Quality by Design-Based Optimization of Silymarin–Pioglitazone Nanoemulsion for Enhanced Topical Delivery

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Abstract:

The primary objective of the present study was to develop and optimize a nanoemulsion formulation of silymarin and pioglitazone for enhanced topical delivery of antidiabetic effect using Quality by Design (QbD) approach. Both drugs exhibited poor aqueous solubility and low bioavailability in water and also had low bioavailability, which required the advanced development of a nanocarrier system that could enhance drug permeation and therapeutic efficacy. Organoleptic evaluation, solubility analysis, Fourier-transform infrared spectroscopy (FTIR) were conducted as preformulation studies to evaluate the physical properties and compatibility of the drug. Capryol 90 was optimized as the oil phase, and Tween 80 and Transcutol P as the surfactant and co-surfactant, respectively. The pseudo-ternary phase diagrams were prepared with various Smix ratios to determine the nanoemulsion region and 2:1 Smix ratio showed the best formulation of nanoemulsion. Central Composite Design (CCD) based QbD approach was used to optimize formulation variables and oil concentration, Smix concentration were selected as the independent variables and particle size, polydispersity index (PDI), and drug loading were selected as dependent response. Optimized nanoemulsion had nanosized globules, narrow size distribution and high drug loading efficiency. Developed models were found to be appropriate and significant through statistical analysis. Further, transmission electron microscopy (TEM) studies showed spherical and uniform nano-sized droplets. The optimized formulation demonstrated excellent physicochemical stability in terms of physicochemical stability and as formulation characteristics for topical application. In conclusion, the developed nanoemulsion system of silymarin and pioglitazone showed great potential to be an advanced nanocarrier system for more efficient topical antidiabetic treatment.

Keywords: Silymarin; Pioglitazone; Nanoemulsion; Quality by Design (QbD); Topical Delivery; Antidiabetic Therapy

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1. Introduction

Diabetes mellitus is a long-term chronic metabolic condition that is defined by elevated blood glucose levels due to a decrease in insulin production, insulin resistance or a combination of both. Diabetes is becoming a major healthcare problem because it is known to be linked to serious complications like neuropathy, nephropathy, cardiovascular disease and delayed healing of wounds (1). Traditional oral antidiabetic medications have been found to have poor bioavailability, extensive first-pass metabolism, and side effects, thus limiting therapeutic efficiency. Nano-emulsion based drug delivery systems have been developed as excellent vehicles for improving the solubility, stability and permeation of poorly water-soluble drugs. Both silymarin and pioglitazone have strong antioxidant and antidiabetic properties, while

pioglitazone is an insulin sensitizer that enhances the utilization of glucose. Topical application of these drugs in a nano-emulsion system could enhance the permeation of the drugs through the skin and its therapeutic effect with controlled drug release (2, 3)

1.1 Diabetes Mellitus and Challenges in Conventional Therapy

Diabetes mellitus (DM) is a chronic metabolic condition that is manifested by chronic hyperglycemia due to either impaired insulin secretion, insulin resistance, or both. The International Diabetes Federation (IDF) reports that diabetes prevalence is continuing to increase at a rapid rate and is a significant public health problem affecting the world globally (4). Long-term uncontrolled diabetes can lead to severe complications including cardiovascular diseases, nephropathy, neuropathy, retinopathy, chronic inflammation, and delayed wound healing. Oxidative stress and inflammatory mediators such as TNF-α, IL-6, and IL-1β are considered major contributors to the progression of diabetic complications (5). Conventional antidiabetic therapies are primarily administered through oral or injectable routes. However, oral delivery systems often suffer from limitations such as poor aqueous solubility, extensive first-pass metabolism, variable gastrointestinal absorption, frequent dosing, and systemic adverse effects, resulting in reduced therapeutic efficacy and patient compliance [4]. Additionally, many antidiabetic drugs exhibit poor permeability and low bioavailability due to their physicochemical properties. Therefore, there is a growing need for advanced drug delivery systems capable of enhancing drug solubility, stability, permeation, and therapeutic performance (6).

1.2 Role of Silymarin and Pioglitazone in Diabetes Management

The flavonolignan complex of Silybum marianu, known as silymarin, has strong anti-inflammatory, hepatoprotective, antioxidant and antidiabetic activity. It has been also reported to lower oxidative stress, normalize glucose metabolism and increase insulin sensitivity in diabetic conditions (7). But its low aqueous solubility and bioavailability limit its therapeutic applications. Pioglitazone is a thiazolidinedione derivative that is a insulin sensitizer which works by stimulating peroxisome proliferator-activated receptor gamma (PPAR-γ), and thus enhances glucose utilization and decreases insulin resistance. However, traditional oral treatment with pioglitazone can cause systemic adverse effects such as oedema, weight gain and cardiovascular side effects. Since both oxidative stress and insulin resistance pathways may be involved in the treatment of diabetes management, it is possible that the combined effect of silymarin and pioglitazone could be beneficial in a single delivery system (3).

1.3 Advantages of Nanoemulsion-Based Topical Delivery

Nanoemulsions are nanosized colloidal dispersions containing a mixture of oil, surfactant, co-surfactant and aqueous phase. Nanoemulsions have many advantages in relation to drug solubility, permeability and stability, because of their small droplet size and high surface area and better thermodynamic properties. Nanoemulsion systems used topically offer many advantages such as absence of first pass metabolism, increased patient compliance, controlled release, greater permeation of drugs through the skin, and decreased systemic toxicity. Also, nano-emulsions can increase the penetration of poorly water-soluble drugs via stratum corneum due to the increase in drug diffusion and skin hydration. The present nano-emulsion systems are promising vehicle for topical delivery of silymarin and pioglitazone in the treatment of diabetes (8).

1.4 Quality by Design (QbD) Approach in Nanoformulation Development

Quality by Design (QbD) is the systematic and science-based approach to pharmaceutical development, suggested by regulatory agencies, that allows to achieve a predetermined control of the product quality and understanding of the process. Identification of critical quality attributes (CQAs), critical process parameters (CPPs) and risk assessment are all part of QbD to optimize formulation performance. Central Composite Design (CCD) is a design used widely for optimization of nano-formulations. Adopting QbD in the formulation development of nanoemulsions enhances formulation robustness, reproducibility, scalability, product consistency and reduces variability (9).

1.5 Research Gap and Study Objective

Although nanotechnology-based antidiabetic delivery systems have been developed, very little work has been carried out on dual drug nanoemulsion systems comprising natural antioxidants and synthetic insulin sensitizers for topical delivery. Furthermore, optimization of the silymarin–pioglitazone nanoemulsions using systematic QbD approach is still under-studied. Hence, the present study was focused on the development and optimization of a QbD based silymarin–pioglitazone nanoemulsion system for a better topical delivery of the drug with improved

physiochemical characteristics and drug delivery performance.

2. Materials and Methods

2.1 Materials

Both Silymarin and Pioglitazone were purchased as gift samples from the reputed pharmaceutical and certified chemical suppliers. Solubility study was used to select Capryol 90 for use as the oil phase. Tween 80 and Transcutol P were used as surfactant and co-surfactant, respectively, for the preparation of nanoemulsion systems. For the preparation of nanoemulgel formulations, Carbopol 940 was used as a gelling agent. The neutralizing agent used for the preparation of gel was triethanolamine. Other analytical grade solvents and chemicals such as methanol, ethanol, potassium bromide (KBr), phosphate buffer pH 6.8 were purchased from Merck (India). Fresh distilled water was prepared in the laboratory and applied in the present study. All the chemicals and reagents used in the investigation were of analytical grade and used as received.

Table 1. List of Chemicals and Excipients Used in the Study

Material/Excipient	Function	Source
Silymarin	Antioxidant and antidiabetic drug	Gift sample
Pioglitazone	Insulin sensitizer	Certified supplier
Capryol 90	Oil phase	Gattefossé, France
Tween 80	Surfactant	Gattefossé, France
Transcutol P	Co-surfactant	Gattefossé, France
Carbopol 940	Gelling agent	Lubrizol, USA
Triethanolamine	Neutralizing agent	Merck, India
Methanol	Solvent	Merck, India
Potassium bromide (KBr)	FTIR sample preparation	Merck, India
Distilled water	Aqueous phase	Laboratory prepared

Table 2. List of Instruments and Equipment Used in the Study

Instrument/Equipment	Model/Manufacturer
UV–Visible Spectrophotometer	UV-1900i, Shimadzu, Japan
FTIR Spectrophotometer	Nicolet iS5, Thermo Scientific
Differential Scanning Calorimeter	DSC 6000, PerkinElmer
Particle Size Analyzer	Beckman Coulter
Transmission Electron Microscope	TOPCON 002B
pH Meter	pH 510, Eutech Instruments
Brookfield Viscometer	Brookfield Engineering Laboratories
Magnetic Stirrer	Remi Equipments, India
Centrifuge	Remi Equipments, India
Stability Chamber	Remi Equipments, India

2.2 Preformulation Studies

2.2.1 Organoleptic Evaluation

The organoleptic properties of Silymarin and Pioglitazone were evaluated to test its physical appearance and its organoleptic properties. Color, appearance, texture and odor of the drugs were visually inspected in normal daylight. The observed properties were noted and compared with the standard properties reported for the drugs to establish the purity and identity of the drugs.

2.2.2 Melting Point Determination

The melting points of Silymarin and Pioglitazone were obtained by capillary fusion method. A small amount of each drug was put into a capillary tube which was closed at one end. The capillary tubes were loaded in a digital melting point apparatus and the temperature range in which the drug samples started to melt and the complete melt was noted. The melting point determination was done to determine the purity and thermal behavior of the drugs.

2.2.3 Drug–Excipient Compatibility Study (FTIR)

To examine the compatibility between Silymarin, Pioglitazone and formulation excipients, Fourier Transform InfraRed (FTIR) spectroscopy was used. The FTIR spectrum of pure drugs and physical mixture of the drugs were recorded by using FTIR spectrophotometer. In order to obtain the sample data, each sample was mixed with potassium bromide (KBr), pressed into a pellet and scanned across the

spectral range 4000–500 cm^{-1}. The resulting spectra were compared for any possible physicochemical interactions between the drugs and excipients by the presence of characteristic functional peaks, peak shift, disappearance or formation of peaks.

2.2.4 PXRD Analysis

To investigate the crystalline nature, the samples of Silymarin, Pioglitazone and optimized nanoemulsion were subjected to powder X-ray diffraction (PXRD) analysis. Powder X-ray diffraction with appropriate operating conditions was used to analyse the samples. Diffraction patterns were captured within the appropriate range and the identified peaks were analyzed and compared to find any changes in crystallinity and/or crystallization of drugs to amorphous form during the formulation process.

2.3 Solubility Study

The solubility of Silymarin and Pioglitazone was studied in various oils, surfactants and co-surfactants to choose the suitable components for the formulation of Nanoemulsion. The emoticin was added to a variety of excipients, each with different quantities, in glass vials and mixed well with a vortex mixer. Mixtures were shaken in the water bath shaker at 37 ± 0.5 °C for 72 h for equilibrium. The sample was centrifuged at 10,000 rpm for 10 min to separate undissolved drug particles from the sample. Supernatants were filtered, appropriately diluted and then analyzed using UV-Visible spectrophotometer. The solubility values were reported in terms of mg/mL.

2.4 Construction of Pseudo-Ternary Phase Diagram

The aqueous titration method was used to build pseudo-ternary phase diagrams to determine the nanoemulsion region and to find the optimum ratio of the oil, surfactant, co-surfactant, and aqueous phase. Capryol 90 was used as the oil phase, while Tween 80 and Transcutol P were used as surfactant and co-surfactant, respectively. Various Smix ratios (1:1, 1:2 and 2:1) were formulated and combined with oil at various ratios. The distilled water was added dropwise with continuous magnetic stirring to obtain transparent and stable nanoemulsion systems. The formulations were visually examined for clarity, phase separation, turbidity and uniformity. The formulations with clear and stable appearance were considered as being in the region of the nanoemulsion.

2.5 Quality by Design (QbD)-Based Optimization

2.5.1 Selection of Independent and Dependent Variables

A preliminary study and pseudo-ternary phase diagram analysis were used to determine the independent variables, which in this case were the concentration of Capryol 90 (oil phase) and the ratio of Smix. The dependent responses of the nanoemulsion system were chosen as particle size (PS), polydispersity index (PDI) and drug loading (DL).

2.5.2 Central Composite Design (CCD)

The nanoemulsion was optimized based on the Central Composite Design (CCD) method using Design-Expert® software (Version 13, Stat-Ease Inc., USA). Thirteen experimental runs were produced to test the effect of the independent variables on the selected responses. The experimental design facilitated systematic optimization and prediction of the formulation variables, in order to obtain an optimized nanoemulsion system with desired physicochemical properties.

2.5.3 Statistical Analysis and Model Validation

Design-Expert® software was used for analysis of experimental data from CCD. Analysis of variance (ANOVA) was used to check the significance and adequacy of developed models. The effect of the formulation variables on the selected responses was analysed using regression coefficients (R^2), p values and response surface plots. The optimized formulation was checked by comparing the values predicted and the experimental ones.

2.6 Preparation of Silymarin–Pioglitazone Nanoemulsion

The optimized nanoemulsion formulation was developed by spontaneous emulsification technique. A certain amount of Silymarin and Pioglitazone was weighed and dissolved in selected oil phase (Capryol 90). Smix was made by mixing Tween 80 and Transcutol P in the optimized ratio. The prepared Smix was then slowly added to oil phase with continuous magnetic stirring to prepare a homogeneous isotropic mixture. Distilled water was then added dropwise, while stirring to create the nanoemulsion spontaneously. Visual assessment of transparency, homogeneity and phase stability was conducted for the prepared nanoemulsion.

2.7 Characterization of Nanoemulsion

2.7.1 Particle Size Analysis

The optimized nanoemulsion was analyzed for its particle size in terms of Dynamic Light Scattering (DLS) using particle size analyzer. The dilute formulation was appropriate as it was diluted with

distilled water to prevent the multiple scattering effect when analyzed. The average droplet size was measured and the testing was done at 25 °C.

2.7.2 Polydispersity Index (PDI)

The polydispersity index (PDI) of nanoemulsion was also determined at the same time during its particle size analysis by DLS technique. The uniformity and homogeneity of droplet size distribution in the formulation were assessed using PDI values.The PDI values were used to assess the uniformity and homogeneity of droplet size distribution within the formulation. The lower values of PDI were meant to high formulation stability and uniformity.

2.7.3 Drug Loading Efficiency

The drug loading efficiency of the optimized nanoemulsion was evaluated by diluting the known quantity of formulation with distilled water and spectrophotometric analysis was done using UV-VIS spectrophotometer at appropriate wavelength for Silymarin and Pioglitazone. The concentration of the drugs was determined by their calibration curve equations.

2.7.4 Zeta Potential Analysis

To assess the surface charge and physical stability of the optimized nanoemulsion, zeta potential analysis was done using the zeta sizer instrument which is based on the principle of electrophoretic light scattering technique. The formulation was appropriately diluted with distilled water for analysis and measurements were conducted at ambient temperature.

2.7.5 Transmission Electron Microscopy (TEM)

The optimized nanoemulsion droplets were characterized for their internal structure and morphology through Transmission Electron Microscopy (TEM). The diluted nanoemulsion was dropped onto a carbon coated copper grid followed by negative staining with phosphotungstic acid solution. The sample was dried at room temperature and then examined under TEM operating condition at appropriate voltage. Morphology, shape and distribution pattern of nano size droplets were observed and recorded.

3. Results and Discussion

3.1 Preformulation Studies

3.1.1 Organoleptic Properties

The organoleptic evaluation of the extracts of Silymarin and Pioglitazone was carried out to examine their physical appearance, color, texture and smell. Silymarin was seen as a yellowish powder with characteristic odour while Pioglitazone was seen as a white crystalline powder with odorless nature. The characteristics obtained were compared to the reported standard characteristics and were found to be consistent with the characteristics of the selected drugs, thereby confirming the purity and identity of the selected drugs.

Table 3. Organoleptic Characteristics of Silymarin and Pioglitazone

Parameter	Silymarin	Pioglitazone
Color	Yellowish powder	White to off-white powder
Odor	Slight characteristic odor	Faint odor
Appearance	Fine powder	Crystalline powder

3.1.2 Melting Point Analysis

The melting point determination of Silymarin and Pioglitazone were performed by capillary fusion method for drug purity and thermal property. The melting point range of Silymarin was found to be in the range of 158–162 °C and the melting point range of Pioglitazone was found to be in the range of 184–188 °C. The melting points obtained were in accordance with the literature values, which showed the purity and suitability of the drugs to develop them into formulations.

Table 4. Melting Point Analysis of Silymarin and Pioglitazone

Drug	Observed Melting Point (°C)	Reported Melting Point (°C)
Silymarin	158–162°C	158–163°C
Pioglitazone	183–187°C	183–187°C

3.1.3 FTIR Analysis

FTIR spectroscopy was used to assess the compatibility of Silymarin, Pioglitazone and formulation excipients. The FTIR spectra of the pure drugs and physical mixture showed no shifting or

disappearance of any functional peaks, which indicated that there was no major physicochemical interaction between the formulation components. The characteristic peaks for Silymarin were O–H stretching, C=O stretching and aromatic C=C stretching vibrations and for Pioglitazone were N–H stretching, C=O stretching and C–N functional groups. Major peaks were preserved in the physical mixture indicating compatibility of the selected excipients with the drugs.

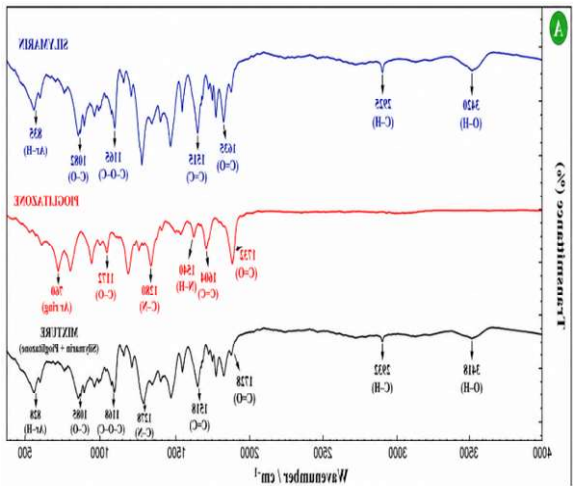

Figure 1. FTIR Spectrum of Pure Silymarin, Pure Pioglitazone, Drug–Drug Physical Mixture

Table 5. Characteristic FTIR Peaks of Silymarin and Pioglitazone

Functional Group	Silymarin (cm^{-1})	Pioglitazone (cm^{-1})	Physical Mixture (cm^{-1})
O–H stretching	3420	–	3418
C–H stretching (aliphatic/aromatic)	2925	2958	2932
C=O stretching	1635	1732	1728
Aromatic C=C stretching	1515	1604	1518
N–H bending	–	1540	1538
C–N stretching	–	1280	1278
C–O–C stretching	1165	1172	1168
Phenolic C–O stretching	1082	–	1085
Aromatic C–H bending (out-of-plane)	835	760	828

3.2 Solubility Study

The solubility of Silymarin and Pioglitazone was studied in various oils, surfactants and co-surfactants to identify the suitable formulation ingredients for formulation of nanoemulsion. In the oils investigated, Capryol 90 showed the highest solubility for both the drugs. The ability of a surfactant to emulsify was assessed by tween 80 and the drug solubilization capacity of co-surfactant was evaluated by Transcutol P with excellent properties for both. Thus, Capryol 90, Tween 80 and Transcutol P were chosen for additional studies on nanoemulation formulation.

Table 6. Solubility of Silymarin and Pioglitazone in Various Excipients

S. No.	Vehicle	Category	Solubility (mg/mL) Mean ± SD
1	Capryol 90	Oil	5.82 ± 0.85
2	Castor Oil	Oil	4.12 ± 0.93
3	Olive Oil	Oil	2.25 ± 0.45
4	Corn Oil	Oil	3.20 ± 0.75
5	Cotton Seed Oil	Oil	4.18 ± 0.69
6	Tween 80	Surfactant	6.12 ± 0.58
7	Lauroglycol 90	Surfactant	3.15 ± 0.93
8	Cremophor EL	Surfactant	2.17 ± 0.54
9	Labrasol	Co-surfactant	3.20 ± 0.79
10	Transcutol P	Co-surfactant	4.20 ± 0.88

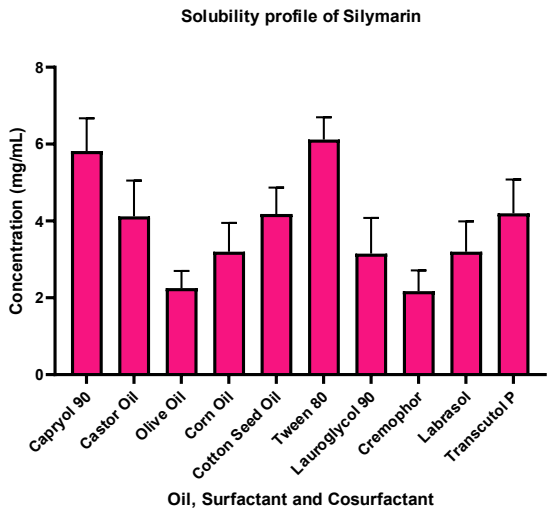

Figure 2. Solubility Profile of Silymarin and Pioglitazone in Various Oils, Surfactants, and Co-surfactants

3.3 Pseudo-Ternary Phase Diagram Analysis

Different Smix ratios, 1:1, 1:2 and 2:1 were used to construct the pseudo-ternary phase diagrams and to assess the efficiency of the formulation ingredients for the formation of the nanoemulsion region. Formulations with the 2:1 Smix ratio had a relatively wide nanoemulsion region and showed excellent physicochemical parameters such as a small particle size, low polydispersity index, and high drug loading efficiency. Hence, 2:1 Smix ratio was chosen to be further optimized and formulated.

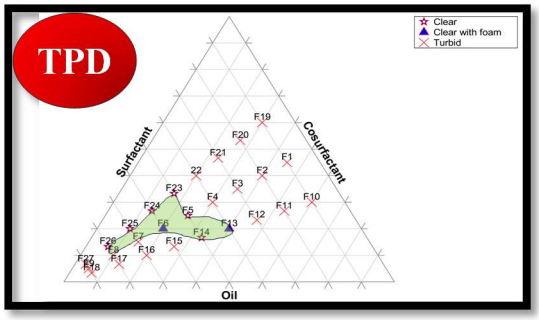

Figure 3. Pseudo-ternary Phase Diagram (PTPD) of Capryol 90, Tween 80, and Transcutol P Showing Nanoemulsion Region for Optimization of Silymarin–Pioglitazone Loaded Nanoemulsion Formulation. Clear formulations are represented by stars, clear formulations with foam by triangles, and turbid formulations by crosses.

Table 7. Composition and Visual Observation of Formulations Used for Construction of Pseudo-Ternary Phase Diagram (□ = Clear nanoemulsion region, ▲ = Clear formulation with foam formation, □ = Turbid/phase-separated formulation)

Formulation	Oil (Capryol 90, µL)	Smix (µL)	Water (µL)	Smix Ratio (Tween 80: Transcutol P)	Observation
F1	100	700	200	1:1	□
F2	200	600	200	1:1	□
F3	300	500	200	1:1	□
F4	400	400	200	1:1	□
F5	500	300	200	1:1	□
F6	600	200	200	1:1	▲
F7	700	150	150	1:1	□
F8	800	100	100	1:1	□
F9	900	50	50	1:1	□
F10	100	650	250	1:2	□
F11	200	550	250	1:2	□
F12	300	450	250	1:2	□
F13	400	350	250	1:2	▲
F14	500	250	250	1:2	□

F15	600	200	200	1:2	□
F16	700	150	150	1:2	□
F17	800	100	100	1:2	□
F18	900	50	50	1:2	□
F19	100	750	150	2:1	□
F20	200	650	150	2:1	□
F21	300	550	150	2:1	□
F22	400	450	150	2:1	□
F23	500	350	150	2:1	□
F24	600	250	150	2:1	□
F25	700	200	100	2:1	□
F26	800	150	50	2:1	□
F27	900	100	0	2:1	□

3.4 Optimization by Central Composite Design

3.4.1 Perturbation Plot Analysis

Design-Expert® software was used to generate the perturbation plots to see the effect of individual formulation variables on the selected response parameters of the developed nanoemulsion system. The perturbation plots gave a graphical view of the sensitivity of each response to variation in the formulation variables with the other variables held at fixed values. In the current study, the concentration of Capryol 90 (oil phase) was included as factor A, and the concentration of Smix (tween 80 and Transcutol P) as factor B. The perturbation plot of particle size showed that the particle size gradually increased with the increase of the oil concentration, while the particle size decreased with the increase of Smix concentration because the emulsification efficiency was enhanced and interfacial tension was decreased. Likewise, the perturbation plot of PDI showed that there was an increase in the heterogeneity of the formulation with the increase of oil concentration; and there was a decrease in the PDI value with the increase of Smix concentration, creating a more uniform nanoemulsion system.

The perturbation plots corresponding to the drug loading of Silymarin and Pioglitazone showed that both the concentration of oil and Smix showed positive effect on the incorporation of the drugs in the nanoemulsion system. Capryol 90 was found to give improved solubilization capacity of the lipophilic drugs and Tween 80 and Transcutol P gave improved emulsification and stabilization of the drug loaded droplets. At very high concentrations, however, it was noticed that the system reached a slight plateau, which could be due to the saturation of the system and may be possible destabilization of the nanoemulsion. The overall conclusion of the perturbation analysis was that both independent variables were found to significantly affect the physicochemical properties of the generated nanoemulsion system and also helped in verifying the optimum formulation region for the further optimization.

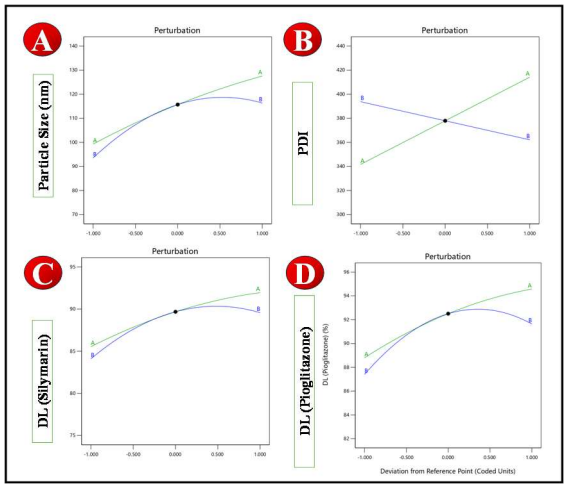

Figure 4. Perturbation plots illustrating the effect of formulation variables, namely oil phase (Capryol 90) and Smix (Tween 80 : Transcutol P), on the response parameters of the developed nanoformulation. (A) Effect on particle size (PS), (B) Effect on polydispersity index (PDI), (C) Effect on drug loading (DL) of Silymarin, and (D) Effect on drug loading (DL) of Pioglitazone.

3.4.2 3D Response Surface Plot Analysis

The effect of formulation variables on the selected response parameters were further studied by three-dimensional (3D) response surface plots produced with Design-Expert® software. The response surface

plots gave a visual understanding of the interaction effect between formulation variables and the overall effect of the formulation variables on the physicochemical characteristics of the nanoemulsion system. The factor A in this study was the Capryol 90 concentration and the factor B was the Smix concentration of Tween 80 and Transcutol P. The response surface plot of particle size showed that a higher concentration of oil led to a greater particle size because of the higher internal phase viscosity, corresponding to a decrease in the efficiency of emulsification. On the other hand, the particle size decreased as the concentration of Smix increased, indicating that this increase caused the interfacial tension to decrease and spontaneous formation of nano emulsion to take place. The region of optimization was observed at intermediate concentrations of oil and Smix at which the minimum particle size was achieved. The 3D response surface plot showed that the oil concentration had a positive effect on the PDI value because droplet heterogeneity was greater with increasing concentration of oil, while increasing the concentration of Smix had a negative effect, showing droplet uniformity and decreasing the PDI value with increasing Smix concentration. The surfactant–co-surfactant system proved to be effective in keeping the nano-sized droplets stable from aggregation and hence stabilizing the formulations, resulting in the homogeneity of the formulations. The response surface plot for drug loading of Silymarin showed that the drug loading in the nanoemulsion system was increased with an increase in the concentration of Capryol 90 and Smix. Drug loading efficiency was also improved, possibly due to improved solubilization in the oil phase and stabilization by Tween 80 and Transcutol P, for the same reason, in the case of Pioglitazone. The curved response surfaces indicated the presence of interactive and quadratic effects among the formulation variables. The resulting response surface plots were able to provide the optimized formulation area that yielded stable nano-sized droplets with narrow size distribution and high drug loading efficiency.

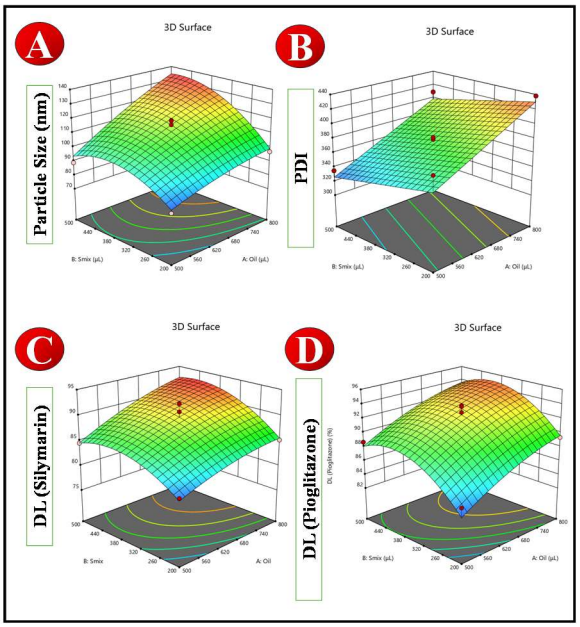

Figure 5. Three-dimensional (3D) response surface plots showing the influence of formulation variables on the physicochemical properties of the developed nanoemulsion system. (A) Effect on particle size (PS), (B) Effect on polydispersity index (PDI), (C) Effect on drug loading of Silymarin, and (D) Effect on drug loading of Pioglitazone**.**

After initial screening using pseudo-ternary phase diagram, a Central Composite Design (CCD) was used to optimize the Silymarin–Pioglitazone nanoemulsion system. Oil and Smix were chosen as the key formulation factors to be used as independent variables. All factors were investigated at two levels lower (−1) and upper (+1). From preliminary experiments and PTPD results, following lower and upper limits were set for the formulation variables:

Table 8. Based on the TPD screening lower limit and higher limit for optimization through CCD.

Factor	**Minimum (µL)**	**Maximum (µL)**
Oil (Capryol 90)	500	800
Smix (Tween 80 : Transcutol P, 2:1)	200	500

DoE

The optimization of developed formulation was performed by using Central Composite Design (CCD) to assess the effect of the independent formulation variables on the selected response parameters. ANOVA was used for statistical analysis of the effect of the formulation variables on the particle size (PS),

polydispersity index (PDI), drug loading of Silymarin and drug loading of Pioglitazone. The regression coefficients (R2), F-calculated values and p values obtained are summarized in Table 3.12. The results of the statistical analysis showed that the models developed were adequate and reliable as the experimental values were found to have good correlation with the proposed values for all the responses studied. All the R^2 values were good (R^2 = 0.9589 for PS, 0.9041 for PDI, 0.9303 for DL (Silymarin) and 0.9278 for DL (Pioglitazone)), suggesting good model fitting and reliability of the optimization design. The independent variables selected were found to have significant influence on the measured responses as indicated by higher R^2 values. The model had an R^2 value of 0.9589, F-calculated value of 2.86 and p-value of 0.1678 for particle size (PS) which showed that the model explained the variation in particle size well. Similarly, the PDI model exhibited R^2 value of 0.9041, a calculated F value of 2.70, and a p value of 0.1780, thus indicating a good level of predictability of the formulation variables about the distribution pattern of globules. The regression properties of the drug loading models were also good. The drug loading of Silymarin was found to have an R^2 value of 0.9303 with p value of 0.8607, whereas drug loading of Pioglitazone was found to have an R^2 value of 0.9278 with p value of 0.4448.

Table 9. ANOVA Analysis overview for CCD Batches

Response variable	Regression Parameters		P Value
	R^2	F_{cal}	
PS	0.9589	2.86	0.1678
PDI	0.9041	2.70	0.1780
DL (Silymarin	0.9303	0.2459	0.8607
DL (Pioglitazone)	0.9278	1.10	0.4448

3.5 Characterization of Optimized Nanoemulsion

3.5.1 Particle Size and PDI

The optimized nanoemulsion formulation exhibited nanosized droplets with narrow size distribution. The small particle size and low PDI suggested the uniformity of droplets and increased formulation stability. The results obtained indicated the optimized formulation would be suitable for topical drug delivery applications. It has been determined that the polydispersity index (PDI) is 0.300 ± 0.02 which is a narrow and uniform droplet size distribution. The PDI value below 0.5 implies homogeneity of formulation and proves that there is not a major degree of aggregation or a wide particle size distribution. The relatively low PDI shows the efficiency of the surfactant–co-surfactant system used in the preparation of a monodispersed nanoemulsion. Dynamic light scattering (DLS) technique was used to determine the particle size and polydispersity index (PDI) of the optimized Silymarin–Pioglitazone nanoemulsion. The optimized nanoemulsion formulation had a mean particle size (Z-average) of 108.97 ± 31.21 nm and a PDI value of 0.300 ± 0.02 that showed nanosized droplets with a narrow size distribution. The particle size obtained was found to be in the required range and it was observed to be uniformly distributed which showed successful formation of nanoemulsion with uniform globule size distribution. The PDI value of less than 0.5 indicated good homogeneity and stability of the nanoemulsion system with minimal aggregation. This is probably due to the efficient emulsification and stabilizing effect that the used surfactant–co-surfactant system (Tween 80 and Transcutol P) provides, which results in a narrow particle size distribution. The nanosized droplets could increase the surface area available for drug diffusion, thus aiding in drug permeation and formulation properties in topical delivery applications.

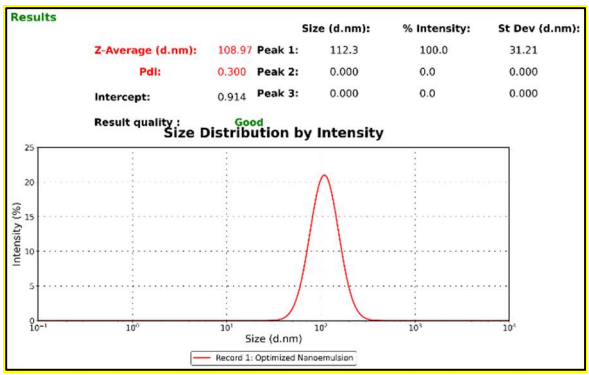

Figure 6. Particle Size Distribution of Optimized Nanoemulsion

3.5.2 Drug Loading Efficiency

The drug loading efficiency of the optimized Silymarin–Pioglitazone nanoemulsion was determined to evaluate the amount of drug successfully incorporated within the nanoemulsion system. High drug loading is considered important for improving therapeutic efficacy and minimizing drug loss during formulation development. The optimized nanoemulsion exhibited satisfactory drug loading efficiency for both Silymarin and Pioglitazone, indicating effective solubilization of the drugs within the oil phase and proper stabilization by the

surfactant–co-surfactant system. The drug loading efficiency of Silymarin was found to be 89.42 ± 1.24%, whereas Pioglitazone showed a drug loading efficiency of 91.18 ± 1.08%. The high drug loading values may be attributed to the excellent solubilization capacity of Capryol 90 along with the emulsification efficiency of Tween 80 and Transcutol P. The results indicated efficient incorporation of both drugs into the nanoemulsion droplets with minimal drug precipitation or leakage. Overall, the optimized nanoemulsion demonstrated excellent drug loading characteristics, which may contribute to improved drug delivery performance and enhanced topical therapeutic efficacy.

3.5.3 Zeta Potential

The zeta potential analysis revealed adequate surface charge of the optimized nanoemulsion system, indicating good colloidal stability and reduced droplet aggregation. The obtained zeta potential value confirmed the physical stability of the developed formulation.

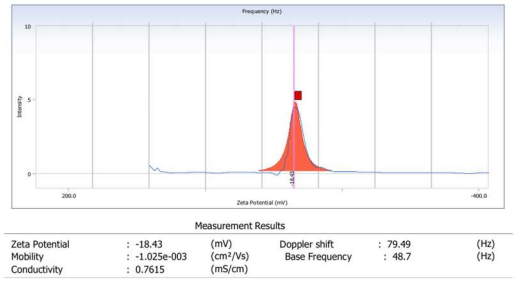

Figure 7. Zeta potential distribution of the optimized Silymarin–Pioglitazone nanoemulsion exhibiting a mean zeta potential of −18.43 mV, suggesting adequate electrostatic stabilization and physical stability of the nanoemulsion system.

The **zeta potential of the optimized nanoemulsion was −18.43 ± 0.75 mV**, indicating moderate electrostatic stability of the system. The negative surface charge generated sufficient repulsive forces between the droplets, thereby minimizing droplet aggregation and coalescence. Although non-ionic surfactants such as Tween 80 primarily provide steric stabilization, the observed zeta potential further contributed to maintaining the physical stability of the nanoemulsion.

3.5.4 TEM Analysis

The optimized nanoemulsion formulation was analyzed by transmission electron microscopy (TEM) to assess the morphology and structural properties of the optimized formulation. The nano-sized droplets observed in TEM micrograph (Figure X) were found to be evenly distributed, mostly spherical in shape and were not found to be aggregating. The physical stability of the formulation was good as the globules observed were discrete and well dispersed at a nano size. The presence of the spherical droplet appearance indicated efficient emulsification and stabilization of the selected Smix system which consisted of Tween 80 and Transcutol P. Moreover, the particle size in the TEM image was in good agreement with the particle size obtained by dynamic light scattering (DLS). The presence of no crystalline aggregates or irregular particles shape suggested the successful encapsulation and uniform distribution of Silymarin and Pioglitazone in the nanoemulsion droplets. Overall, TEM results validated the development of a stable nanoemulsion system having a spherical shape with a uniform particle size distribution, with excellent physicochemical properties and appropriate for improved drug delivery.

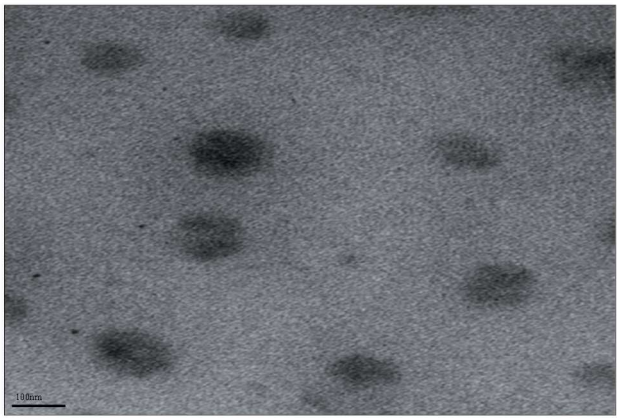

Figure 8. Transmission electron microscopy (TEM)

4. Conclusion

In the present study, Silymarin–Pioglitazone nanoemulsion system has been successfully developed and optimized for a topical drug delivery system using a Quality by Design (QbD) approach. The compatibility and suitability of the selected drugs and excipients were confirmed by preformulation studies necessary to make development of the nanoemulsion possible. Capryol 90, Tween 80 and Transcutol P were found to be suitable formulation excipients from solubility studies, and the nanoemulsion region obtained with the pseudo-ternary phase diagram analysis was most stable at Smix ratio of 2:1. Central Composite Design (CCD) was successfully used to optimize the formulation variables and optimized nanoemulsion showed good physicochemical properties such as nano sized globules, low

polydispersity index, and high drug loading efficiency. Optimized formulation showed a particle size of 108.97 nm with PDI value of 0.300, which means the formulation had good droplet uniformity and stability. The Zeta Potential analysis showed good colloidal stability and TEM analysis showed spherical and uniform distribution of nano size droplets without any aggregation. Physicochemical properties of developed Silymarin–Pioglitazone nanoemulsion were found to be good and also have great potential for use in topical delivery system applications as an advanced nanocarrier system. The optimization approach by QbD also ensured the robustness of the formulation which further guaranteed that the formulation was reproducible and reliable; hence, the developed nanoemulsion could be considered as a promising platform for drug delivery via the topical route.

7. References

1. DeFronzo RA, Ferrannini E, Groop L, Henry RR, Herman WH, Holst JJ, et al. Type 2 diabetes mellitus. Nat Rev Dis Primers. 2015;1(1):15019 10.1038/nrdp.2015.19
2. Hefnawy A, Abdelhamid AS, Abdelaziz MM, Elzoghby AO, Khalil IA. Recent advances in nano-based drug delivery systems for treatment of liver cancer. J Pharm Sci. 2024;113(11):3145-72 10.1016/j.xphs.2024.08.012
3. Shakeel F, Shafiq S, Haq N, Alanazi FK, Alsarra IA. Nanoemulsions as potential vehicles for transdermal and dermal delivery of hydrophobic compounds: an overview. Expert opinion on drug delivery. 2012;9(8):953-74
4. Magliano DJ, Boyko EJ, Atlas ID. COVID-19 and diabetes. IDF DIABETES ATLAS [Internet] 10th edition: International Diabetes Federation; 2021.
5. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013;93(1):137-88 10.1152/physrev.00045.2011
6. Chatterjee S, Davies MJ. Current management of diabetes mellitus and future directions in care. Postgrad Med J. 2015;91(1081):612-21 10.1136/postgradmedj-2014-133200
7. Surai PF. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. Antioxidants. 2015;4(1):204-47
8. Solans C, Solé I. Nano-emulsions: Formation by low-energy methods. Current opinion in colloid & interface science. 2012;17(5):246-54
9. Singh B, Sharma T, Saini S, Kaur R, Jain A, Raza K, et al. Systematic Development of Drug Nanocargos Using Formulation by Design (FbD): An Updated Overview. Crit Rev Ther Drug Carrier Syst. 2020;37(3):229-69 10.1615/CritRevTherDrugCarrierSyst.2020032040