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Formulation design and optimization of triclosan loaded nanoparticles for enhanced drug delivery across gingival sulcus by Resolution IV modeling of Design Expert®

Abstract— The aim of this study was to design and systematically optimize triclosan loaded nanoparticles (TCS-loaded NPs) formulation for the treatment of periodontal disease. Triclosan (TCS) is a broad spectrum antimicrobial agent that has been used in the treatment of the disease. The free drug has poor aqueous solubility and therefore may encounter permeability problems when applied to the oral cavity. Resolution IV model of Design-Expert® software (version 10) was used for the design of experiment and optimization of TCS-loaded NPs. The nanoparticles (NPs) were prepared using the solvent displacement method. Effect of factors that were investigated include drug-polymer ratio, surfactant concentration, stirring speed, stirring duration, and drug-polymer injection rate. Particle size, zeta potential, polydispersity index (PDI) and entrapment efficiency (EE) were the critical quality attributes selected for the study. Desirability function determined by the software for optimized TCS-loaded NPs was 0.704. The observed particle size, PDI, zeta potential and EE of the optimized TCS-loaded NPs was found to be 135 ± 2.3 nm, 0.1 ± 0.012, -30 ± -4 mV and 75 ± 6%, respectively. It was found that particle size increases by elevating the concentration of polymer and decreases with an increase in surfactant concentration and stirring speed. Zeta potential was found to increase when surfactant concentration was reduced. Both surfactant concentration and drug to polymer ratio were found to negatively affect PDI while % EE was positively influenced by the increase in polymer concentration and decrease in surfactant concentration. The use of Design-Expert® software helped in identifying suitable levels of critical quality parameters for preparing improved NPs formulation for delivery of TCS into the periodontal pocket.

***Keywords* — Design-Expert®, Resolution IV, design of experiment, periodontal disease, triclosan**

1 INTRODUCTION

Designing and formulating a pharmaceutical product requires an in depth understanding of the theory involved in the formulation, as well as the targeted process parameters and ranges for such parameters and each excipient that will be used in the formulation [1]. Furthermore, understanding the relationship between independent and dependent variables is important for predicting the best formulation ingredients and processes. Formulation and optimization principles involve a series of logical steps by controlling variables carefully and switching one factor at a time until a product which meets the suitable quality attributes is obtained. In conventional formulation studies, manual changing of one-factor-at-a-time while keeping the other factors constant is generally adopted. However, this approach has several limitations such as increasing the project cost by conducting a large number of experiments, is very laborious and may not detect certain vital interactions [2,3]. Design of experiments using an advanced formulation software like Design-Expert® can be a turning point in overcoming these limitations. The Design-Expert® method can save cost and time, as well as provide the necessary information that will enable a better understanding of the relationship between variables studied [3]. These benefits have attracted the attention of many researchers in recent years to employed Design-Expert® software modelings such as Resolution IV and V designs, Box-Behnken Design, Factorial Design, Plackett Burman Design, Central Composite Design e.t.c., in designing and developing various nanoparticulate drug delivery systems with a great success [4–12]. For example, Resolution V design of Design-Expert® was used by Avasatthi et al. (2015) to fabricate a novel nanogel formulation of methotrexate for localized treatment of psoriasis disease [2]. Similarly, Box–Behnken Design of Design-Expert® was employed by Sukhbir et al. (2016) to

develop nanospheres loaded with nefopam hydrochloride for neuropathic pain [13], and in the current year by Patil et al. (2018) to formulate nano-carriers loaded with natamycin for ocular applications [5]. All of these investigations found Design-Expert® software as a useful tool in achieving the desired quality characteristics of the nano-formulations.

Clinically, periodontal disease affects the tooth supporting structures such as gums, periodontal ligament, alveolar bone and cementum, and if left untreated may result in tooth loosening and loss [14–16]. The main area involved in the disease pathology is the gingival sulcus (crevice that surrounds the tooth at its bottom), and it is located between the tooth and the free gingival margin [17,18]. An increase in depth of the gingival sulcus will lead to the formation of periodontal pockets which is one of the major clinical consequences of periodontal disease. Many intra-pocket drug delivery systems have been developed for the treatment of the disease [19,20]. However, poor penetration of the junctional epithelium – a circular arrangement of epithelial cells occurring at the base of the gingival sulcus – has limited the effectiveness of these delivery systems [16]. Delivering a therapeutic agent across the gingival sulcus to the underlying connective tissue should be a key desirable property for any ideal drug delivery system intended for localized treatment of periodontal disease. Colloidal drug carrier systems such as polymeric NPs could be used to provide site-specific or targeted drug delivery, combined with optimal drug release profiles [21].

Herein, we present a novel optimization strategy for possible enhancement of TCS delivery across the gingival sulcus by development of poly-ε-caprolactone (PCL) NPs loaded with the drug and optimizing the NPs using Resolution IV experimental design. Resolution IV Design is a type of a Regular Two-Level Factorial Design Builder which can be used for regular fractional factorial, as well as two-level full factorial designs. One can use this model to investigate 2 to 21 factors through 4 to 512 runs. It is a good choice for formulation screening as the design is developed to avoid any interference of two-factor interactions in the main effects. Similar models of Design-Expert® software were used by other researchers to design and optimize formulations meant for delivery into the periodontal pocket. For instance, a thermoreversible and syringeable periodontal sol of metronidazole benzoate and serratiopeptidase has been developed for localized application into the periodontal pocket, by using Design-Expert® software modeling [22]. Srivastava et al. (2016) also formulated in situ gelling and syringeable nanoemulgels loaded with ketoprofen for intra-pocket delivery in the treatment of periodontitis, by Box–Behnken Design of Design-Expert® software [23]. The selected drug candidate, i.e. TCS, is a broad spectrum antimicrobial agent which has established efficacy against many plaque-forming bacteria, the main cause of the disease [24]. PCL was selected as the candidate polymer to prepare the NPs because of its advantages such as biodegradability, biocompatibility and high permeability to small drug molecules. In addition, the polymer undergoes degradation in a slow manner, which is a desirable property for prolonged release drug delivery systems [18,25,26].

2 MATERIALS AND METHODS

2.1 Materials

The TCS used in this study was purchased from Bio Basic Canada Inc. (Markham Ontario, Canada). PCL (with a molecular weight of ~ 14,000) and Kolliphor® P188 were purchased from Sigma-Aldrich, USA. High performance liquid chromatography (HPLC) grade acetonitrile and methanol was purchased from Merck (Darmstadt, Germany). AR grade acetone was from QReC® Asia, (Selangor, Malaysia). Distilled water was produced in-house by the Favorit W4L water system (Genristo Ltd, England).

2.2 Experimental Design

Experimental design and statistical evaluation can ease, as well as enhance the quality of experimentation, by evaluating and identifying the most important parameters and interactions of such parameters that are likely to occur [2]. Resolution IV statistical design was applied for TCS-loaded NPs formulation to evaluate the influence of formulation processes and parameters (such as stirring speed and rate, injection volume and concentrations of the used excipients) in two different extreme levels (Table I). The significant effect of these formulation independent variables in Table I were investigated on the dependent variables viz. Particle Size (Response 1), Zeta Potential (Response 2), Polydispersity Index (Response 3) and % Entrapment Efficiency (Response 4), using Design-Expert® 10.0.6.0 software. The design generated 12 experiments as shown in Table II.

Table I: Factors and their level in Resolution IV Design

	Levels	
Factors	–1	+1
A = Drug-Polymer ratio (w/w)	1:2	1:6
B = Surfactant concentration (% w/v)	0.1	0.6
C = Stirring speed (rpm)	500	1200
D = Stirring duration (h)	2	24
E = Drug-Polymer injection rate (drops/min)	20	60

Table II: Resolution IV experimental design batches for TCS-loaded NPs formulations

Run	Factor A	Factor B	Factor C	Factor D	Factor E
	Drug-Polymer ratio (w/w)	Surfactant concentration (% w/v)	Stirring speed (rpm)	Stirring duration (h)	Drug-Polymer injection rate (drops/min)
1	1:2	0.1	500	24	60
2	1:6	0.1	1200	2	60
3	1:6	0.6	500	24	60
4	1:6	0.1	1200	24	20
5	1:2	0.1	1200	24	20
6	1:2	0.6	1200	24	60
7	1:6	0.6	1200	2	20
8	1:2	0.1	1200	2	20
9	1:2	0.6	500	24	20
10	1:2	0.6	500	2	60
11	1:6	0.1	500	2	20
12	1:6	0.6	500	2	60

Table III: Results of experimental trials for Resolution IV Design

Run	Factors					Responses			
	A Drug-Polymer ratio (w/w)	B Surfactant concentration (% w/v)	C Stirring speed (rpm)	D Stirring duration (h)	E Drug-Polymer injection rate (drops/min)	Particle size (nm)	Zeta potential (mV)	Polydispersity index	Entrapment efficiency (%)
1	1:2	0.1	500	24	60	124.8	-29	0.112	75.5
2	1:6	0.1	1200	2	60	135.6	-24.3	0.109	76.5
3	1:6	0.6	500	24	60	170	-25.9	0.105	90.2
4	1:6	0.1	1200	24	20	142.6	-22.7	0.146	64.9
5	1:2	0.1	1200	24	20	95.5	-26.4	0.126	44.6
6	1:2	0.6	1200	24	60	105.6	-27.8	0.115	42.2
7	1:6	0.6	1200	2	20	132.8	-19.1	0.111	77.2
8	1:2	0.1	1200	2	20	89.6	-27.2	0.177	56.8
9	1:2	0.6	500	24	20	143.7	-29.7	0.124	83.7
10	1:2	0.6	500	2	60	131.6	-22.8	0.134	76.9
11	1:6	0.1	500	2	20	188.6	-30.1	0.019	94
12	1:6	0.6	500	2	60	187.2	-20.6	0.064	94.2

2.3 Preparation of Nanoparticles

The NPs were prepared according to a modified reported solvent displacement method [18]. An amount of TCS and PCL was weighed accurately and then dissolved in acetone by mild heating under magnetic stirring until a clear solution was obtained. This clear drug-polymer solution was injected drop-wise into 50 mL aqueous solution of Kolliphor® P188 under continuous magnetic stirring. The stirring was continued until acetone was evaporated off. The developed NPs suspension was centrifuged at 12,000 rpm for 1 h. The pellet was separated from the supernatant by decantation, and then washed twice with distilled water and dried using a rotary evaporator (EYELA N-1000, Japan). The supernatant was used for the determination of percentage entrapment efficiency (% EE). All the batches were prepared based on the Resolution IV experimental design that was generated by Design-Expert® (Table II).

2.4 Particle size, polydispersity index and zeta potential measurements

The hydrodynamic diameters (sizes) and its distribution, PDI and zeta potential of the developed TCS-loaded NPs were measured in a polystyrene cuvette, by dynamic light scattering at 25 °C at a 90º angle using a Zetasizer 1000 HSA (Malvern Instruments, UK), which uses a laser at a wavelength of 633 nm. Zeta potential measurement was carried out in a folded capillary cell using a Zetasizer Nano Series (Nano-Z, Malvern Instruments, UK).

2.5 Determination of percentage entrapment efficiency of triclosan

An indirect method was employed for the determination of % EE of TCS for the developed NPs. Briefly, the supernatant collected after centrifugation of NPs suspension (at 12,000 rpm for 1 h) was diluted with methanol. The amount of the unloaded TCS available in the supernatant was quantified by using an in-house HPLC method which was developed according to the protocols of our published HPLC methods (27–29). The following formula was used for the calculation of % EE.

EE (%) = [(Amount of TCS added in the formulation – Amount present in the supernatant) / Amount of TCS added in the formulation] X 100

3 RESULTS AND DISCUSSION

3.1 Optimization of TCS-loaded NPs

The results of experimental trials generated by Design-Expert® software are presented in Table III, and all the 12 batches that were proposed yielded NPs. Two-factor interactions were adopted under randomized factorial design in order to assess all main effects, as well as to determine the factors and interaction points that influence the responses, i.e. particle size, PDI, zeta potential and % EE of TCS-loaded NPs.

Figure 1 showed the response surface plots of the actual relationships which occurred between some of the formulation factors and response variables. An increase in particle size was observed when the level of PCL in the formulation was raised. On the other hand, the particle size decreases with an increase in the level of factor B and C (Figure 1a). This could be as a result of the surfactant-induced reduction in surface and interfacial tension between the two phases used (aqueous and organic) [30], and the effect of internal frictional forces exerted by magnetic steering at a higher rate (31), leading to smaller NPs sizes [30–32]. This result is well in agreement with those reported by Emami et al. (2014) and Sukhbir et al. (2016) who also observed a significant reduction in NPs size with an increase in surfactant concentration and stirring speed [13,31]. PDI value increases in direct proportion to an increase in the level of factor B and C (Figure 1b), while the zeta potential was found to be negatively affected by the increase in the level of these two factors (Figure 1c).

All the PDI values for the 12 batches are within the acceptable limit of < 4 for polydisperse system [33]. The results of the experimental design further revealed that an increase in the level of factor A and C will lead to rise and fall of the % EE, respectively. The positive effect on % EE may probably have occurred as a result of the ability of PCL to encapsulate large amounts of TCS due to an increase in the mass of PCL, while the decrease may have occurred because of dislodgment of TCS (that were superficially attached on the outer surface of NPs) into the main stream of the continuous phase when the stirring speed was at high levels, according to the described phenomenon [13,34]. Moreover, a reported study showed that polymer concentration and stirring speed can significantly influence the % EE [31] and this fact has been confirmed by the present study.

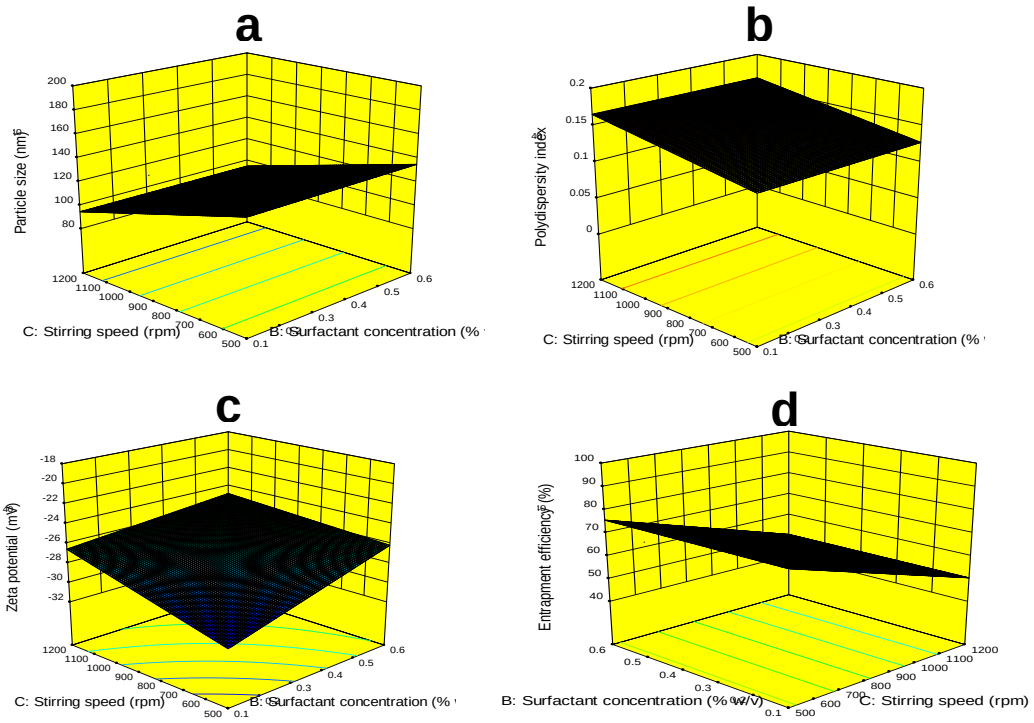
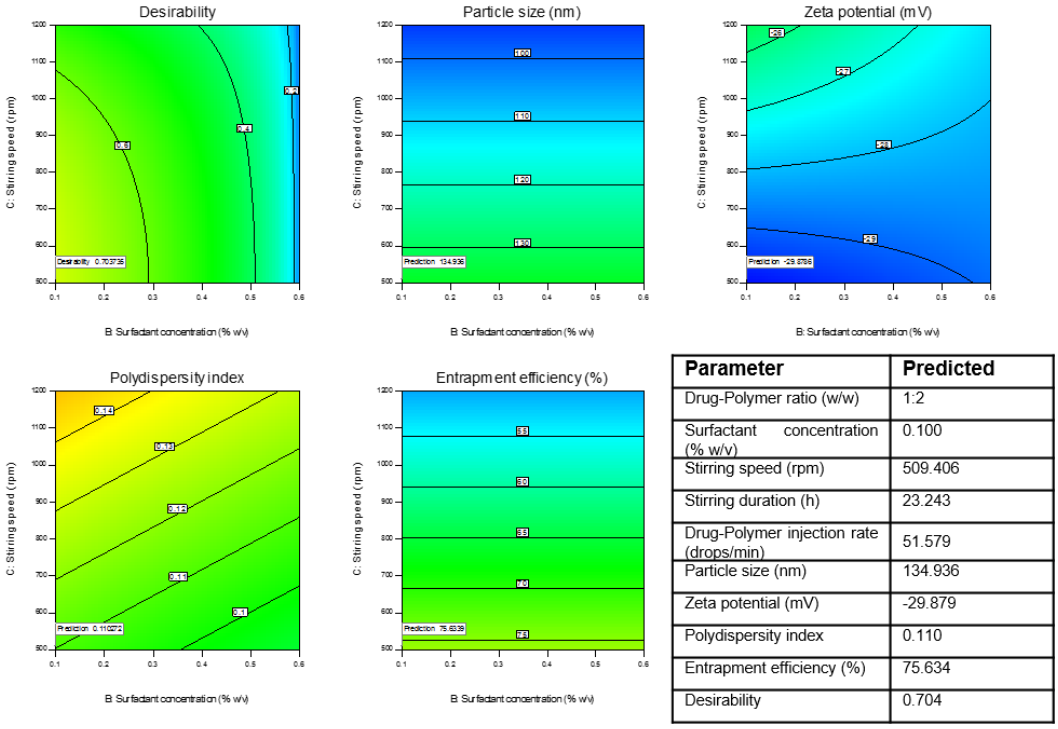


Figure 1: Response surface plots for the design indicating the effect of formulation factors on the responses



Parameter	Predicted
Drug-Polymer ratio (w/w)	1:2
Surfactant concentration (% w/v)	0.100
Stirring speed (rpm)	509.406
Stirring duration (h)	23.243
Drug-Polymer injection rate (drops/min)	51.579
Particle size (nm)	134.936
Zeta potential (mV)	-29.879
Polydispersity index	0.110
Entrapment efficiency (%)	75.634
Desirability	0.704

Figure 2: Contour plots for the design indicating the effect of formulation factors on responses, and the predicted values, i.e. optimized formula

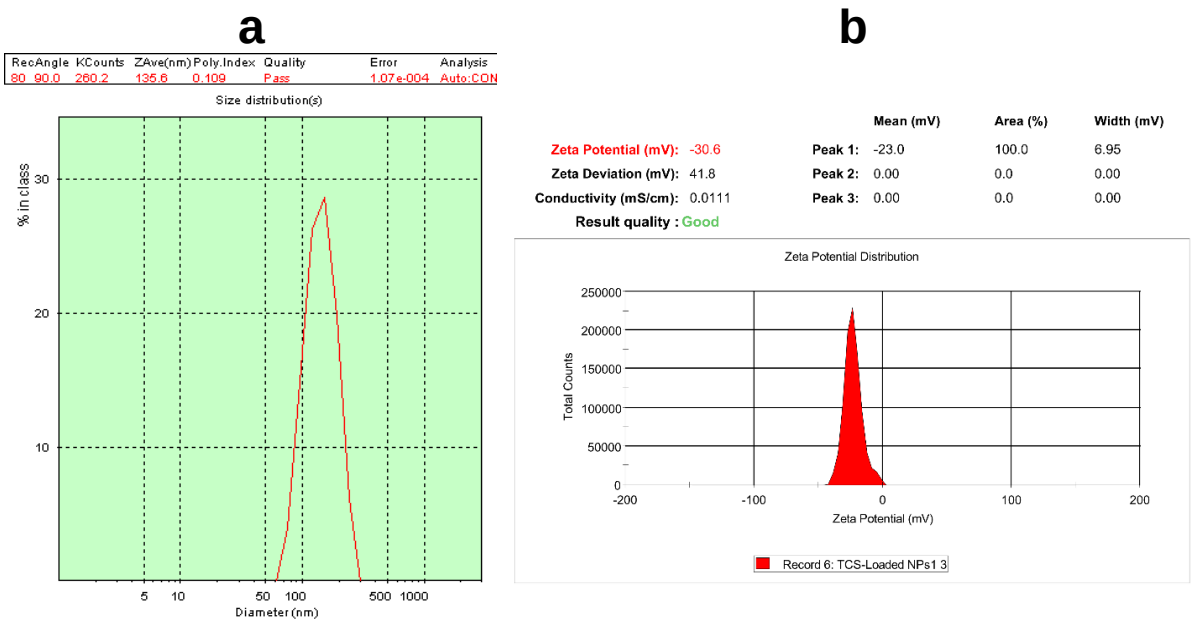


Figure 3: Optimized TCS-loaded NPs results indicating particle size of 135.6 nm and PDI of 0.109 (a), and zeta potential of -30.6 mV (b)

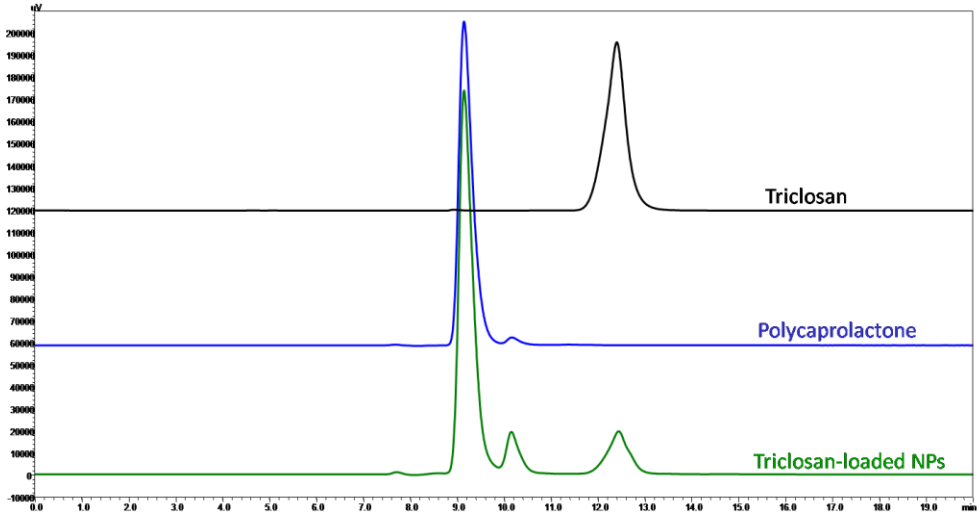


Figure 4: Chromatograms of the standard samples of TCS, PCL and TCS-loaded NPs for the used HPLC method

After defining the goal of the formulation constrains, where particle size and surfactant concentration were set to the minimum while % EE was set to the maximum, the Design-Expert® generated optimized formula (Figure 2) for the preparation of TCS-loaded NPs' optimal formulation. Replicates of the optimized formulation were prepared according to the optimized formula, and all the necessary characterizations were made. There was an acceptable agreement between the predicted values and the experimental values, and this has confirmed the reliability of the used optimization model.

3.2 Characterization of TCS-loaded NPs

The developed optimized TCS-loaded NPs were characterized and the results for particle size, PDI and zeta potential are presented in Figure 3, while that of HPLC is presented in Figure 4.

The particle size of the developed NPs is one of the key variables that was aimed to be

controlled to the minimum considering the desired application of the formulation. Larger particles that are greater than 500 nm in diameter may not permeate through the junctional epithelium to deliver the drug at the site of action. The average NPs size (135 ± 2.3 nm) of the optimized formulation falls within the targeted value, and as predicted by the software, there was no significant difference between the predicted and the observed value. Although NPs was described as structures that are in the size range of 1 to 100 nm in diameter [35], in pharmaceutical context however, all particles or structures that are less than 1 µm in diameter are recognized as NPs [36,37].

Correct zeta potential and PDI are important quality attributes which indicate stability of colloidal systems. The found zeta potential value of the optimized formulation indicated adequate surface charge of the NPs. It is well established that colloidal systems with pronounced zeta potential values (whether negative or positive) tend to stabilize the suspended particles because electrostatic repulsion between particles with similar electrical charge will prevent the aggregation of the particles within the dispersion system [31,38]. PDI value of suspensions that are less than 0.4 indicates moderate particle size distribution which may not aggregate or sediment quickly. Both the predicted and observed PDI values of the optimized NPs were around 0.1, which confirmed a suitable particle distribution of the formulation.

% EE is an important variable which indicates the loaded drug release and the overall efficacy of the formulation development process. EE above 70% was found for all the replicates of the optimized TCS-loaded NPs, indicating satisfactory encapsulation of the drug.

4 CONCLUSION

The application of Resolution IV design with a two-level five-factor modeling pattern turned out to be a useful tool for the development and optimization of TCS-loaded NPs prepared by solvent displacement method. The design has adequately revealed the influence of different levels of independent variables selected on the responses under study. The poor permeation characteristic of free TCS across gingival sulcus has been enhanced by encapsulating it in a biocompatible polymer that resulted in NPs of around 135 nm. This NPs formulation displayed desirable physicochemical properties that are suitable for intra-pocket application in the treatment of periodontal disease. Therefore, it is concluded that the use of Design-Expert® software may help in identifying the critical quality parameters for preparing TCS-loaded NPs formulation for improved delivery into the periodontal pocket.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

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