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Formulation optimization of scutellarin-loaded HP-β-CD/chitosan nanoparticles using response surface methodology with Box–Behnken design

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ABSTRACT

The aim of this paper is to investigate and optimize the preparation of scutellarin (SCU)-loaded HP-β-CD/chitosan (CS) nanoparticles (CD/CS-SCU-NPs). CD/CS-SCU-NPs were prepared by ionic cross-linking method and the process and formulation variables were optimized using response surface methodology (RSM) with a three-level, three factor Box–Behnken design (BBD). The independent variables were the added amounts of CS, sodium tripolyphosphate (TPP) and Pluronic F-68 during the preparation. Dependent variables (responses) were particle size and entrapment efficiency. Mathematical equations and respond surface plots were used to correlate independent and dependent variables. The preparation process and formulation variables were optimized to achieve minimum particle size and maximum entrapment efficiency by calculating the overall desirability value (OD). The optimized NP formulation was characterized for particle size, PDI, zeta potential, entrapment efficiency and *in vitro* drug release. According to the results, an optimized CD/CS-SCU-NP formulation was prepared. Results for particle size, PDI, zeta potential and entrapment efficiency were found to be around 200 nm, 0.5, 25 mV, and 70% respectively. For *in vitro* study, the release of SCU from the NPs exhibited a biphasic release and was in accordance with Higuchi equation. The optimized preparation was simple with the probability for industrialization. The combination use of RSM, BBD and overall desirability values could provide a promising application for incorporating CD into CS nanoparticles as drug delivery carrier and help develop lab-scale procedures.

1. Introduction

It is estimated that there are 31 million stroke survivors around the world and about 6 million deaths are due to cerebrovascular diseases [1]. Scutellarin (SCU) is the primary effective constituent in breviscapine extracted from Chinese herb of *Erigeron breviscapus* (Vant.) Hand-Mazz [2]. Various pharmacological studies have demonstrated that SCU has a various of protective effects including dilate blood vessel, improve microcirculation, decrease the viscosity of blood, and has the bioactivities associated with protective effects in the brain and

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heart, hypercholesterolemia suppression, platelet aggregation inhibition, fibrinolysis and anticoagulation [3]. Its main clinical use is for the coronary heart disease, angina, myocardial ischemia and cerebral thrombosis. However, it is hard to be absorbed due to its poor solubility in both aqueous and organic media so its bioavailability is very low. Its absolute bioavailability in Beagle dog when administered orally was rarely 0.2%–0.75% [4]. Pharmacokinetic studies showed that the elimination half-life of SCU in dog plasma was only 1.0 min [5]. Therefore, finding a new dosage form for SCU is necessary for the ischemic cerebrovascular disease treatment.

The development of dosage forms of poorly water-soluble drugs is always difficult, especially for drugs that are poorly soluble in both aqueous and organic media. Studies on nanotechnology have opened up potential applications for drug brain-targeting [6–8]. For the preparation of nanoparticles, chitosan (CS) is used as a bioadhesive polymer since it is non-toxic, biodegradable, biocompatible, mucoadhesive and is able to transiently open the tight junctions of the intestinal barriers [9]. The free amino groups of CS can induce an intermolecular or intramolecular ionic gelation reaction with sodium tripolyphosphate (TPP) when preparing the chitosan nanoparticles [10–12] (Fig. 1).

Cyclodextrin (CD) can form inclusion complexes with a variety of drugs which can increase solubility, improve chemical and physical stability and/or enhance absorption of the drug [13]. 2-Hydroxypropyl-β-CD (HP-β-CD), a hydrophilic cyclodextrin derivative, is the cheapest, most used and has been extensively studied in pharmaceutical research [14]. According to the advantages of both HP-β-CD and CS, combining HP-β-CD with CS as a drug carrier to prepare nanoparticles may lead to a carrier that possesses the advantages of CD including inclusion, size specificity and transport properties as well as mucoadhesive properties of CS. In recent years, drug delivery systems based on CS and CD NPs have been widely investigated for hydrophilic/hydrophobic drug and macromolecular drug [15]. Trapani et al. [16] prepared glutathione loaded CS/sulfobutyl ether-β-CD NPs for oral administration. However, the drug loaded nanoparticles could only be released with enzymes in simulated gastric and intestinal media.

The aims of this study were to prepare a novel SCU-loaded HP-β-CD/CS nanoparticles (CD/CS-SCU-NPs) by ion cross-linking reaction and optimize the formulation variables using the response surface methodology (RSM): a three-level, three factor Box–Behnken design (BBD). The CD/CS-SCU-NPs were characterized in terms of particle size, entrapment efficiency and *in vitro* release behavior. This kind of drug delivery system of SCU has not been reported previously.

2. Materials and methods

2.1. Materials

SCU (>98%) was purchased from Wuhan Yuancheng Gongchuang Science and Technology Co. Ltd, China; CS (medium molecular weight) was supplied by Aldrich Chemical Company; HP-β-CD was purchased from Wacker Chemical Corp.; Pluronic F-68 was provided by BASF Cooperation; TPP was purchased from Sigma-Aldrich; methanol and acetonitrile were of HPLC grade and purchased from Merck Chemicals Co. All other reagents and solvents were of analytical grade.

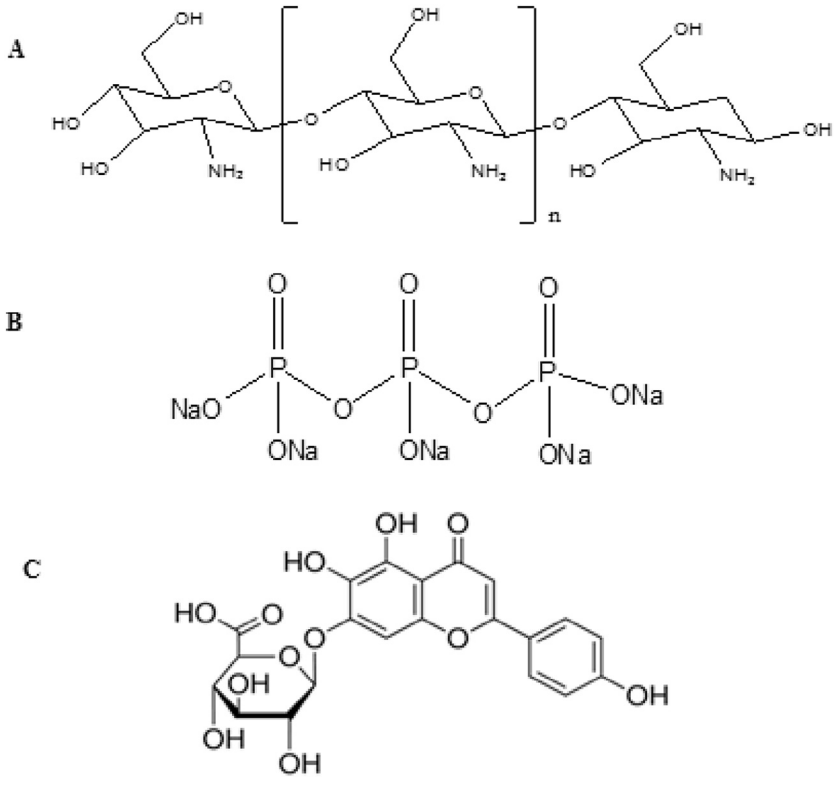

Fig. 1 – Chemical structures of CS (A), TPP (B) and SCU(C).

2.2. Preparation of CD/CS-SCU-NPs

The CD/CS-SCU-NPs were prepared by a modified ionic cross-linking method reported previously [17,18]. Briefly, SCU was dissolved in 2 ml of methanol; certain amount of pluronic F-68 as a stabilizer was added into 5 ml of CS solution which was obtained by dissolving CS into 0.2% acetic acid solution [19] and mixed well. Sodium hydroxide (1 mol/l) was used to adjust the pH to 4.5. The SCU solution was dropped into the CS solution with magnetic stirrer (500 rpm). Then 1 ml of TPP solution containing certain amount of HP-β-CD was added drop by drop (20–40 drop/min) into the mixture with magnetic stirrer. After forming completely dispersed particles, the dispersions were sonicated using a sonic dismembrator (Fisher Scientific Co., Model 500) for about 30 min. Then the mixture was stirred with magnetic stirrer for about 2h to evaporate the organic solvent. The obtained nanoparticle suspension was filtered through 0.45 μm filter membrane to remove the unincorporated SCU aggregates, and the drug-loaded nanoparticle suspensions were obtained.

2.3. Single factor evaluation

2.3.1. Influence of CS and TPP concentrations

A series of CS solutions with different concentrations (5 ml) were prepared and their pH values were adjusted using sodium hydroxide (1 mol/l) to 4.5. TPP solutions with different concentrations (1 ml) were then added drop by drop with magnetic stirrer (500 rpm). NP formation in the mixture was observed.

2.3.2. Influence of CS/TPP mass ratio

The concentrations of CS, TPP and SCU were kept as 2 mg/ml and the amount of pluronic F-68 was kept as 30 mg. The pH was kept at 4.5. The different CS/TPP mass ratios (1:1, 3:1, 5:1, 7:1) were used to prepare the CD/CS-SCU-NPs. Products of different formulations were tested for its particle size, PDI, zeta potential and entrapment efficiency.

2.3.3. Influence of the pH of CS solution

The other preparation conditions of NPs were kept the same. The different pH values of CS solution (pH 4.0, 4.5, 5.0, 6.0) were adjusted by sodium hydroxide (1 mol/l) and were used to prepare the CD/CS-SCU-NPs. Products of different formulations were tested for its particle size, PDI, zeta potential and entrapment efficiency.

2.3.4. Influence of the added amount of HP-β-CD

The other preparation conditions of NPs were kept the same. The different added amounts of HP-β-CD (0, 10, 20, 40 mg) were used to prepare the CD/CS-SCU-NPs. Products of different formulations were tested for their particle size, PDI, zeta potential and entrapment efficiency.

2.3.5. Influence of the added amount of Pluronic F-68

The other preparation conditions of NPs were kept the same. The different added amounts of Pluronic F-68 (0, 30, 40, 50 mg) were used to prepare the CD/CS-SCU-NPs. Products of different formulations were tested for their particle size, PDI, zeta potential and entrapment efficiency.

2.3.6. Influence of the SCU concentration

The other preparation conditions of NPs were kept the same. The different SCU concentrations (1, 2, 3, 4 mg/ml) were added into the formulation. Products of different formulations were tested for their particle size, PDI, zeta potential and entrapment efficiency.

2.4. Formulation optimization

In this section, BBD was specifically selected since it is a popular template for RSM which requires only three levels of each process factor and with only a few runs, all possible combinations could be covered. In this design, the experimental region is assumed to be a cube, and experiments are performed at points corresponding to midpoint of each edge and replicated experiments at the center of this multidimensional cube [20]. The complete design consisted of 17 experimental points that included 12 factor points and 5 replications at the center point. The nonlinear quadratic model generated by the design is as follows:

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{11}A^2 + b_{22}B^2 + b_{33}C^2$$

where Y denotes the measured response (dependant variable) associated with each factor-level combination; A, B, and C are the independent variables. The coefficients of the polynomial equation were represented by b_0 (intercept), b_1, b_2, and b_3 (linear effects), b_{11}, b_{22}, and b_{33} (quadratic effects), and b_{12}, b_{13}, and b_{23} (interaction effects). The terms AB, BC, AC and A^2, B^2, C^2 represent the interaction and quadratic terms, respectively. Based on the preliminary experiments, the added amounts of TPP (A, mg), CS (B, mg) and pluronic F-68 (C, mg) were selected as main factors, while particle size (Y_1) and entrapment efficiency (Y_2) were selected as two response factors [21]. The response surface design table of the three factors and three levels were shown in Table 1. Design Expert (Version 9.0.6) was used to analyze the experimental data and perform multiple regressions to obtain the coefficients of the quadratic polynomial model. The quality of the fitted model was expressed by the coefficient of determination R^2, and its statistical significance was determined by F-test. Three batches of CD/CS-SCU-NPs prepared using the optimal formulation were used for verification.

2.5. Characterization of CD/CS-SCU-NPs

2.5.1. Size and zeta potential

Photon correlation spectroscopy (Zetasizer 3000; Malvern Instruments, Malvern, UK) was used to measure particle size and

Table 1 – Factors and levels for formulation optimization.

Factors	Levels		
	−1	0	1
A: TPP added (mg)	1	2	3
B: CS added (mg)	10	15	20
C: Pluronic F-68 added (mg)	30	40	50
Y_1: Particle size (nm)			
Y_2: Entrapment efficiency (%)			

zeta potential [22]. All samples were diluted with appropriate distilled water for measurements. Z-average particle size, polydispersity index (PDI), and zeta potential were measured in triplicate at room temperature.

2.5.2. *Determination of entrapment efficiency*

The entrapment efficiency was determined by centrifugation method as reported previously [22,23]. 1 ml of CD/CS-SCU-NPs was centrifuged at 15,000 rpm for 15 min (4 °C) and the clear supernatant solutions were obtained. After dilution by 10-fold methanol, concentrations of SCU in the supernatant (free drug) were determined by HPLC consisting of a pump (Model LC-20A, Shimadzu, Japan), a reversed-phase C_{18} column (150 × 4.5 mm, 3 μm) maintained at room temperature, and a variable wavelength UV detector (Model SPD-20A, Shimadzu, Japan) at 335 nm. The mobile phase was composed of 30% acetonitrile and 70% water (using phosphoric acid to adjust pH to 2.5) and was delivered at a flow rate of 0.5 ml/min [24,25]. The injection volume was 20 μl by an autosampler and the retention time was 4.5 min. The method was validated for SCU assay, according to ICH guidelines, with respect to specificity, linearity ($R^2 > 0.999$), precision (intra-day R.S.D. < 0.41 and inter-day R.S.D. < 0.38%), and accuracy (recoveries between 100.0% and 100.5%). The entrapment efficiency (EE %) can be calculated by the following formula:

$$EE\,(\%) = (C_{total} - C_{free})/C_{total} \times 100\%$$

where C_{free} is the SCU concentration in the supernatant after centrifugation while C_{total} is the initial amount of drug added during the preparation of CD/CS-SCU-NPs.

2.5.3. *In vitro release*

In vitro SCU release from CD/CS-SCU-NPs and free SCU were evaluated using dialysis bag diffusion technique [22]. Dialysis bag with a molecular weight cutoff of 350 Da (Sigma-Aldrich Co.) were filled with 2 ml of CD/CS-SCU-NP suspension or 2 mg free SCU and then placed into 150 ml PBS (pH 6.8) containing 2% EDTA-2Na as an antioxidant. *In vitro* SCU release was performed at 37 °C with the rotation speed at 300 rpm. 1 ml samples were collected at predetermined time points (0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h), then the withdrawn samples were replaced with the same volume of fresh release medium to maintain a constant total volume. The samples were filtered with filter cartridge (PTFE 0.45 μm) before assayed by HPLC for SCU at 335 nm.

The release kinetics of SCU from CD/CS-SCU-NPs in PBS (pH 6.8) containing 2% EDTA-2Na was evaluated by different mathematical models such as the First model, Higuchi and Korsmeyer-Peppas Equation.

3. Results and discussion

3.1. *Single factor evaluation*

3.1.1. *Influence of CS and TPP concentrations*

Results in Table 2 showed that the concentrations of CS and TPP could have large impact on the NP formation. When their concentrations were too low (≤0.5 mg/ml), the ion cross-linking reaction to form NPs would not happen thus the final product would become a clear solution. NPs started to form when their concentrations increased to ≥1 mg/ml. But when the concentrations of CS and TPP were too high, the NPs were also difficult to be formed. Probably it was because when the concentration of CS was too high, the ionic strength of CS solution was increased. The repulsion of amino groups in CS could be bent and gathered the CS molecules. As a result, adding too much TPP might promote a further aggregation of CS thus leads to a precipitation.

Table 2 – Influence of CS and TPP concentration on the formation of nanoparticles.

Concentration of CS (mg/ml)	Concentration of TPP (mg/ml)				
	0.5	1. 0	1.5	2.0	2.5
0.5	–	√	×	×	×
1.0	–	√	×	×	×
1.5	–	√	√	√	√
2.0	–	-	√	√	√
2.5	–	-	√	√	√

where –: clear solution, cannot form NPs; √: can form NPs; ×: turbid solution with precipitation.

3.1.2. *Influence of CS/TPP mass ratio*

Table 3 shows that the NP formation could be influenced by CS/TPP mass ratio. When the CS/TPP mass ratio was low (1:1), the NPs were not stable and easy to precipitate. As the CS/TPP mass ratio increased from 3:1 to 7:1, the NPs could be formed. The lowest particle size (294.8 nm) came out when the CS/TPP mass ratio was 5:1. It might be due to the fact that two CS molecules and a TPP molecule could form the closest structure, thus the minimum particle size could be obtained. Moreover, the PDI values increased as the CS/TPP mass ratio increased. For zeta potential, it represents the stability of colloidal dispersions in the products. The higher the value, the more stable the particles would be. As shown in Table 3, all zeta potential values were over 20 except the one with 3:1 CS/TPP mass ratio which was only 4.10. For entrapment efficiency, as the CS/TPP mass ratio increased, the entrapment efficiency decreased. That is because less amount of TPP was added in the formulation; the number of NH_3^+ groups in CS which take part in the reaction could be decreased. However, for the highest entrapment efficiency (CS/TPP mass ratio 3:1), its particle size was almost twice as the other two and its zeta potential value was only 4.1, meaning that this formulation had larger particle

Table 3 – Influence of CS/TPP mass ratio on the particle characteristics.

CS/TPP mass ratio	Particle size (nm)	PDI	Zeta potential	EE (%)
1:1	precipitation	–	–	–
3:1	616.5	0.121	4.10	71.67 ± 0.46
5:1	294.8	0.144	26.9	48.15 ± 0.72
7:1	338.3	0.152	28.4	39.02 ± 0.16

Table 4 – Influence of the pH of CS solution on the particle characteristics.

pH	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
4.0	290.3	0.140	27.3	42.49 ± 0.37
4.5	301.5	0.187	23.7	54.28 ± 0.15
5.0	740.7	0.886	7.54	82.56 ± 0.04
6.0	1102.0	0.946	4.48	64.62 ± 0.02

size and was not stable. Thus considering all the conditions, the optimum CS/TPP mass ratio was 5:1.

3.1.3. *Influence of the pH value of solution*

The effects of the pH of solution on the characteristics of the CD/CS-SCU-NPs were shown in Table 4. According to the table, the particle size increased as the pH value increased. When pH value was less than 4.0, the NPs were difficult to be formed mainly because most of the phosphate groups in TPP are occupied by H^+, thus the electrostatic attraction between phosphate groups in TPP and NH_3^+ group in CS was blocked. The zeta potential value decreased with the increase of pH value while the entrapment efficiency increased as the pH value increased but decreased when pH value was 6.0. That is because the pH value could directly influence the solubility and stability of SCU. SCU has only one carboxyl group but several hydroxyl groups. Thus under alkaline condition, SCU solubility could be increased while its stability could be decreased which would lead to an apparent hydroxylation of SCU. Considering that the most stable pH value for SCU is around 2–5, as a result, the optimum pH value of CS solution for the formulation should be at pH 4.5.

3.1.4. *Influence of the added amount of HP-β-CD*

The effects of the added amount of HP-β-CD on the characteristics of the CD/CS-SCU-NPs were shown in Table 5. The particle sizes, zeta potential and entrapment efficiency were all found to have a slight increase as the HP-β-CD added amount increased, indicating that the presence of HP-β-CD had no critical impact on the NP formation process. However, by adding HP-β-CD, the tight connections between CS particles could be affected thus leads to an increase of particle size. At the same time, more drugs could be encapsulated into HP-β-CD which could increase the entrapment efficiency of the nanoparticles. In conclusion, the optimum concentration for HP-β-CD was 40 mg.

Table 5 – Influence of HP-β-CD concentration on the particle characteristics.

HP-β-CD added (mg)	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
0	301.5	0.187	23.7	54.28 ± 0.15
10	316.2	0.213	26.6	61.59 ± 0.09
20	329.3	0.195	27.3	65.49 ± 0.37
40	362.3	0.182	28.7	67.07 ± 0.07

Table 6 – Influence of the Pluronic F-68 amount on the particle characteristics.

Plutonic F-68 added (mg)	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
0	815.0	0.998	27.6	61.83 ± 0.12
30	362.3	0.182	28.7	67.07 ± 0.07
40	312.3	0.386	25.8	70.38 ± 0.05
50	199.4	0.451	23.3	71.08 ± 0.04

3.1.5. *Influence of the added amount of pluronic F-68*

In ionic cross-linking method, an excellent surfactant should be able to be adsorbed on the surface of the newly formed NPs to prevent aggregation. According to our previous experiment, Pluronic F-68 as a stabilizer showed good impact on the stability of NPs which could assist the separation of nanoparticles thus increases the stability of the nanoparticle system. The results were shown in Table 6. The particle size decreased as the added amount of Pluronic F-68 into the formulation increased. Without the addition of Pluronic F-68, the prepared nanoparticles were easy to aggregate thus leading to an increase of particle size. The entrapment efficiency had a slight increase which might be due to the addition of surfactant which could increase the solubility of SCU. Considering the particle size and stability, 40 mg was the optimum amount of Pluronic F-68 which could be added into the nanoparticles.

3.1.6. *Influence of the SCU concentration*

The effects of the SCU concentration on the characteristics of the CD/CS-SCU-NPs were shown in Table 7. The particle size as well as the PDI values increased as the amount of added SCU increased while the zeta potential value was decreased. It might due to the fact that the added drugs could be entrapped into the nanoparticles which could hinder the cross-linking of the polymers. Moreover, some drugs could be absorbed onto the surface of the nanoparticles. The carboxyl group of SCU and the amino group of CS could have weak ionic reaction on the surface of nanoparticles thus the number of NH_3^+ could be decreased which leads to a decrease of the zeta potential value. The entrapment efficiency was increased as the drug amount increased probably because some drugs were encapsulated into the CD. However, when the drug concentration is over 4.0 mg/ml, the entrapment efficiency decreased. It is because only a certain amount of drugs could be encapsulated in the NPs. Considering the particle size and stability, 3.0 mg/ml was the maximum amount of SCU which could be loaded in the nanoparticles.

Table 7 – Influence of the SCU concentration on the NPs characteristics.

SCU concentration (mg/ml)	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
1.0	290.3	0.140	27.3	42.49 ± 0.37
2.0	312.3	0.386	25.8	70.38 ± 0.05
3.0	364.1	0.200	20.6	81.83 ± 0.12
4.0	489.7	0.328	12.9	77.59 ± 0.05

Table 8 – Results of response surface design.

Run	A	B	C	Y_1 (Size, nm)	Y_2 (EE, %)	OD
	TPP added (mg)	CS added (mg)	Pluronic F-68 added (mg)			
1	0	–1	1	350.2	64.86	0.780
2	0	1	–1	522.7	54.52	0.480
3	1	0	1	320.0	42.35	0.599
4	0	–1	–1	194.9	54.57	0.840
5	0	0	0	386.8	67.43	0.754
6	1	–1	0	168.8	65.39	0.975
7	1	0	–1	293.5	31.35	0.475
8	–1	0	–1	489.0	17.11	0.096
9	–1	1	0	682.1	20.23	0
10	–1	–1	0	348.8	15.83	0
11	0	0	0	385.3	66.78	0.752
12	1	1	0	528.1	67.99	0.547
13	0	1	1	515.3	53.96	0.487
14	0	0	0	386.4	67.88	0.758
15	0	0	0	385.0	68.09	0.761
16	0	0	0	387.7	67.55	0.754
17	-1	0	1	510.5	19.71	0.158

3.2. *Formulation optimization and verification*

A systematic optimization was carried out using RSM for estimating the effect of formulation variables (A, B, and C) on dependent variables (Y_1 and Y_2). A three-factor, three-level BBD statistical experimental design was used to optimize the formulation variables and the response surface methodology required 17 experiments. The experimental data, including independent variables along with their low (–), medium (0), and high (+) levels and responses for all 17 experimental runs were summarized in Table 8 [26]. The value of overall desirability (OD) was calculated by Hassan method [27]. For particle size, the smaller the size, the better the nanoparticles, thus $d_1 = d_{min} = (Y_{max} - Y_i)/(Y_{max} - Y_{min})$ while for entrapment efficiency the bigger entrapment efficiency, the better the nanoparticles, thus $d_2 = d_{max} = (Y_i - Y_{min})/(Y_{max} - Y_{min})$. As a result, the value of OD is equal to $OD = (d_1 \times d_2)^{1/2}$.

The experimental results were fitted into non-linear response surface model. The polynomial models for OD can be represented by the equation:

$$OD = 0.74 + 0.29A - 0.13B + 0.017C - 0.11AB + 0.16AC + 0.017BC - 0.35A^2 - 0.080C^2$$

$$(R^2 = 0.9477, \text{Adj } R^2 = 0.8954, F = 18.11, P = 0.0002).$$

The 3D response surface graphs of the effect between each factor were shown in Fig. 2. Fig. 2A displays the combined effect of the added amounts TPP and CS on OD by keeping the added amount of pluronic F-68 at 40 mg. The TPP concentration showed a synergistic effect on OD while chitosan concentration did not exhibit any significant effect. Nevertheless, there is a significant interaction between the two variables. The plot in Fig. 2B revealed the combined effect of the added amounts of TPP and the pluronic F-68 while keeping the added amount of CS at 10 mg on OD. The TPP concentration showed a synergistic effect on OD while pluronic F-68 concentration did not exhibit any significant effect. The maximum point was lying at the intermediate value of the two variables which indicated that there was a significant interaction between the two variables [27]. The response surface plot in Fig. 2C displayed the combined effect of the added amounts of CS and pluronic F-68 by keeping the added amount of TPP at 2 mg on OD. The chitosan concentration showed an antagonistic effect on OD while pluronic F-68 concentration did not exhibit any significant effect.

By means of this method, there were 10 solutions to obtain smaller particle size and higher entrapment efficiency. One of the optimal conditions obtained were the added amounts of 2.5 mg TPP, 10.1 mg CS and 40.0 mg pluronic F-68 with the predicted OD of 0.985.

3.3. *Characterization of CD/CS-SCU-NPs*

According to the results stated above, an optimized result from the response surface design experiments using Design Expert software based on the effects of three factors was described as follows: SCU (6 mg) was dissolved in 2 ml of methanol; Pluronic F-68 (40 mg) was dissolved in 5 ml of CS solution (2.02 mg/ml), and sodium hydroxide was used to adjust the pH of the mixture to 4.5. The SCU solution was dropped into the CS solution with magnetic stirrer (500 rpm). Then HP-β-CD (40 mg) and TPP (2.5 mg) were dissolved in 1 ml of distilled water and the mixture was added drop by drop (20–40 drops/min). To check the suitability of the model equation, three batches of NPs were prepared according to the optimized formulation. Then particle size, PDI, zeta potential and entrapment efficiency of each batch were determined. The results shown in Table 9 were in good agreement with the former study done by Wei et al. [22], suggesting that the optimization was reliable and reasonable.

3.4. *In vitro release study*

Fig. 3 shows the percent release of SCU from CD/CS-SCU-NPs prepared using the optimal formulations for verification. The

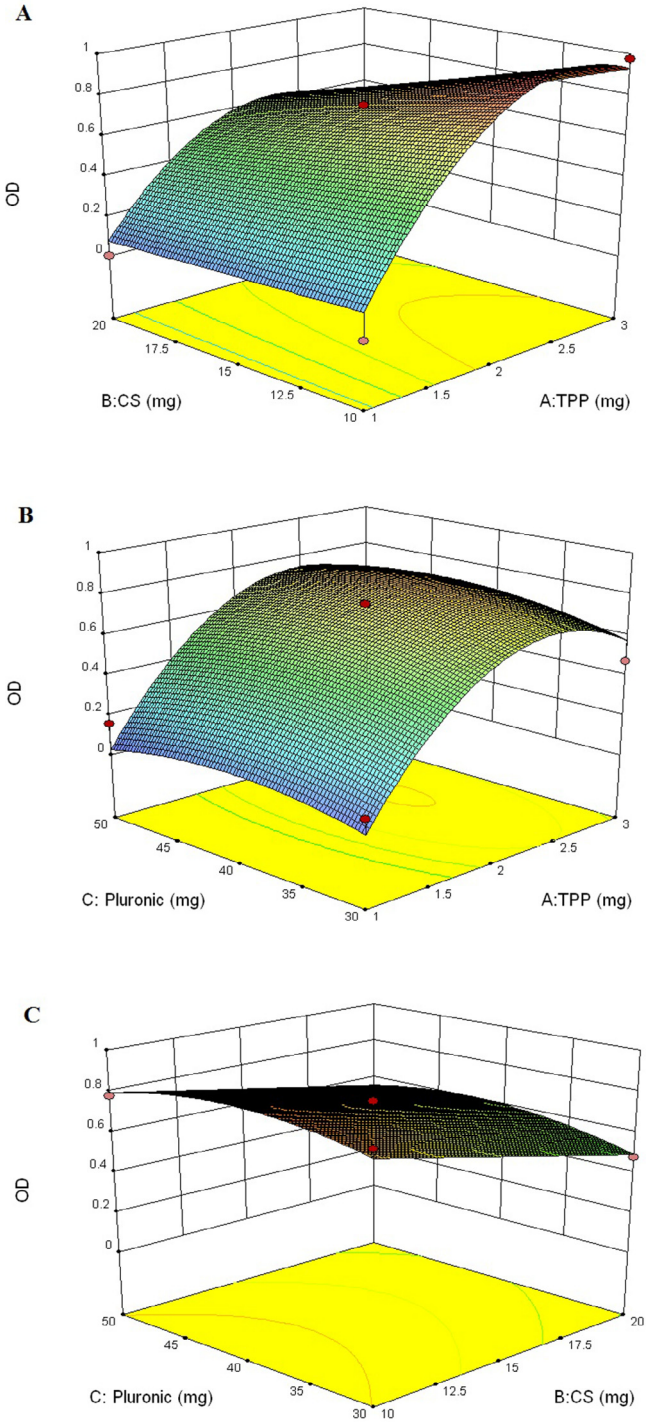

Fig. 2 – 3D response surface graphs of the effect of factor *A* & *B* on OD value (A), factor *A* & *C* on OD value (B) and factor *B* & *C* on OD value (C).

SCU release profiles in pH 6.8 exhibited a biphasic manner, an initial fast release phase followed by a slower release phase. In the first 8 h, the drug cumulative release value was 54.8%. After 24 h, almost 80% of SCU was released. On the contrary, the drug release from free SCU was fast and completed after 4 h. Similar phenomena had been observed in a previous study [22].

Table 9 – Results of optimal experimental condition for preparation of D/CS-SCU-NPs.

Batch	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
1	239.5	0.545	26.0	73.41 ± 1.31
2	230.7	0.669	23.4	69.52 ± 0.15
3	225.2	0.658	25.9	70.10 ± 0.92

Table 10 – Release kinetics parameters of CD/CS-SCU-NPs according to different kinetic models.

Model	Equation	R^2
Zero order	$Q = 3.628t + 14.561$	0.8147
First order	$Ln\ (1 - Q) = -0.0642t + 4.0394$	0.7125
Higuchi	$Q = 19.6237t^{1/2} - 2.4455$	0.9792
Korsmeyer-Peppas	$\ln Q = 1.4734 \ln t + 2.3355$	0.7901

The drug release from polymer modified nanoparticles is a rather complicated process. It can be affected by many factors such as polymer degradation, molecular weight and the binding affinity between the polymer and drug. Several important kinetic models such as zero-order equation, first-order equation, Higuchi's square root of time equation and Korsmeyer-Peppas equation were selected to fit the experimental data from Fig. 3. Table 10 shows the release equations obtained from these kinetic models. It was clearly seen in Table 10 that Higuchi's square-root of time model ($R^2 = 0.9769$) showed significantly better fitting than zero-order model ($R^2 = 0.8147$), first-order model ($R^2 = 0.7125$) and Korsmeyer-Peppas model ($R^2 = 0.7901$). The best fit was obtained with the Higuchi's equation, which suggested that SCU release was controlled both by diffusion and degradation.

4. Conclusion

In this study, the RSM based on the BBD combined with the overall desirability value was successfully used to optimize the

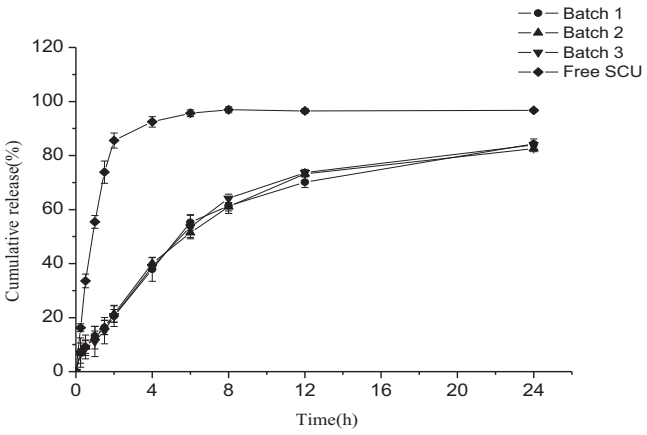

Fig. 3 – Release profiles of SCU from the CD/CS-SCU-NPs prepared under the optimal experimental condition and free SCU (*n* = 3).

preparation process and formulation variables of CD/CS-SCU-NPs. The experimental values under the optimum conditions were mostly close to the predicted values. It was demonstrated by the results that the particle sizes of the optimum formulation were all around 200 nm, PDI values around 0.5, zeta potential values around 25 mV and entrapment efficiency around 70%, indicating that the products of the optimum formulation had a nano-grade particle size, were stable in the solution and around 70% of the SCU could be entrapped into the nanoparticles. The drug release behavior from the CD/CS-SCU-NPs exhibited a biphasic pattern with an initial fast release phase followed by a slower release phase. The NPs were proved to be successful in prolonging drug release compared with free SCU and the SCU released from the NPs was in accordance with the Higuchi's model. All these results indicated that the CD/CS-SCU-NPs had higher entrapment efficiency and promising controlled release profiles. Thus, incorporating CD into CS nanoparticles might be a potential alternative for drug delivery system. The combination of RSM and BBD can provide an insight into a lab-scale pharmaceutical formulation studies in which the optimal formulation could be easily obtained, leading to a saving of experimental time and materials.

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