

### Data obtained from Design Expert Software

#### Build Information

|                        |                   |                |            |
|------------------------|-------------------|----------------|------------|
| <b>File Version</b>    | 22.0.1.0          |                |            |
| <b>Study Type</b>      | Response Surface  | <b>Subtype</b> | Randomized |
| <b>Design Type</b>     | Central Composite | <b>Runs</b>    | 20         |
| <b>Design Model</b>    | Quadratic         | <b>Blocks</b>  | No Blocks  |
| <b>Build Time (ms)</b> | 1.0000            |                |            |

#### Factors

| Factor | Name    | Units | Type    | SubType    | Minimum | Maximum | Coded Low  | Coded High | Mean  | Std. Dev. |
|--------|---------|-------|---------|------------|---------|---------|------------|------------|-------|-----------|
| A      | [BGE]   | mM    | Numeric | Continuous | 10.00   | 100.00  | -1 ↔ 25.00 | +1 ↔ 75.00 | 50.50 | 21.88     |
| B      | pH      |       | Numeric | Continuous | 2.60    | 10.60   | -1 ↔ 5.00  | +1 ↔ 9.00  | 6.96  | 1.84      |
| C      | Voltage | kV    | Numeric | Continuous | 5.00    | 23.00   | -1 ↔ 10.00 | +1 ↔ 18.00 | 14.00 | 3.91      |

#### Responses

| Response | Name           | Units | Observations | Minimum | Maximum | Mean | Std. Dev. | Ratio  |
|----------|----------------|-------|--------------|---------|---------|------|-----------|--------|
| R1       | R1             |       | 20.00        | 0       | 4.3     | 1.91 | 0.9307    | N/A    |
| R2       | R2             |       | 20.00        | 0.01    | 5.76    | 1.24 | 1.71      | 576.00 |
| R3       | R3             |       | 20.00        | 0.5     | 3.5     | 2.04 | 0.6810    | 7.00   |
| R4       | Migration Time | min   | 20.00        | 2.2     | 8       | 3.90 | 1.46      | 3.64   |

R1: Resolution between Metformin (first peak) and Phenformin (second peak)

R1: Resolution between Phenformin (second peak) and Mexiletine (third peak)

R1: Resolution between Mexiletine (third peak) and Ranitidine (fourth peak)

**Optimization Table**

|     |     |            | Factor 1 | Factor 2 | Factor 3  | Response 1 | Response 2 | Response 3 | Response 4     |
|-----|-----|------------|----------|----------|-----------|------------|------------|------------|----------------|
| Std | Run | Space Type | A:[BGE]  | B:pH     | C:Voltage | R1         | R2         | R3         | Migration Time |
|     |     |            | mM       |          | kV        |            |            |            | min            |
| 18  | 1   | Center     | 50       | 7        | 14        | 1.47       | 0.6        | 2.5        | 3.64           |
| 4   | 2   | Factorial  | 75       | 9        | 10        | 1.92       | 2.6        | 1.4        | 5.04           |
| 1   | 3   | Factorial  | 25       | 5        | 10        | 1.7        | 1.5        | 2.5        | 4.9            |
| 20  | 4   | Center     | 50       | 7        | 14        | 1.55       | 0.5        | 2.2        | 3.65           |
| 19  | 5   | Center     | 50       | 7        | 14        | 1.62       | 0.095      | 2.01       | 3.68           |
| 12  | 6   | Axial      | 50       | 10.6     | 14        | 2.82       | 5.76       | 0.9        | 4.39           |
| 13  | 7   | Axial      | 50       | 7        | 5         | 3.14       | 0.01       | 2.94       | 8              |
| 5   | 8   | Factorial  | 25       | 5        | 18        | 1.17       | 0.6        | 1.8        | 2.5            |
| 11  | 9   | Axial      | 50       | 2.6      | 14        | 4.3        | 4.4        | 3.5        | 5.8            |
| 16  | 10  | Center     | 50       | 7        | 14        | 1.19       | 0.15       | 2.11       | 3.57           |
| 8   | 11  | Factorial  | 75       | 9        | 18        | 1.31       | 4.58       | 0.5        | 2.56           |
| 6   | 12  | Factorial  | 75       | 5        | 18        | 2.76       | 0.2        | 1.7        | 2.9            |
| 17  | 13  | Center     | 50       | 7        | 14        | 1.76       | 0.15       | 2.72       | 3.71           |
| 9   | 14  | Axial      | 10       | 7        | 14        | 0          | 0.3        | 2.5        | 2.2            |
| 2   | 15  | Factorial  | 75       | 5        | 10        | 3.11       | 0.4        | 1.87       | 5.73           |
| 10  | 16  | Axial      | 100      | 7        | 14        | 1.3        | 0.7        | 1.7        | 2.86           |
| 7   | 17  | Factorial  | 25       | 9        | 18        | 1.5        | 1.1        | 1.62       | 2.31           |
| 14  | 18  | Axial      | 50       | 7        | 23        | 1.65       | 0.22       | 1.86       | 2.33           |
| 15  | 19  | Center     | 50       | 7        | 14        | 1.8        | 0.3        | 2.39       | 3.86           |
| 3   | 20  | Factorial  | 25       | 9        | 10        | 2.18       | 0.6        | 2.15       | 4.44           |

**Factors**

| Factor | Name    | Level | Low Level | High Level | Std. Dev. | Coding |
|--------|---------|-------|-----------|------------|-----------|--------|
| A      | [BGE]   | 75.00 | 25.00     | 75.00      | 0.0000    | Actual |
| B      | pH      | 9.00  | 5.00      | 9.00       | 0.0000    | Actual |
| C      | Voltage | 13.73 | 10.00     | 18.00      | 0.0000    | Actual |

**Point Prediction**

Two-sided Confidence = 95% Population = 99%

| Solution 1 of 56 Response | Predicted Mean | Predicted Median | Std Dev  | SE Mean   | 95% CI low for Mean | 95% CI high for Mean | 95% TI low for 99% Pop | 95% TI high for 99% Pop |
|---------------------------|----------------|------------------|----------|-----------|---------------------|----------------------|------------------------|-------------------------|
| R1 (Met and Phen)         | 1.54368        | 1.54368          | 0.193411 | 0.111289  | 1.29571             | 1.79164              | 0.548314               | 2.53904                 |
| R2 (Phen and Mex)         | 3.46659        | 3.46659          | 0.30845  | 0.177483  | 3.07113             | 3.86205              | 1.87919                | 5.05399                 |
| R3 (Mex and Ran)          | 1.31736        | 1.31736          | 0.433401 | 0.184343  | 0.926573            | 1.70815              | -0.58706               | 3.22179                 |
| Migration Time (min)      | 3.71159        | 3.71159          | 0.130252 | 0.0749471 | 3.5446              | 3.87859              | 3.04127                | 4.38192                 |

**Confirmation Location #1**

| [BGE] | pH | Voltage |
|-------|----|---------|
| 75    | 9  | 13.7298 |

**Confirmation**

Two-sided Confidence = 95%

| Solution 1 of 56 Response | Predicted Mean | Predicted Median | Observed | Std Dev  | n | SE Pred  | 95% PI low | Data Mean | 95% PI high |
|---------------------------|----------------|------------------|----------|----------|---|----------|------------|-----------|-------------|
| R1 (Met and Phen)         | 1.54368        | 1.54368          |          | 0.193411 | 2 | 0.176321 | 1.15081    |           | 1.93654     |
| R2 (Phen and Mex)         | 3.46659        | 3.46659          |          | 0.30845  | 2 | 0.281195 | 2.84005    |           | 4.09313     |
| R3 (Mex and Ran)          | 1.31736        | 1.31736          |          | 0.433401 | 2 | 0.357632 | 0.559216   |           | 2.07551     |
| Migration Time (min)      | 3.71159        | 3.71159          |          | 0.130252 | 2 | 0.118743 | 3.44702    |           | 3.97617     |

## Coefficients Table

p-value shading:  $p < 0.05$   $0.05 \leq p < 0.1$   $p \geq 0.1$

|                             | Intercept | A                    | B                    | C                    | AB                  | AC            | BC            | A <sup>2</sup>     | B <sup>2</sup>     | C <sup>2</sup>     |
|-----------------------------|-----------|----------------------|----------------------|----------------------|---------------------|---------------|---------------|--------------------|--------------------|--------------------|
| <b>R1 (Met and Phen)</b>    | 1.56413   | <b>0.386803</b>      | -<br><b>0.201755</b> | <b>-0.30469</b>      | -<br><b>0.43125</b> | 0.03125       | -<br>0.05125  | <b>-0.283684</b>   | <b>0.486736</b>    | <b>0.167491</b>    |
| <b>p-values</b>             |           | <b>&lt; 0.0001</b>   | <b>0.0022</b>        | <b>&lt; 0.0001</b>   | <b>&lt; 0.0001</b>  | 0.6574        | 0.4708        | <b>0.0001</b>      | <b>&lt; 0.0001</b> | <b>0.0003</b>      |
| <b>R2 (Phen and Mex)</b>    | 0.320569  | <b>0.32857</b>       | <b>0.796648</b>      | 0.102207             | <b>0.8725</b>       | <b>0.2725</b> | <b>0.4475</b> | -<br>0.00312549    | <b>1.2072</b>      | -<br>0.0455386     |
| <b>p-values</b>             |           | <b>0.0026</b>        | <b>&lt; 0.0001</b>   | 0.1887               | <b>&lt; 0.0001</b>  | <b>0.0315</b> | <b>0.0021</b> | 0.9668             | <b>&lt; 0.0001</b> | 0.3759             |
| <b>R3 (Mex and Ran)</b>     | 2.03973   | -<br><b>0.275817</b> | -<br><b>0.464186</b> | -<br><b>0.260966</b> |                     |               |               |                    |                    |                    |
| <b>p-values</b>             |           | <b>0.0274</b>        | <b>0.0006</b>        | <b>0.0208</b>        |                     |               |               |                    |                    |                    |
| <b>Migration Time (min)</b> | 3.65191   | <b>0.297031</b>      | -<br><b>0.217877</b> | <b>-1.24676</b>      | -0.0475             | -0.0975       | 0.0775        | <b>-0.38564</b>    | <b>0.32678</b>     | <b>0.286134</b>    |
| <b>p-values</b>             |           | <b>&lt; 0.0001</b>   | <b>&lt; 0.0001</b>   | <b>&lt; 0.0001</b>   | 0.3266              | 0.0603        | 0.1233        | <b>&lt; 0.0001</b> | <b>&lt; 0.0001</b> | <b>&lt; 0.0001</b> |

## Model Comparison Statistics

|                          | R1 (Met and Phen) | R2 (Phen and Mex) | R3 (Mex and Ran) | Migration Time (min) |
|--------------------------|-------------------|-------------------|------------------|----------------------|
| <b>PRESS</b>             | 1.28              | 5.44              | 4.92             | 1.19                 |
| <b>-2 Log Likelihood</b> | -22.82            | -4.15             | 18.85            | -38.64               |
| <b>BIC</b>               | 7.13              | 25.80             | 30.83            | -8.68                |
| <b>AICc</b>              | 21.62             | 40.29             | 29.52            | 5.81                 |

### Coded Equation

|                      | R1 (Met and Phen) | R2 (Phen and Mex) | R3 (Mex and Ran) | Migration Time (min) |
|----------------------|-------------------|-------------------|------------------|----------------------|
| <b>Intercept</b>     | 1.56              | 0.3206            | 2.04             | 3.65                 |
| <b>A</b>             | 0.3868            | 0.3286            | -0.2758          | 0.2970               |
| <b>B</b>             | -0.2018           | 0.7966            | -0.4642          | -0.2179              |
| <b>C</b>             | -0.3047           | 0.1022            | -0.2610          | -1.25                |
| <b>AB</b>            | -0.4312           | 0.8725            |                  | -0.0475              |
| <b>AC</b>            | 0.0313            | 0.2725            |                  | -0.0975              |
| <b>BC</b>            | -0.0513           | 0.4475            |                  | 0.0775               |
| <b>A<sup>2</sup></b> | -0.2837           | -0.0031           |                  | -0.3856              |
| <b>B<sup>2</sup></b> | 0.4867            | 1.21              |                  | 0.3268               |
| <b>C<sup>2</sup></b> | 0.1675            | -0.0455           |                  | 0.2861               |

### Fit Statistics

|                                | R1 (Met and Phen) | R2 (Phen and Mex) | R3 (Mex and Ran) | Migration Time (min) |
|--------------------------------|-------------------|-------------------|------------------|----------------------|
| <b>Std. Dev.</b>               | 0.1934            | 0.3084            | 0.4334           | 0.1303               |
| <b>Mean</b>                    | 1.91              | 1.24              | 2.04             | 3.90                 |
| <b>C.V. %</b>                  | 10.11             | 24.91             | 21.21            | 3.34                 |
| <b>R<sup>2</sup></b>           | 0.9773            | 0.9828            | 0.6589           | 0.9958               |
| <b>Adjusted R<sup>2</sup></b>  | 0.9568            | 0.9674            | 0.5950           | 0.9921               |
| <b>Predicted R<sup>2</sup></b> | 0.9221            | 0.9018            | 0.4414           | 0.9707               |
| <b>Adeq Precision</b>          | 30.3065           | 26.9547           | 10.4331          | 62.0644              |

### Constraints

| Name           | Goal        | Lower Limit | Upper Limit | Lower Weight | Upper Weight | Importance |
|----------------|-------------|-------------|-------------|--------------|--------------|------------|
| A:[BGE]        | maximize    | 25          | 75          | 1            | 1            | 3          |
| B:pH           | maximize    | 5           | 9           | 1            | 1            | 3          |
| C:Voltage      | is in range | 10          | 18          | 1            | 1            | 3          |
| R1             | maximize    | 0           | 4.3         | 1            | 1            | 3          |
| R2             | maximize    | 0.01        | 5.76        | 1            | 1            | 3          |
| R3             | maximize    | 0.5         | 3.5         | 1            | 1            | 3          |
| Migration Time | minimize    | 2.2         | 8           | 1            | 1            | 3          |

## Solutions

56 Solutions found

| Number   | [BGE]         | pH           | Voltage       | R1           | R2           | R3           | Migration Time | Desirability |                 |
|----------|---------------|--------------|---------------|--------------|--------------|--------------|----------------|--------------|-----------------|
| <b>1</b> | <b>75.000</b> | <b>9.000</b> | <b>13.730</b> | <b>1.544</b> | <b>3.467</b> | <b>1.317</b> | <b>3.712</b>   | <b>0.593</b> | <b>Selected</b> |
| 2        | 75.000        | 9.000        | 13.735        | 1.543        | 3.468        | 1.317        | 3.710          | 0.593        |                 |
| 3        | 75.000        | 9.000        | 13.690        | 1.547        | 3.458        | 1.320        | 3.725          | 0.593        |                 |
| 4        | 75.000        | 9.000        | 13.771        | 1.540        | 3.475        | 1.315        | 3.698          | 0.593        |                 |
| 5        | 75.000        | 9.000        | 13.828        | 1.535        | 3.487        | 1.311        | 3.680          | 0.593        |                 |
| 6        | 74.999        | 8.999        | 13.644        | 1.551        | 3.448        | 1.323        | 3.739          | 0.593        |                 |
| 7        | 75.000        | 9.000        | 13.976        | 1.523        | 3.517        | 1.301        | 3.632          | 0.593        |                 |
| 8        | 75.000        | 9.000        | 14.036        | 1.518        | 3.530        | 1.297        | 3.613          | 0.593        |                 |
| 9        | 74.999        | 9.000        | 14.068        | 1.515        | 3.536        | 1.295        | 3.603          | 0.593        |                 |
| 10       | 74.967        | 9.000        | 14.166        | 1.509        | 3.555        | 1.289        | 3.573          | 0.593        |                 |
| 11       | 74.797        | 9.000        | 13.679        | 1.553        | 3.447        | 1.323        | 3.732          | 0.593        |                 |
| 12       | 75.000        | 9.000        | 13.131        | 1.599        | 3.342        | 1.356        | 3.913          | 0.593        |                 |
| 13       | 74.662        | 9.000        | 13.649        | 1.559        | 3.434        | 1.326        | 3.745          | 0.593        |                 |
| 14       | 74.975        | 9.000        | 12.898        | 1.624        | 3.291        | 1.372        | 3.996          | 0.592        |                 |
| 15       | 74.448        | 9.000        | 14.208        | 1.518        | 3.538        | 1.292        | 3.571          | 0.592        |                 |
| 16       | 75.000        | 9.000        | 12.838        | 1.629        | 3.280        | 1.376        | 4.017          | 0.592        |                 |
| 17       | 74.204        | 9.000        | 13.800        | 1.557        | 3.444        | 1.322        | 3.705          | 0.592        |                 |
| 18       | 74.999        | 8.988        | 13.549        | 1.558        | 3.404        | 1.332        | 3.769          | 0.592        |                 |
| 19       | 74.244        | 9.000        | 14.164        | 1.526        | 3.519        | 1.297        | 3.589          | 0.592        |                 |
| 20       | 74.122        | 9.000        | 13.988        | 1.543        | 3.478        | 1.310        | 3.646          | 0.592        |                 |
| 21       | 74.999        | 9.000        | 14.976        | 1.452        | 3.720        | 1.236        | 3.333          | 0.592        |                 |
| 22       | 74.999        | 8.986        | 14.401        | 1.488        | 3.575        | 1.277        | 3.498          | 0.592        |                 |
| 23       | 75.000        | 9.000        | 12.504        | 1.666        | 3.208        | 1.397        | 4.139          | 0.592        |                 |
| 24       | 75.000        | 8.980        | 14.156        | 1.505        | 3.514        | 1.294        | 3.572          | 0.592        |                 |
| 25       | 73.659        | 9.000        | 13.661        | 1.582        | 3.389        | 1.337        | 3.761          | 0.592        |                 |
| 26       | 74.999        | 9.000        | 15.237        | 1.437        | 3.772        | 1.219        | 3.260          | 0.591        |                 |
| 27       | 75.000        | 9.000        | 15.381        | 1.429        | 3.801        | 1.210        | 3.222          | 0.591        |                 |
| 28       | 75.000        | 9.000        | 15.412        | 1.427        | 3.807        | 1.208        | 3.213          | 0.591        |                 |
| 29       | 75.000        | 9.000        | 15.697        | 1.413        | 3.863        | 1.189        | 3.139          | 0.591        |                 |
| 30       | 73.162        | 9.000        | 14.998        | 1.493        | 3.632        | 1.255        | 3.364          | 0.590        |                 |
| 31       | 75.000        | 9.000        | 11.969        | 1.729        | 3.093        | 1.432        | 4.342          | 0.590        |                 |
| 32       | 72.255        | 9.000        | 13.590        | 1.620        | 3.309        | 1.357        | 3.809          | 0.590        |                 |
| 33       | 75.000        | 8.962        | 14.857        | 1.453        | 3.617        | 1.253        | 3.359          | 0.590        |                 |
| 34       | 71.895        | 9.000        | 14.055        | 1.588        | 3.385        | 1.330        | 3.666          | 0.590        |                 |
| 35       | 72.086        | 9.000        | 15.151        | 1.508        | 3.607        | 1.257        | 3.343          | 0.589        |                 |
| 36       | 74.996        | 9.000        | 16.353        | 1.388        | 3.990        | 1.146        | 2.979          | 0.589        |                 |
| 37       | 71.288        | 9.000        | 14.875        | 1.542        | 3.514        | 1.284        | 3.433          | 0.589        |                 |
| 38       | 75.000        | 9.000        | 11.566        | 1.781        | 3.005        | 1.459        | 4.501          | 0.589        |                 |

|    |        |       |        |       |       |       |       |       |  |
|----|--------|-------|--------|-------|-------|-------|-------|-------|--|
| 39 | 74.998 | 9.000 | 16.504 | 1.383 | 4.019 | 1.136 | 2.944 | 0.588 |  |
| 40 | 72.455 | 9.000 | 12.433 | 1.734 | 3.082 | 1.430 | 4.210 | 0.588 |  |
| 41 | 70.942 | 9.000 | 12.960 | 1.710 | 3.123 | 1.412 | 4.044 | 0.587 |  |
| 42 | 75.000 | 8.912 | 13.344 | 1.564 | 3.211 | 1.363 | 3.824 | 0.587 |  |
| 43 | 74.997 | 9.000 | 17.126 | 1.370 | 4.137 | 1.096 | 2.810 | 0.586 |  |
| 44 | 71.645 | 9.000 | 12.181 | 1.782 | 2.995 | 1.455 | 4.317 | 0.586 |  |
| 45 | 71.230 | 9.000 | 12.260 | 1.782 | 2.994 | 1.455 | 4.293 | 0.586 |  |
| 46 | 75.000 | 9.000 | 17.437 | 1.366 | 4.195 | 1.076 | 2.748 | 0.585 |  |
| 47 | 72.335 | 9.000 | 17.435 | 1.425 | 4.042 | 1.105 | 2.808 | 0.584 |  |
| 48 | 74.792 | 9.000 | 17.774 | 1.369 | 4.245 | 1.056 | 2.689 | 0.584 |  |
| 49 | 75.000 | 9.000 | 10.738 | 1.897 | 2.822 | 1.513 | 4.848 | 0.584 |  |
| 50 | 71.363 | 9.000 | 17.541 | 1.444 | 4.006 | 1.109 | 2.808 | 0.583 |  |
| 51 | 75.000 | 9.000 | 10.574 | 1.922 | 2.785 | 1.523 | 4.919 | 0.583 |  |
| 52 | 75.000 | 9.000 | 10.468 | 1.938 | 2.761 | 1.530 | 4.966 | 0.582 |  |
| 53 | 71.505 | 9.000 | 17.995 | 1.439 | 4.093 | 1.078 | 2.724 | 0.582 |  |
| 54 | 71.122 | 9.000 | 17.999 | 1.447 | 4.071 | 1.082 | 2.731 | 0.581 |  |
| 55 | 64.872 | 9.000 | 18.000 | 1.552 | 3.704 | 1.151 | 2.832 | 0.574 |  |
| 56 | 49.106 | 5.678 | 10.573 | 2.241 | 0.471 | 2.580 | 5.245 | 0.322 |  |

Factor Coding: Actual

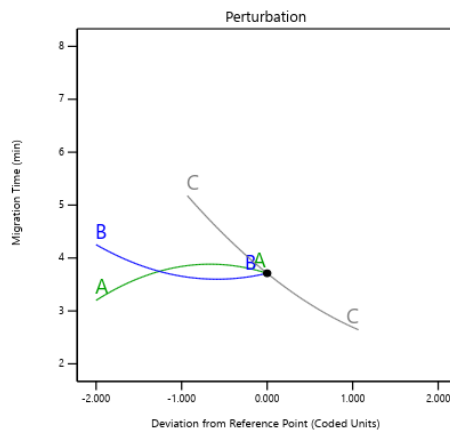
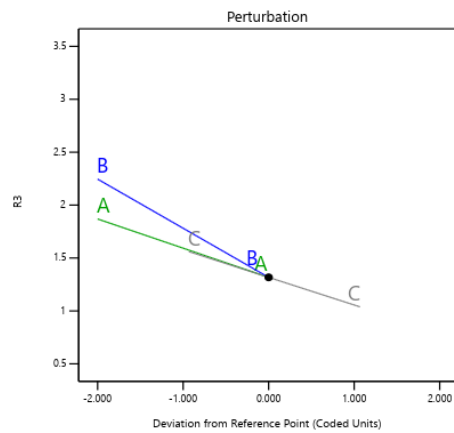
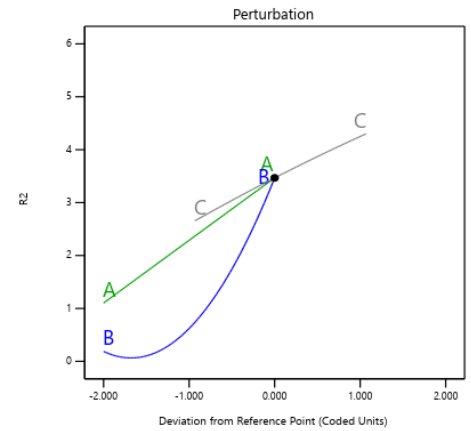
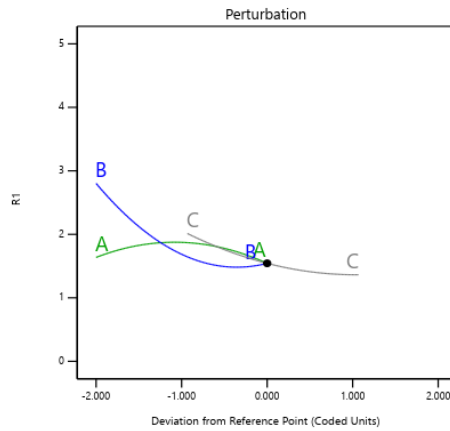
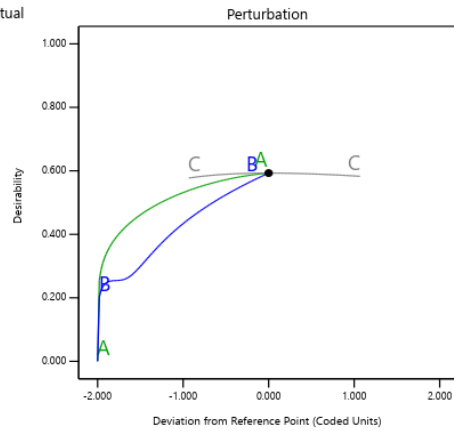
All Responses

Actual Factors:

A = 75

B = 9

C = 13.7298



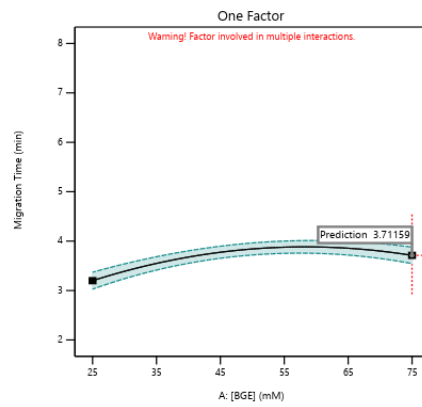
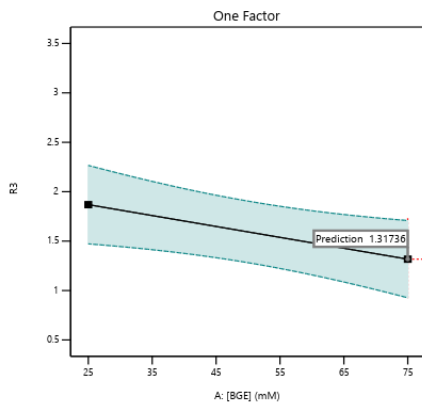
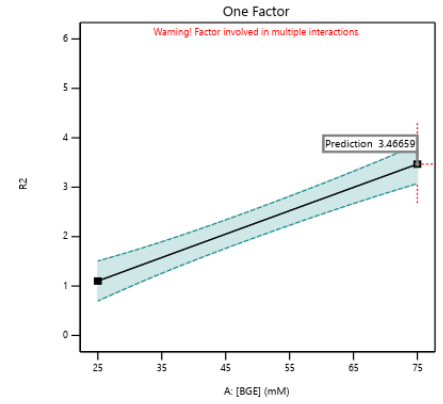
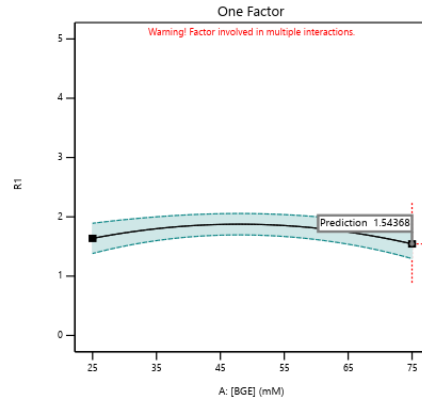
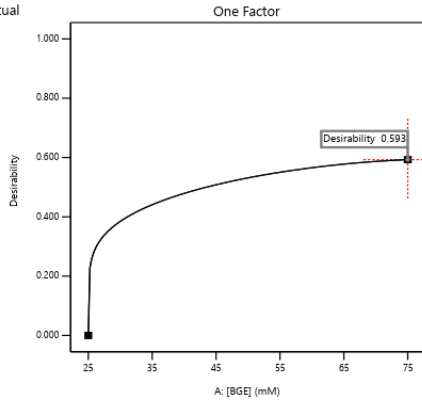
Factor Coding: Actual

All Responses

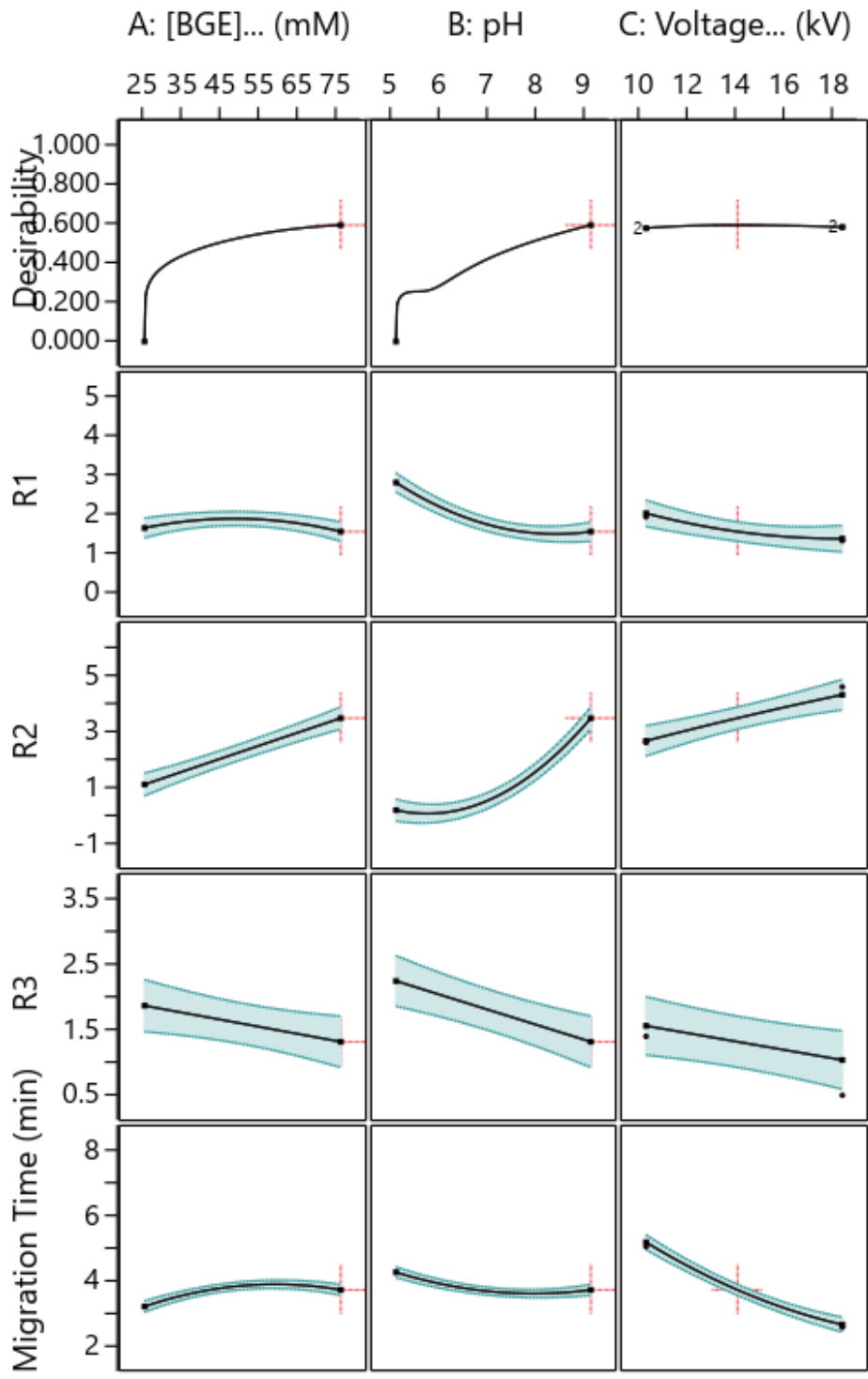
Actual Factors:

B = 9

C = 13.7298



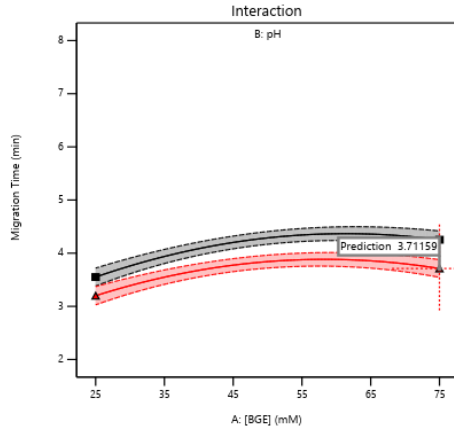
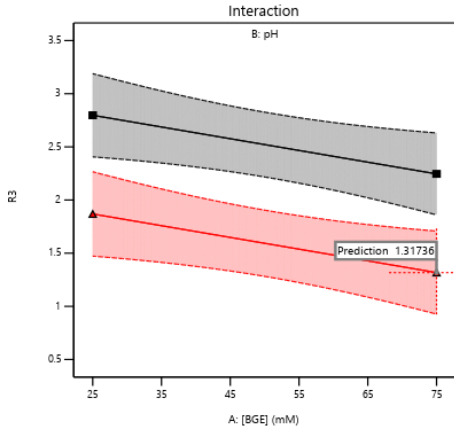
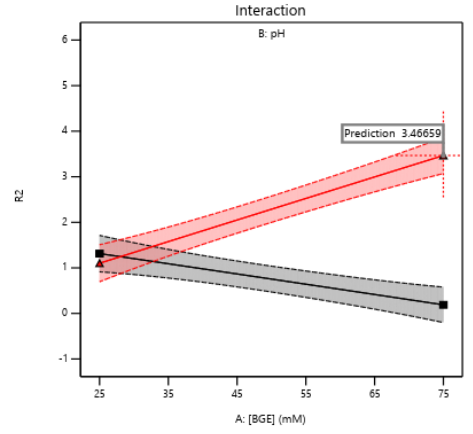
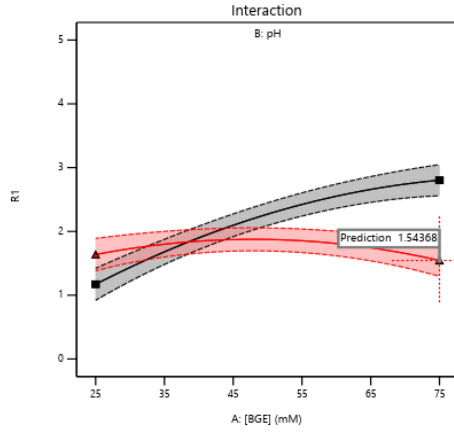
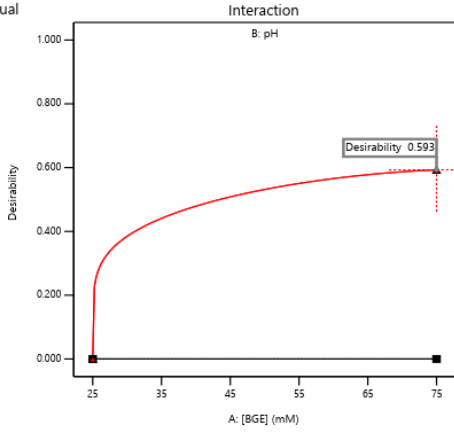




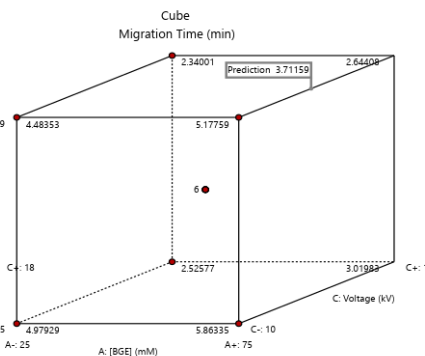
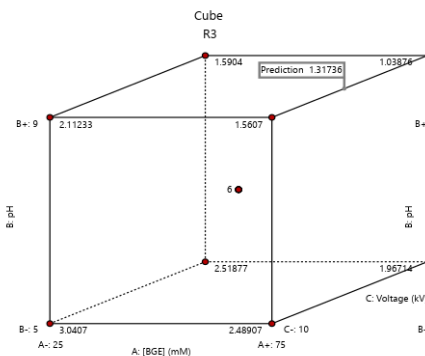
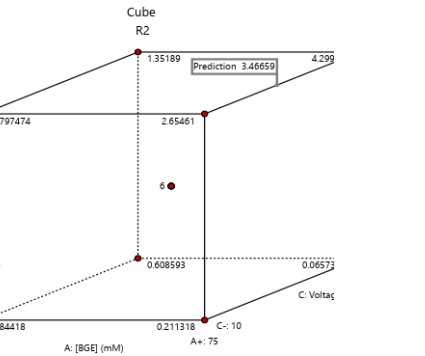
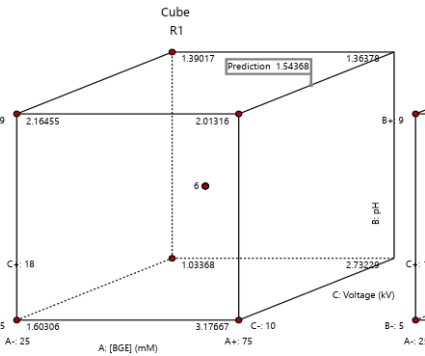
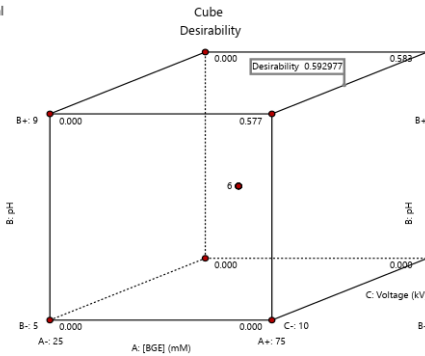
Factor Coding: Actual  
All Responses

Actual Factor:  
C = 13.7298

■ B- 5  
▲ B+ 9



Factor Coding: Actual  
All Responses



Factor Coding: Actual

All Responses

0 1

Actual Factor:

C = 13.7298

