**Supplementary data:**

A central-composite-face centered design was applied including five factors. Three concentration levels for each variable were used including a maximum (1), a minimum (-1) and a centre point (0). The model contained 96 experimental runs and six additional controls were implemented to improve the model like zero values for each factor. When fitting the model, the histogram plot revealed the normal distribution of the tested factors and the residual normal probability plot was evaluated to determine outliers, which were excluded to improve the model. In addition, coefficient plot analysis was used to evaluate the significance of the single, quadratic and interactive model terms. Non-significant terms were also removed from the model. Single response values were not included in the measurement to improve R2 (model fit) and Q2 (model prediction power).

The results were modelled with a polynomial equation to determine the effect of each factor and to predict the response of non-sampling points. Therefore, the central-composite-face centered design was fitted to a second order polynomial function as described below:

$Y=β\_{0}+\sum\_{}^{}β\_{i}x\_{i}+\sum\_{}^{}β\_{ii}x\_{i}^{2}+\sum\_{}^{}β\_{ij}x\_{i}x\_{j}$ (1)

Where Y is the response, $β\_{0}$ is the offset term, $β\_{i} $the linear coefficient, $β\_{ii} $the quadratic coefficient, $β\_{ij} $the interaction coefficient and $x\_{i} $and $x\_{j}$ are the independent variables.

For the best setting of each factor, coefficient and response contour plots were created to facilitate model interpretation and to predict the optimum values. The data were fitted to the second order polynomial equation (1) by non-linear regression analysis and resulted in the following model equations for monomer recovery:

$Y=185.48+0.12x\_{2}+0.11x\_{3}-0.01x\_{4}+0.03x\_{2}^{2}-0.09x\_{3}^{2}-0.04x\_{4}^{2}+0.06x\_{2}x\_{3}-0.01x\_{4}x\_{5}- -0.14 x\_{3}^{2}x\_{4} $

Where $x\_{1}$ is the coded value for trehalose, $x\_{2}$ for sorbitol, $x\_{3}$ for arginine, $x\_{4}$ for PS20 and $x\_{5}$ for HBC: The regression analysis led to a coefficient for the SE-HPLC data of R2=0.844. The coefficient blots represent the data variability and the significance of model terms that can have single, quadratic or interaction effects.

***Supplementary table 1: Worksheet of the DoE approach***To fit the model, outliers for recovery were identified in the residual normal probability plot and were excluded to improve the model (grey shaded values).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Exp No** | **Run Order** | **Trehalose [%]** | **Sorbitol [%]** | **Arginine [%]** | **PS20 [%]** | **HBC [%]** | **Recovery [%]** |
| 2 | 27 | 6 | 2.75 | 2.75 | 0.005 | 3.5 | 62.18 |
| 4 | 5 | 6 | 5 | 0.5 | 0.005 | 3.5 | 57.44 |
| 6 | 14 | 6 | 2.75 | 0.5 | 0.005 | 3.5 | 44.07 |
| 8 | 26 | 6 | 0.5 | 5 | 0.005 | 3.5 | 43.62 |
| 10 | 12 | 6 | 0.5 | 2.75 | 0.005 | 3.5 | 37.63 |
| 12 | 16 | 6 | 0.5 | 0.5 | 0.005 | 3.5 | 50.96 |
| 14 | 23 | 6 | 2.75 | 5 | 0.005 | 3.5 | 74.09 |
| 16 | 22 | 6 | 5 | 5 | 0.005 | 3.5 | 83.49 |
| 18 | 20 | 3.25 | 2.75 | 2.75 | 0.027 | 0.35 | 63.92 |
| 19 | 3 | 3.25 | 5 | 0.5 | 0.027 | 0.35 | 69.17 |
| 20 | 9 | 3.25 | 2.75 | 0.5 | 0.027 | 0.35 | 48.71 |
| 21 | 17 | 3.25 | 0.5 | 5 | 0.027 | 0.35 | 44.96 |
| 22 | 25 | 3.25 | 0.5 | 2.75 | 0.027 | 0.35 | 67.92 |
| 23 | 21 | 3.25 | 0.5 | 0.5 | 0.027 | 0.35 | 86.23 |
| 24 | 2 | 3.25 | 2.75 | 5 | 0.027 | 0.35 | 82.47 |
| 25 | 13 | 3.25 | 5 | 5 | 0.027 | 0.35 | 84.1 |
| 26 | 6 | 6 | 2.75 | 2.75 | 0.05 | 3.5 | 60.48 |
| 27 | 11 | 6 | 5 | 0.5 | 0.05 | 3.5 | 64.42 |
| 1 | 10 | 6 | 2.75 | 0.5 | 0.05 | 3.5 | 55.76 |
| 3 | 4 | 6 | 0.5 | 5 | 0.05 | 3.5 | 34.9 |
| 5 | 24 | 6 | 0.5 | 2.75 | 0.05 | 3.5 | 71.5 |
| 7 | 15 | 6 | 0.5 | 0.5 | 0.05 | 3.5 | 70.92 |
| 9 | 7 | 6 | 2.75 | 5 | 0.05 | 3.5 | 74.03 |
| 11 | 18 | 6 | 5 | 5 | 0.05 | 3.5 | 64.63 |
| 13 | 8 | 0.5 | 2.75 | 2.75 | 0.005 | 1.92 | 67.46 |
| 15 | 1 | 0.5 | 5 | 0.5 | 0.005 | 1.92 | 75.81 |
| 17 | 19 | 0.5 | 2.75 | 0.5 | 0.005 | 1.92 | 41.16 |
| 28 | 28 | 0.5 | 0.5 | 5 | 0.005 | 1.92 | 38 |
| 29 | 29 | 0.5 | 0.5 | 2.75 | 0.005 | 1.92 | 45.39 |
| 30 | 30 | 0.5 | 0.5 | 0.5 | 0.005 | 1.92 | 41.86 |
| 31 | 31 | 0.5 | 2.75 | 5 | 0.005 | 1.92 | 61.21 |
| 32 | 32 | 0.5 | 5 | 5 | 0.005 | 1.92 | 86.15 |
| 33 | 33 | 0.5 | 2.75 | 2.75 | 0.05 | 0.35 | 65.55 |
| 34 | 34 | 0.5 | 5 | 0.5 | 0.05 | 0.35 | 75.82 |
| 35 | 35 | 0.5 | 2.75 | 0.5 | 0.05 | 0.35 | 39.61 |
| 36 | 36 | 0.5 | 0.5 | 5 | 0.05 | 0.35 | 37.63 |
| 37 | 37 | 0.5 | 0.5 | 2.75 | 0.05 | 0.35 | 62.89 |
| 38 | 38 | 0.5 | 0.5 | 0.5 | 0.05 | 0.35 | 47.57 |
| 39 | 39 | 0.5 | 2.75 | 5 | 0.05 | 0.35 | 52.86 |
| 40 | 40 | 0.5 | 5 | 5 | 0.05 | 0.35 | 85.21 |
| 41 | 41 | 0.5 | 2.75 | 2.75 | 0.027 | 1.92 | 64.71 |
| 42 | 42 | 0.5 | 5 | 0.5 | 0.027 | 1.92 | 74.29 |
| 43 | 43 | 0.5 | 2.75 | 0.5 | 0.027 | 1.92 | 38.02 |
| 44 | 44 | 0.5 | 0.5 | 5 | 0.027 | 1.92 | 45.24 |
| 45 | 45 | 0.5 | 0.5 | 2.75 | 0.027 | 1.92 | 97.03 |
| 46 | 46 | 0.5 | 0.5 | 0.5 | 0.027 | 1.92 | 87.13 |
| 47 | 47 | 0.5 | 2.75 | 5 | 0.027 | 1.92 | 82.65 |
| 48 | 48 | 0.5 | 5 | 5 | 0.027 | 1.92 | 84.32 |
| 49 | 49 | 0.5 | 2.75 | 2.75 | 0.005 | 3.5 | 66.33 |
| 50 | 50 | 0.5 | 5 | 0.5 | 0.005 | 3.5 | 69.82 |
| 51 | 51 | 0.5 | 2.75 | 0.5 | 0.005 | 3.5 | 38.52 |
| 52 | 52 | 0.5 | 0.5 | 5 | 0.005 | 3.5 | 35.27 |
| 53 | 53 | 0.5 | 0.5 | 2.75 | 0.005 | 3.5 | 67.57 |
| 54 | 54 | 0.5 | 0.5 | 0.5 | 0.005 | 3.5 | 88.92 |
| 55 | 55 | 0.5 | 2.75 | 5 | 0.005 | 3.5 | 86.83 |
| 56 | 56 | 0.5 | 5 | 5 | 0.005 | 3.5 | 84.79 |
| 57 | 57 | 0.5 | 2.75 | 2.75 | 0.05 | 3.5 | 68.85 |
| 58 | 58 | 0.5 | 5 | 0.5 | 0.05 | 3.5 | 70.43 |
| 59 | 59 | 0.5 | 2.75 | 0.5 | 0.05 | 3.5 | 33.9 |
| 60 | 60 | 0.5 | 0.5 | 5 | 0.05 | 3.5 | 30.08 |
| 61 | 61 | 0.5 | 0.5 | 2.75 | 0.05 | 3.5 | 46.15 |
| 62 | 62 | 0.5 | 0.5 | 0.5 | 0.05 | 3.5 | 85.61 |
| 63 | 63 | 0.5 | 2.75 | 5 | 0.05 | 3.5 | 77.05 |
| 64 | 64 | 0.5 | 5 | 5 | 0.05 | 3.5 | 83.42 |
| 65 | 65 | 0.5 | 2.75 | 2.75 | 0.005 | 0.35 | 69.08 |
| 66 | 66 | 0.5 | 5 | 0.5 | 0.005 | 0.35 | 77.31 |
| 67 | 67 | 0.5 | 2.75 | 0.5 | 0.005 | 0.35 | 41.63 |
| 68 | 68 | 0.5 | 0.5 | 5 | 0.005 | 0.35 | 32.57 |
| 69 | 69 | 0.5 | 0.5 | 2.75 | 0.005 | 0.35 | 60.47 |
| 70 | 70 | 0.5 | 0.5 | 0.5 | 0.005 | 0.35 | 36.08 |
| 71 | 71 | 0.5 | 2.75 | 5 | 0.005 | 0.35 | 83.91 |
| 72 | 72 | 0.5 | 5 | 5 | 0.005 | 0.35 | 88.88 |
| 73 | 73 | 3.25 | 2.75 | 2.75 | 0.05 | 1.92 | 69.17 |
| 74 | 74 | 3.25 | 5 | 0.5 | 0.05 | 1.92 | 72.58 |
| 75 | 75 | 3.25 | 2.75 | 0.5 | 0.05 | 1.92 | 45.26 |
| 76 | 76 | 3.25 | 0.5 | 5 | 0.05 | 1.92 | 26.67 |
| 77 | 77 | 3.25 | 0.5 | 2.75 | 0.05 | 1.92 | 89.9 |
| 78 | 78 | 3.25 | 0.5 | 0.5 | 0.05 | 1.92 | 43.46 |
| 79 | 79 | 3.25 | 2.75 | 5 | 0.05 | 1.92 | 57.7 |
| 80 | 80 | 3.25 | 5 | 5 | 0.05 | 1.92 | 83.54 |
| 81 | 81 | 6 | 2.75 | 2.75 | 0.05 | 0.35 | 72.4 |
| 82 | 82 | 6 | 5 | 0.5 | 0.05 | 0.35 | 74.44 |
| 83 | 83 | 6 | 2.75 | 0.5 | 0.05 | 0.35 | 44.86 |
| 84 | 84 | 6 | 0.5 | 5 | 0.05 | 0.35 | 30.41 |
| 85 | 85 | 6 | 0.5 | 2.75 | 0.05 | 0.35 | 75.24 |
| 86 | 86 | 6 | 0.5 | 0.5 | 0.05 | 0.35 | 49.57 |
| 87 | 87 | 6 | 2.75 | 5 | 0.05 | 0.35 | 62.98 |
| 88 | 88 | 6 | 5 | 5 | 0.05 | 0.35 | 82.78 |
| 89 | 89 | 6 | 2.75 | 2.75 | 0.005 | 0.35 | 69.16 |
| 90 | 90 | 6 | 5 | 0.5 | 0.005 | 0.35 | 74.53 |
| 91 | 91 | 6 | 2.75 | 0.5 | 0.005 | 0.35 | 43.04 |
| 92 | 92 | 6 | 0.5 | 5 | 0.005 | 0.35 | 49.39 |
| 93 | 93 | 6 | 0.5 | 2.75 | 0.005 | 0.35 | 43.34 |
| 94 | 94 | 6 | 0.5 | 0.5 | 0.005 | 0.35 | 93.86 |
| 95 | 95 | 6 | 2.75 | 5 | 0.005 | 0.35 | 62.1 |
| 96 | 96 | 6 | 5 | 5 | 0.005 | 0.35 | 87.58 |
| 97 | 97 | 3.25 | 0 | 0 | 0 | 0 | 60.28 |
| 98 | 98 | 0 | 2.75 | 0 | 0 | 0 | 59.17 |
| 99 | 99 | 0 | 0 | 2.75 | 0 | 0 | 47.84 |
| 100 | 100 | 0 | 0 | 0 | 0.027 | 0 | 71.83 |
| 101 | 101 | 0 | 0 | 0 | 0 | 1.92 | 66.57 |
| 102 | 102 | 0 | 0 | 0 | 0 | 0 | 61.79 |