# Supplementary information

**Table S1** Summary result of regression analysis for model fitting.

| Response | *p-value* | $R^2$ | $R^2_{adjusted}$ | $R^2_{predicted}$ | Lack of fit | Remark |
|---|---|---|---|---|---|---|
| *Response $Y_1$ (particle size)* | | | | | | |
| *Linear* | $\leq 0.0001$ | 0.9636 | 0.9537 | 0.9321 | 0.1334 | Suggested |
| *2FI* | 0.4999 | 0.9725 | 0.9518 | 0.8954 | 0.1191 | - |
| *Quadratic* | 0.1048 | 0.9912 | 0.9752 | 0.8741 | 0.1868 | - |
| Statistically significant factors for $Y_1$ ($p < 0.05$) are $X_1$, $X_2$ and $X_3$ | | | | | | |
| *Response $Y_2$ (zeta potential)* | | | | | | |
| *Linear* | $\leq 0.0001$ | 0.9563 | 0.9444 | 0.9106 | 0.3358 | Suggested |
| *2FI* | 0.5692 | 0.9656 | 0.9398 | 0.8339 | 0.2957 | - |
| *Quadratic* | 0.6174 | 0.9752 | 0.9306 | 0.6557 | 0.2206 | - |
| Statistically significant factors for $Y_2$ ($p < 0.05$) are $X_1$, *and* $X_2$ | | | | | | |
| *Response $Y_3$ (EE)* | | | | | | |
| *Linear* | $< 0.0001$ | 0.9435 | 0.9281 | 0.8968 | 0.2042 | - |
| *2FI* | 0.4150 | 0.9597 | 0.9294 | 0.8601 | 0.1939 | - |
| *Quadratic* | 0.0133 | 0.9945 | 0.9846 | 0.9504 | 0.6548 | Suggested |
| Statistically significant factors for $Y_3$ ($p < 0.05$) are $X_1$, $X_2$ and $X_3$ | | | | | | |

**Notes**: $X_1$ = TPP (mg/mL), $X_2$ = Pluronic® F-127 (% w/v), $X_3$ = HHC (mg/mL), $Y_1$ = particle size (nm), $Y_2$ = zeta potential (mV), and $Y_3$ = EE (%).

**Equation:**

The equation below explained the physical meaning and significance necessary for the second-order polynomial model used in response surface analysis, given as equations S1-S3.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Where $Y$ is response (dependent variable), $\beta_0$ is intercept, $\beta_1 - \beta_{33}$ are regression coefficients computed from the observed value of $Y$ from experiments, and $X_1$, $X_2$ and $X_3$ are factors (independent variables). The terms ($X_1X_2$, $X_1X_3$, and $X_2X_3$) and ($X_1^2$, $X_2^2$, and $X_3^2$) represent the interaction and quadratic terms, respectively [1]. The mathematic model equation was used to evaluate the effect of each factor on the responses and determine the optimum setting of these variables to achieve optimum response [2]. "

**Equation:**

**Equation S1** The multiple linear regression for the response particle size ($Y_1$)

Particle size ($Y_1$) = + 239.87 + 84.87$X_1$ + 56.12$X_2$ + 17.00$X_3$

**Equation S2** The multiple linear regression for the response zeta potential ($Y_1$)

Zeta potential ($Y_2$) = + 31.07 - 9.00$X_1$ – 1.63$X_2$ - 0.625$X_3$

**Equation S3** The multiple linear regression for the response EE ($Y_3$)

EE ($Y_3$) = + 75.00 + 10.62$X_1$ – 3.25$X_2$ + 14.63$X_3$ + 2.51$X_1X_2$ - 1.75$X_1X_3$ + 1.50$X_2X_3$ - 1.12$X_1^2$ – 0.875$X_2^2$ - 5.13$X_3^2$

Where $Y$ is response, $X_1$ = TPP (mg/mL), $X_2$ = Pluronic® F-127 (% w/v), $X_3$ = HHC (mg/mL), $Y_1$ = particle size (nm), $Y_2$ = zeta potential (mV), and $Y_3$ = EE (%).

**References**

1. Motwani, S.K.; Chopra, S.; Kohli, T.K.; Ahmad, F.J.; Khar, R.K. Chitosan-sodium alginate nanoparticles as submicroscopic reservoirs for ocular delivery: formulation, optimisation and *in vitro* characterisation. *Eur. J. Pharm. Biopharm*, **2008**, *68* (2008), 513–525. doi: 10.1016/j.ejpb.2007.09.009. Epub 2007 Sep 25.

2. Khuri, A.I.; Mukhopadhyay, S. Response surface methodology. *WIREs Comp Stat*, **2010**, *2*, 128-149. doi.org/10.1002/wics.73.