine is hydrophobic, particle size is a crucial factor to consider when analyzing the nanoemulsion. It was discovered that the Model F-value of 907.56 indicates that the model is significant. P-values of less than 0.0500 suggest that model terms are significant (Table 2). As a result, all model term

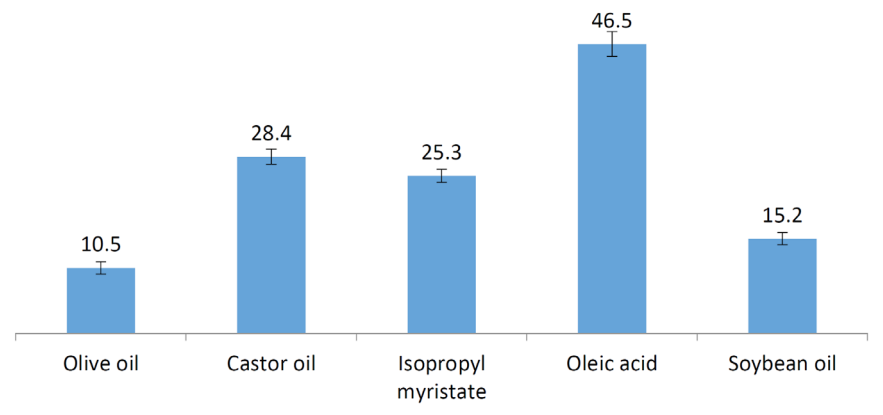

Figure 1. Solubility (mg/mL) of ETD in various oils (n=3, ±SD)

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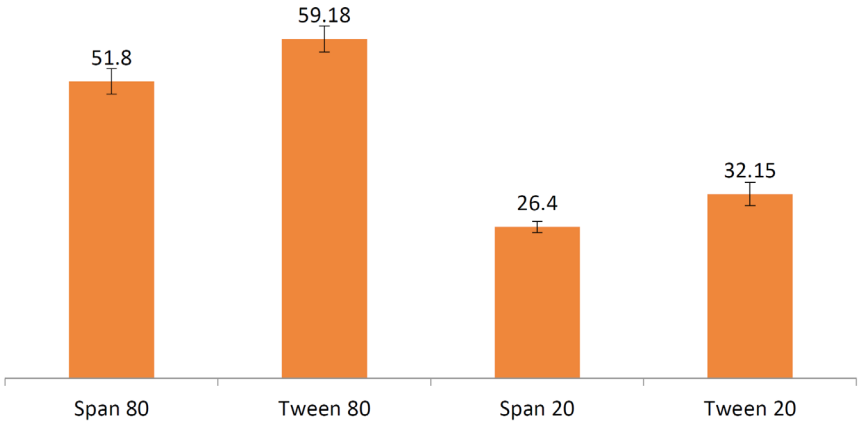

Figure 2. Solubility (mg/mL) of ETD in various surfactants (n=3, ±SD)

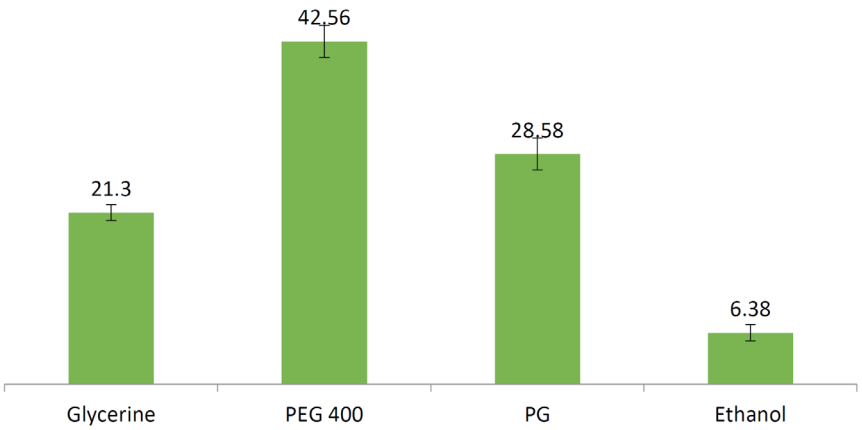

Figure 3. Solubility (mg/mL) of ETD in various co-surfactants (n=3, ±SD)

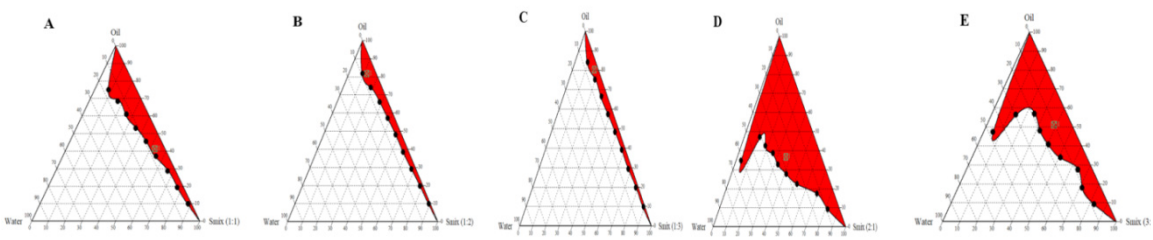

Figure 4. Pseudoternary phase diagram of ETD NE containing Oleic acid, Tween 80 and PEG 400 in ratio of (A) 1:1 (B) 1:2 (C) 1:3 (D) 2:1 (E) 3:1

values were determined to be within the acceptability limit, indicating that all formulation components (oil and Smix) were significant for response Y1 (particle size), as indicated in equation 3.

$$Y_1 = 172.16 + 45.78X_1 + 3.88X_2 - 0.3500X_1X_2 - 9.88X_1^2 + 9.42X_2^2 \ldots. \text{Eq. (3)}$$

According to equation (3), the size of the particles increases as the concentration of oil does, but the size of the particles decreases when the concentration of

Smix increases. Figure 5 illustrates the impact of oil and Smix, provides more explanation.

3.3.2. Impact of independent factors on zeta potential

The stability of dispersed oil globules in nanoemulsions is influenced by their surface charge. The surface charge of dispersed particles in NEs is controlled by the zeta potential. The model is significant, as indicated by the Model F-value of 526.39 (Table 5). P-values below 0.0500 imply the significance of the model terms. Equation 4 shows that all formulation parameters significantly impacted the response Y2 (zeta potential).

Y1 = -27.56-5.60X1-1.90X2+0.0750X1X2-0.2667X12-1.07X22 …. Eq. (4)

Concentration of oil and S_{mix} decreases zeta potential decreases. Effect of these variables can be further explained by response surface plots as represented in Figure 6.

3.4. Thermodynamic testing of ETD NE

On the basis of the pseudoternary phase diagram and optimization formulation, thermodynamic stability testing was performed on batches of 2:1 Smix ratio. Formulation batches that stay stable and show

Table 3. Variables and observed responses in Central Composite design

Batches	Factor 1	Factor 2	Response 1	Response 2
	X_1:Oil (%v/v)	X_2:Smix (%v/v)	Y_1: Particle Size (nm)	Y_1: Zeta Potential (mV)
F1	0	0	171.0	-27.7
F2	-1	0	115.7	-22.1
F3	-1	1	130.5	-25.2
F4	1	0	210.0	-33.4
F5	0	1	186.1	-30.7
F6	0	-1	178.2	-26.4
F7	-1	-1	122.1	-21.5
F8	1	1	220.0	-36.2
F9	1	-1	213.0	-32.8

Table 4. ANOVA for Quadratic model for particle size

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	13040.36	5	2608.07	907.56	< 0.0001	significant
A-Oil	12576.68	1	12576.68	4376.47	< 0.0001	
B-Smix	90.48	1	90.48	31.49	0.0112	
AB	0.4900	1	0.4900	0.1705	0.7074	
A^2	195.36	1	195.36	67.98	0.0037	
B^2	177.35	1	177.35	61.71	0.0043	
Residual	8.62	3	2.87			
Cor Total	13048.98	8				

Response 1: Particle size, Y_1

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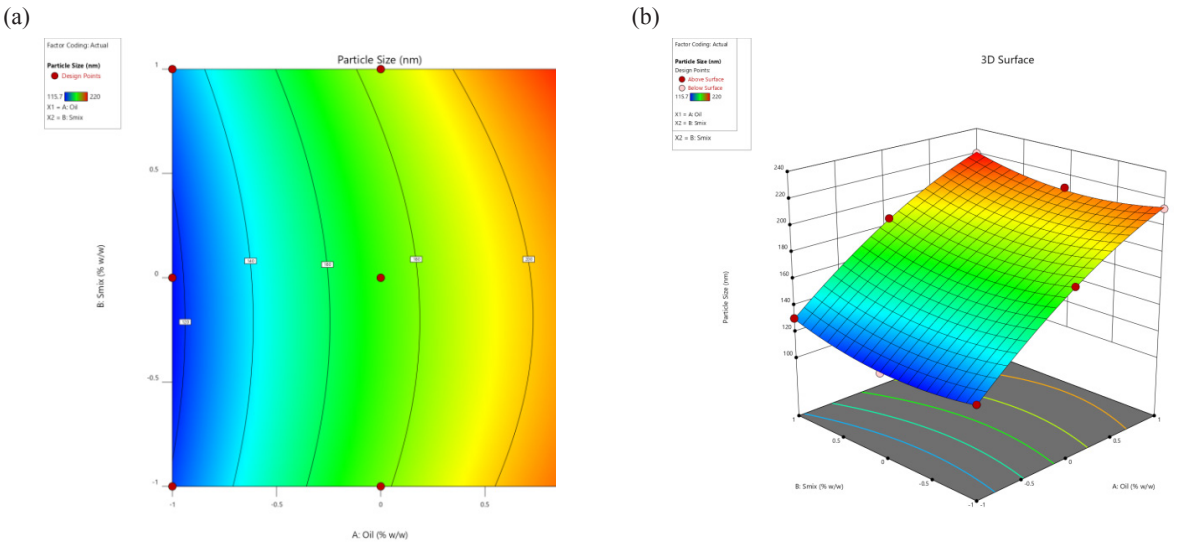

Figure 5. 2D Contour plot (a) and 3D response surface plot (b) for evaluating influence of Oil (X1) and Smix (X2) on Particle size (Y1)

Table 5. ANOVA for Quadratic model for zeta potential

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	212.26	5	42.45	526.39	0.0001	significant
A-Oil	188.16	1	188.16	2333.10	< 0.0001	
B-Smix	21.66	1	21.66	268.57	0.0005	
AB	0.0225	1	0.0225	0.2790	0.6339	
A^2	0.1422	1	0.1422	1.76	0.2762	
B^2	2.28	1	2.28	28.22	0.0130	
Residual	0.2419	3	0.0806			
Cor Total	212.50	8				

Response 2: Zeta Potential, Y_2

no signs of instability were chosen for further studies. Based on thermodynamic stability testing, oleic acid in the range of 10-20% and Smix (Tween 80 and PEG 400) in the range of 35-55% were employed.

3.5. Determination of NE type

Dye test was performed for all the NE formulation and which were shows the o/w type of NE they were chosen for further study.

The diluted nanoemulsions which were shows the clear and o/w type of NE, they were chosen for further study.

3.6. Preparation of ETD loaded chitosan based nanogel

Chitosan is used in nanogel formulations as a gelling agent to retain the formulation on the skin for a longer period of time. As a result, an attempt has been made to convert optimal formulation to ETD loaded chitosan based nanogel to improve the sticky behavior of formulation by adding chitosan. In the final formulation triethanolamine is added for pH adjustment. The addition of chitosan at 1% w/w results in good particle size, PDI, and zeta potential. As a result, it was chosen for future research.

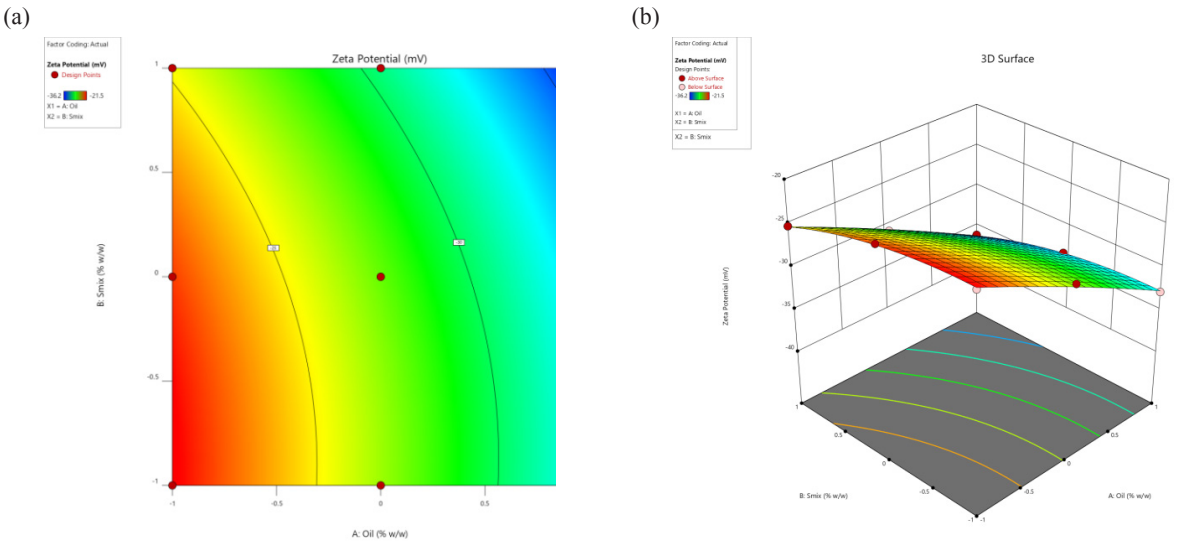

Figure 6. 2D Contour plot (a) and 3D response surface plot (b) for evaluating influence of Oil (X_1) and S_{mix} (X_2) on Zeta potential (Y2)

3.7. Evaluation of ETD NE and ETD loaded chitosan based nanogel

3.7.1. Particle Size, Polydispersity index (PDI) and Zeta potential

Figure 7a depicts the optimized formulation (F8) ETD NE with a particle size of 220.0 nm and a PDI of 0.363. The tiny particle size and PDI of the dispersed oil phase imply faster release through the formulation due to greater surface area, which increases absorption rate. The particle size and PDI of ETD-loaded chitosan-based nanogel were measured to be 230.4 nm and 0.376, respectively (Figure 7b). The additional S-shaped line is the cumulative undersize distribution, used to understand how particle sizes accumulate across the sample.

The optimized formulation (F8) ETD NE zeta potential was found to be -36.2 mV (Figure 8a). The presence of a negative zeta potential in a droplet of nanoemulsion shows that the system was stable. Because there was no charge on the particles, there was no flocculation, and so the ETD NE was stable. The zeta potential of an ETD loaded chitosan based nanogel was determined to be -37.1 mV (Figure 8b). Because of the carboxyl ions in chitosan, the zeta potential of ETD loaded chitosan based nanogel was altered to the negative.

3.7.2. Transmission electron microscope (TEM)

The morphology of ETD NE and ETD loaded chitosan based nanogels was determined using TEM of an optimal formulation. Figure 9 depicts a photomicrograph of ETD NE (a) and ETDloaded chitosan based nanogel (b). It is obvious that the oil globules are spherical in shape and scattered in the aqueous phase.

3.7.3. FTIR spectroscopy

Figure 10 displays the FTIR spectra of native ETD (a) ETD-loaded nanogel (b). The FTIR spectrum of ETD exhibited typical IR bands at, 3392 (NH stretch), 1656 (C=O), 1465 (C=C), 1021 (C-N) and 744 (C-H) (Figure 10a). ETD-loaded nanogel FTIR spectrum showed characteristic bands as follows (Figure 10b): 3397 (NH stretch), 2921 (C=O), 1644 (C=C), 1249 (C-N) and 837 (C-H). The characteristic peaks of native ETD are reflected in ETD-loaded nanogel.

3.7.4. Measurement of pH, viscosity and Spreadability

The pH of ETD NE and ETD loaded chitosan based nanogel was 6.2 and 7.1, respectively. The pH values of ETD NE and ETD loaded chitosan based nanogel were determined to be within the skin pH range, allowing for the least amount of skin irritation.

The addition of chitosan altered the flowability of ETD NE. The viscosity of ETD NE and ETD load-

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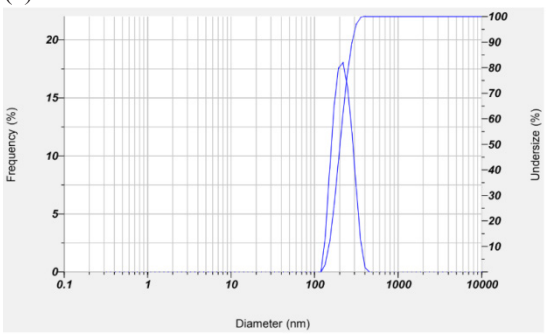
(a)



(b)

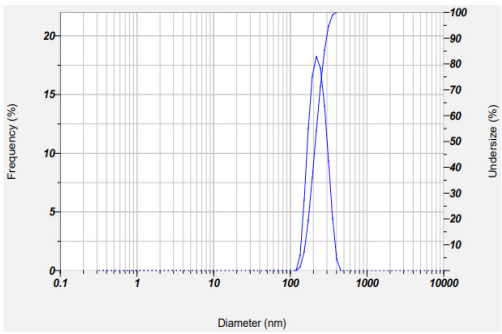
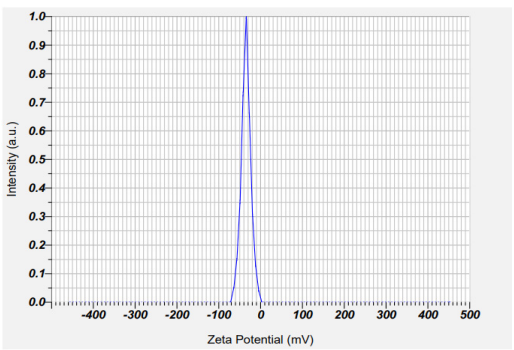


Figure 7. Particle size of optimized ETD NE (a) and optimized ETD loadedchitosan based nanogel (b)

(a)



(b)

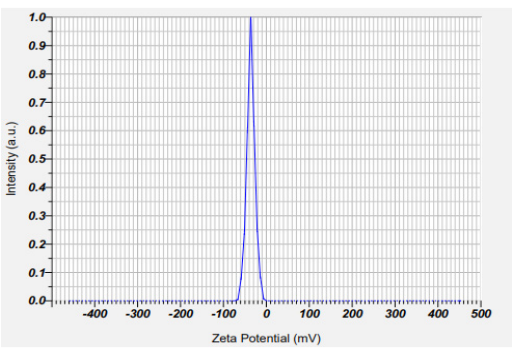
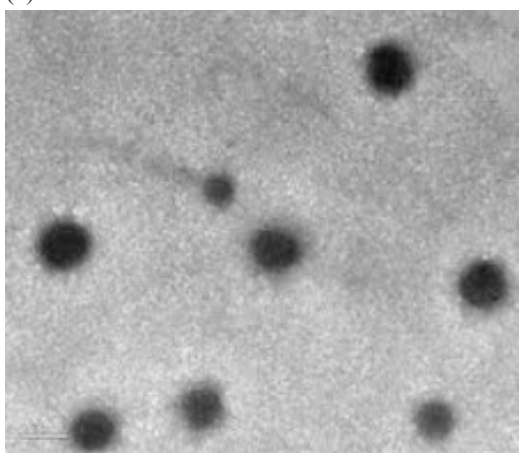


Figure 8. Zeta potential of optimized ETD NE (a) and optimized ETD loaded chitosan based nanogel (b)

(a)



(b)

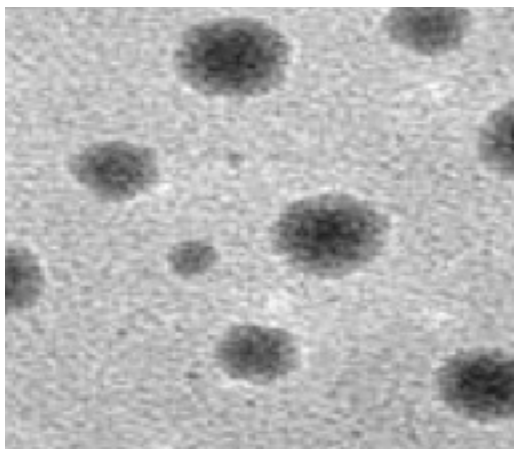


Figure 9. TEM images of optimized ETD NE (A) and (B) ETD loaded chitosan based nanogel

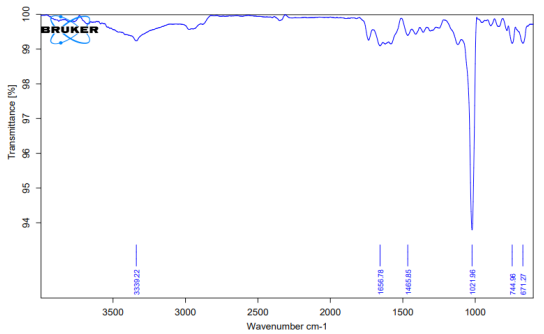
ed chitosan nanogel was found to be 1265.3 cP and 4347.2 cP respectively, suggesting that the viscosity of the formulation increases with increasing water content, making the preparation more appropriate for topical administration.

The spreadability of ETD NE and ETD loaded chitosan based nanogels was measured utilizing a spreadability instrument and found to be 22.22 Gm.cm/sec and 40.00 Gm.cm/sec, respectively.

3.7.5. Extrudability

Extrudability study was not applicable for ETD nanoemulsion due to very less consistency of NE formulations. The average extrudability ETD loaded chitosan based nanogel was found to be 15 g/cm^2, which shows within the acceptable range.

(a)



(b)

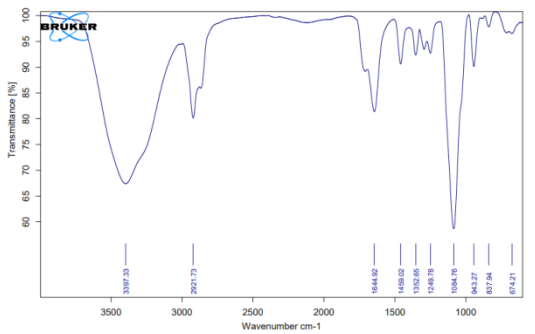


Figure 10. FTIR spectra of ETD (a) and ETD loaded chitosan based nanogel (b)

3.7.6. Percentage transmittance

A percentage transmission of 98.62 for ETD NE indicated clear dispersion, however no transmittance was observed for ETD loaded chitosan based nanogel due to the presence of corresponding adhesive or gelling component in the formulations.

3.7.7. In-vitro drug release

The release profile of ETD from NE and chitosan based nanogel through the dialysis bag at various time intervals is shown in Figure 11. The figure depicts a graph of % cumulative medication release versus time. The release of ETD from NE was determined to be 78.93±3.30% after 8 hours, and 73.75±2.89% from the chitosan-based nanogel. A slight decrease in ETD release was observed from ETD loaded chitosan based nanogel formulation compared to ETD NE, which could be explained by the presence of chitosan network over oil globules in ETD loaded chitosan based nanogel, which acts as a barrier for drug and retards drug diffusion from formulation.

ETD loaded chitosan based nanogel release data was fitted to several kinetic models (Figure 12). ETD release was found to be zero order for ETD loaded chitosan based nanogel, with R^2 values of 0.9962 as shown in Figure 12(A). This suggests that the release of ETD from established formulations is not affected by drug concentration in the formulation. TheR^2 value for other drug release models such asfirst order release, Higuchi, Korsmeyer-Peppas and Hixon-Crowell model were found to be0.9662, 0.9201, 0.9861 and 0.9851 respectively.

3.8. Short term stability studies

On visual inspection, optimized ETD NE and ETD loaded chitosan-based nanogels showed no drug precipitation, creaming, phase separation, or flocculation. For two months, an optimized formulation of ETD NE and ETD loaded chitosan-based nanogel was assessed for short-term stability experiments. The particle size, PDI, and zeta potential of ETD formulations were evaluated. Table 6 shows the results of the short-term stability tests, which indicate that both formulations are stable.

4. Conclusion

ETD loaded chitosan based nanogel was successfully formulated by spontaneous emulsification method. Nanogel formulation shows that they can be implemented as tool to enhance bioavailability of poorly water drugs.The current work demonstrates that the therapeutic efficacy of drugs like etodolac that are not very water soluble can be enhanced by nanogel formulation. The etodolac's slow release demonstrated that the drug stays localized for an extended length of time, allowing for skin-specific drug targeting and the viability of employing a dermatological formulation to reduce pain.

Conflict of Interest

The authors have no conflicts of interests.

Statement of Contribution of Researchers

Concept, Design – J.A.M.; Experimental work, Writing – S.S.K.; Review and editing of the manuscript – J.A.M.

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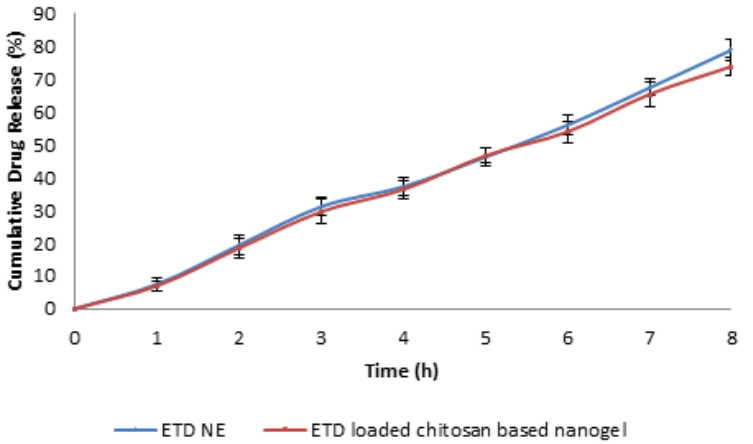

Figure 11. *In-vitro* release of ETD from ETD NE and ETD loaded chitosan based nanogel (n=3, ±SD)

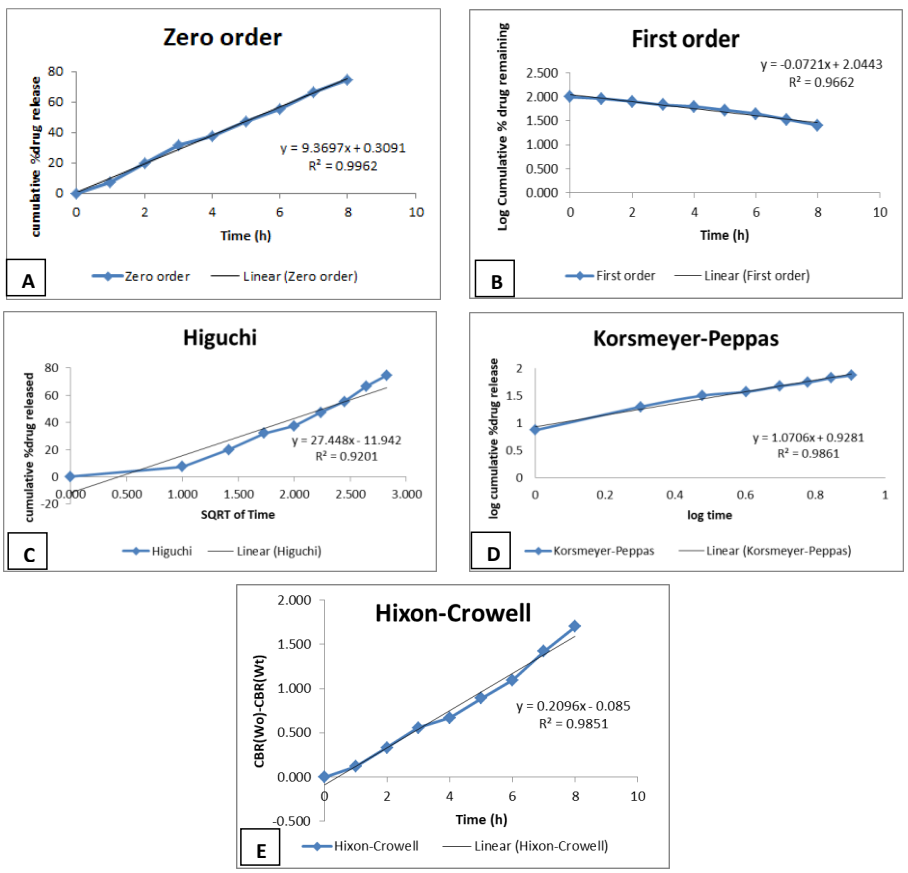

Figure 12. Release kinetics models for ETD loaded chitosan based nanogel: Zero order (A), First order (B), Higuchi (C), Korsmeyer-Peppas (D), Hixon-Crowell (E).

Table 6. Short term stability studies of optimized ETD NE and ETD loaded chitosan based nanogel

Parameters	ETD NE			ETD loaded chitosan based nanogel		
	Initial	1 Month	2 Month	Initial	1 Month	2 Month
Particle size (nm) 220		222.4	226.8	230.4	245.7	255.8
Polydispersity index	0.363	0.367	0.370	0.376	0.381	0.392
Zeta potential (mV)	-36.2	-36.6	-36.8	-37.1	-37.5	-37.8

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