Original Article

Implementation of Quality by Design (QbD) Approach in Development of QCT-SMEDDS with Combination of AgNPs for Diabetic Foot Ulcer Management

Reena Badhwar[1], Ruchi Singh[1], Tinku[2,3], Harvinder Popli[1,*]

[1]Department of Pharmaceutics, Delhi Pharmaceutical Science and Research University, New Delhi, INDIA.
[2]School of Medical and Allied Sciences, G.D. Goenka University, Gurgaon, Haryana, INDIA.
[3]School of Pharmaceutical Education and Research, Jamia Hamdard (Deemed to be University), Hamdard Nagar, New Delhi, INDIA.

ABSTRACT

Introduction: Quercetin (QCT) is a flavonoid with antioxidant, potential free radical scavenger, and strong anti-inflammatory activities. These properties of QCT promote wound healing process. **Objectives:** The present work to investigate the diabetic wound healing efficacy of QCT- loaded self-micro emulsifying drug delivery system (QCT-SMEDDS) and Silver nanoparticles (AgNPs) in combination. **Materials and Methods:** The QCT-SMEDDS were develop and optimized using D-Optimal design mixture by observing numerous critical qualities attributes (CQAs). A pseudo ternary phase diagram was constructed using Transcutol HP, PEG 400 and oleic acid. The developed formulation evaluated for *in vivo* and stability studies. Further, prepared formulation was characterized for globules size, *in vitro* drug release, antimicrobial, *ex vivo* permeation, skin deposition, and pharmacokinetics studies in diabetic wound model. The optimized formulation showed globules size, polymer dispersive index (PDI) and zeta potential of QCT-SMEDDS were found to be 101.7nm with PDI 0.17 and -29.2 mV respectively with improved release rate (92%) due to enhanced solubility of QCT in the form of SMEDDS. Silver nanoparticles (AgNPs) were combined with QCT-SMEDDS to enhance the antimicrobial effects and for better wound healing. The combination of QCT and AgNPs were developed in an emulgel and various physiochemical characterization and evaluation of emulgel were carried out. *In vitro, ex vivo* studies using dialysis membrane and Strat-M respectively. *In-vivo* experiment including % wound contraction and histopathological studies (rate of re-epithelization) of emulgel and marketed gel were performed using diabetic excision wound model. **Results:** Presented that emulgel has better wound contraction and rate of re-epithelialization as compared to marketed gel. Further histopathological study of skin including rate of re-Epithelization were carried out and found that this gel help to stimulates fibroblast and granulocytosis formation that leads to eschar and keratin layer formation which is a good sign of healing. **Conclusion:** The present study that the AgNPs-QCT incorporated emulgel has been projected as a potential substitute for the topical delivery of QCT in the treatment of diabetic foot ulcer (DFU).

Key words: Diabetic foot ulcer, QCT-SMEDDS, Silver nanoparticles, Strat-M, Wound contraction, Histopathology.

INTRODUCTION

Diabetic foot ulcer (DFU) is one of the most common and severe complications of diabetes. Due to local and systemic factors, the healing process does not progress towards proliferation and maturation phases. Severe infection inhibits the synthesis of cytokines, proteins, and growth factors which affects the proliferation, migration of fibroblast and keratinocytes formation.[1] *S. aureus*, *E. coli* and *P. aeruginosa* are the common infective organisms which are the leading cause of mortality, amputation and morbidity of the DFU.[2]

Submission Date: 17-10-2021;
Revision Date: 08-11-2021;
Accepted Date: 28-11-2021

DOI: 10.5530/ijper.55.4.220

Correspondence:
Prof. Harvinder Popli
Department of Pharmaceutics, Delhi Pharmaceutical Science and Research University, Mehrauli Badarpur Road, Sector-3 Pushp Vihar, New Delhi-110017, INDIA.
Phone no: +919899700704
E-mail: tinkujhajjar87@gmail.com, popli.harvinder@gmail.com

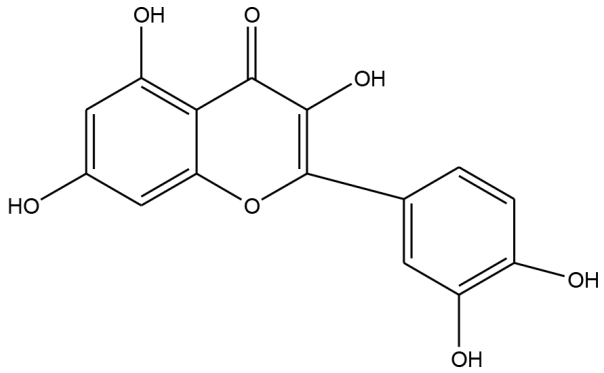
www.ijper.org

DFU is one of the major complication of Diabetes Mellitus (DM) that decrease the quality of life and becomes major cause of patient hospitalization.[3] Around 61.2 million public are diabetic and it is estimated to augment to 101.2 million by 2030 in India. The DFU patients mortality is double than a non DFU diabetic person. The prevalence rate of DFU among diabetic population has reached from 3% to 13% globally. It is estimated that at every 30 sec, one lower limb is surgically removed due to diabetic wounds.[4]

Management of DFU includes wound debridement, offloading the ulcer's extracellular matrix protein (ECM) extracts, bioengineered skin substitutes, negative pressure therapy, hyperbaric oxygen and growth factors such as platelet derived growth factor, epidermal growth factor and fibroblast growth factors.[5] But the overall approaches are focused on closure of wounds rather than skin regeneration that leads towards re-epithelialization. The re-epithelialization process can be separated from infective wound process because sometimes re-epithelialization is occurred but the dermis and adjacent tissues are not healed still completely, that stimulates the reoccurrence of infection and inflammation resulting again wound formation.[6]

So, research on DFU is the need of the hour due to prevalence of diabetes in world. Multiple approaches are being studied by the researcher but the effective delivery of the drug on the wound site is limited. Demands for the new technologically products for treatment of DFU is rising globally. So, we focus on the development of a topical delivery system incorporated with flavonoid because novel drug delivery systems loaded with flavonoids is the most preferred these days, due to minimum side effects, less toxicity, increased solubility and bioavailability.[7] Flavonoids possess wide range of pharmacological properties such as anti-inflammatory, antioxidant, apoptosis and antiproliferative. All these activities can be directly linked to the healing of wound. Flavone's act as antioxidants and protects reactive oxygen species (ROS) formation.[8]

Quercetin (QCT) [2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4-Hchromen-4-one)] is a strong anti-oxidant agent and found to stimulate angiogenesis and proliferation of epithelial cells and fibroblasts (Figure 1). It decreased inflammatory cells, increased fibroblast proliferation, re-epithelization and quality of healing in diabetic wounds.[9] It also helps to decrease tumour necrosis factor alpha (TNF-a), Interleukin -1-beta (IL-1b), increased Interleukin 10 (IL-10), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-b1) expressions, elevates the blood vessels formation and promotes the neuronal regeneration.



2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one

Figure 1: Chemical structure of Quercetin (QCT).

Figure 2: Pictographic representation of synthesized AgNPs.

QCT presents limited capability to penetrate the skin and poor water solubility. To reach therapeutic levels in the organism, QCT requires a carrier with high loading capacity and good adherence to the skin.[10] Besides the drawbacks associated with the physicochemical properties of QCT, another major hindrance is the stratum corneum (SC) hinders drug absorption through the skin resulting in low bioavailability. To overcome these issues, drug delivery systems have been designed for QCT topical transport based on self -micro emulsifying drug delivery system (SMEDDS).

Silver nanoparticles (AgNPs) are considered promising option for the treatment of DFU due to its strong antimicrobial action.[11] The greatest barrier to healing is represented by the biofilm, which is highly resistance to conventional antimicrobial therapies.[12] AgNPs has received significant attention because of the emergence of antibiotic-resistant strains and its low tendency to develop resistance[13] including wound dressings.

Therefore, recent researches are focus on the development of new and effective wound care materials.

Table 1: QTPP elements for QCT- SMEDDS.

QTPP Attributes	Targets	Justification
Dosage form	SMEDDS incorporated into gel	To improve the solubility and permeability of QCT at the site of action and for ease of application
Dosage type	Sustain release	Will lead to longer duration of action hence enhanced therapeutic effects
Dosage strength	QCT 1.5 mg	Based on the antimicrobial study
Route of administration	Topical	Recommended route for local action for wound healing
Appearance	Clear transparent/ translucent	Ease of application and patient compliance
Mode of Delivery	Emulgel	The SMEDDS can be easily incorporated into a hydrogel for ease of application, improved patient compliance, portability, and manufacturing ease
Stability	At least 6 months as per ICH guidelines Q1A (R2)	To maintain the therapeutic potential of the drug during the storage period

Table 2: CQA for QCT- SMEDDS and their justification.

Quality Attributes of the Drug Product		Target	Is this a CQA?	Justification
Physical Attributes	Colour	Acceptable to patients	No	Colour, odour, and appearance were not considered as critical, as these are not directly linked to the efficacy and the safety of dosage form
	Odour	No unpleasant order		
	Appearance	Acceptable to patients		
Assay and drug content		90%-110% of saturated concentration in the solubilizing phase	Yes	Drug content or Assay is an important parameter for safety and efficacy of the formulation. It is important for product performance. Material attributes and process parameters can affect the assay of the formulation. However as the developed system is a homogenous dispersion containing drug in the solubilized form in a mixture of oil, surfactant, and co surfactant, hence it is regarded as moderately critical
Emulsification Time		Low	Yes	Lower values of emulsification time help in ease of formation of SMEDDS hence was considered as critical
In-vitro Release		>90% at end of 8 hr.	Yes	*In-vitro* release is an important parameter that will determine the availability of the drug at the site of action, thereby affecting safety and efficacy, hence considered as critical CQA
Ex-vivo skin retention		Higher skin retention	Yes	The product under development is for local action at the site of wound. In order for higher efficacy, increased retention is required at the wound site. Hence considered as CQA
Globule size		100-150 nm	Yes	Particle size affects the physicochemical, drug release, and is considered as a benchmark of stability of the formulation. Smaller globule size allows better solubility, high surface area and better permeation at the therapeutic site hence it was regarded as highly critical
Zeta Potential			Yes	Higher value of zeta potential may lead to disaggregation of particles. Important for stability and determination of shelf life of the SMEDDS formulation which will affect the safety and efficacy of the product
%Transmittance		>90%	Yes	%Transmittance is a critical and fundamental attribute in the formulation of SMEDDS as it represents the optical birefringence and homogeneity of the formulation thereby affecting the efficacy of SMEDDS

The nano particles are also well-known to stimulate the healing process by facilitating suitable movement through the different healing phases.[14] To date, focuses have been made on the efficacy of AgNPs in treating the diabetic wound.

SMMEDS has dragged attention as an effective drug delivery approach due to focus on lipid-based preparations. This emulsifying delivery system is an isotropic mixture (drug (s), oils, surfactant, and co-surfactants) with high thermodynamically stability.[15] This system is transparent with a droplet size range of 50–250 nm. The concentration of excipients used to form SMEEDS has direct influence on the ME globules size that further can affect the *in-vitro* and *in-vivo*/*ex-vivo* results. Now a days, SMEEDS are used in topical and transdermal drug delivery system in the form of gel matrix developed using different polymers such as carbomer-934, carrageenan, xanthan gum and chitosan.[16] The ME containing gel matrix is called emulgel which are more preferable for topical delivery than ME alone.[17]

MATERIALS AND METHODS

Materials

Silver nitrate ($AgNO_3$) (high M.W., > 99%), Carbopol 934 (CP-934) (High M.W., > 75%), Transcutol HP (T-HP), Labrasol 90, Labrafac 90 and Tocopherols were procured from Gattefosse (Saint-Priest, France). Triethanolamine, ethanol, absolutemethanol, methanol (HPLC grade), polyvinylpyrrolidone (PVP - MW 40000), oleic acid, polyethylene glycol (PEG-400), Tween 80, glycerol was purchased from CDH Chemical Company (New Delhi, India). Quercetin (>99.99%) was purchased from Link Biotech (New Delhi, India). Mineral salt broth and nutrient agar were obtained from Himedia Chemicals, India.

Solubility Assessment of QCT in oils, surfactants and co surfactants

The solubility of QCT base was ascertained by dissolving excess quantity (1g) of drug in 2 ml of various solvents like ethanol, methanol, acetone and chloroform, coconut oil, oleic acid, olive oil, egg oil, rice bran oil, arachis oil, capryol 90 and lauroglycol 90 with buffer pH 7.4. The mixture was stored in a stoppered vial and a vortex mixer was used for proper mixing, vials were kept aside at 25±1°C for 3 days to get equilibrium. Later on 72 hr., samples were centrifuged at 2500-3000 rpm for 10-15 min.[18] A membrane filter (0.45µm) was used to filter resultant supernatant of samples and UV-vis spectrophotometer (UV1800, Shimadzu Japan), was used to determine the drug concentration in each solvent.

QbD Based Development and Optimization of QCT- SMEDDS

QCT- SMEDDS were formulated with an aim to improve QCT solubility and permeability for topical delivery in order to improve its efficacy for the treatment of DFU. A systematic QbD based approach was used for development of QCT-SMEDDS. Initial Quality risk assessment studies were carried out to identify factors affecting Quality of QCT- SMEDDS and Ishikawa fish bone diagram was created to identify CPAs and CMAs. REM was constructed for identifying the CPAs and CMAs. Further, formulation was optimized by employing three factor three levels D-Optimal mixture design.

Development and characterization of QCT-SMEDDS

Construction of Pseudo Ternary Phase diagram

The different ratio of oil (oleic acid) surfactants (T-HP), co-surfactant (PEG 400) was utilized to examine the phase behavior by applying titration method. The Smix ratios of T-HP and PEG-400 were altered 1:1, 1:0.5 and 1:2 to construct a three ternary phase diagrams.[19] To construct this diagram at each S_{mix} ratio, the mixtures containing water and S_{mix} were prepared with volume ratios ranging between 1:9 and 9:1, and further titrated drop by drop using oil phase under continuous magnetic stirring at ambient temperature till turbidity appearance. In the same way, volume ratios of oily phase and S_{mix} (1:9 to 9:1) were changed and titrated with water. Then, the maximum ME area of the ternary system was noted as a triangular phase diagrams using an online software.

Quality Risk Assessment of Formulation Variables of QCT- SMEDDS

The risk assessment studies were carried out to identify the CMAs and/or CPPs for QCT loaded SMEDDS plausibly affecting the CQAs of drug product. Ishikawa fish-bone diagram was constructed by employing to Minitab 17 software to find out the cause-effect relationship among the product and process variables. In addition, risk estimation matrix (REM) was constructed for qualitative analysis of risk levels to MA and/or PP.[20]

Formulation, evaluation and optimization of QCT-SMEDDS (D-optimal mixture design)

Formulation of QCT- SMEDDS

On the basis of preliminary studies, the critical formulation attributes (CFAs) that affect the QCT-SMEDDS were identified. D-optimal design was further

selected for the systematic optimization employing Design Expert® software 13.0.1 (M/s Stat-Ease, Minneapolis, USA). The selected quantity of CFAs such as oleic acid (X_1), T-HP (X_2) and PEG 400 (X_3) were studied at three levels each viz. low (−1), medium (0) and high (+1) equidistant from each other.[21] Sixteen formulations were developed taking into account the vertex points, edges and interior surface of the selected mixture design without replicates. The design matrix represents the number of different factors along with factors combinations. The developed QCT-SMEDDS were further investigated for globules size (nm), drug release ($Q8_{hr}$) and % transmittance and % permeation as the response variables.

Response surface mapping

The understanding of the dependence, inter-dependence and co-variation among the variables studied and their interactions is facilitated by constructing response surface diagrams to map the responses over the entire experimental domain. The 3-D response surfaces and their 2-D contour plots, constructed for various CQAs viz. globule size, transmittance, $Q8_{hr}$ and % permeation.[21]

Characterization of QCT- SMEDDS

The prepared QCT-SMEDDS were further characterized by globules size, zeta potential, transmittance, thermodynamic stability and cloud point measurement.

Globules size and Zeta potential determination

1ml of QCT-SMEDDS was diluted up to 100 times with distilled water in volumetric flask and shaken properly for proper dispersion. Further, the globule size and zeta potential was determined using Zeta Sizer (Malvern Instruments, DTS version 4.10). The procedure was completed in triplicate within range −120 to 120 V and mean value was reported as the final results.[22]

Transmittance (%)

QCT-SMEDDS (1g) was emulsified with 200 ml phosphate buffer solution (PBS) at pH-7.4 and methanol. Transparency, % transmittance and turbidity was determined at λ_{max} (650 nm) using UV- spectrophotometer (UV1800, Shimadzu, Japan). Moreover, the pH of QCT-SMEEDS was also measured at 25°C using pH meter.[23]

Thermodynamic stability

Temperature changes in QCT-SMEDDS was evaluated heating cooling cycle, centrifugation test and freeze thaw cycle.[24] The QCT- SMEDDS were stored at different temperature conditions like heating (40°C) cooling (4°C) cycles for 48 hr. Then subjected to centrifuge at 4000 rpm for 25- 30 min. In addition, the QCT-SMEEDS were stored at -20°C and -70°C and thawing at 25°C for 48 hr. at each different temperature environment. The samples were tested for turbidity and presence of any phase separation

Cloud point measurement

For the determination of cloud point of formulation, 0.5 g of QCT-SMEDDS was dispersed in 250 ml of PBS (pH 6.8) and allowed to stabilize. The dispersion was then subjected to heating under water bath with gradually increase in temperature. The temperature at which the turbidity was observed was considered as cloud point.[25]

Synthesis of AgNPs

Sodium borohydride ($NaBH_4$) and silver nitrate ($AgNO_3$) were used to make AgNPs using a chemical process.[26] Both solutions were prepared at a concentration of 0.24 % w/v $NaBH_4$ and 3.4 %w/v $AgNO_3$ respectively. The chilled ($NaBH_4$) solution (50µl) was then added drop wise to the $AgNO_3$ solution at continuous stirring for 45 min at 750 rpm. To avoid aggregation and for long-term stability, a small amount of polyvinyl pyrrolidine (PVP) (1% w/v) was added (Figure 2).

Preparation of AgNPs and QCT- SMEDDS (EMULGEL)

Forthe preparation of gel base, the Carbopol-934 was used as a gelling agent. 1% w/v Carbopol-934 was mixed in water under continuous stirring at 250 rpm for 30 min. The pH of the gel was measured using digital pH meter (Eutech Instruments, India) and maintained at 6.5 with the addition of triethanolamine. The developed AgNPs and QCT-SMEDDS were loaded into already prepared gel base at 500 rpm using magnetic stirrer to formulate AgNPs–QCT (Emulgel). Tocopherol was added in to the emulgel as an antioxidant and chemo protective substance. Hence, emulgel was formulated with combination of AgNPs and QCT- SMEDDS[27] and compared with marketed gel.

Characterization and evaluation of emulgel

Viscosity

The viscosity of emulgel and marketed gel containing AgNPs was determined using Brookfield viscometer (AMETEK Model DV II+ Pro, India). Prior to viscosity measurement, equilibrium state was achieved of the samples within 25-30 min at 25°C. Further, sample was subjected in the sample holder and allowed to the perpendicular spindle immersion for the viscosity reading in centipoises (cps).[28]

Physical Parameters

Extrudability of an emulgel is measure of the force or / weight in (g) required to drag out the 0.5 cm ribbon of sample from the collapsible aluminium tube in 10 sec. For extrudability measurement, a closed collapsible tube containing hydrogel was pressed firmly from the crimped end for 10 sec.[29] The extruded amount of formulation was weighed, and then the % of extruded formulations was calculated. The procedure was performed in triplicate and finally grades were allotted in the form of fair, good and excellent.

Drug content

Emulgel (1g) and marketed gel was dissolved with methanol (50 ml) and solutions was kept in bath sonicator for proper mixing nearly for 20 min followed by filtration through membrane filter (0.45 µm). The concentration of QCT and AgNPs was measured by the HPLC method,[30] for QCT and validated UV method for Ag^{+} ion.

Antimicrobial efficacy

Antimicrobial efficacy of control, QCT-SMEDDS, AgNPs, AgNPs + QCT, marketed gel and emulgel was tested against *E. coli* and *S. aureus* using disk diffusion method. The prepared media (Mueller Hilton Agar) was inoculated with *S. aureus* and *E. coli* and was carefully striked with sterilized loop. Both bacteria strain were treated with different formulations and placed overnight at 37°C in incubator for appropriate growth of microbes and to avoid the moisture in plates. Zone of inhibition (ZOI) was measured against both bacterial strain for findings the antimicrobial strength.[31]

In-vitro release study in Franz Diffusion Cell (dialysis membrane)

The release rate of emulgel and marketed gel was evaluated using dialysis membrane (Himedia Dialysis membrane with 21.5 mm diameter, 12000-14000 kDa) in Franz diffusion cell. The dialysis bag was activated and washed in running tap water for 3-4 hr. and bag was soaked in diffusion medium (PBS, pH 7.4) for 12 hr. The receptor compartments of Franz diffusion cell having 10 mL media (37 ± 0.5°C) with 300 rpm at continues stirring. The donor compartment was filled with 2 g of sample and at predetermined specific time, 1 mL aliquot was withdrawn from the receptor compartment and the same amount of fresh medium was transferred into receptor compartment to maintain the sink condition. The samples were filtered and analysed by HPLC and UV spectroscopy. To understand the release mechanism of QCT from AgNPs, data were fitted in different release kinetic models like zero-order, first order, Korsmeyer Peppas, Hixon Crowell and Higuchi and the model with the highest value of correlation coefficient (*R2*) was taken as the best fit model.[32]

Ex-vivo Permeation study In Franz Diffusion Cell (Strat-M membrane)

Ex-vivo permeation of emulgel and marketed gel were studied using Strat-M (Merck Millipore, Darmstadt, Germany). The Franz diffusion cells (Dolphin Pharmacy Instruments Pvt. Ltd., India) with the surface area (3.14 cm^2) and positioned at 32°C using a thermostat water bath and receiver compartment volume (10 ml) were used. It was placed between the donor and receptor compartments of Franz diffusion cells. The receptor compartments were filled with (PBS, pH 7.4) media and kept at continuous stirring 500 rpm fixed at 32°C. The donor chambers were filled with an amount (1g) with testing samples in different cells. At the specific time intervals 0.5, 1, 2, 4, 6, 8 hr. of the receptor media (1ml) was withdrawn from each sample and immediately replaced with an equal amount of fresh PBS to maintain the sink condition by avoiding air entrapment and the samples were filtered using membrane filter (0.45µm). All the experiments were performed in triplicate. The released amount of QCT and AgNPs into the receptor media was determined by HPLC and UV techniques respectively. The flux of QCT and AgNPs (Jss, mg/cm^2/hr.) was calculated from the slope of the linear portion of the cumulative amount permeated through the membrane per unit area versus the time plot. In addition, the drug retention was also calculated. Further, using ultrapure water this membrane was washed thrice, dried, chopped, and macerated into ethanol (5 ml) for a day. The solution was filtered and the filtrate of QCT and AgNPs were analysed using a HPLC and UV method,[33] respectively.

In-vivo evaluation of wound healing efficacy

In-vivo wound healing efficacy of emulgel and marketed gel were performed on diabetic induced Swiss albino mice (30-40g) of 7 to 9 weeks old with approved number (IHBTP-11/2014 and IHBT6-MAR2015, DPSRU, New Delhi) as per the CPCSEA guidelines with registration number (1381/GO/ReBiBt/S/2010/CPCSEA).The wound healing efficacy of emulgel, control group and marketed gel were performed on excision diabetic wound model. These tested samples were categorized into three different groups; Diabetic control (group I), marketed gel (group II), and emulgel (group III).

Induction of Diabetes

Streptozotocin (STZ) (4 mg) was administered through intraperitoneal (i.p.) route for next consecutive 4 days for induction of diabetes. During this period, all the mice were provided with 10 % sucrose solution and on the 6th day of experiment, 10 % sucrose solution was replaced with normal water. Last dose of STZ on 9th day, all mice were kept on fasting for 6 hr. to monitor their fasting blood glucose level by using glucometer (AGM-4000 India). Mice having blood glucose level (250 mg/dl) considered diabetic and selected for further study. Animals were anesthetized using diethyl ether before wound creation. One day before the experiment, the dorsal skin hair was trimmed using electric calliper. A circular biopsy punch was used for the creation of excision wound with diameter of (9mm× 3mm) and visualized under microscope.

The emulgel and marketed gel were applied on open wound. Finally, the wound healing parameters like % wound contraction was monitored on four-day interval (0, 4th, 9th, and 14th, day) and % wound contraction was calculated using following formula.[34]

$$\%\ \text{wound contraction} = \frac{A_0 - A_t}{A_t} \times 100$$

Where, A_0 = baseline wound area (initial), and A_t = area of wound at the after-time point

Histopathological examination

To examine the rate of re-epithelization, the skin of euthanized experimental animals was collected and stored in formalin solution (10%) for one day. After washing, samples were fixed into paraffin at (60°C) for 24 hr. The isolated skin tissue was stained with hematoxylin and eosin (H&E) dye and was observed under microscope. The % re-epithelialization was calculated using following formula.[35]

$$\%\ \text{Re-Epitheliazation} = \frac{D_0 - D_u}{D_u} \times 100$$

Where, D_0 is the initial diameter of wound and Du the length of the un-epithelialized tissue.

Stability studies

Emulgel was stored into three different temperature conditions i.e. 2 – 8°C (Refrigerator), 30 ± 2°C/65% RH (room temperature), 40±2°C/75% RH (high temperature) for the period of 6 months as per the ICH-Q1A, R^2 guidelines (ICH, 2003)[36].

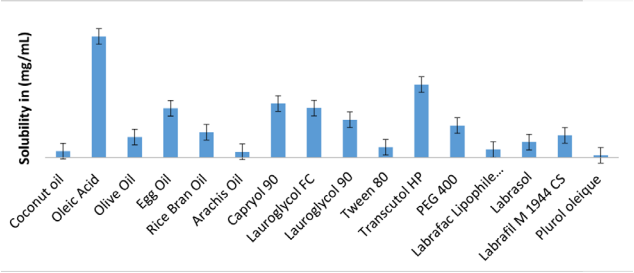


Figure 3: Solubility of QCT into various solvents.

RESULTS AND DISCUSSIONS

Solubility assessment of QCT

The drug showed good solubility in oils, surfactants and co-surfactants as shown (Figure 3) due to lipophilic nature. For a successful SMEDDS formulation unit dose should be soluble in 200µl of the oil. If the quantity of oil exceeds this amount the size of unit dose will exceed the permissible limits. So, drug better solubility in oils successful QCT-SMEDDS could be formulated.

QbD based development and optimization of QCT- SMEEDS

In spite of the promising properties, QCT suffers from poor water solubility and the inability to penetrate the skin. QCT shows water solubility of less than 0.5 µg/ml and higher solubility in polar organic solvents (2mg/ml in ethanol). Due to its poor solubility in water, the amount of QCT available at the site of action from the topical gel is limited, thereby affecting its efficacy. QCT also has a partition coefficient of 1.82 ± 0.32 due to the presence of nonpolar groups in its structure. But despite this log P, QCT polar hydroxyl groups hinder its skin penetration capacity. To overcome the problem of low solubility and permeability at the site of action, efforts were made to formulate QCT-SMEDDS so as to improve its solubility and thereby increase the amount of drug available at the site of action as well as to improve its permeability so that it can penetrate deeper into the wound site for faster wound healing activity.

Identification of QTPP and CQAs for QCT- SMEDDS

The QTPP is a prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account the safety and efficacy of the drug product (Table 1 and 2). CQA is a physical, chemical, biological characteristic that should be within an appropriate limit to ensure the desired product quality.

Quality risk assessment studies of QCT-SMEDDS

A risk assessment study was carried out to identify the Critical Materials Attributes (CMAs) Critical Process Parameters (CPPs) for the evaluation of QCT and excipients. Fish-bone diagram or Ishikawa diagram was depicted (Figure 4) for identifying and grouping of the causes and sub-causes affecting the CQAs of QCT SMEDDS. Subsequently, the primary exercise was performed for identifying the prominent few input variables, from the plausible so many termed as CMAs and CPPs by creating REM. In this technique, the low, medium and high levels were assigned to each unit operation.[37] The REM constructed for QCT -SMEDDS (Table 3). The Table 4 illustrates that the amount of oil, surfactant and co-solvent are high- risk parameters for the CQAs i.e., globules size, % transmittance, $Q8_{hr}$ and % permeation. Further, initial risk assessment studies employing REM also verified that the MAs like amount of oil, surfactant and co-surfactant were highly critical owing to the high risk allied with them on CQAs.

Construction of Pseudo-ternary phase diagram

The pseudo-ternary phase diagrams constructed using oleic acid, T–HP, and PEG 400 with the occurrence of a stable ME area that is the combination of Oleic acid: (T-HP: PEG 400) S_{mix} (1:1) had maximum water uptake capacity, resulting in maximum ME area for the QCT-SMEDDS shown in Figure 5. However, a considerable reduction in the ME region was noticed, when the ratio of Smix was increased to 1:2. This may be attributed to the lesser concentration of surfactant available for decreasing the interfacial tension between the oil globules owing to the presence of higher concentration of co-solvent i.e., PEG 400. In addition, no significant ME region was observed, as probably the surfactant was not able to sufficiently reduce the o/w interfacial tension in the reduced concentration of the co-surfactant.[38] Further, when the S_{mix} ratio was further reduced to 1:0.5 a decrease in the ME region was observed vis-a-vis 1:1, indicating that the optimum ME region has been achieved in the latter case. In addition, it was noticed that the presence of drug in the pseudo-ternary phase diagram experiments neither produce any change in the ME area nor affected the self-emulsification performance of the system. So, the pseudo-ternary phase diagram (s) showed that the Oleic acid: (T-HP: PEG 400) S_{mix} (1:1) resulted maximum and stable ME area. Further, this ratio was considered in selecting the levels of these (MAs) during the screening studies.

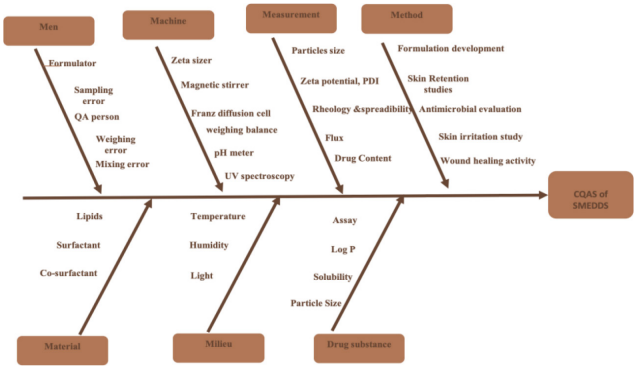


Figure 4: Ishikawa Fishbone diagram for QCT-SMEDDS.

Formulation and evaluation of QCT- SMEDDS by D-optimal mixture design

Based on the results of solubility studies and ternary plots diagram, it was decided to formulate (QCT-SMEDDS) applying an optimization experimental design i.e., 3-factor 3-level D-optimal mixture design as Table 4. In pharmaceutical formulations with multiple excipients, the characteristics of the finished product usually depend not so much on the quantity of each substance present but also on their proportions. The mixture designs have often been described as experimental designs for formulation and optimization. However, in case the domain is irregular in shape, the design has been recommended to be used.[39] The designs are non-classical experimental designs generated using computer algorithms, based on the D-optimum criterion, i.e., the principle of minimization of variance and covariance

Table 3: Initial Risk Estimation Matrix (REM) for SMEDDS of QCT.

Drug Product CQA	Conc. of Oil US	Conc. of Surfactant US	Conc. of co Surfactant US	Water US	ME stirring Time US	ME stirring Speed US
Globule Size (nm)	3 4 High	2 4 High	2 4 High	2 3 Medium	2 3 Medium	2 3 Medium
Zeta potential	3 4 High	2 3 Medium	2 3 Medium	2 3 Medium	1 3 Low	1 3 Low
PDI	3 4 High	2 4 High	2 4 High	2 3 Medium	1 3 Low	1 3 Low
%Transmittance	3 4 High	3 4 High	3 4 High	2 3 Medium	1 3 Low	1 3 Low
Q 8_{hr}	3 4 High	3 4 High	3 4 High	2 3 Medium	1 3 Low	1 3 Low
% Permeation	3 4 High	3 4 High	3 4 High	1 3 Low	1 3 Low	1 3 Low

of parameters. One of the ways of obtaining such a design is by the use of exchange algorithms using computers. These designs can be continuous i.e., more design points can be added to it subsequently, and the experimentation can be carried out in stages.[20]

Response surface mapping

The understanding of the dependence, inter-dependence and co-variation among the variables studied and their interactions are facilitated by constructing response surface diagrams to map the responses over the entire experimental domain. The 3-D response surfaces and their 2-D contour plots are depicted in Figure 6-9. Figure 6(a) shows a remarkable influence of all the studied CMAs, i.e., PEG400, Oleic acid and T- HP on the CQA, i.e., globule size (nm). An increase in the value of globule size was observed with increase in the levels of oleic acid, whereas an opposite trend was observed with T-HP. The combined interaction effect of all the three excipients revealed the lowest value of globule size at low levels of oleic acid, PEG 400 and intermediate levels of T- HP, as is clearly observed from the Figure 6 (b). As shown in Figure 7 (a) the 3D response surface plot for transmittance exhibited low transmittance value with increase in the levels of oleic acid, whereas an opposite trend was observed with T-HP and PEG 400. The combined interaction effect of all the three excipients revealed the highest value of transmittance at low levels of oleic acid, PEG 400 and high levels of T - HP, as is clearly observed from the Figure 7 (b). As depicted in Figure 8 (a) for $Q8_{hr}$ exhibited a curvilinear decreasing trend on increasing the concentration of oleic acid, while PEG 400 showed a slight decreasing trend. On the other hand, with increase in the T-HP concentration, a curvilinear increase in the values of $Q8_{hr}$ min was initially observed followed by a plateau phase. The higher values of $Q8_{hr}$ were observed, at higher levels of T-HP, and lower levels of Oleic acid and PEG 400, respectively as shown in Figure 8 (b).The % permeation effect was

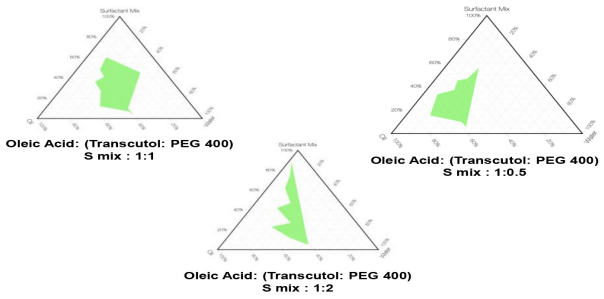


Figure 5: Phase behaviour observed using Oleic acid: (Transcutol HP: PEG 400) S_{mix} (1:1), Oleic acid: (Transcutol HP: PEG 400) S_{mix} (1:0.5) and Oleic acid: (Transcutol HP: PEG 400) S_{mix} (1:2).

Table 4: Summary of overall parameters of all the formulations prepared as per the experimental design.

	Component 1	Component 2	Component 3	Response 1	Response 2	Response 3	Response 4
Run	A: oil	B: Surfactant	C: Co surfactant	Globule size	Transmittance	Q_8 hr	% permeate
				nm	%	%	%
1	317.098	365.585	317.317	125	96	93.1	21.65
2	400	350	250	196	82	79.1	4.12
3	326.772	395.755	277.473	138	94	91.6	28.54
4	300	400.083	299.917	108	98	95.9	37.34
5	400	350	250	194	83	78.7	4.64
6	350.556	350.64	298.804	162	89	86.21	13.67
7	300	450	250	98	97	97.3	47
8	300	400.083	299.917	106	98	96.1	37.87
9	349.344	400.08	250.576	148	91	87.9	26.78
10	350.556	350.64	298.804	170	88	86.98	13.21
11	300	350	350	118	97	94.3	18.56
12	367.247	366.793	265.96	176	86	83.67	17.54
13	349.344	400.08	250.576	151	91	88.4	38.1
14	300	424.668	275.332	102	97	96.8	42
15	350.556	350.64	298.804	168	88	86.87	13.98
16	324.952	425.048	250	133	95	92.12	42.33

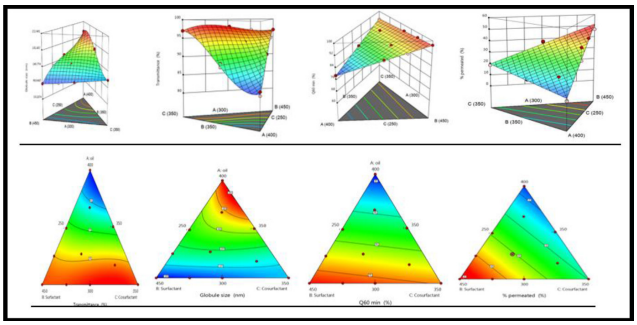


Figure 6: 3D and 2D-response surface plots portraying the influence of Oleic acid, PEG 400 and Transcutol HP A) globule size (Dnm)B) Transmittance C) TQ60 min D) % Permeation.

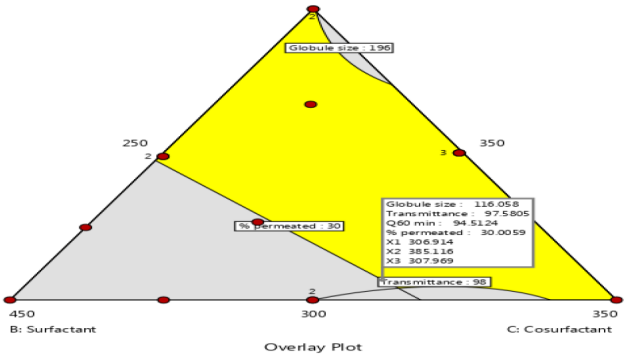


Figure 7: 2-D overlay plot indicating design space for QCT-SMEDDS.

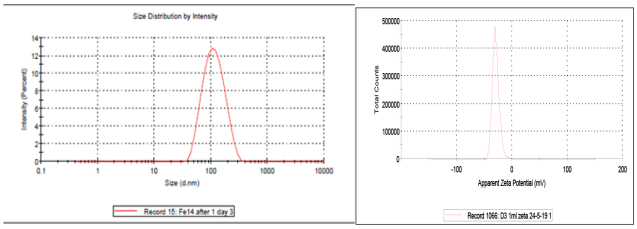


Figure 8: A) Particle size of QCT-SMEDDS B) Zeta Potential of QCT-SMEDDS.

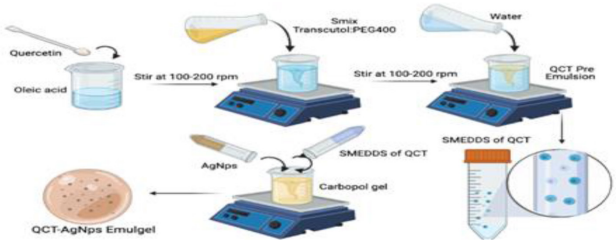


Figure 9: Schematic representation for the preparation of emulgel.

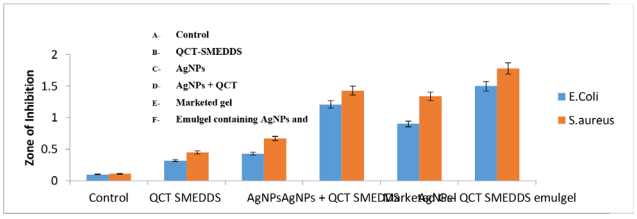


Figure 10: Antimicrobial activity of APIs, optimized AgNPs-QCT formulations and marketed formulation.

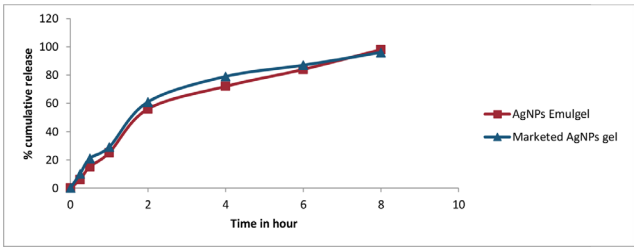


Figure 11: % Cumulative release of AgNPs.

shown in Figure 9 (a) with a remarkable influence of oleic acid as compare to T-HP. A decrease in the values of permeation was observed with increase in the level of oleic acid, while T-HP shows negligible influence on the value of % permeation. The combined interaction effect of all the three excipients exhibited decreasing values of % permeation at higher levels of oleic acid and low levels of T-HP and PEG 400,[21] as is clearly evident from the given Figure 9 (b).

Search for optimized formulation

The optimized formulation was embarked upon by mathematical optimization using numerical desirability function by trading off of various CQAs for attaining the desired goals, i.e., minimization of globule size, maximum transmittance, $Q8_{hr}$, and minimum of % permeation. The constraints were further narrowed down to finally demarcate the design space in the overlay plot, as shown in Figure 10. The overlay contour plot between the three factors across 2D-experimental domains shows the desirable optimal design space region in yellow color surrounded with grey color region, also called as the knowledge space.[40] The optimized formulation, comprising of oleic acid: 306.91 mg, PEG 400: 307.96 mg and T- HP: 385.11 mg, exhibited globules size (116.05 nm), % permeation (30.00 μgml^{-1}), % transmittance (97.58%), and $Q8_{hr}$ (94.51).

Characterization of optimized QCT- SMEDDS

Globule size and zeta potential

The globule size and zeta potential affect the biopharmaceutical, physicochemical, drug release, and stability of the QCT- SMEDDS. The result showed that

as the Smix ratio increases, the globule size decreases. Zeta potential values of QCT-SMEDDS dispersion were dependent on the ratios of S_{mix} and oil.(22)The globule size and PDI of prepared QCT- SMEDDS was found to be 101.7 ± 5.67nm, PDI 0.172 respectively (Figure 11) whereas the zeta potential of QCT-SMEDDS was found to be -31.7mV indicates a high negative surface charge on QCT-SMEDDS which in turn indicates higher stability because of the anticipated surface repulsion between similarly charged particles (Figure 12).

Transmittance (%) and thermodynamic stability

Among all the design matrix runs the utmost percentage transmittance was found to be (99.6 ±0.3). The transmittance (%) value close to 100% is a sign of clear and homogenous and transparent ME formation. The tested samples were found to be thermodynamically stableas shown in Table 5. The heating-cooling cycles may cause coalescence in oil particles by halting the surfactant concentration as well as may disturb the dispersed and continuous phases result in ME cracking.[23]

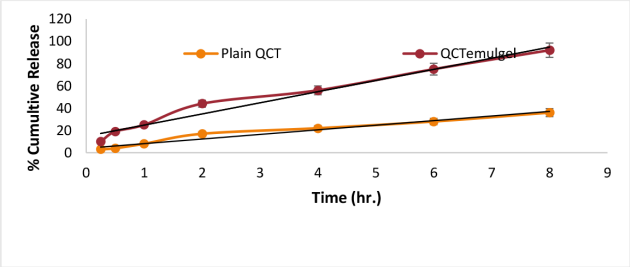


Figure 12: % Cumulative release of QCT.

Table 5: Thermodynamic stability testing of the optimized QCT- SMEDDS.

Tests	Observations of change in formulation properties
Heating cooling cycle	Transparent without any turbidity
Centrifugation	Phase separation was not noticed
Freeze thaw test	Absence of any type precipitation, colour appearance and turbidity

Cloud point determination

Formulations should exhibit a cloud point greater than 37.5°C to retain their self-emulsification property. In this study, the cloud point value as mean ± SD (n=3) of the QCT-SMEDDS was found to be 65.0 ± 0.34°C, which signifies QCT-SMEDDS stability at physiologic temperature.

Characterization and evaluation (Emulgel vs. Marketed gel)

For comparing the effectiveness of the prepared nano formulations i.e. emulgel containing was compared to marketed gel for various characterization parameters.

Physical parameters

The physical parameters (colour, extrudability, homogeneity, viscosity, and surface appearance) were described in Table 6. Viscosity of gels is directly proportional to the concentration and molecular weight of the polymer. The Carbopol-934 within 1% concentration showed better results w.r.t. Viscosity of prepared emulgel while the oil concentration has the negative impact on gel viscosity. Moreover, the hydrophilic nature of T-HP also has positive effect on viscosity of emulgel due to its hydrated chain length linked together with strong hydrogen bonding. The extruded amount of formulation from the tube after applying force was found to be very good. The grading was done for extrudability of the formulations as (+ fair, ++ good and +++ excellent).[29]

Drug Content

The drug content of emulgel and marketed gel are described in Table 7 which was carried out by sophisticated technique i.e. UV spectroscopy and HPLC method.

Antimicrobial efficacy

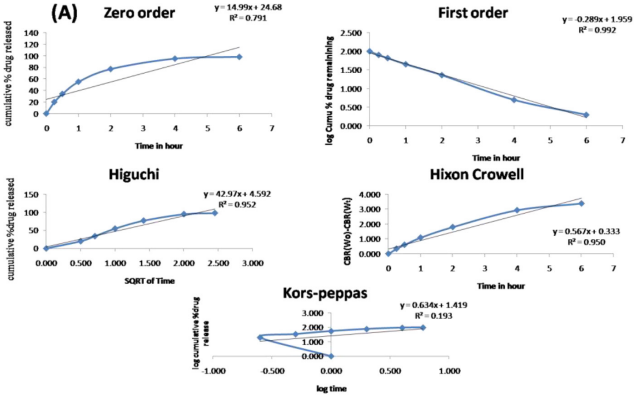
Emulgel exhibited the highest ZOI in both E. coli and S. aureus as compared with the others formulations as depicted in (Figure 13). Synergetic effect was observed

Table 6: Physical parameters (colour, extrudability, homogeneity and surface appearance) of Formulations.

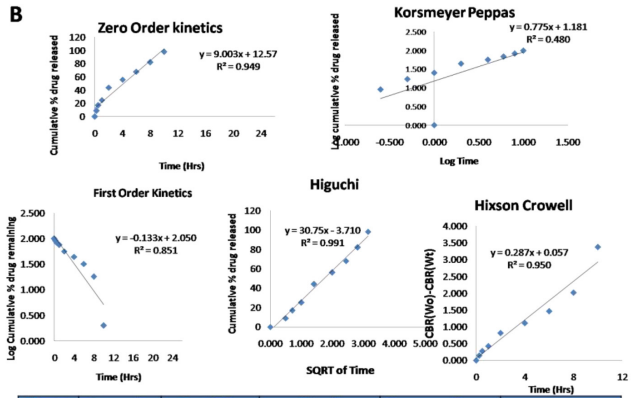
Formulations	Colour	Extrudability	Homogeneity	Viscosity	Surface appearance
Emulgel containing AgNPs and QCT- SMEDDS	Yellowish bright	+++	Very Good	123.4 mPas	Smooth
Marketed formulation containing AgNPs	White	+++	Very Good	121.5 mPas	Smooth

(+ fair, ++ good and +++ Excellent)

Table 7: Drug content.			
Sr.No	**Formulation**	**% Drug content**	
1	Emulgel Containing AgNPs and QCT -SMEDDS	QCT	99.13± 0.13
		Ag+	99.78 ± 0.29
2	Marketed formulation containing AgNPs	Ag+	99.47± 0.31



Model	Zero- order	First Order	Higuchi	Korsmeyer Peppas	Hixon Crowell
R^2	0.791	0.992	0.952	0.950	0.193



Model	Zero- order	First Order	Higuchi	Korsmeyer Peppas	Hixon Crowell
R^2	0.949	0.851	0.991	0.480	0.950

Figure 13: Release kinetics fit model for A); AgNPs and, B); QCT from optimize AgNPs – QCT emulgel.

when QCT and AgNPs were given in combined form as compared to alone. The higher ZOI of emulgel could be due to the high solubility of QCT in the form of SMEDDS. In previous studies, QCT has already been reported as a potential antimicrobial agent. In the combination form of AgNPs with QCT showed significant antimicrobial activity as compare to multidrug-resistant bacterial diseases (S. aureus and Shigella).When compared with marketed gel the emulgel showed better microbial. Our results suggested QCT-SMEDDS loaded into AgNPs and embedded into emulgel matrices shows better antimicrobial efficacy as compared with others formulations against broad-spectrum bacteria.[41]

In-vitro release study using Franz Diffusion cell (dialysis membrane)

Drug release studies from gel are not only a significant step during the developmental of new formulation but also a usual quality control test for assuring uniformity of the finished product. Figure 14,15 displayed the *in-vitro* release studies of emulgel (QCT-SMEDDS and QCT- AgNPs), plane QCT and marketed gel. The findings indicated that QCT and AgNPs from all the formulations were released in a sustained manner. The QCT and AgNPs encapsulated within the core of matrices was released at the end of 8 hr. and a net drug release of AgNPs was 92% from the emulgel and marketed gel whereas, the release of plain QCT was 30% and 95% from the QCT-emulgel which could be due to the enhanced solubility of QCT which is encapsulated in the form of QCT-SMEDDS in the optimized formulation. This sustained release behaviour could be due retarding effect of the polymeric matrix of gelling agent i.e., Carbopol-934. This slow-release behaviour of the gel matrices is beneficial for topical application which could provide long-term effect after single application. Furthermore, to understand the AgNPs, and QCT release mechanism from the emulgel and marketed gel release pattern was fitted into various kinetic models. From kinetic models, it was observed that AgNPs from emulgel followed the first-order model with R^2 value 0.992 (Figure 16-a,b) whereas QCT from

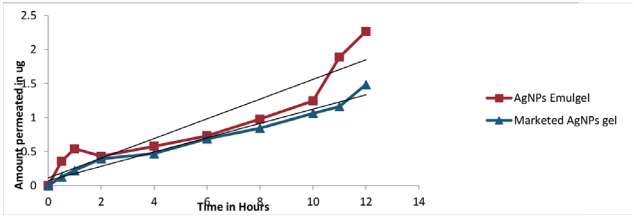


Figure 14: Amount of Ag+ permeated from AgNPs emulgel, and marketed AgNPs gel.

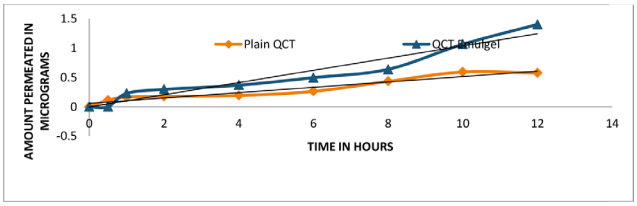


Figure 15: Amount of QCT permeated from Plain QCT and QCT- emulgel.

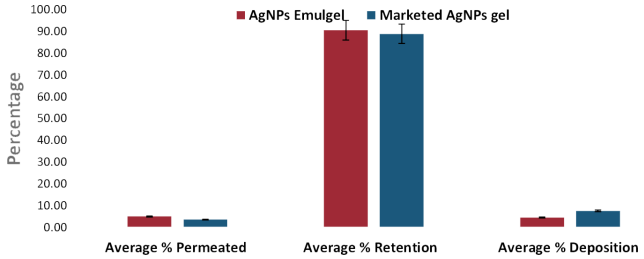


Figure 16: Average Percent retention of Ag+ through Strat-M.

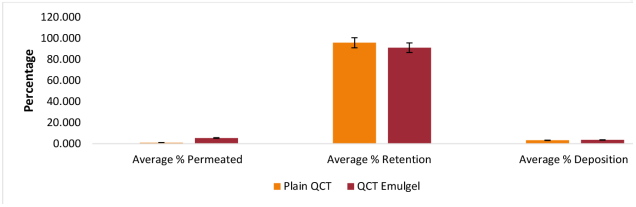


Figure 17: Average Percent retention of QCT through Strat-M.

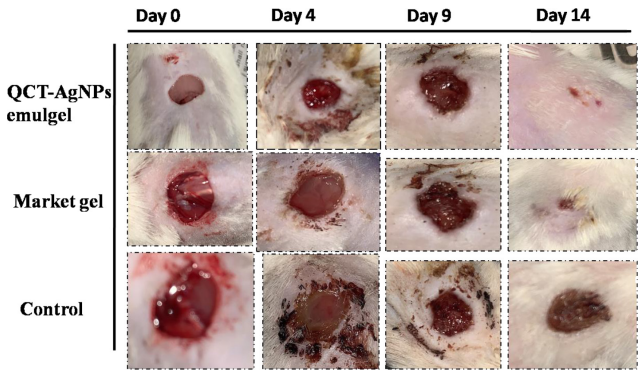
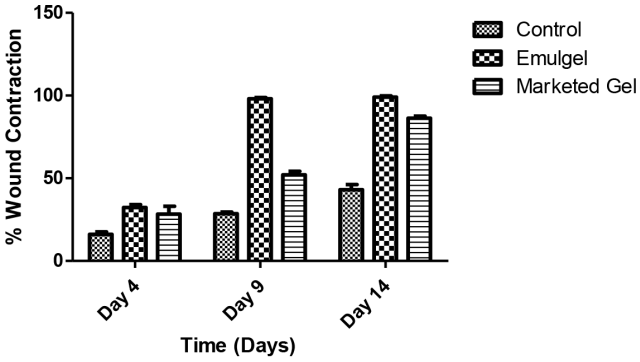


Figure 18: Representative images of excision wound tissue sections of diabetic mice group treated with Control, emulgel, and marketed gel on day 0 to day 14.

the emulgel followed Higuchi model with R^2 value 0.997 showing that the drug release process was controlled by diffusion.

Ex-vivo permeation study in Franz Diffusion Cell (Strat-M membrane)

The permeation potential of emulgel, marketed gel and plain QCT were studied using Strat-M. It was employed between the donor and receptor compartment of Franz diffusion cells. The main advantage of Strat-M is low variability resulting in more reliable data. The permeation profiles of AgNPs and QCT through Strat-M from emulgel and marketed gelare shown (Figure 17 and 18) respectively. In addition, the amount of AgNPs and QCT retained in the membrane from various formulations at the end of the study period is illustrated (Figure 19 and 20) respectively. Further, the



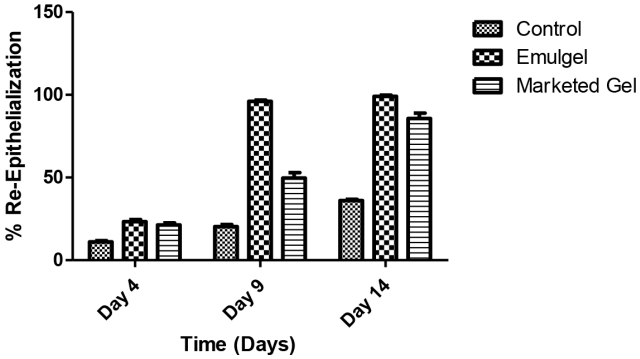


Figure 19: A) % Wound closure and B) % Re -Epithelialization for *in -vivo* wound healing experiments.

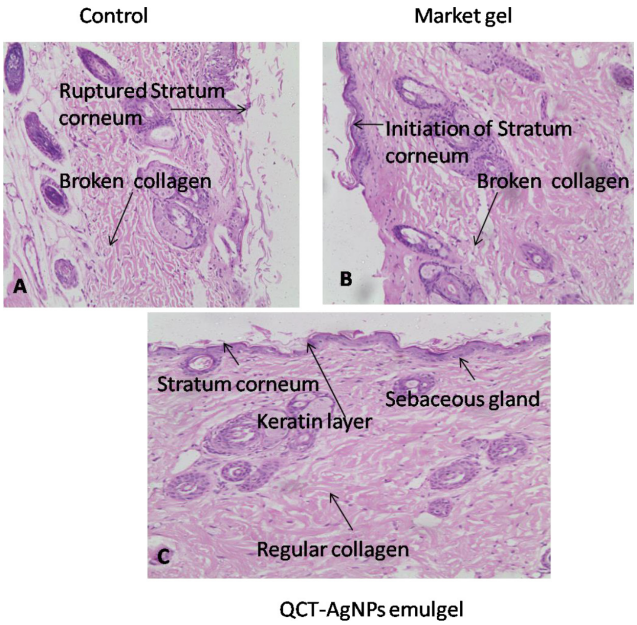


Figure 20: Histopathological photographs of epithelial tissues A) Control B) marketed gel and C) Emulgel.

flux values (Jss, mg/cm^2/hr.) for both AgNPs and QCT from all the formulations are also shown (Table 8). For AgNPs there was not much difference in the release rate of emulgel and marketed formulations. However, only

30% of plain QCT drug was released at the end of 8 hr. whereas, 95% was released from emulgel. This might be due to the formation of SMEDDS as we know QCT is a hydrophobic API and exhibits poor solubility which is evident from the release rate of plain QCT. Emulgel exhibited around 96.75%retention through Strat-M. It was well within the limit for permeation of topical formulation i.e. less than 30%.[42]

In-vivo wound healing study

The wound healing treatment of the emulgel was completed in 14 days shown (Figure 20A). It might be due to the improved solubility of QCT in the form of QCT-SMEDDS which were encumbered in emulgel and leads to provide better permeation of the drug at therapeutic site. It was observed that mice treated with QCT-AgNPs emulgel significantly recovered from wound as compared with diabetic control and marketed gel after 14 days of the treatment. The wound contraction activity was found much better with QCT-AgNPs emulgel (group-III) in contrast to diabetic control (group-I) and marketed gel (group-II) (Figure 20-A). When compared with marketed gel, both the formulations showed higher wound contraction (%) suggesting the synergetic effect of AgNPs-QCT in our formulation while marketed gel comprised of AgNPs only. Various studies have been conducted to elucidate the mechanism of action of QCT in diabetic wound healing[43] Both QCT and AgNPs have been reported to have antibacterial effect via

Table 8: Flux of AgNPs and QCT through Strat-M.

Formulations	Flux of AgNPs	Flux of QCT
QCT- AgNPs emulgel	0.227129338	0.364353312
Marketed AgNPs gel	0.195583596	---
Plain QCT	---	0.070977918

Table 9 (A): Stability data of AgNPs-QCT emulgel. Data are expressed as mean ± SD (*n* = 3).

	Storage Condition	0 Day	1st month	3rd Month	6th month
% Assay	30±2°C/65%RH (QCT)	98.46±0.23	98.46±0.23	97.32±0.98	97.32±0.98
	30±2°C/65%RH (Ag)	99.12±0.11	98.65±0.21	97.32±0.98	98.24±0.82
	40±2°C/75±5%RH (QCT)	98.46±0.23	98.65±0.21	97.32±0.98	98.33±0.76
	40±2°C/75±5%RH (Ag)	99.12±0.11	98.54±0.6	99.16±0.32	101.14±0.81
	2-8°C (Refrigerator) (QCT)	98.46±0.23	98.15±0.3	98.21±0.46	98.13±0.68
	2-8°C (Refrigerator) (Ag)	99.12±0.11	98.52±0.71	99.31±0.81	99.43±0.32

Table 9 (B): Other important stability parameters of AgNPs- QCT emulgel.

Parameter	Storage Condition	0 Day	1st month	3rd Month	6th month
Visual Appearance	30±2°C/65%RH	Bright yellow	Bright yellow	Bright yellow	Bright yellow
	40±2°C/75±5%RH	Bright yellow	Bright yellow	Bright yellow	Bright yellow
	2-8°C (Refrigerator)	Bright yellow	Bright yellow	Bright yellow	Bright yellow
pH	30±2°C/65%RH	6.5	6.4	6.2	6.4
	40±2°C/75±5%RH	6.5	6.0	5.7	6.0
	2-8°C (Refrigerator)	6.5	6.2	6.1	6.3
Leakage	30±2°C/65%RH	Not Found			
	40±2°C/75±5%RH				
	2-8°C (Refrigerator)				
Texture	30±2°C/65%RH	Smooth	Smooth	Smooth	Smooth
	40±2°C/75±5%RH	Smooth	Smooth	Smooth	Smooth
	2-8°C (Refrigerator)	Smooth	Smooth	Smooth	Smooth
Phase separation	30±2°C/65%RH	Not found			
	40±2°C/75±5%RH				
	2-8°C (Refrigerator)				

destroying bacterial cell wall, changing cell permeability, and affecting nucleic acid and protein synthesis.

Histopathological examination of skin was performed and observed the higher generation of epithelium and dermis layer. In addition, rate of re-epithelization was noted significantly in emulgel with comparison to diabetic control group (Figure 20, B). Comparatively, AgNPs-QCT emulgel showed higher rate of re-epithelization w.r.t marketed gel. In addition, a good sign of wound healing was observed in 14 days with emulgel indicating the synergistic effect of QCT and AgNPs. The rate of re-epithelization and other healing parameters of (A) Control, (B) marketed gel, and (C) emulgel (Figure 20-A, B, C). This could be due to antioxidant and anti-inflammatory activity of QCT and in addition anti-inflammatory and antimicrobial activity of AgNPs.[35] More importantly, QCT have been reported to enhance fibroblast cells proliferation and cell migration towards wound which could be another plausible mechanism in better wound healing in compared to marketed gel and diabetic control group. Taken together, our results show the synergetic effect of QCT and AgNPs when QCT was loaded into AgNPs and dispersed in the emulgel matrices.

Stability studies

The developed AgNPs- QCT emulgel showed very good stability at all three different conditions. No phase separation or any change in visual appearance was observed. The data for stability testing are illustrated in Table 7 A, B.

CONCLUSION

The present study aimed to improve the QCN solubility via developing QCT-SMEDDS and further incorporation into carbopol-934 gel loaded with AgNPs and hence formulation of QCN-AgNPs emulgel employing Quality by Design (QbD) paradigm. Hence, the QCN-SMEDDS based QCN-AgNPs emulgel has been projected as a potential substitute for the topical delivery of QCN in the treatment of diabetic foot ulcer.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to DPSRU, New Delhi for providing with a working environment.

CONFLICT OF INTEREST

The authors report no conflict of interest.

ABBREVIATIONS

QCT: Quercetin; **SMEDDS:** Self-micro emulsifying drug delivery system; **AgNPs:** Silver nanoparticles; **CQAs:** Critical Qualities Attributes; **T-HP:** Transcutol HP; **PDI:** Dispersive index; **DFU:** Diabetic foot ulcer; **DM:** Diabetes Mellitus; **ECM:** Extracellular matrix protein; **TNF-α:** Tumour necrosis factor alpha; **IL-1β:** Interleukin -1-beta; **IL-10:** Increased Interleukin 10; **VEGF:** Vascular endothelial growth factor; **TGF-β1:** Transforming growth factor beta; **SC:** Stratum corneum; **ME:** Micro emulsion; **CP-934:** Carbopol 934; **Q8hr:** Release in 8 hours; **PBS:** Phosphate buffer solution; **ZOI:** Zone of inhibition; **QTPP:** Quality Target Product Parameters; **CMAs:** Critical Material Attributes; **CPPs:** Critical Process Parameters.

Compliance with ethical standards

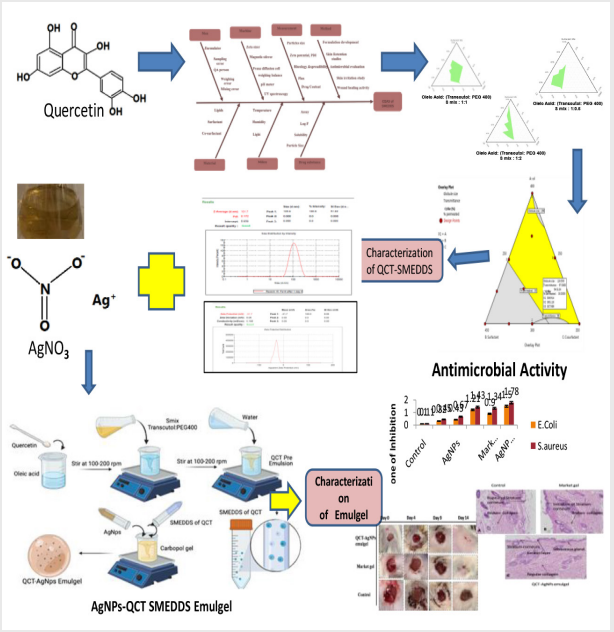
The animal study protocol was approved by the "Delhi Pharmaceutical Sciences and Research University, New Delhi, India Animal Ethics Committee" (Register Number: 1381/GO/ReBiBt/S/2010) and "CPCSEA, Government of India".

REFERENCES

1. Edwards R, Harding KG. Bacteria and wound healing. Curr Opin Infect Dis. 2004;17(2):91-6. doi: 10.1097/00001432-200404000-00004, PMID 15021046.
2. Jneid J, Lavigne JP, La Scola B, Cassir N. The diabetic foot microbiota: A review. Hum Microbiome J. 2017;5-6:1-6. doi: 10.1016/j.humic.2017.09.002.
3. Jupiter DC, Thorud JC, Buckley CJ, Shibuya N. The impact of foot ulceration and amputation on mortality in diabetic patients. I: From ulceration to death, a systematic review. Int Wound J. 2016;13(5):892-903. doi: 10.1111/iwj.12404, PMID 25601358.
4. Vijayakumar V, Samal SK, Mohanty S, Nayak SK. Recent advancements in biopolymer and metal nanoparticle-based materials in diabetic wound healing management. Int J Biol Macromol. 2019;122:137-48. doi: 10.1016/j.ijbiomac.2018.10.120, PMID 30342131.
5. Everett E, Mathioudakis N. Update on management of diabetic foot ulcers. Ann N Y Acad Sci. 2018;1411(1):153-65. doi: 10.1111/nyas.13569, PMID 29377202.
6. Gosain A, DiPietro LA. Aging and wound healing. World J Surg. 2004;28(3):321-6. doi: 10.1007/s00268-003-7397-6, PMID 14961191.
7. Nagula RL, Wairkar S. Recent advances in topical delivery of flavonoids: A review. J Control Release. 2019;296:190-201. doi: 10.1016/j.jconrel.2019.01.029, PMID 30682442.
8. Wang Y, Shao T, Wang J, Huang X, Deng X, Cao Y, *et al*. An update on potential biomarkers for diagnosing diabetic foot ulcer at early stage. Biomed Pharmacother. 2021;133:110991. doi: 10.1016/j.biopha.2020.110991, PMID 33227713.
9. Hatahet T, Morille M, Hommoss A, Devoisselle JM, Müller RH, Bégu S. Quercetin topical application, from conventional dosage forms to nanodosage forms. Eur J Pharm Biopharm. 2016;108:41-53. doi: 10.1016/j.ejpb.2016.08.011, PMID 27565033.
10. Rothwell JA, Day AJ, Morgan MRA. Experimental determination of octanol–water partition coefficients of quercetin and related flavonoids. J Agric Food Chem. 2005;53(11):4355-60. doi: 10.1021/jf0483669, PMID 15913295.
11. Salomoni R, Léo P, Montemor AF, Rinaldi BG, Rodrigues MFA. Antibacterial effect of silver nanoparticles in *Pseudomonas aeruginosa*. Nanotechnol Sci Appl. 2017;10:115-21. doi: 10.2147/NSA.S133415, PMID 28721025.

12. Kalishwaralal K, BarathManiKanth S, Pandian SRK, Deepak V, Gurunathan S. Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. Colloids Surf B Biointerfaces. 2010;79(2):340-4. doi: 10.1016/j.colsurfb.2010.04.014, PMID 20493674.
13. Burdușel AC, Gherasim O, Grumezescu AM, Mogoantă L, Ficai A, Andronescu E. Biomedical applications of silver nanoparticles: An up-to-date overview. Nanomaterials (Basel). 2018;8(9):1-25. doi: 10.3390/nano8090681, PMID 30200373.
14. Senthil B, Devasena T, Prakash B, Rajasekar A. Non-cytotoxic effect of green synthesized silver nanoparticles and its antibacterial activity. J Photochem Photobiol B. 2017;177(October):1-7. doi: 10.1016/j.jphotobiol.2017.10.010, PMID 29028495.
15. Kamboj S, Rana V. Quality-by-design based development of a self-microemulsifying drug delivery system to reduce the effect of food on nelfinavir mesylate. Int J Pharm. 2016;501(1-2):311-25. doi: 10.1016/j.ijpharm.2016.02.008, PMID 26854426.
16. Peltola S, Saarinen-Savolainen P, Kiesvaara J, Suhonen TM, Urtti A. Microemulsions for topical delivery of estradiol. Int J Pharm. 2003;254(2):99-107. doi: 10.1016/s0378-5173(02)00632-4, PMID 12623186.
17. Valenta C, Schultz K. Influence of carrageenan on the rheology and skin permeation of microemulsion formulations. J Control Release. 2004;95(2):257-65. doi: 10.1016/j.jconrel.2003.11.020, PMID 14980774.
18. Aithal GC, Nayak UY, Mehta C, Narayan R, Gopalkrishna P, Pandiyan S, *et al*. Localized *in situ* nanoemulgel drug delivery system of quercetin for periodontitis: Development and computational simulations. Molecules. 2018;23(6). doi: 10.3390/molecules23061363, PMID 29882751.
19. Fahmy UA, Ahmed OAA, Hosny KM. Development and evaluation of avanafil self-nanoemulsifying drug delivery system with rapid onset of action and enhanced bioavailability. AAPS Pharm Sci Tech. 2015;16(1):53-8. doi: 10.1208/s12249-014-0199-3, PMID 25168449.
20. Namjoshi S, Dabbaghi M, Roberts MS, Grice JE, Mohammed Y. Quality by design: Development of the quality target product profile (QTPP) for semisolid topical products. Pharmaceutics. 2020;12(3):1-11. doi: 10.3390/pharmaceutics12030287, PMID 32210126.
21. Beg S, Katare OP, Saini S, Garg B, Khurana RK, Singh B. Solid self-nanoemulsifying systems of olmesartan medoxomil: Formulation development, micromeritic characterization, *in vitro* and *in vivo* evaluation. Powder Technol. 2016;294:93-104. doi: 10.1016/j.powtec.2016.02.023.
22. Sharma G, Wilson K, Van der Walle CF, Sattar N, Petrie JR, Ravi Kumar MNV. Microemulsions for oral delivery of insulin: Design, development and evaluation in streptozotocin induced diabetic rats. Eur J Pharm Biopharm. 2010;76(2):159-69. doi: 10.1016/j.ejpb.2010.07.002, PMID 20655382.
23. Singh AK, Chaurasiya A, Awasthi A, Mishra G, Asati D, Khar RK, *et al*. Oral bioavailability enhancement of exemestane from self-microemulsifying drug delivery system (SMEDDS). AAPS Pharm Sci Tech. 2009;10(3):906-16. doi: 10.1208/s12249-009-9281-7, PMID 19609837.
24. Akula S, Gurram AK, Devireddy SR. Self-microemulsifying drug delivery systems: An attractive strategy for enhanced therapeutic profile. Int Sch Res Notices. 2014;2014:964051. doi: 10.1155/2014/964051, PMID 27382619.
25. Elnaggar YSR, El-Massik MA, Abdallah OY. Self-nanoemulsifying drug delivery systems of tamoxifen citrate: Design and optimization. Int J Pharm. 2009;380(1-2):133-41. doi: 10.1016/j.ijpharm.2009.07.015, PMID 19635537.
26. Banne SV, Patil MS, Kulkarni RM, Patil SJ. Synthesis and characterization of silver Nano particles for EDM applications. Mater Today Proc. 2017;4(11):12054-60. doi: 10.1016/j.matpr.2017.09.130.
27. Miastkowska M, Kulawik-Pióro A, Szczurek M. Nanoemulsion gel formulation optimization for burn wounds: Analysis of rheological and sensory properties. Processes. 2020;8(11):1-26. doi: 10.3390/pr8111416.
28. Worachun N, Opanasopit P, Rojanarata T, Ngawhirunpat T. Development of ketoprofen microemulsion for transdermal drug delivery. AMR. 2012;506:441-4. doi: 10.4028/www.scientific.net/AMR.506.441.
29. Aiyalu R, Govindarjan A, Ramasamy A. Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model. Braz J Pharm Sci. 2016;52(3):493-507. doi: 10.1590/s1984-82502016000300015.
30. Thakur K, Mahajan A, Sharma G, Singh B, Raza K, Chhibber S, *et al*. Implementation of Quality by Design (QbD) approach in development of silver sulphadiazine loaded egg oil organogel: An improved dermatokinetic profile and therapeutic efficacy in burn wounds. Int J Pharm. 2020;576:118977. doi: 10.1016/j.ijpharm.2019.118977, PMID 31870953.
31. Mekkawy AI, El-Mokhtar MA, Nafady NA, Yousef N, Hamad MA, El-Shanawany SM, *et al*. *In vitro* and *in vivo* evaluation of biologically synthesized silver nanoparticles for topical applications: Effect of surface coating and loading into hydrogels. Int J Nanomedicine. 2017;12:759-77. doi: 10.2147/IJN.S124294, PMID 28176951.
32. Fathalla D, Youssef EMK, Soliman GM. Liposomal and ethosomal gels for the topical delivery of anthralin: Preparation, comparative evaluation and clinical assessment in psoriatic patients. Pharmaceutics. 2020;12(5). doi: 10.3390/pharmaceutics12050446, PMID 32403379.
33. Mohamed LA, Kamal N, Elfakhri KH, Ibrahim S, Ashraf M, Zidan AS. Application of synthetic membranes in establishing bio-predictive IVPT for testosterone transdermal gel. Int J Pharm. 2020;586(March):119572. doi: 10.1016/j.ijpharm.2020.119572.
34. Nehete MN, Nipanikar S, Kanjilal AS, Kanjilal S, Tatke PA. Comparative efficacy of two polyherbal creams with framycetin sulfate on diabetic wound model in rats. J Ayurveda Integr Med. 2016;7(2):83-7. doi: 10.1016/j.jaim.2015.09.004, PMID 27449205.
35. Singla R, Soni S, Patial V, Kulurkar PM, Kumari ASM, Padwad YS, *et al*. Cytocompatible anti-microbial dressings of *Syzygium cumini* cellulose nanocrystals decorated with silver nanoparticles accelerate acute and diabetic wound healing [sci rep] [internet]. Sci Rep. 2017;7(1):10457. doi: 10.1038/s41598-017-08897-9, PMID 28874762.
36. Vol. 41. ICH. In: International Conference on Harmonization. Geneva: International Federation of Pharmaceutical Manufacturers Associations; 2003. Q1A (R2) Stability Testing of New Drug Substances and Products.
37. Beg S, Kaur R, Khurana RK, Rana V, Sharma T, Singh B. QbD-based development of cationic self-nanoemulsifying drug delivery systems of paclitaxel with improved biopharmaceutical attributes. AAPS Pharm Sci Tech. 2019;20(3):118. doi: 10.1208/s12249-019-1319-x, PMID 30790136.
38. Singh B, Singh R, Bandyopadhyay S, Kapil R, Garg B. Optimized nanoemulsifying systems with enhanced bioavailability of carvedilol. Colloids Surf B Biointerfaces. 2013;101:465-74. doi: 10.1016/j.colsurfb.2012.07.017, PMID 23010056.
39. Holm R 1. IHM Jensen JS. Optimization of self-microemulsifying drug delivery systems (SMEDDS) using a D-optimal design and the desirability function. Drug Dev Ind Pharm. 2006;32(9):1025-32.
40. Negi P, Singh B, Sharma G, Beg S, Raza K, Katare OP. Phospholipid microemulsion-based hydrogel for enhanced topical delivery of lidocaine and prilocaine: QbD-based development and evaluation. Drug Deliv. 2016;23(3):951-67. doi: 10.3109/10717544.2014.923067, PMID 24892623.
41. Quintero-Quiroz C, Acevedo N, Zapata-Giraldo J, Botero LE, Quintero J, Zárate-Trivinõ D, *et al*. Optimization of silver nanoparticle synthesis by chemical reduction and evaluation of its antimicrobial and toxic activity. Biomater Res. 2019;23(1):27. doi: 10.1186/s40824-019-0173-y, PMID 31890269.
42. Joshi M, Patravale V. Nanostructured lipid carrier (NLC) based gel of celecoxib. Int J Pharm. 2008;346(1-2):124-32. doi: 10.1016/j.ijpharm.2007.05.060, PMID 17651933.
43. Tran HA, Ly KL, Fox KE, Tran PA, Nguyen TH. Immobilization of antimicrobial silver and antioxidant flavonoid as a coating for wound dressing materials. Int J Nanomedicine. 2019;14:9929-39. doi: 10.2147/IJN.S230214, PMID 31908450.

PICTORIAL ABSTRACT



Cite this article: Badhwar R, Singh R, Tinku, Popli H. Implementation of Quality by Design (QbD) Approach in Development of QCT-SMEDDS with Combination of AgNPs for Diabetic Foot Ulcer management. Indian J of Pharmaceutical Education and Research. 2021;55(4):1207-23.