Supplementary Data

For Journal of Chromatography and Separation Techniques

Evaluation of driving forces for protein partition in PEG–salt aqueous two-phase systems and optimization by design of experiments

Anna Glyk, Dörte Solle, Thomas Scheper and Sascha Beutel[*]

Institute of Technical Chemistry, Leibniz University of Hannover, Callinstr. 5, 30167 Hannover, Germany

Supplementary Figures

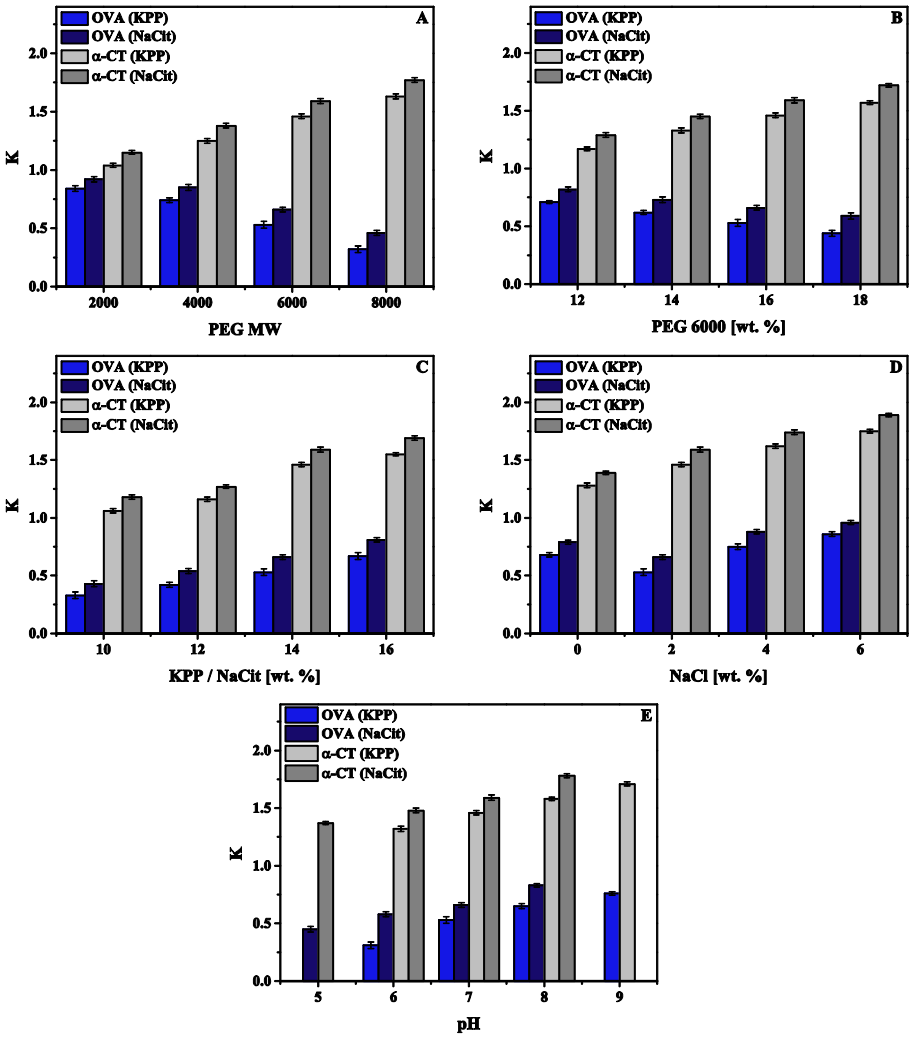

Fig. S1 Influence of increasing PEG MW (A), PEG 6000 concentration (B), KPP/NaCit concentration (C), NaCl concentration (D) and pH value (E) on the partitioning behavior (*K*) of OVA and α-CT (0.1 wt. %) in different PEG–KPP/NaCit ATPS, respectively.

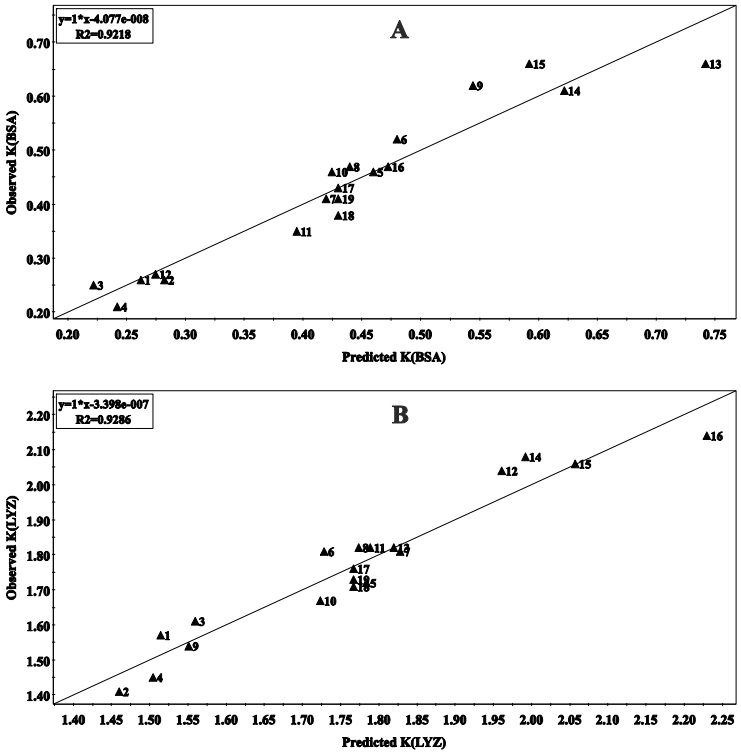

Fig. S2 Relationship between the observed and predicted values of K_{BSA} (A) and K_{LYZ} (B) based on the results of the 2^{5-1} fFD considering the partitioning behavior of BSA and LYZ (0.1 wt. %) in different PEG–KPP/NaCit ATPS, respectively. Numbers inside the figure are experimental assay numbers.

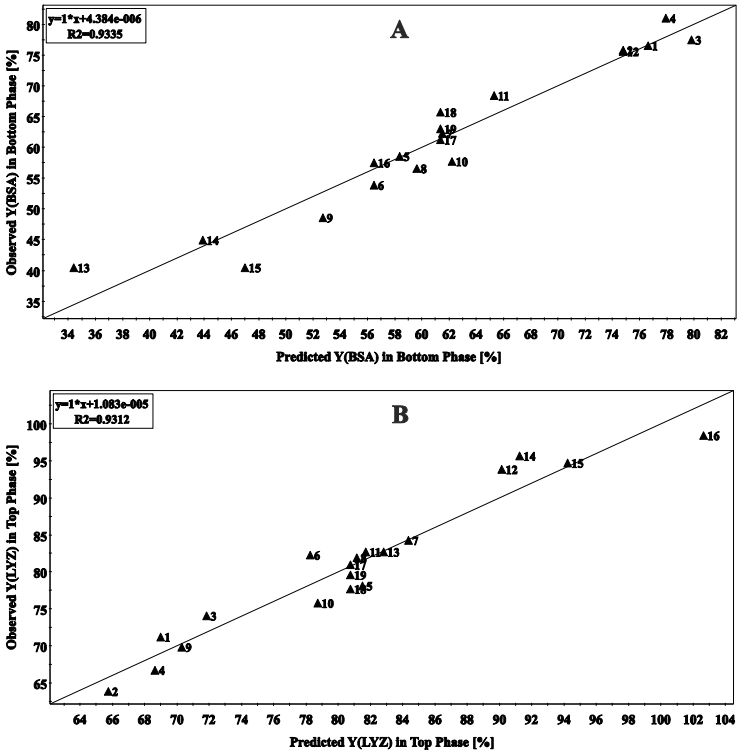

Fig. S3 Relationship between the observed and predicted values of $Y_{BP\text{-}BSA}$ % (A) and $Y_{TP\text{-}LYZ}$ % (B) based on the results of the 2^{5-1} fFD considering the recovery yield of BSA and LYZ (0.1 wt. %) in the bottom/top phase in different PEG–KPP/NaCit ATPS, respectively. Numbers inside the figure are experimental assay numbers.

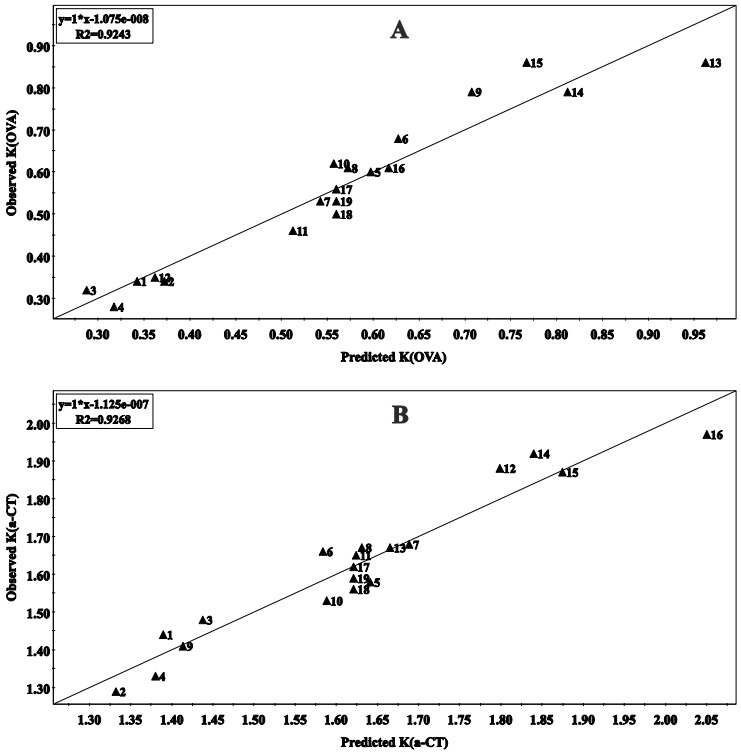

Fig. S4 Relationship between the observed and predicted values of K_{OVA} (A) and $K_{\alpha\text{-}CT}$ (B) based on the results of the 2^{5-1} fFD considering the partitioning behavior of OVA and α-CT (0.1 wt. %) in different PEG–KPP/NaCit ATPS, respectively. Numbers inside the figure are experimental assay numbers.

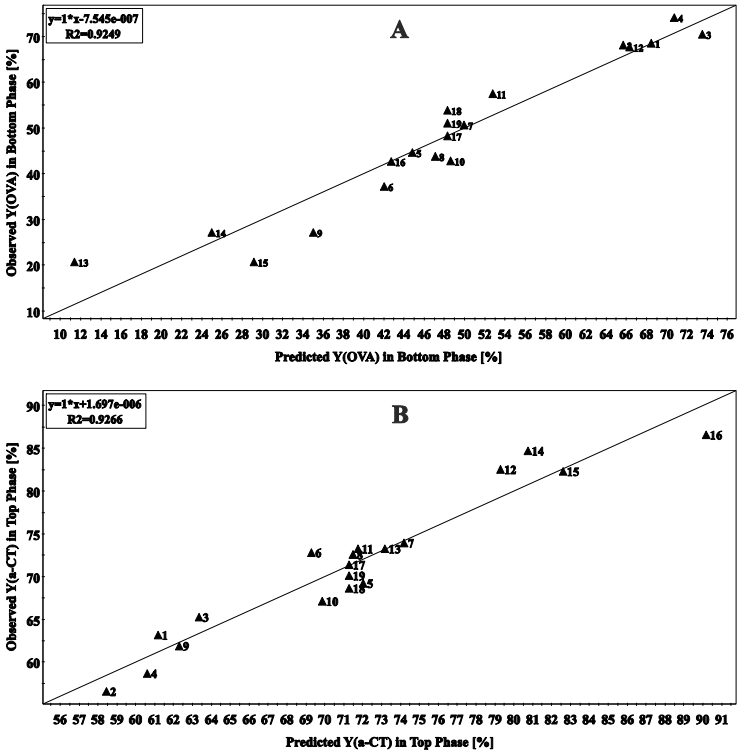

Fig. S5 Relationship between the observed and predicted values of $Y_{BP\text{-}OVA}$ % (A) and $Y_{TP\text{-}\alpha\text{-}CT}$ % (B) based on the results of the 2^{5-1} fFD considering the recovery yield of OVA and α-CT (0.1 wt. %) in the bottom/top phase in different PEG–KPP/NaCit ATPS, respectively. Numbers inside the figure are experimental assay numbers.

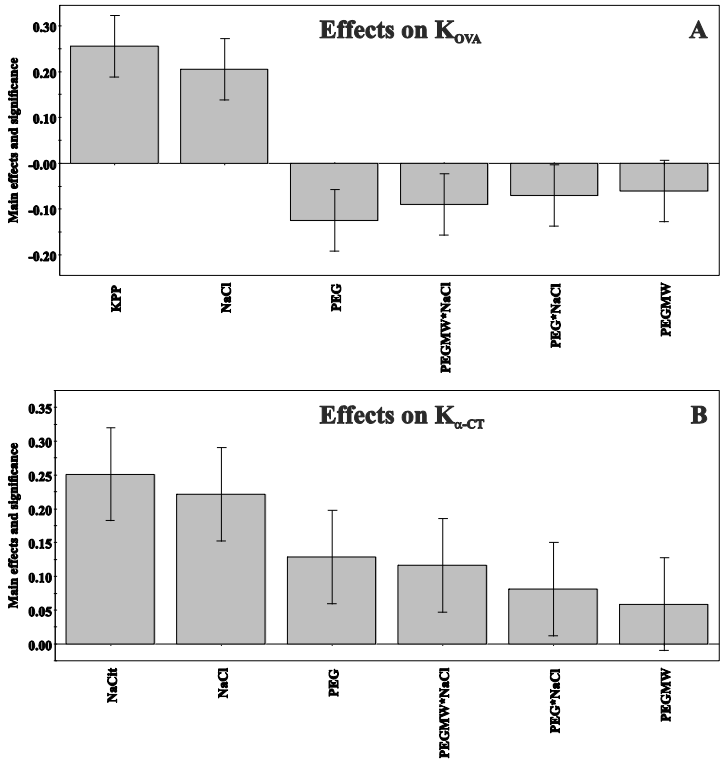

Fig. S6 Main effects of parameters on the partitioning behavior of OVA and α-CT (0.1 wt. %), K_{OVA} (A) and $K_{\alpha\text{-}CT}$ (B), in different PEG–KPP/NaCit ATPS and their significance based on the results of the 2^{5-1} fFD, respectively. For abbreviations, see Table 1.

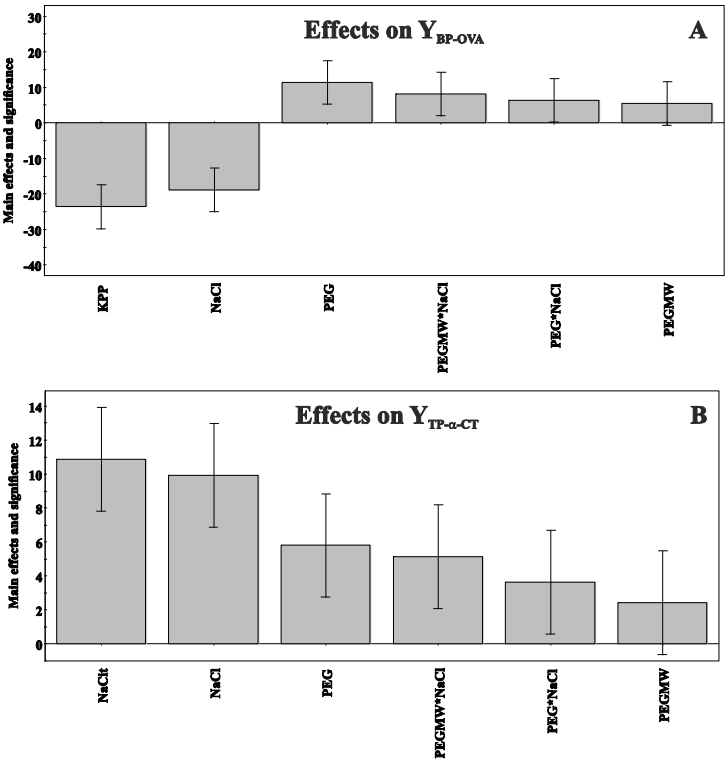

Fig. S7 Main effects of parameters on the recovery yield of OVA and α-CT (0.1 wt. %) in the bottom/top phase, $Y_{BP\text{-}OVA}$ (A) and $Y_{TP\text{-}\alpha\text{-}CT}$ (B), in different PEG–KPP/NaCit ATPS and their significance based on the results of the 2^{5-1} fFD, respectively. For abbreviations, see Table 1.

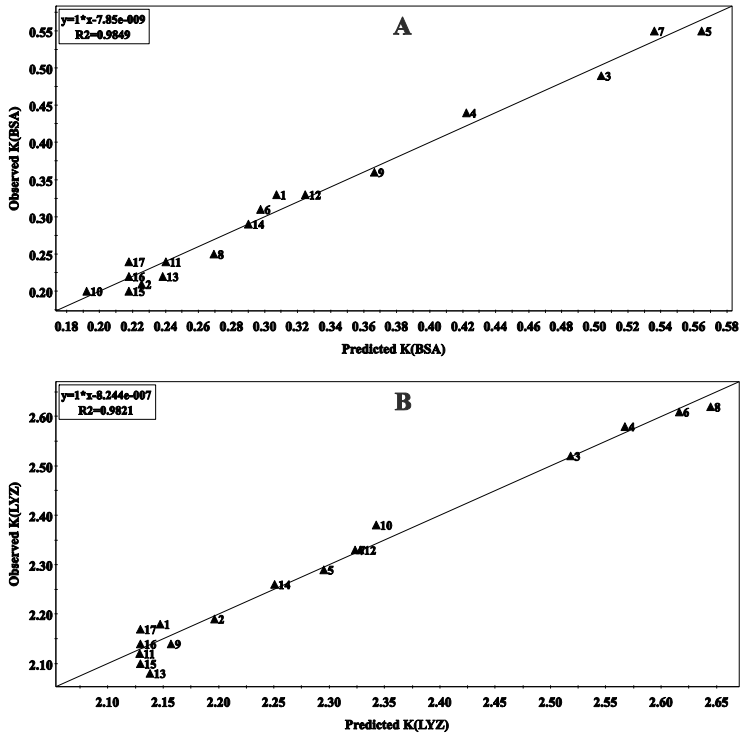

Fig. S8 Relationship between the observed and predicted values of K_{BSA} (A) and K_{LYZ} (B) based on the results of the 2^3 CCF design considering the partitioning behavior of BSA and LYZ (0.1 wt. %) in different PEG 6000–KPP/NaCit ATPS at pH 7, respectively. Numbers inside the figure are experimental assay numbers.

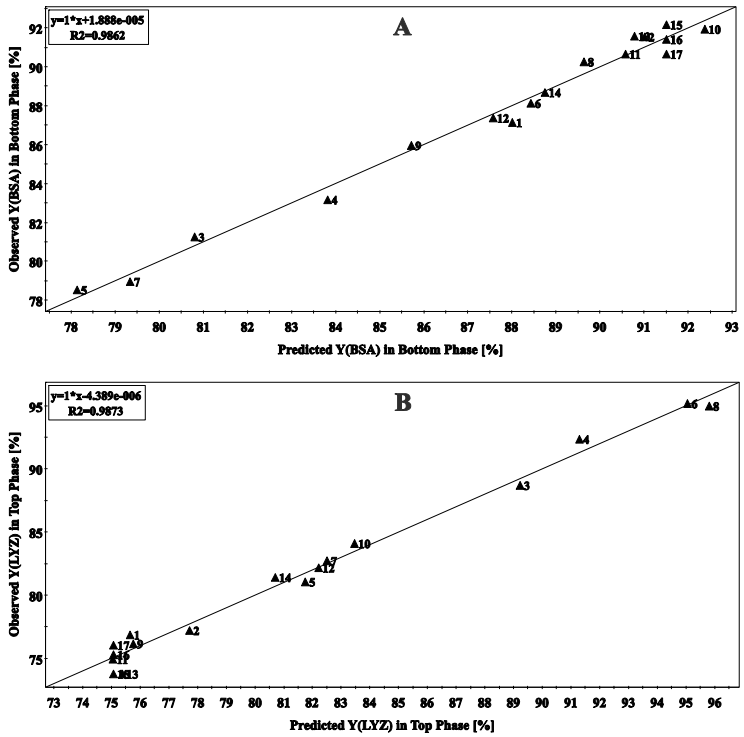

Fig. S9 Relationship between the observed and predicted values of $Y_{BP\text{-}BSA}$, % (A) and $Y_{TP\text{-}LYZ}$, % (B) based on the results of the 2^3 CCF design considering the recovery yield of BSA and LYZ (0.1 wt. %) in the bottom/top phase in different PEG 6000–KPP/NaCit ATPS at pH 7, respectively. Numbers inside the figure are experimental assay numbers.

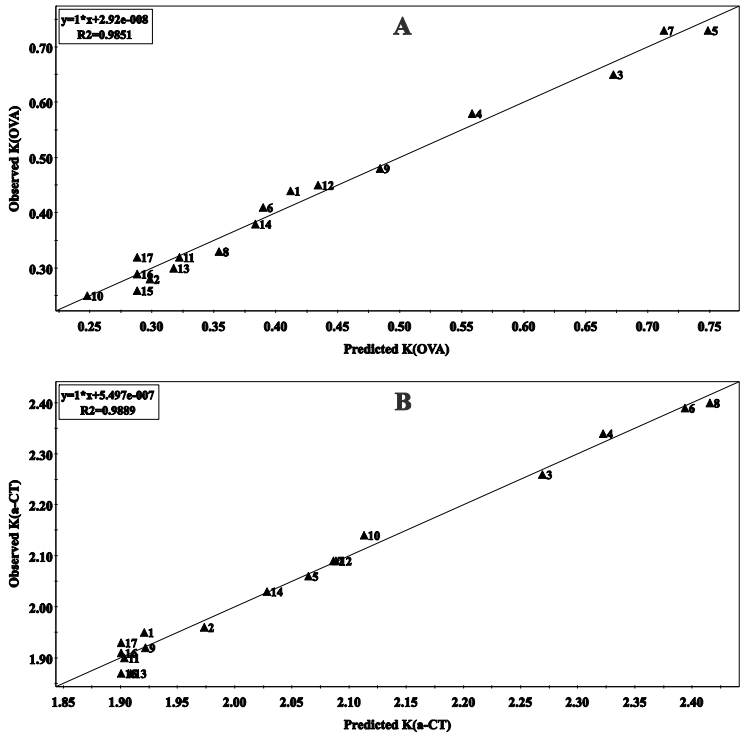

Fig. S10 Relationship between the observed and predicted values of K_{OVA} (A) and $K_{\alpha\text{-}CT}$ (B) based on the results of the 2^3 CCF design considering the partitioning behavior of OVA and α-CT (0.1 wt. %) in different PEG 6000–KPP/NaCit ATPS at pH 7, respectively. Numbers inside the figure are experimental assay numbers.

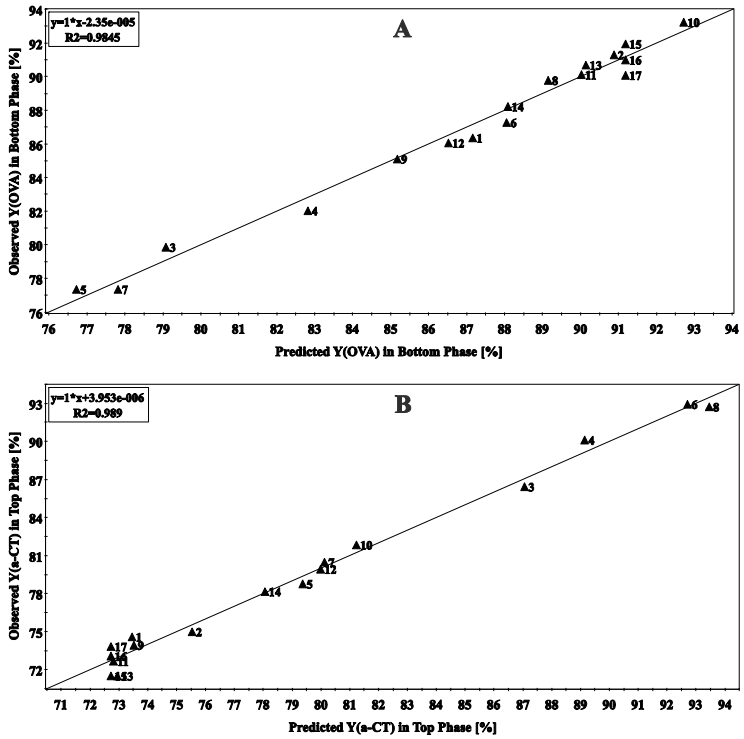

Fig. S11 Relationship between the observed and predicted values of $Y_{BP\text{-}OVA}$, % (A) and $Y_{TP\text{-}\alpha\text{-}CT}$, % (B) based on the results of the 2^3 CCF design considering the recovery yield of OVA and α-CT (0.1 wt. %) in the bottom/top phase in different PEG 6000–KPP/NaCit ATPS at pH 7, respectively. Numbers inside the figure are experimental assay numbers.

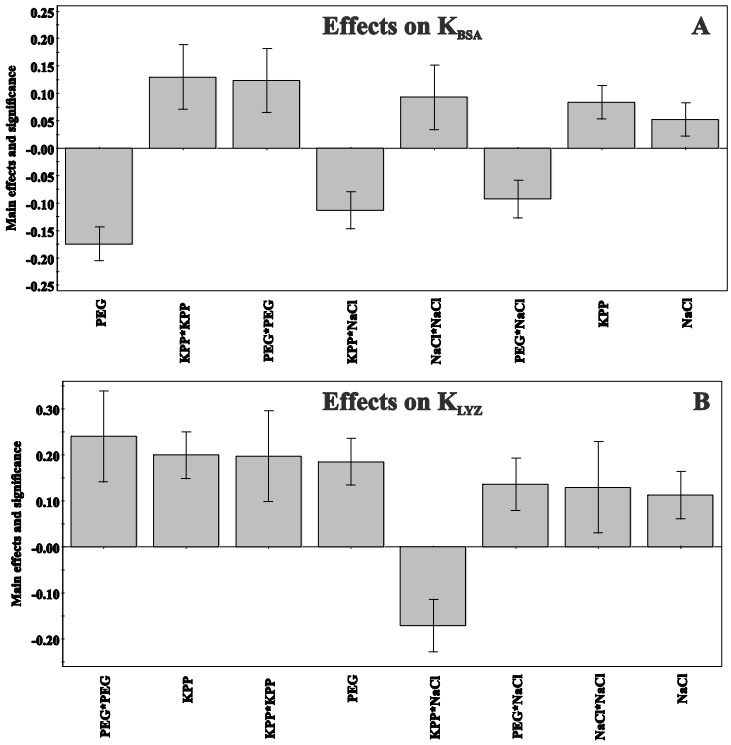

Fig. S12 Main effects of parameters on the partitioning behavior of BSA and LYZ (0.1 wt. %), K_{BSA} (A) and K_{LYZ} (B), in different PEG 6000–KPP/NaCit ATPS at pH 7 and their significance based on the results of the 2^3 CCF design, respectively. For abbreviations, see Table 4.

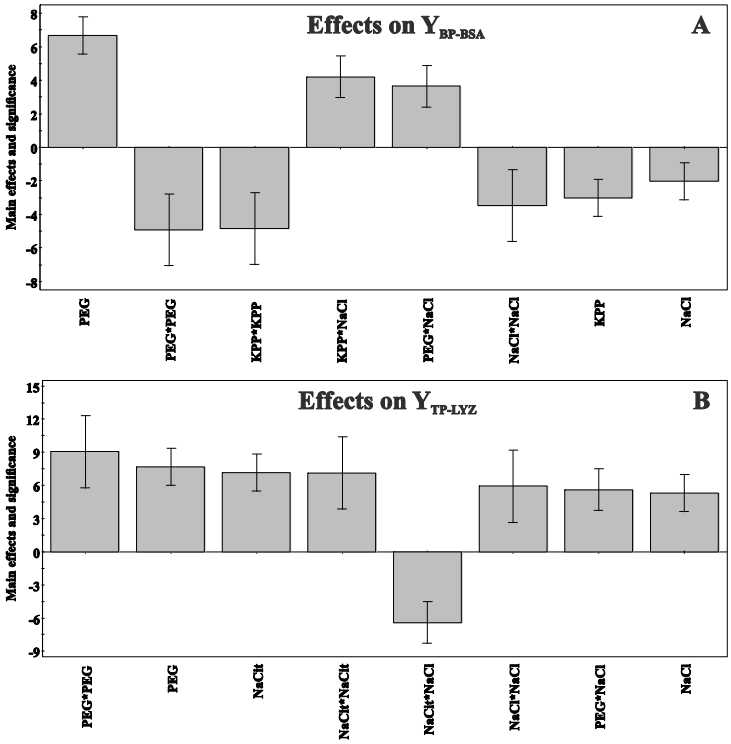

Fig. S13 Main effects of parameters on the recovery yield of BSA and LYZ (0.1 wt. %) in the bottom/top phase, Y_{BP-BSA} (A) and Y_{TP-LYZ} (B), in different PEG 6000–KPP/NaCit ATPS at pH 7 and their significance based on the results of the 2^3 CCF design, respectively. For abbreviations, see Table 4.

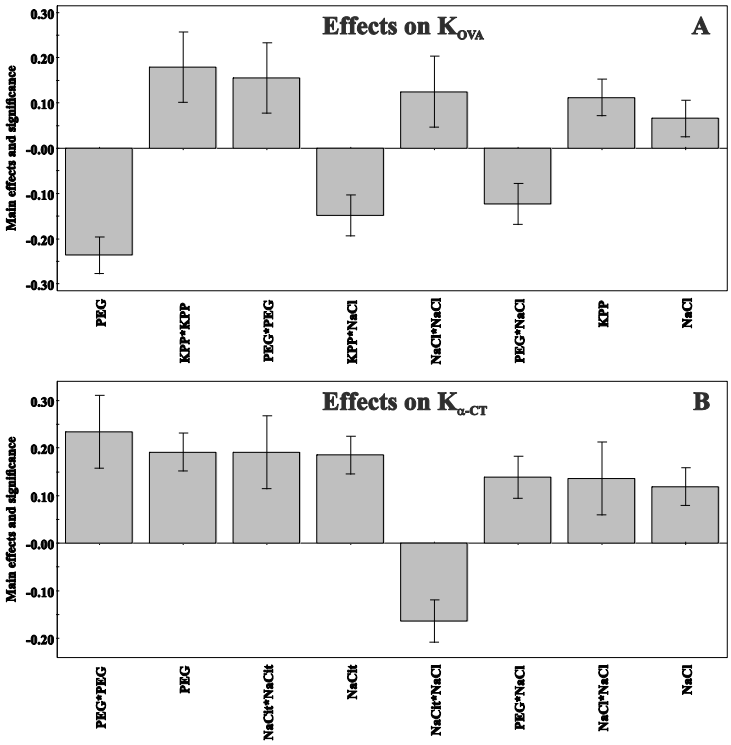

Fig. S14 Main effects of parameters on the partitioning behavior of OVA and α-CT (0.1 wt. %), K_{OVA} (A) and $K_{\alpha\text{-}CT}$ (B), in different PEG 6000–KPP/NaCit ATPS at pH 7 and their significance based on the results of the 2^3 CCF design, respectively. For abbreviations, see Table 4.

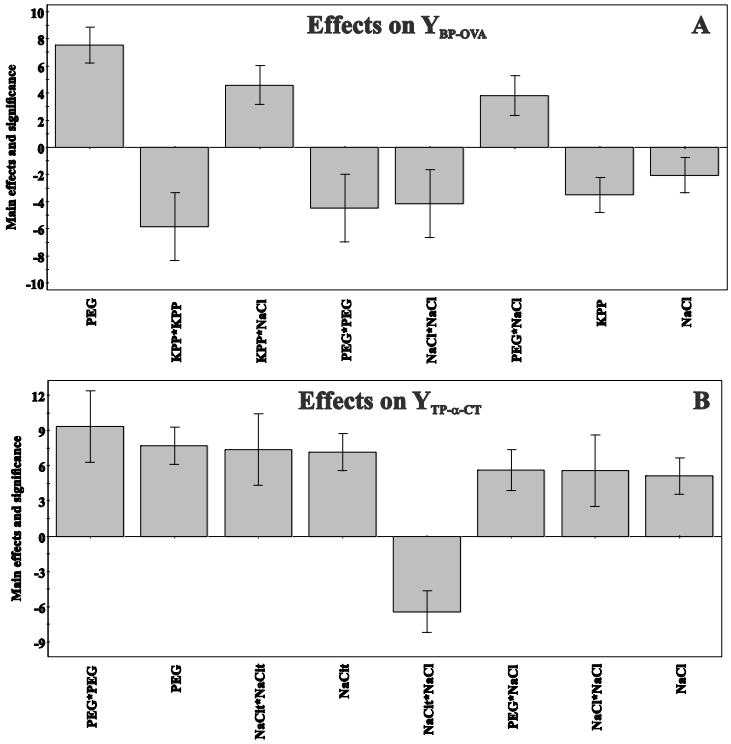

Fig. S15 Main effects of parameters on the recovery yield of OVA and α-CT (0.1 wt. %) in the bottom/top phase, $Y_{BP\text{-}OVA}$ (A) and $Y_{TP\text{-}\alpha\text{-}CT}$ (B), in different PEG 6000–KPP/NaCit ATPS at pH 7 and their significance based on the results of the 2^3 CCF design, respectively. For abbreviations, see Table 4.

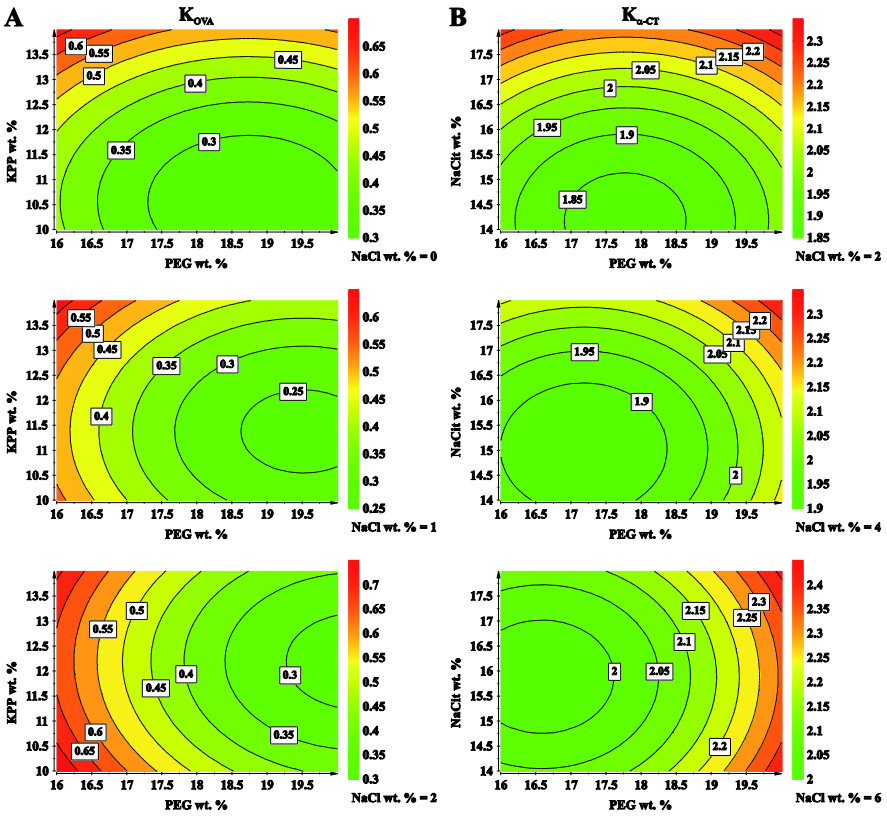

Fig. S16 Response contour plots showing the effects of the significant operating parameters (PEG, KPP/NaCit and NaCl concentration) on the partitioning behavior of OVA and α-CT (0.1 wt. %), K_{OVA} (A) and $K_{\alpha\text{-}CT}$ (B), in different PEG 6000–KPP/NaCit ATPS at pH 7 obtained by the response surface methodology (RSM) based on the results of the 2^3 CCF design, respectively. The numbers inside the figures represent the constant response (K_{OVA} and $K_{\alpha\text{-}CT}$) along each line. The color range from green to red corresponds to increase in responses.

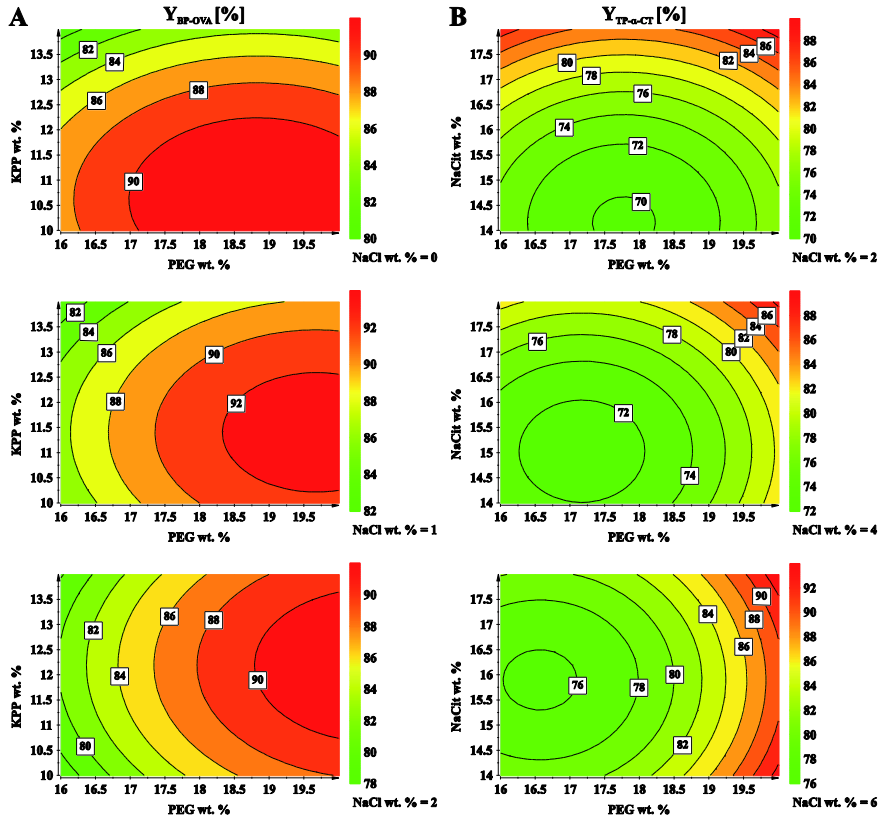

Fig. S17 Response contour plots showing the effects of the significant operating parameters (PEG, KPP/NaCit and NaCl concentration) on the recovery yield of OVA and α-CT (0.1 wt. %) in the bottom/top phase, $Y_{BP\text{-}OVA}$ (A) and $Y_{TP\text{-}\alpha\text{-}CT}$ (B), in different PEG 6000–KPP/NaCit ATPS at pH 7 obtained by the response surface methodology (RSM) based on the results of the 2^3 CCF design, respectively. The numbers inside the figures represent the constant percentage of response ($Y_{BP\text{-}OVA}$, % and $Y_{TP\text{-}\alpha\text{-}CT}$, %) along each line. The color range from green to red corresponds to increase in responses.

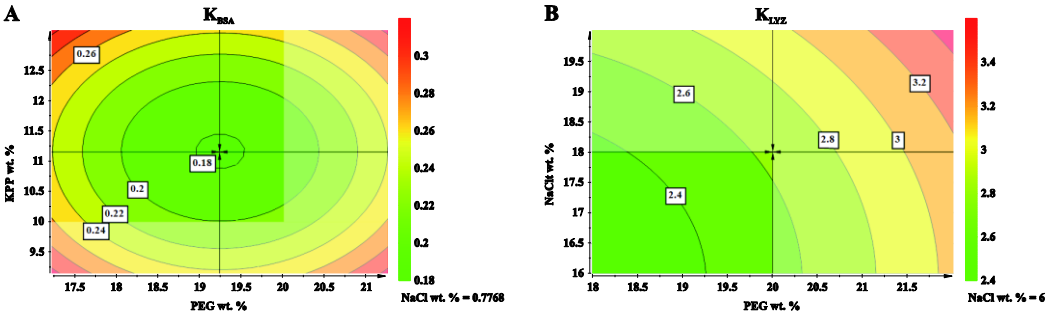

Fig. S18 Response contour plots showing the predicted optimum operating parameters (PEG, KPP/NaCit and NaCl concentration) providing an optimal partitioning of BSA and LYZ (0.1 wt. %), K_{BSA} (A) and K_{LYZ} (B), in PEG 6000–KPP/NaCit ATPS at pH 7 obtained by the response optimizer tool in MODDE 9.1 based on the results of the 2^3 CCF design, respectively. The numbers inside the figures represent the constant response (K_{BSA} and K_{LYZ}) along each line. The color range from green to red corresponds to increase in responses. The grey shaded area indicates model extrapolation. For details, see Table 7.

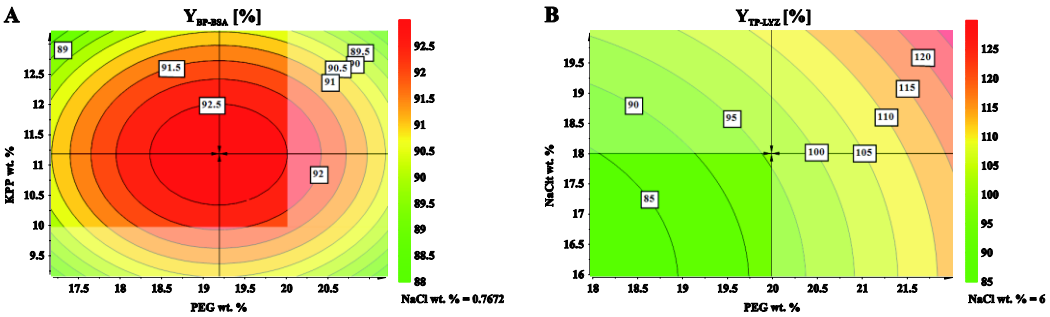

Fig. S19 Response contour plots showing the predicted optimum operating parameters (PEG, KPP/NaCit and NaCl concentration) providing a maximal recovery yield of BSA and LYZ (0.1 wt. %) in the bottom/top phase, $Y_{BP\text{-}BSA}$ (A) and $Y_{TP\text{-}LYZ}$ (B), in PEG 6000–KPP/NaCit ATPS at pH 7 obtained by the response optimizer tool in MODDE 9.1 based on the results of the 2^3 CCF design, respectively. The numbers inside the figures represent the constant percentage response ($Y_{BP\text{-}BSA}$, % and $Y_{TP\text{-}LYZ}$, %) along each line. The color range from green to red corresponds to increase in responses. The grey shaded area indicates model extrapolation. For details, see Table 7.

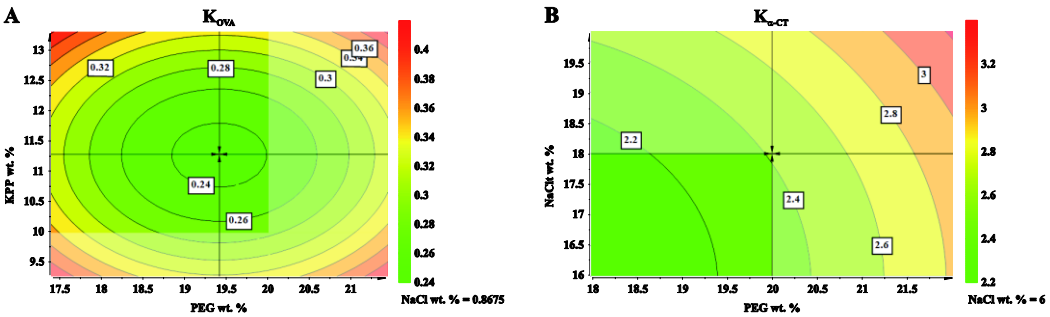

Fig. S20 Response contour plots showing the predicted optimum operating parameters of PEG, KPP/NaCit and NaCl concentration providing an optimal partitioning of OVA and α-CT (0.1 wt. %), K_{OVA} (A) and $K_{\alpha\text{-}CT}$ (B), in PEG 6000–KPP/NaCit ATPS at pH 7 obtained by the response optimizer tool in MODDE 9.1 based on the results of the 2^3 CCF design, respectively. The numbers inside the figures represent the constant response (K_{OVA} and $K_{\alpha\text{-}CT}$) along each line. The color range from green to red corresponds to increase in responses. The grey shaded area indicates model extrapolation. For details, see Table S20.

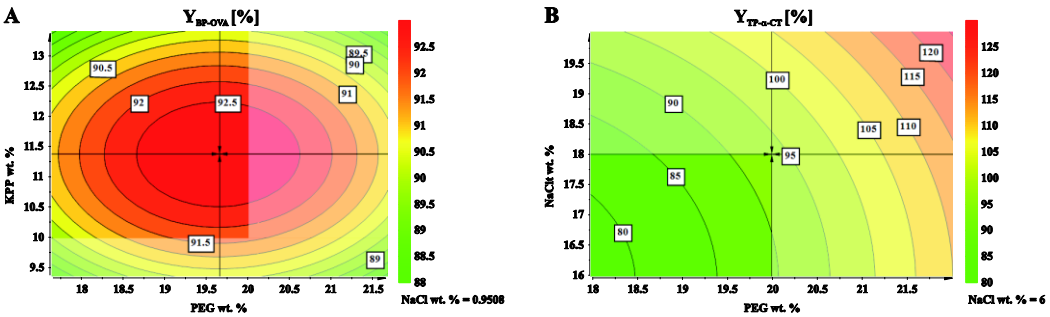

Fig. S21 Response contour plots showing the predicted optimum operating parameters of PEG, KPP/NaCit and NaCl concentration providing a maximal recovery yield of OVA and α-CT (0.1 wt. %) in the bottom/top phase, $Y_{BP\text{-}OVA}$ (A) and $Y_{TP\text{-}\alpha\text{-}CT}$ (B), in PEG 6000–KPP/NaCit ATPS at pH 7 obtained by the response optimizer tool in MODDE 9.1 based on the results of the 2^3 CCF design, respectively. The numbers inside the figures represent the constant percentage response ($Y_{BP\text{-}OVA}$, % and $Y_{TP\text{-}\alpha\text{-}CT}$, %) along each line. The color range from green to red corresponds to increase in responses. The grey shaded area indicates model extrapolation. For details, see Table S20.

Supplementary Equations

Reduced linear/first-order regression model for all response variables expressed by the following equations **S1–S8** (in uncoded values):

$$K_{BSA} = 0.4295 - 0.0236\,X_1 - 0.0448\,X_2 + 0.0931\,X_3 + 0.0742\,X_4 - 0.0311\,X_1{*}X_4 - 0.0244\,X_2{*}X_4 \quad \textbf{(S1)}$$

$$K_{LYZ} = 1.7668 + 0.0277\,X_1 + 0.0666\,X_2 + 0.1267\,X_3 + 0.1161\,X_4 + 0.0506\,X_1{*}X_4 + 0.0428\,X_2{*}X_4 \quad \textbf{(S2)}$$

$$K_{OVA} = 0.5595 - 0.0283\,X_1 - 0.0589\,X_2 + 0.1202\,X_3 + 0.0966\,X_4 - 0.0400\,X_1{*}X_4 - 0.0311\,X_2{*}X_4 \quad \textbf{(S3)}$$

$$K_{\alpha\text{-}CT} = 1.6211 + 0.0277\,X_1 + 0.0607\,X_2 + 0.1184\,X_3 + 0.1043\,X_4 + 0.0517\,X_1{*}X_4 + 0.0361\,X_2{*}X_4 \quad \textbf{(S4)}$$

$$Y_{BP\text{-}BSA}\,(\%) = 61.3679 + 1.7949\,X_1 + 3.7111\,X_2 - 8.6267\,X_3 - 6.3816\,X_4 + 2.5156\,X_1{*}X_4 + 2.0956\,X_2{*}X_4 \quad \textbf{(S5)}$$

$$Y_{TP\text{-}LYZ}\,(\%) = 80.7600 + 1.2280\,X_1 + 3.3705\,X_2 + 5.8985\,X_3 + 5.3858\,X_4 + 2.5911\,X_1{*}X_4 + 1.9000\,X_2{*}X_4 \quad \textbf{(S6)}$$

$$Y_{BP\text{-}OVA}\,(\%) = 48.3116 - 2.5432\,X_1 - 5.3799\,X_2 - 11.1369\,X_3 - 8.9178\,X_4 + 3.6367\,X_1{*}X_4 + 2.8089\,X_2{*}X_4 \quad \textbf{(S7)}$$

$$Y_{TP\text{-}\alpha\text{-}CT}\,(\%) = 71.2737 + 1.1396\,X_1 + 2.7353\,X_2 + 5.1230\,X_3 + 4.6787\,X_4 + 2.2856\,X_1{*}X_4 + 1.6167\,X_2{*}X_4 \quad \textbf{(S8)}$$

Reduced quadratic/second-order regression model for all response variables expressed by the following equations **S9–S16** (in uncoded values):

$$K_{BSA} = 0.2175 - 0.0689\,X_1 + 0.0333\,X_2 + 0.0206\,X_3 + 0.0386\,X_1^2 + 0.0405\,X_2^2 + 0.0291\,X_3^2 - 0.0290\,X_1{*}X_3 - 0.0352\,X_2{*}X_3 \quad \textbf{(S9)}$$

$$K_{LYZ} = 2.1294 + 0.0733\,X_1 + 0.0789\,X_2 + 0.0446\,X_3 + 0.0752\,X_1^2 + 0.0617\,X_2^2 + 0.0392\,X_3^2 + 0.0406\,X_1{*}X_3 - 0.0535\,X_2{*}X_3 \quad \textbf{(S10)}$$

$$K_{OVA} = 0.2881 - 0.0935\,X_1 + 0.0444\,X_2 + 0.0261\,X_3 + 0.0486\,X_1^2 + 0.0562\,X_2^2 + 0.0391\,X_3^2 - 0.0384\,X_1{*}X_3 - 0.0462\,X_2{*}X_3 \quad \textbf{(S11)}$$

$$K_{\alpha\text{-}CT} = 1.9007 + 0.0756\,X_1 + 0.0732\,X_2 + 0.0469\,X_3 + 0.0730\,X_1^2 + 0.0596\,X_2^2 + 0.0423\,X_3^2 + 0.0432\,X_1{*}X_3 - 0.0511\,X_2{*}X_3 \quad \textbf{(S12)}$$

$$Y_{BP\text{-}BSA} = 91.4994 + 2.6312\,X_1 - 1.1880\,X_2 - 0.8012\,X_3 - 1.5336\,X_1^2 - 1.5126\,X_2^2 - 1.0813\,X_3^2 + 1.1395\,X_1{*}X_3 + 1.3134\,X_2{*}X_3 \quad \textbf{(S13)}$$

$$Y_{TP\text{-}LYZ} = 875.0729 + 3.0442\,X_1 + 2.8315\,X_2 + 2.0991\,X_3 + 2.8284\,X_1^2 + 2.2243\,X_2^2 + 1.8554\,X_3^2 + 1.7551\,X_1{*}X_3 - 2.0029\,X_2{*}X_3 \quad \textbf{(S14)}$$

$$Y_{BP\text{-}OVA} = 91.1843 + 2.9781\,X_1 - 1.3781\,X_2 - 0.8107\,X_3 - 1.4007\,X_1^2 - 1.8225\,X_2^2 - 1.2958\,X_3^2 + 1.1896\,X_1{*}X_3 + 1.4323\,X_2{*}X_3 \quad \textbf{(S15)}$$

$$Y_{TP\text{-}\alpha\text{-}CT} = 72.7125 + 3.0490\,X_1 + 2.8360\,X_2 + 2.0229\,X_3 + 2.9116\,X_1^2 + 2.3063\,X_2^2 + 1.7451\,X_3^2 + 1.7579\,X_1{*}X_3 - 2.0061\,X_2{*}X_3 \quad \textbf{(S16)}$$

Supplementary Tables

Table S1 Design matrix of the 2^{5-1} fFD and corresponding results for OVA and α-CT.

Run	X_1	X_2	X_3	X_4	X_5	K_{OVA}	$K_{\alpha\text{-}CT}$	$Y_{BP\text{-}OVA}$ (%)	$Y_{TP\text{-}\alpha\text{-}CT}$ (%)
01	4000	14	12	4	6	0.79	1.41	27.12	61.87
02	4000	18	16	0	8	0.53	1.68	50.71	73.95
03	4000	18	12	4	8	0.46	1.65	57.59	73.27
04	8000	18	12	4	6	0.35	1.88	67.73	82.57
05	8000	14	16	0	8	0.68	1.66	37.30	72.85
06	6000	16	14	2	7	0.53	1.59	51.13	70.11
07	4000	14	16	0	6	0.60	1.58	44.68	69.26
08	4000	18	16	4	6	0.86	1.87	20.72	82.33
09	8000	18	16	0	6	0.61	1.67	43.76	72.57
10	8000	18	16	4	8	0.61	1.97	42.72	86.58
11	4000	14	16	4	8	0.86	1.67	20.71	73.28
12	6000	16	14	2	7	0.56	1.62	48.37	71.38
13	8000	14	12	0	6	0.34	1.29	68.15	56.59
14	4000	18	12	0	6	0.32	1.48	70.50	65.27
15	6000	16	14	2	7	0.50	1.56	53.90	68.63
16	8000	14	16	4	6	0.79	1.92	27.16	84.69
17	4000	14	12	0	8	0.34	1.44	68.65	63.14
18	8000	18	12	0	8	0.28	1.33	74.18	58.71
19	8000	14	12	4	8	0.62	1.53	42.84	67.15

X_1: PEG MW; X_2: PEG concentration (wt. %); X_3: KPP/NaCit concentration (wt. %); X_4: NaCl concentration (wt. %); X_5: pH. OVA/α-CT concentration is fixed to 0.1 wt. %.

Table S2 Experimental design of the steepest ascent/descent and corresponding results for OVA and α-CT.

Run	X_1	X_2	X_3	K_{OVA}	$Y_{BP\text{-}OVA}$ (%)	Run	X_1	X_2	X_3	$K_{\alpha\text{-}CT}$	$Y_{TP\text{-}\alpha\text{-}CT}$ (%)
01	16.0	14.0	2.0	0.51	52.97	01	16.0	14.0	2.0	1.61	65.88
02	16.4	13.6	1.8	0.48	62.34	02	16.4	14.4	2.4	1.65	67.23
03	16.8	13.2	1.6	0.43	71.75	03	16.8	14.8	2.8	1.71	69.36
04	17.2	12.8	1.4	0.38	82.48	04	17.2	15.2	3.2	1.77	73.61
05	17.6	12.4	1.2	0.34	86.23	05	17.6	15.6	3.6	1.83	76.37
06	**18.0**	**12.0**	**1.0**	**0.30**	**90.52**	**06**	**18.0**	**16.0**	**4.0**	**1.94**	**80.12**
07	18.4	11.6	0.8	0.35	84.16	07	18.4	16.4	4.4	1.86	78.62
08	18.8	11.2	0.6	0.44	69.51	08	18.8	16.8	4.8	1.81	74.53
09	19.2	10.8	0.4	0.47	65.17	09	19.2	17.2	5.2	1.76	71.44
10	19.6	10.4	0.2	0.54	49.05	10	19.6	17.6	5.6	1.64	66.78

X_1: PEG concentration (wt. %); X_2: KPP/NaCit concentration (wt. %); X_3: NaCl concentration (wt. %). OVA/α-CT concentration is fixed to 0.1 wt. %. Furthermore, the PEG MW and pH are fixed to PEG 6000 and pH 7, respectively. While the factors levels in run 01 were used as the center-points in screening experiments, the factor levels shown in bold (run 06) were chosen as the new center-points for subsequent optimization experiments of OVA and α-CT, respectively.

Table S3 Design matrices of the 2^3CCF design and corresponding results for OVA and α-CT.

Run	X_1	X_2	X_3	K_{OVA}	$Y_{BP\text{-}OVA}$ (%)	Run	X_1	X_2	X_3	$K_{\alpha\text{-}CT}$	$Y_{TP\text{-}\alpha\text{-}CT}$ (%)
01	18	12	2	0.38	88.22	01	18	16	6	2.03	78.16
02	16	14	2	0.73	77.37	02	16	18	6	2.09	80.47
03	16	12	1	0.48	85.12	03	16	16	4	1.92	73.92
04	16	14	0	0.65	79.85	04	16	18	2	2.26	86.45
05	18	12	1	0.29	91.01	05	18	16	4	1.91	73.06
06	18	14	1	0.45	86.05	06	18	18	4	2.09	79.94
07	20	10	2	0.41	87.29	07	20	14	6	2.39	92.95
08	18	10	1	0.32	90.11	08	18	14	4	1.90	72.68
09	16	10	0	0.44	86.36	09	16	14	2	1.95	74.59
10	18	12	1	0.26	91.94	10	18	16	4	1.87	71.53
11	20	10	0	0.28	91.32	11	20	14	2	1.96	74.97
12	20	12	1	0.25	93.25	12	20	16	4	2.14	81.86
13	20	14	0	0.58	82.02	13	20	18	2	2.34	90.09
14	18	12	1	0.32	90.08	14	18	16	4	1.93	73.82
15	20	14	2	0.33	89.77	15	20	18	6	2.40	92.71
16	18	12	0	0.30	90.70	16	18	16	2	1.87	71.53
17	16	10	2	0.73	77.37	17	16	14	6	2.06	78.79

X_1: PEG concentration (wt. %); X_2: KPP/NaCit concentration (wt. %); X_3: NaCl concentration (wt. %). OVA/α-CT concentration is fixed to 0.1 wt. %. Furthermore, PEG MW and pH are fixed to PEG 6000 and pH 7, respectively.

Table S4 Regression coefficients and their *p*-values for K_{BSA} and K_{LYZ} in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Term/Variable (K_{BSA})	Coefficient	*p*-value	Term/Variable (K_{LYZ})	Coefficient	*p*-value
Constant	0.4295	$5.91*10^{-14*}$	Constant	1.7668	$1.41*10^{-19*}$
PEG MW (X_1)	-0.0236	0.0616	PEG MW (X_1)	0.0277	0.1080
PEG (X_2)	-0.0448	0.0020^{*}	PEG (X_2)	0.0666	0.0013^{*}
KPP (X_3)	0.0931	$3.14*10^{-6*}$	NaCit (X_3)	0.1267	$4.03*10^{-6*}$
NaCl (X_4)	0.0742	$2.97*10^{-5*}$	NaCl (X_4)	0.1161	$9.74*10^{-6*}$
PEG MW*NaCl (X_1*X_4)	-0.0311	0.0137^{*}	PEG MW*NaCl (X_1*X_4)	0.0506	0.0056^{*}
PEG*NaCl (X_2*X_4)	-0.0244	0.0426^{*}	PEG*NaCl (X_2*X_4)	0.0428	0.0147^{*}

The coefficients are calculated by fitting the obtained data with eq 4, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. K_{BSA}: $R^2 = 0.9218$; $R^2_{adj} = 0.8827$; $Q^2 = 0.7384$. K_{LYZ}: $R^2 = 0.9286$; $R^2_{adj} = 0.8928$; $Q^2 = 0.7448$. *Significant at 95 % confidence level.

Table S5 Regression coefficients and their *p*-values for K_{OVA} and $K_{\alpha\text{-}CT}$ in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Term/Variable (K_{OVA})	Coefficient	*p*-value	Term/Variable ($K_{\alpha\text{-}CT}$)	Coefficient	*p*-value
Constant	0.5595	$4.39*10^{-14*}$	Constant	1.6211	$1.79*10^{-19*}$
PEG MW (X_1)	-0.0283	0.0754	PEG MW (X_1)	0.0277	0.0882
PEG (X_2)	-0.0589	0.0016^{*}	PEG (X_2)	0.0607	0.0016^{*}
KPP (X_3)	0.1202	$2.66*10^{-6*}$	NaCit (X_3)	0.1184	$4.07*10^{-6*}$
NaCl (X_4)	0.0966	$2.36*10^{-5*}$	NaCl (X_4)	0.1043	$1.45*10^{-5*}$
PEG MW*NaCl (X_1*X_4)	-0.0400	0.0128^{*}	PEG MW*NaCl (X_1*X_4)	0.0517	0.0032^{*}
PEG*NaCl (X_2*X_4)	-0.0311	0.0423^{*}	PEG*NaCl (X_2*X_4)	0.0361	0.0247^{*}

The coefficients are calculated by fitting the obtained data with eq 4, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. K_{OVA}: $R^2 = 0.9243$; $R^2_{adj} = 0.8865$; $Q^2 = 0.7442$. $K_{\alpha\text{-}CT}$: $R^2 = 0.9268$; $R^2_{adj} = 0.8902$; $Q^2 = 0.7434$. *Significant at 95 % confidence level.

Table S6 Regression coefficients and their *p*-values for $Y_{BP\text{-}BSA}$ and $Y_{TP\text{-}LYZ}$ in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Term/Variable ($Y_{BP\text{-}BSA}$)	Coefficient	*p*-value	Term/Variable ($Y_{TP\text{-}LYZ}$)	Coefficient	*p*-value
Constant	61.3679	$6.61*10^{-17*}$	Constant	80.7600	$1.62*10^{-19*}$
PEG MW (X_1)	1.7949	0.0762	PEG MW (X_1)	1.2280	0.1216
PEG (X_2)	3.7111	0.0017^{*}	PEG (X_2)	3.3705	0.0006^{*}
KPP (X_3)	-8.6267	$7.58*10^{-7*}$	NaCit (X_3)	5.8985	$3.75*10^{-6*}$
NaCl (X_4)	-6.3816	$1.65*10^{-5*}$	NaCl (X_4)	5.3858	$9.38*10^{-6*}$
PEG MW*NaCl (X_1*X_4)	2.5156	0.0137^{*}	PEG MW*NaCl (X_1*X_4)	2.5911	0.0029^{*}
PEG*NaCl (X_2*X_4)	2.0956	0.0334^{*}	PEG*NaCl (X_2*X_4)	1.9000	0.0181^{*}

The coefficients are calculated by fitting the obtained data with eq 4, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. $Y_{BP\text{-}BSA}$: $R^2 = 0.9335$; $R^2_{adj} = 0.9003$; $Q^2 = 0.7629$. $Y_{TP\text{-}LYZ}$: $R^2 = 0.9312$; $R^2_{adj} = 0.8969$; $Q^2 = 0.7530$. *Significant at 95 % confidence level.

Table S7 Regression coefficients and their *p*-values for $Y_{BP\text{-}OVA}$ and $Y_{TP\text{-}\alpha\text{-}CT}$ in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Term/Variable ($Y_{BP\text{-}OVA}$)	**Coefficient**	***p*-value**	**Term/Variable ($Y_{TP\text{-}\alpha\text{-}CT}$)**	**Coefficient**	***p*-value**
Constant	48.3116	$9.03*10^{-14*}$	Constant	71.2737	$1.89*10^{-19*}$
PEG MW (X_1)	2.5432	0.0806	PEG MW (X_1)	1.1396	0.1093
PEG (X_2)	5.3799	0.0017^{*}	PEG (X_2)	2.7353	0.0013^{*}
KPP (X_3)	-11.1369	$2.40*10^{-6*}$	NaCit (X_3)	5.1230	$5.03*10^{-6*}$
NaCl (X_4)	-8.9178	$2.23*10^{-5*}$	NaCl (X_4)	4.6787	$1.25*10^{-5*}$
PEG MW*NaCl (X_1*X_4)	3.6367	0.0135^{*}	PEG MW*NaCl (X_1*X_4)	2.2856	0.0032^{*}
PEG*NaCl (X_2*X_4)	2.8089	0.0452^{*}	PEG*NaCl (X_2*X_4)	1.6167	0.0231^{*}

The coefficients are calculated by fitting the obtained data with eq 4, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. $Y_{BP\text{-}OVA}$: R^2 = 0.9249; R^2_{adj} = 0.8874; Q^2 = 0.7461. $Y_{TP\text{-}\alpha\text{-}CT}$: R^2 = 0.9266; R^2_{adj} = 0.8898; Q^2 = 0.7430. *Significant at 95 % confidence level.

Table S8 ANOVA table for K_{BSA} and K_{LYZ} in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Source (K_{BSA})	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	19	3.8658	0.2035		
Constant	1	3.5045	3.5045		
Total corrected	18	0.3613	0.0201		
Regression	6	0.3331	0.0555	23.5832	**0.000**
Residual	12	0.0282	0.0024		
Lack of fit (model error)	10	0.0270	0.0027	4.2597	**0.205**
Pure error (replicate error)	2	0.0013	0.0006		
Source (K_{LYZ})	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	19	60.0817	3.1622		
Constant	1	59.3129	59.3129		
Total corrected	18	0.7688	0.0427		
Regression	6	0.7139	0.1190	25.9959	**0.000**
Residual	12	0.0549	0.0046		
Lack of fit (model error)	10	0.0537	0.0054	8.4721	**0.110**
Pure error (replicate error)	2	0.0013	0.0006		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. K_{BSA}: R^2 = 0.9218; R^2_{adj} = 0.8827; Q^2 = 0.7384. K_{LYZ}: R^2 = 0.9286; R^2_{adj} = 0.8928; Q^2 = 0.7448.

Table S9 ANOVA table for K_{OVA} and $K_{\alpha\text{-}CT}$ in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Source (K_{OVA})	DF	SS	MS (variance)	*F*-value	*p*-value
Total	19	6.5499	0.3447		
Constant	1	5.9472	5.9472		
Total corrected	18	0.6027	0.0335		
Regression	6	0.5571	0.0929	24.437	**0.000**
Residual	12	0.0456	0.0038		
Lack of fit (model error)	10	0.0438	0.0044	4.8661	**0.182**
Pure error (replicate error)	2	0.0018	0.0009		
Source ($K_{\alpha\text{-}CT}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	19	50.5854	2.6624		
Constant	1	49.9284	49.9284		
Total corrected	18	0.6570	0.0365		
Regression	6	0.6089	0.1015	25.3222	**0.000**
Residual	12	0.0481	0.0040		
Lack of fit (model error)	10	0.0463	0.0046	5.1435	**0.174**
Pure error (replicate error)	2	0.0018	0.0009		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. K_{OVA}: $R^2 = 0.9243$; $R^2_{adj} = 0.8865$; $Q^2 = 0.7442$. $K_{\alpha\text{-}CT}$: $R^2 = 0.9268$; $R^2_{adj} = 0.8902$; $Q^2 = 0.7434$.

Table S10 ANOVA table for $Y_{BP\text{-}BSA}$ and $Y_{TP\text{-}LYZ}$ in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Source ($Y_{BP\text{-}BSA}$)	DF	SS	MS (variance)	*F*-value	*p*-value
Total	19	74334.8	3912.36		
Constant	1	71554.4	71554.4		
Total corrected	18	2780.43	154.468		
Regression	6	2595.58	432.597	28.083	**0.000**
Residual	12	184.85	15.4042		
Lack of fit (model error)	10	174.548	17.4548	3.3886	**0.249**
Pure error (replicate error)	2	10.3021	5.1510		
Source ($Y_{TP\text{-}LYZ}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	19	125628	6611.99		
Constant	1	123921	123921		
Total corrected	18	1706.4	94.7999		
Regression	6	1589.06	264.843	27.0852	**0.000**
Residual	12	117.338	9.7782		
Lack of fit (model error)	10	111.845	11.1845	4.0718	**0.213**
Pure error (replicate error)	2	5.4936	2.7468		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. $Y_{BP\text{-}BSA}$: $R^2 = 0.9335$; $R^2_{adj} = 0.9003$; $Q^2 = 0.7629$. $Y_{TP\text{-}LYZ}$: $R^2 = 0.9312$; $R^2_{adj} = 0.8969$; $Q^2 = 0.7530$.

Table S11 ANOVA table for $Y_{BP\text{-}OVA}$ and $Y_{TP\text{-}\alpha\text{-}CT}$ in the reduced linear/first-order model obtained by the 2^{5-1} fFD after backward elimination of model terms/variables.

Source ($Y_{BP\text{-}OVA}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	19	49459	2603.1		
Constant	1	44346.2	44346.2		
Total corrected	18	5112.79	284.044		
Regression	6	4729.03	788.172	24.6464	**0.000**
Residual	12	383.751	31.9793		
Lack of fit (model error)	10	368.461	36.8461	4.8195	**0.184**
Pure error (replicate error)	2	15.2905	7.6452		
Source ($Y_{TP\text{-}\alpha\text{-}CT}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	19	97795.8	5147.15		
Constant	1	96518.8	96518.8		
Total corrected	18	1276.98	70.9431		
Regression	6	1183.2	197.2	25.2344	**0.000**
Residual	12	93.7766	7.8147		
Lack of fit (model error)	10	89.9881	8.9988	4.7505	**0.186**
Pure error (replicate error)	2	3.7886	1.8943		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. $Y_{BP\text{-}OVA}$: $R^2 = 0.9249$; $R^2_{adj} = 0.8874$; $Q^2 = 0.7461$. $Y_{TP\text{-}\alpha\text{-}CT}$: $R^2 = 0.9266$; $R^2_{adj} = 0.8898$; $Q^2 = 0.7430$.

Table S12 Regression coefficients and their *p*-values for K_{BSA} and K_{LYZ} in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Term/Variable (K_{BSA})	**Coefficient**	***p*-value**	**Term/Variable (K_{LYZ})**	**Coefficient**	***p*-value**
Constant	0.2175	$8.50*10^{-9*}$	Constant	2.1294	$6.90*10^{-15*}$
PEG (X_1)	-0.0689	$1.01*10^{-6*}$	PEG (X_1)	0.0733	$3.22*10^{-5*}$
KPP (X_2)	0.0333	0.0002^{*}	NaCit (X_2)	0.0789	$1.89*10^{-5*}$
NaCl (X_3)	0.0206	0.0042^{*}	NaCl (X_3)	0.0446	0.0010^{*}
PEG*PEG (X_1^2)	0.0386	0.0013^{*}	PEG*PEG (X_1^2)	0.0752	0.0005^{*}
KPP*KPP (X_2^2)	0.0405	0.0009^{*}	NaCit*NaCit (X_2^2)	0.0617	0.0018^{*}
NaCl*NaCl (X_3^2)	0.0291	0.0064^{*}	NaCl*NaCl (X_3^2)	0.0392	0.0164^{*}
PEG*NaCl (X_1*X_3)	-0.0290	0.0002^{*}	PEG*NaCl (X_1*X_3)	0.0406	0.0006^{*}
KPP*NaCl (X_2*X_3)	-0.0352	$6.01*10^{-5*}$	NaCit*NaCl (X_2*X_3)	-0.0535	0.0001^{*}

The coefficients are calculated by fitting the obtained data with eq 5, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. K_{BSA}: $R^2 = 0.9849$; $R^2_{adj} = 0.9699$; $Q^2 = 0.8572$. K_{LYZ}: $R^2 = 0.9821$; $R^2_{adj} = 0.9641$; $Q^2 = 0.8969$. *Significant at 95 % confidence level.

Table S13 Regression coefficients and their *p*-values for K_{OVA} and $K_{\alpha\text{-}CT}$ in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Term/Variable (K_{OVA})	Coefficient	*p*-value	Term/Variable ($K_{\alpha\text{-}CT}$)	Coefficient	*p*-value
Constant	0.2881	$8.59*10^{-9*}$	Constant	1.9007	$2.20*10^{-15*}$
PEG (X_1)	-0.0935	$8.53*10^{-7*}$	PEG (X_1)	0.0756	$3.82*10^{-6*}$
KPP (X_2)	0.0444	0.0002^{*}	NaCit (X_2)	0.0732	$4.87*10^{-6*}$
NaCl (X_3)	0.0261	0.0053^{*}	NaCl (X_3)	0.0469	0.0001^{*}
PEG*PEG (X_1^2)	0.0486	0.0017^{*}	PEG*PEG (X_1^2)	0.0730	0.0001^{*}
KPP*KPP (X_2^2)	0.0562	0.0007^{*}	NaCit*NaCit (X_2^2)	0.0596	0.0004^{*}
NaCl*NaCl (X_3^2)	0.0391	0.0060^{*}	NaCl*NaCl (X_3^2)	0.0423	0.0035^{*}
PEG*NaCl (X_1*X_3)	-0.0384	0.0002^{*}	PEG*NaCl (X_1*X_3)	0.0432	$9.26*10^{-5*}$
KPP*NaCl (X_2*X_3)	-0.0462	$6.51*10^{-5*}$	NaCit*NaCl (X_2*X_3)	-0.0511	$2.80*10^{-5*}$

The coefficients are calculated by fitting the obtained data with eq 5, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. K_{OVA}: R^2 = 0.9851; R^2_{adj} = 0.9702; Q^2 = 0.8649. $K_{\alpha\text{-}CT}$: R^2 = 0.9889; R^2_{adj} = 0.9777; Q^2 = 0.9099. *Significant at 95 % confidence level.

Table S14 Regression coefficients and their *p*-values for $Y_{BP\text{-}BSA}$ and $Y_{TP\text{-}LYZ}$ in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Term/Variable ($Y_{BP\text{-}BSA}$)	Coefficient	*p*-value	Term/Variable ($Y_{TP\text{-}LYZ}$)	Coefficient	*p*-value
Constant	91.4994	$2.77*10^{-17*}$	Constant	75.0729	$4.11*10^{-15*}$
PEG (X_1)	2.6312	$6.94*10^{-7*}$	PEG (X_1)	3.0442	$5.93*10^{-6*}$
KPP (X_2)	-1.1880	0.0002^{*}	NaCit (X_2)	2.8315	$1.02*10^{-5*}$
NaCl (X_3)	-0.8012	0.0029^{*}	NaCl (X_3)	2.0991	$8.95*10^{-5*}$
PEG*PEG (X_1^2)	-1.5336	0.0007^{*}	PEG*PEG (X_1^2)	2.8284	0.0002^{*}
KPP*KPP (X_2^2)	-1.5126	0.0008^{*}	NaCit*NaCit (X_2^2)	2.2243	0.0010^{*}
NaCl*NaCl (X_3^2)	-1.0813	0.0057^{*}	NaCl*NaCl (X_3^2)	1.8554	0.0031^{*}
PEG*NaCl (X_1*X_3)	1.1395	0.0001^{*}	PEG*NaCl (X_1*X_3)	1.7551	0.0001^{*}
KPP*NaCl (X_2*X_3)	1.3134	$5.00*10^{-5*}$	NaCit*NaCl (X_2*X_3)	-2.0029	$5.20*10^{-5*}$

The coefficients are calculated by fitting the obtained data with eq 5, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. $Y_{BP\text{-}BSA}$: R^2 = 0.9862; R^2_{adj} = 0.9724; Q^2 = 0.8669. $Y_{TP\text{-}LYZ}$: R^2 = 0.9873; R^2_{adj} = 0.9745; Q^2 = 0.9024. *Significant at 95 % confidence level.

Table S15 Regression coefficients and their *p*-values for $Y_{BP\text{-}OVA}$ and $Y_{TP\text{-}\alpha\text{-}CT}$ in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Term/Variable ($Y_{BP\text{-}OVA}$)	Coefficient	*p*-value	Term/Variable ($Y_{TP\text{-}\alpha\text{-}CT}$)	Coefficient	*p*-value
Constant	91.1843	$1.02*10^{-16*}$	Constant	72.7125	$2.95*10^{-15*}$
PEG (X_1)	2.9781	$9.15*10^{-7*}$	PEG (X_1)	3.0490	$3.37*10^{-6*}$
KPP (X_2)	-1.3781	0.0003^{*}	NaCit (X_2)	2.8360	$5.81*10^{-6*}$
NaCl (X_3)	-0.8107	0.0065^{*}	NaCl (X_3)	2.0229	$6.91*10^{-5*}$
PEG*PEG (X_1^2)	-1.4007	0.0033^{*}	PEG*PEG (X_1^2)	2.9116	0.0001^{*}
KPP*KPP (X_2^2)	-1.8225	0.0007^{*}	NaCit*NaCit (X_2^2)	2.3063	0.0005^{*}
NaCl*NaCl (X_3^2)	-1.2958	0.0051^{*}	NaCl*NaCl (X_3^2)	1.7451	0.0028^{*}
PEG*NaCl (X_1*X_3)	1.1896	0.0003^{*}	PEG*NaCl (X_1*X_3)	1.7579	$7.80*10^{-5*}$
KPP*NaCl (X_2*X_3)	1.4323	$8.41*10^{-5*}$	NaCit*NaCl (X_2*X_3)	-2.0061	$3.02*10^{-5*}$

The coefficients are calculated by fitting the obtained data with eq 5, and the *p*-values (probability of error/probability values) are calculated by comparing the *F*-value of each model term relative to the *F*-value in the standard *F*-table for the corresponding number of degrees of freedom of parameter and residual. $Y_{BP\text{-}OVA}$: R^2 = 0.9845; R^2_{adj} = 0.9689; Q^2 = 0.8767. $Y_{TP\text{-}\alpha\text{-}CT}$: R^2 = 0.9890; R^2_{adj} = 0.9780; Q^2 = 0.9061. *Significant at 95 % confidence level.

Table S16 ANOVA table for K_{BSA} and K_{LYZ} in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Source (K_{BSA})	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	1.9649	0.1156		
Constant	1	1.7344	1.7344		
Total corrected	16	0.2305	0.0144		
Regression	8	0.2270	0.0284	65.3724	**0.000**
Residual	8	0.0035	0.0004		
Lack of fit (model error)	6	0.0027	0.0004	1.1136	**0.544**
Pure error (replicate error)	2	0.0008	0.0004		
Source (K_{LYZ})	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	90.2046	5.3062		
Constant	1	89.6542	89.6542		
Total corrected	16	0.5504	0.0344		
Regression	8	0.5405	0.0676	54.7418	**0.000**
Residual	8	0.0099	0.0012		
Lack of fit (model error)	6	0.0074	0.0012	1.0009	**0.578**
Pure error (replicate error)	2	0.0025	0.0012		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. K_{BSA}: $R^2 = 0.9849$; $R^2_{adj} = 0.9699$; $Q^2 = 0.8572$. K_{LYZ}: $R^2 = 0.9821$; $R^2_{adj} = 0.9641$; $Q^2 = 0.8969$.

Table S17 ANOVA table for K_{OVA} and $K_{\alpha\text{-}CT}$ in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Source (K_{OVA})	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	3.4600	0.2035		
Constant	1	3.0494	3.0494		
Total corrected	16	0.4106	0.0257		
Regression	8	0.4045	0.0506	66.2203	**0.000**
Residual	8	0.0061	0.0008		
Lack of fit (model error)	6	0.0043	0.0008	0.7978	**0.649**
Pure error (replicate error)	2	0.0018	0.0009		
Source ($K_{\alpha\text{-}CT}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	73.0429	4.2966		
Constant	1	72.5125	72.5125		
Total corrected	16	0.5304	0.0332		
Regression	8	0.5245	0.0656	88.7771	**0.000**
Residual	8	0.0059	0.0007		
Lack of fit (model error)	6	0.0040	0.0007	0.7217	**0.680**
Pure error (replicate error)	2	0.0019	0.0009		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. K_{OVA}: $R^2 = 0.9851$; $R^2_{adj} = 0.9702$; $Q^2 = 0.8649$. $K_{\alpha\text{-}CT}$: $R^2 = 0.9889$; $R^2_{adj} = 0.9777$; $Q^2 = 0.9099$.

Table S18 ANOVA table for $Y_{BP\text{-}BSA}$ and $Y_{TP\text{-}LYZ}$ in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Source ($Y_{BP\text{-}BSA}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	130830	7695.88		
Constant	1	130498	130498		
Total corrected	16	332.234	20.7646		
Regression	8	327.649	40.9561	71.4482	**0.000**
Residual	8	4.5858	0.5732		
Lack of fit (model error)	6	3.4152	0.5692	0.9725	**0.587**
Pure error (replicate error)	2	1.1706	0.5853		
Source ($Y_{TP\text{-}LYZ}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	113971	6704.18		
Constant	1	113325	113325		
Total corrected	16	845.711	52.8569		
Regression	8	834.931	104.366	77.4508	**0.000**
Residual	8	10.7801	1.3475		
Lack of fit (model error)	6	8.0593	1.3432	0.9873	**0.582**
Pure error (replicate error)	2	2.7209	1.3604		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. $Y_{BP\text{-}BSA}$: $R^2 = 0.9862$; $R^2_{adj} = 0.9724$; $Q^2 = 0.8669$. $Y_{TP\text{-}LYZ}$: $R^2 = 0.9873$; $R^2_{adj} = 0.9745$; $Q^2 = 0.9024$.

Table S19 ANOVA table for $Y_{BP\text{-}OVA}$ and $Y_{TP\text{-}\alpha\text{-}CT}$ in the reduced quadratic/second-order polynomial model obtained by the 2^3 CCF design after backward elimination of model terms/variables.

Source ($Y_{BP\text{-}OVA}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	128876	7580.94		
Constant	1	128470	128470		
Total corrected	16	406.461	25.4038		
Regression	8	400.149	50.0186	63.3909	**0.000**
Residual	8	6.3124	0.7891		
Lack of fit (model error)	6	4.5826	0.7638	0.8831	**0.617**
Pure error (replicate error)	2	1.7298	0.8649		
Source ($Y_{TP\text{-}\alpha\text{-}CT}$)	**DF**	**SS**	**MS (variance)**	***F*-value**	***p*-value**
Total	17	107659	6332.9		
Constant	1	106812	106812		
Total corrected	16	846.977	52.9360		
Regression	8	837.671	104.7709	90.0169	**0.000**
Residual	8	9.3057	1.1632		
Lack of fit (model error)	6	6.5848	1.0975	0.8067	**0.646**
Pure error (replicate error)	2	2.7209	1.3604		

DF: degrees of freedom; SS: sum of squares; MS: mean squares (variance) (= SS/DF); *F*-value: *F*-value of variance source (= $MS_{regression}/MS_{residual}$); *p*-value: probability of error/probability value. $Y_{BP\text{-}OVA}$: $R^2 = 0.9845$; $R^2_{adj} = 0.9689$; $Q^2 = 0.8767$. $Y_{TP\text{-}\alpha\text{-}CT}$: $R^2 = 0.9890$; $R^2_{adj} = 0.9780$; $Q^2 = 0.9061$.

Table S20 Experimental validation of the quadratic model and corresponding results for OVA and α-CT.

Optimized input process parameters			Predicted	Experimental	Predicted	Experimental
X_1	X_2	X_3	K_{OVA}	K_{OVA}	$K_{\alpha\text{-}CT}$	$K_{\alpha\text{-}CT}$
19.42	11.28	0.87	0.23	0.25 ± 0.03	–	–
20.00	18.00	6.00	–	–	2.42	2.41 ± 0.02
Optimized input process parameters			**Predicted**	**Experimental**	**Predicted**	**Experimental**
X_1	X_2	X_3	$Y_{BP\text{-}OVA}$ (%)	$Y_{BP\text{-}OVA}$ (%)	$Y_{TP\text{-}\alpha\text{-}CT}$ (%)	$Y_{TP\text{-}\alpha\text{-}CT}$ (%)
19.66	11.37	0.95	93.03	92.81 ± 0.80	–	–
20.00	18.00	6.00	–	–	93.46	92.85 ± 0.81

X_1: PEG concentration (wt. %); X_2: KPP/NaCit concentration (wt. %); X_3: NaCl concentration (wt. %). OVA/α-CT concentration is fixed to 0.1 wt. %. Furthermore, PEG MW and pH are fixed to PEG 6000 and pH 7, respectively.