ANOVA for Response Surface Quadratic Model

 **Supplementary Table 1**: Response1 Particles size(nm) ANOVA for Response Surface Quadratic Model.
 Analysis of variance table [Partial sum of squares - Type III]

 **Sum of** **Mean** **F** **p-value**
 **Source** **Squares** **df** **Square** **Value** **Prob > F**

 Model 3.524E+005 9 39158.33 71.66 < 0.0001 significant

 *A-PLGA(mg/ml)* *10046.53* *1* *10046.53* *18.39* *0.0078*
 *B-TS (mg/ml)* *15216.40* *1* *15216.40* *27.85* *0.0033*
 *C-Poloxamer188(%)* *1.524E+005* *1* *1.524E+005* *278.92* *< 0.0001*
 *AB* *5343.61* *1* *5343.61* *9.78* *0.0260*
 *AC* *35250.06* *1* *35250.06* *64.51* *0.0005*
 *BC* *464.40* *1* *464.40* *0.85* *0.3989*
 *A2* *16989.64* *1* *16989.64* *31.09* *0.0026*
 *B2* *61221.62* *1* *61221.62* *112.04* *0.0001*
 *C2* *45828.25* *1* *45828.25* *83.87* *0.0003*
 Residual 2732.07 5 546.41
 *Lack of Fit* *2416.86* *3* *805.62* *5.11* *0.1680* *not significant*
 *Pure Error* *315.21* *2* *157.60*
 Cor Total 3.552E+005 14

 The Model F-value of 71.66 implies the model is significant. There is only
 a 0.01% chance that a "Model F-Value" this large could occur due to noise.

 Values of "Prob > F" less than 0.0500 indicate model terms are significant.
 In this case A, B, C, AB, AC, A2, B2, C2 are significant model terms.
 Values greater than 0.1000 indicate the model terms are not significant.
 If there are many insignificant model terms (not counting those required to support hierarchy),
 model reduction may improve your model.

 The "Lack of Fit F-value" of 5.11 implies the Lack of Fit is not significant relative to the pure
 error. There is a 16.80% chance that a "Lack of Fit F-value" this large could occur due
 to noise. Non-significant lack of fit is good -- we want the model to fit.

 Std. Dev. 23.38 R-Squared 0.9923
 Mean 565.95 Adj R-Squared 0.9785
 C.V. % 4.13 Pred R-Squared 0.8891

 PRESS 39379.01 Adeq Precision 30.676

 The "Pred R-Squared" of 0.8891 is in reasonable agreement with the "Adj R-Squared" of 0.9785.

 **Supplementary** **Equation 1:**  **Final Equation in Terms of Coded Factors:**

 Particles size(nm) =
 +592.87
 +35.44 \* A
 +43.61 \* B
 -138.03 \* C
 -36.55 \* A \* B
 -93.88 \* A \* C
 -10.77 \* B \* C
 -67.83 \* A2
 +128.77 \* B2
 -111.41 \* C2

  **Final Equation in Terms of Actual Factors:**

 Particles size(nm) =
 -55.88333
 +56.76208 \* PLGA(mg/ml)
 -75.36083 \* TS (mg/ml)
 +1033.81667 \* Poloxamer188(%)
 -0.73100 \* PLGA(mg/ml) \* TS (mg/ml)
 -18.77500 \* PLGA(mg/ml) \* Poloxamer188(%)
 -4.31000 \* TS (mg/ml) \* Poloxamer188(%)
 -0.67833 \* PLGA(mg/ml)2
 +5.15067 \* TS (mg/ml)2
 -445.63333 \* Poloxamer188(%)2

**Supplementary Table 2**: Response 2 Zeta potential (mV) ANOVA for Response Surface Quadratic Model. Analysis of variance table [Partial sum of squares - Type III]

 **Sum of** **Mean** **F** **p-value**
 **Source** **Squares** **df** **Square** **Value** **Prob > F**

 Model 347.96 9 38.66 145.35 < 0.0001 significant

 *A-PLGA(mg/ml)* *23.80* *1* *23.80* *89.49* *0.0002*
 *B-TS (mg/ml)* *63.85* *1* *63.85* *240.02* *< 0.0001*
 *C-Poloxamer188(%)* *2.00* *1* *2.00* *7.52* *0.0407*
 *AB* *15.21* *1* *15.21* *57.18* *0.0006*
 *AC* *9.00* *1* *9.00* *33.83* *0.0021*
 *BC* *123.21* *1* *123.21* *463.20* *< 0.0001*
 *A2* *27.92* *1* *27.92* *104.97* *0.0002*
 *B2* *24.96* *1* *24.96* *93.83* *0.0002*
 *C2* *73.12* *1* *73.12* *274.88* *< 0.0001*
 Residual 1.33 5 0.27
 *Lack of Fit* *1.33* *3* *0.44*
 *Pure Error* *0.000* *2* *0.000*
 Cor Total 349.29 14

 The Model F-value of 145.35 implies the model is significant. There is only
 a 0.01% chance that a "Model F-Value" this large could occur due to noise.

 **Supplementary Equation 2:**  **Final Equation in Terms of Coded Factors:**

 Zeta potential (mV) =
 -27.50
 +1.72 \* A
 +2.83 \* B
 -0.50 \* C
 +1.95 \* A \* B
 -1.50 \* A \* C
 -5.55 \* B \* C
 -2.75 \* A2
 -2.60 \* B2
 -4.45 \* C2

  **Final Equation in Terms of Actual Factors:**

 Zeta potential (mV) =
 -95.20000
 +1.18250 \* PLGA(mg/ml)
 +4.08500 \* TS (mg/ml)
 +62.80000 \* Poloxamer188(%)
 +0.039000 \* PLGA(mg/ml) \* TS (mg/ml)
 -0.30000 \* PLGA(mg/ml) \* Poloxamer188(%)
 -2.22000 \* TS (mg/ml) \* Poloxamer188(%)
 -0.027500 \* PLGA(mg/ml)2
 -0.10400 \* TS (mg/ml)2
 -17.80000 \* Poloxamer188(%)2

**Supplementary Table 3**: Response 3 EE (%)ANOVA for Response Surface Quadratic Model. Analysis of variance table [Partial sum of squares - Type III].

 **Sum of** **Mean** **F** **p-value**
 **Source** **Squares** **df** **Square** **Value** **Prob > F**

 Model 230.08 9 25.56 76.12 < 0.0001 significant

 *A-PLGA(mg/ml)* *18.91* *1* *18.91* *56.31* *0.0007*
 *B-TS (mg/ml)* *2.10* *1* *2.10* *6.26* *0.0544*
 *C-Poloxamer188(%)* *3.64* *1* *3.64* *10.85* *0.0216*
 *AB* *0.040* *1* *0.040* *0.12* *0.7440*
 *AC* *2.500E-003* *1* *2.500E-003* *7.444E-003* *0.9346*
 *BC* *0.20* *1* *0.20* *0.60* *0.4726*
 *A2* *104.37* *1* *104.37* *310.78* *< 0.0001*
 *B2* *35.87* *1* *35.87* *106.80* *0.0001*
 *C2* *93.85* *1* *93.85* *279.46* *< 0.0001*
 Residual 1.68 5 0.34
 *Lack of Fit* *1.55* *3* *0.52* *8.17* *0.1110* *not significant*
 *Pure Error* *0.13* *2* *0.063*
 Cor Total 231.76 14

 The Model F-value of 76.12 implies the model is significant. There is only
 a 0.01% chance that a "Model F-Value" this large could occur due to noise.

 Values of "Prob > F" less than 0.0500 indicate model terms are significant.
 In this case A, C, A2, B2, C2 are significant model terms.
 Values greater than 0.1000 indicate the model terms are not significant.

 **Supplementary Equation 3:**  **Final Equation in Terms of Coded Factors:**

 EE (%) =
 +80.57
 +1.54 \* A
 -0.51 \* B
 +0.67 \* C
 -0.100 \* A \* B
 -0.025 \* A \* C
 -0.22 \* B \* C
 +5.32 \* A2
 +3.12 \* B2
 +5.04 \* C2

  **Final Equation in Terms of Actual Factors:**

 EE (%) =
 +129.66667
 -1.94792 \* PLGA(mg/ml)
 -2.46583 \* TS (mg/ml)
 -37.98333 \* Poloxamer188(%)
 -2.00000E-003 \* PLGA(mg/ml) \* TS (mg/ml)
 -5.00000E-003 \* PLGA(mg/ml) \* Poloxamer188(%)
 -0.090000 \* TS (mg/ml) \* Poloxamer188(%)
 +0.053167 \* PLGA(mg/ml)2
 +0.12467 \* TS (mg/ml)2
 +20.16667 \* Poloxamer188(%)2

**Supplementary Table 4: Fit Summary of different mathematical models for mixed PLNs size.**

|  |  |
| --- | --- |
| **Source** | **Sequential Lack of Fit Adjusted Predicted Rejected /Suggested p-value p -value R-Squared R- Squared**  |
| Linear |  |
| 0.0472 0.0080 0.03640 -0.0633 |
|  |
| 2FI | 0.5267 0.00 0.3278 -1.0164 |
|  |
| Quadratic | 0.0001 0.1680 0.9785 0.8891 Suggested Suggested |
|  |
| Cubic |  0.1680 Aliased |
|  |
|  |  |

**Supplementary Table 5 : Fit Summary of different mathematical models for mixed PLNs zeta potential.**

|  |  |
| --- | --- |
| **Source** | **Sequential Lack of Fit Adjusted Predicted Rejected /Suggested p-value p -value R-Squared R- Squared**  |
| Linear | 0.3324 0.0548 -0.3580 |
|  |
| 2FI | 0.0698 0.4394 0.2655 |
|  |
| Quadratic | 0.0001 0.9880 0.9315 Suggested Suggested |
|   |
| Cubic | 0.0001 Aliased |
|  |

**Supplementary Table 6: Fit Summary of different mathematical models for mixed PLNs entrapment efficiency**

|  |  |
| --- | --- |
| **Source** | **Sequential Lack of Fit Adjusted Predicted Rejected /Suggested p-value p-value R-Squared R- Squared**  |
| Linear |  |
| 0.7313 0.0027 - 0.1373 -0.2489 |
|  |
| 2FI | 0.9997 0.0018 -0.5620 -0.9580 |
|  |
| Quadratic | 0.0001 0.1110 0.9797 0.8916 Suggested |
|  |
| Cubic | 0.1110 Aliased |
|  |



**Supplementary Figure 1:**  **Design space for IBU-PLNs. nanoparticles. Yellow areas contain the feasible settings to obtain desired values for average particle size 217 nm), %EE (93%) and Zeta potential (-35).**



**Supplementary Figure 2: Desirability ramp for optimized conditions for PLNs process conditions.**