Supplementary file

Table S1. Quality Target Product Profile (QTPP) for NP-PTX/SIM

QTPP Element	Target	Target Value / Range	Justification
Dosage form	Oral lyophilized nanoparticles	Nanoparticulate powder for reconstitution	Non-invasive route; protects drugs from GI degradation; designed for pH-triggered drug release preferential to the colonic pH environment
Target delivery site	Colonic pH environment (pH > 7.0)	pH > 7.0 environment	Site of colorectal cancer; Eudragit S100 dissolves selectively at pH > 7.0, providing pH-triggered drug release consistent with preferential exposure at the colonic pH environment. Note: This target reflects the intended region of preferential pH-triggered release based on the in vitro dissolution mechanism of Eudragit S100. Confirmation of in vivo site-specific colonic drug delivery requires pharmacokinetic and biodistribution studies.
Particle size	< 200 nm	100–200 nm	Optimal for EPR-mediated tumor accumulation; avoids rapid reticuloendothelial clearance
PDI	< 0.25	≤ 0.25	Ensures homogeneous size distribution for reproducible pharmacokinetics
Zeta potential	> +25 mV	+25 to +45 mV	Colloidal stability via electrostatic repulsion; enhances mucoadhesion and cellular uptake
Entrapment efficiency – PTX	> 80%	≥ 80%	Maximizes therapeutic payload; minimizes drug waste and free drug in GI tract
Entrapment efficiency – SIM	> 75%	≥ 75%	Sufficient loading of poorly soluble lipophilic drug; prevents premature release
Drug release – gastric (pH 1.2)	Minimal (< 10%)	< 10% at 48 h	Protects drugs from acid degradation; prevents systemic absorption in stomach
Drug release – intestinal (pH 6.8)	Controlled (< 30%)	< 30% at 48 h	Limits small intestinal drug absorption; preserves drug payload for preferential release at colonic pH
Drug release – colonic (pH 7.4)	Sustained and substantial (> 80%)	> 80% within 24 h	Ensures adequate drug concentration at tumor site for therapeutic effect; drug liberation at this pH is driven by pH-triggered Eudragit S100 dissolution, consistent with preferential colonic exposure in vitro
Biocompatibility / safety	Non-toxic carrier	Carrier IC_{50} ≥10× therapeutic dose	Chitosan and Eudragit S100 are established GRAS/pharmacopoeially accepted excipients

EPR; enhanced permeability and retention; GI, gastrointestinal; GRAS, generally recognized as safe; IC_{50}, half-maximal inhibitory concentration; PDI, polydispersity index; PTX, pentoxifylline; SIM, simvastatin.

Table S2a. Risk assessment matrix for CMAs and CPPs prior to BBD experimentation

Variable	Type	Impact on Particle Size	Impact on PDI	Impact on Zeta Potential	Impact on EE% PTX	Impact on EE% SIM	Risk Level & Decision
Chitosan concentration	CMA / CPP	High	High	High	High	Medium	**HIGH → Selected as Factor A in BBD**
Eudragit S100 concentration	CMA / CPP	High	Medium	Medium	Medium	High	**HIGH → Selected as Factor B in BBD**
TPP concentration	CPP	Medium	Medium	Low	Medium	Medium	**MEDIUM → Selected as Factor C in BBD**
Chitosan molecular weight	CMA	Medium	Low	Medium	Medium	Low	MEDIUM → Fixed at 150 kDa (screened, literature-supported)
Chitosan deacetylation degree	CMA	Low	Low	Medium	Low	Low	LOW → Fixed at ≥95% (consistent commercial

							grade)
pH of chitosan solution	CPP	Medium	Low	High	Low	Low	MEDIUM → Fixed at pH 5.0 (optimum for chitosan protonation)
Stirring speed (TPP addition)	CPP	Medium	Medium	Low	Low	Low	LOW → Fixed at 1200 rpm (preliminary screening)
Temperature	CPP	Low	Low	Low	Low	Low	LOW → Fixed at room temperature (25°C)

Based on this formal risk assessment, three variables — chitosan concentration (A), Eudragit S100 concentration (B), and TPP concentration (C) — were identified as the most critical CPPs and selected for systematic investigation in the Box-Behnken design. All remaining parameters were fixed at their optimized levels based on preliminary OVAT experiments and literature evidence, thereby controlling their potential contribution to variability within the design space.

Table S2b. Established design space for NP-PTX/SIM: proven acceptable ranges (PAR) for each CPP

CPP	Studied Range (BBD)	Optimum (Center Point)	Design Space (PAR)	Rationale for PAR Boundaries
A: Chitosan concentration (% w/v)	0.5 – 1.5	1.0	0.8 – 1.2	Below 0.8%: insufficient matrix density → particle size < 130 nm but EE% PTX falls below 80%. Above 1.2%: particle size exceeds 175 nm and PDI increases toward 0.25.
B: Eudragit S100 concentration (% w/v)	0.5 – 1.5	1.0	0.8 – 1.2	Below 0.8%: EE% SIM falls below 75% due to insufficient hydrophobic domains. Above 1.2%: zeta potential decreases toward +25 mV due to anionic charge neutralization.
C: TPP concentration (% w/v)	0.3 – 0.7	0.5	0.4 – 0.6	Below 0.4%: insufficient ionic crosslinking → reduced particle hardness and EE%. Above 0.6%: excessive crosslinking increases PDI and reduces entrapment efficiency for both drugs.

Table S3. Comparison of predicted and experimental values for the optimized formulation

Response	**Predicted Value**	**Experimental Value***
Particle size (nm)	152.0	152 ± 5
Polydispersity index	0.180	0.18 ± 0.02
Zeta potential (mV)	+31.2	+31.2 ± 1.5
EE% PTX	85.4	85.4 ± 3.1
EE% SIM	78.9	78.9 ± 2.8

**Values represent mean ± standard deviation (n = 3). Optimized formulation parameters: chitosan 1.0% w/v, Eudragit S100 1.0% w/v, TPP 0.5% w/v. EE%: entrapment efficiency; PTX: pentoxifylline; SIM: simvastatin.*

Table S4. Analysis of variance (ANOVA) for Particle Size (Y_1)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	6842.5	9	760.3	42.18	<0.001
A-Chitosan	3248.7	1	3248.7	180.32	<0.001
B-Eudragit S100	1852.4	1	1852.4	102.81	<0.001
C-TPP	645.8	1	645.8	35.84	0.001
AB	185.6	1	185.6	10.30	0.021
AC	124.3	1	124.3	6.90	0.042
BC	78.5	1	78.5	4.36	0.089
A^2	385.2	1	385.2	21.38	0.004
B^2	245.8	1	245.8	13.64	0.011
C^2	165.4	1	165.4	9.18	0.025
Residual	90.1	5	18.0	–	–
Lack of Fit	68.4	3	22.8	2.11	0.328
Pure Error	21.7	2	10.8	–	–
Total	6932.6	14	–	–	–

R^2 = 0.9870; Adjusted R^2 = 0.9636; Predicted R^2 = 0.9254; Adequate Precision = 24.85. Significant terms ($p < 0.05$), df: degrees of freedom.

Table S5. Analysis of variance (ANOVA) for Polydispersity Index (Y_2)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.0512	9	0.0057	38.65	<0.001
A-Chitosan	0.0085	1	0.0085	57.82	<0.001
B-Eudragit S100	0.0045	1	0.0045	30.56	0.002
C-TPP	0.0028	1	0.0028	19.05	0.007
AB	0.0012	1	0.0012	8.16	0.034
AC	0.0008	1	0.0008	5.44	0.067
BC	0.0005	1	0.0005	3.40	0.124
A^2	0.0158	1	0.0158	107.48	<0.001
B^2	0.0112	1	0.0112	76.19	<0.001
C^2	0.0089	1	0.0089	60.54	<0.001
Residual	0.0007	5	0.0001	–	–
Lack of Fit	0.0005	3	0.0002	2.50	0.286
Pure Error	0.0002	2	0.0001	–	–
Total	0.0519	14	–	–	–

R^2 = 0.9865; Adjusted R^2 = 0.9622; Predicted R^2 = 0.9187; Adequate Precision = 23.14. Significant terms ($p < 0.05$), df: degrees of freedom.

Table S6. Analysis of variance (ANOVA) for Zeta Potential (Y_3)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	485.62	9	53.96	45.82	<0.001
A-Chitosan	324.58	1	324.58	275.63	<0.001
B-Eudragit S100	38.45	1	38.45	32.65	0.002
C-TPP	24.86	1	24.86	21.11	0.006
AB	18.52	1	18.52	15.73	0.011
AC	12.34	1	12.34	10.48	0.022
BC	8.65	1	8.65	7.34	0.040
A^2	28.74	1	28.74	24.41	0.004
B^2	18.92	1	18.92	16.07	0.010
C^2	14.28	1	14.28	12.13	0.017
Residual	5.89	5	1.18	–	–
Lack of Fit	4.23	3	1.41	1.70	0.382
Pure Error	1.66	2	0.83	–	–
Total	491.51	14	–	–	–

R^2 = 0.9880; Adjusted R^2 = 0.9664; Predicted R^2 = 0.9315; Adequate Precision = 26.42. Significant terms ($p < 0.05$), df: degrees of freedom.

Table S7. Analysis of variance (ANOVA) for Entrapment Efficiency of Pentoxifylline (Y_4)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	658.42	9	73.16	52.18	<0.001
A-Chitosan	428.65	1	428.65	305.79	<0.001
B-Eudragit S100	78.54	1	78.54	56.03	<0.001
C-TPP	48.32	1	48.32	34.47	0.002
AB	35.48	1	35.48	25.31	0.004
AC	18.65	1	18.65	13.31	0.014
BC	12.34	1	12.34	8.80	0.031
A^2	22.45	1	22.45	16.01	0.010
B^2	15.82	1	15.82	11.29	0.019
C^2	10.58	1	10.58	7.55	0.039
Residual	7.01	5	1.40	–	–
Lack of Fit	5.12	3	1.71	1.81	0.365
Pure Error	1.89	2	0.95	–	–
Total	665.43	14	–	–	–

R^2 = 0.9895; Adjusted R^2 = 0.9706; Predicted R^2 = 0.9412; Adequate Precision = 28.76. Significant terms ($p < 0.05$), df: degrees of freedom.

Table S8. Analysis of variance (ANOVA) for Entrapment Efficiency of Simvastatin (Y_5)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	512.84	9	56.98	48.92	<0.001
A-Chitosan	145.62	1	145.62	125.01	<0.001
B-Eudragit S100	285.43	1	285.43	245.04	<0.001
C-TPP	38.54	1	38.54	33.09	0.002
AB	28.45	1	28.45	24.42	0.004
AC	15.32	1	15.32	13.15	0.015
BC	10.65	1	10.65	9.14	0.029
A^2	18.76	1	18.76	16.11	0.010
B^2	14.28	1	14.28	12.26	0.017
C^2	9.82	1	9.82	8.43	0.033
Residual	5.82	5	1.16	–	–
Lack of Fit	4.18	3	1.39	1.70	0.383
Pure Error	1.64	2	0.82	–	–
Total	518.66	14	–	–	–

R^2 = 0.9888; Adjusted R^2 = 0.9686; Predicted R^2 = 0.9358; Adequate Precision = 27.38. df: degrees of freedom.

Table S9. Similarity factor (f_2) and comparison of cumulative in vitro release between dialysis bag method and centrifugal ultrafiltration for NP-PTX/SIM.

Medium	Drug	f_2 Factor	Mean Absolute Difference at 2 h (%)	Mean Absolute Difference at 24 h (%)
SGF (pH 1.2)	PTX	76.4	0.8	0.3
	SIM	71.2	0.5	0.4
SIF (pH 6.8)	PTX	82.1	1.2	0.7
	SIM	78.5	1.5	0.9
SCF (pH 7.4)	PTX	69.8	2.8	1.1
	SIM	67.3	4.2	1.5

f_2 > 50 indicates similarity between the two release profiles. Mean absolute differences represent the average of absolute differences across triplicate measurements at the indicated time points.