Using JMP to Create Experiment Designs with Non-Linear Constraints – Two Examples from the Pharmaceuticals Industry

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Abstract

Experiment Designs provide a powerful planning tool for effective experimentation. Some experimental situations have physical limitations, or *constraints*. JMP's Custom Designer provides for the inclusion of *linear constraints* in experiment designs. Some experiments have *non-linear constraints*, and these are more difficult to work with. If a constraint requires more than just the simple sum of scaled factors it is non-linear and must be linearized to use the JMP Custom Designer[1].

In this presentation you will learn how to use the JMP Custom Designer to create Experiment Designs with non-linear constraints. First you will learn how to create a design with a simple non-linear constraint. Next you will see two interesting examples from the pharmaceuticals industry that use JMP to create experiment designs with complex, non-linear constraints: an osmolality constraint and a solubility constraint.

[1]This statement turned out not to be true. Unfortunately, the constraints in the two examples presented were too strict to work with JMP's Disallowed Combinations feature, so the paper remains in tact. JMP can easily handle the simple laser constraint example, but learning to linearize the constraint is a necessary precursor for understanding the two case studies. The Appendix shows how to deal with the non-linear constraint for the laser example directly.

Introduction

People love to simplify. Nature is perfectly comfortable with complexity. In this paper you will see how to simplify Nature's complexity without losing your grasp on the real world.

Unconstrained Experiment Designs

Design of Experiments is a powerful Statistical technique for extracting the maximum amount of information from the minimum amount of experimental work. It is valuable in industry because it allows experimenters to balance frugality with thoroughness.

A typical designed experiment will vary a number of factors, each from a low to high level. If every combination of factor levels within these high and low limits is allowed, the design is "unconstrained."

Table 1 is an example of an unconstrained experiment design.

	X1	X2	X3	X4	Y
1	-1	-1	-1	-1	•
2	-1	-1	-1	1	•
3	-1	-1	1	-1	•
4	-1	-1	1	1	•
5	-1	1	-1	-1	•
6	-1	1	-1	1	•
7	-1	1	1	-1	•
8	-1	1	1	1	•
9	1	-1	-1	-1	•
10	1	-1	-1	1	•
11	1	-1	1	-1	•
12	1	-1	1	1	•
13	1	1	-1	-1	•
14	1	1	-1	1	•
15	1	1	1	-1	•
16	1	1	1	1	•
17	-1	0	0	0	•
18	1	0	0	0	•
19	0	-1	0	0	•
20	0	1	0	0	•
21	0	0	-1	0	•
22	0	0	1	0	•
23	0	0	0	-1	•
24	0	0	0	1	•
25	0	0	0	0	•
26	0	0	0	0	•
27	0	0	0	0	•

Table 1 – An unconstrained experiment design.

Linear Constraints

An experiment design is “constrained” when it has limitations on the combinations of the factor levels. These limitations come from aspects of the real world.

If these limitations can be expressed mathematically as the sum of scaled factor levels, the constraint is called “linear.”

For example, suppose you want to study combinations of dyes in an ink-jet ink. Each dye can range from 0 to 5% in the ink, however the total amount of dye must be at least 1% in order to be seen and no more than 5% to prevent nozzle clogging.

Mathematically the constraints look like this:

$$\%Dye\,1 + Dye\,2 + Dye3 + Dye\,4 > 1\%$$

$$\%Dye\,1 + Dye\,2 + Dye3 + Dye\,4 < 5\%$$

Clearly an unconstrained experiment design will not work for us here. For instance, it will ask for a combination of dyes that is 5% of each, or 20% total dye. Similarly it will ask for an ink that has no dye at all.

JMP's Custom Designer let's us specify these linear constraints as in Figure 1.

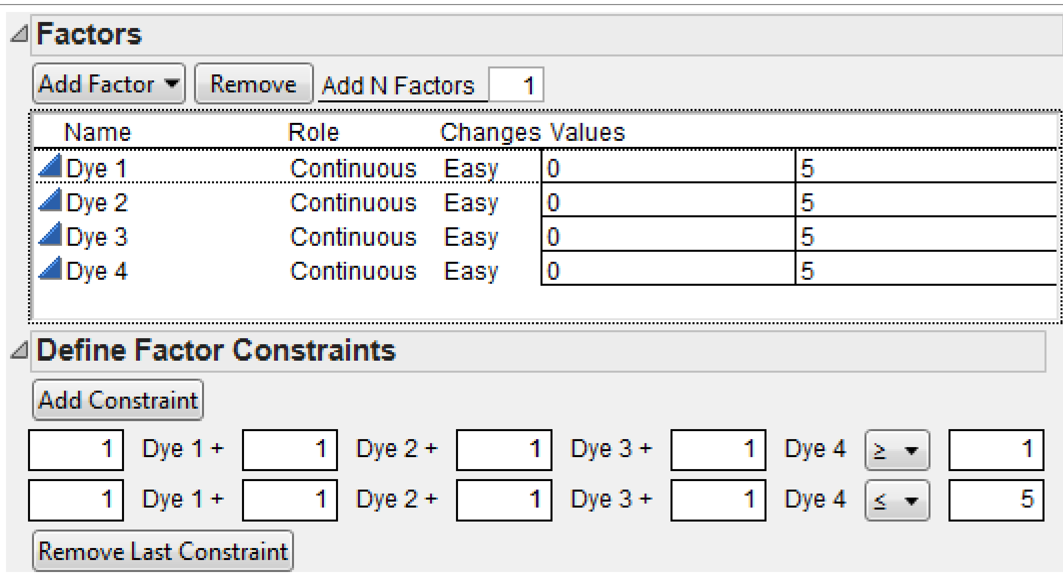

Figure 1 – Linear constraints in JMP.

Table 2 shows a design created for these constraints.

	Dye 1	Dye 2	Dye 3	Dye 4	Total Dye	Y
1	0.9	1.3	1.1	0.4	3.8	•
2	2.8	0.0	0.0	0.0	2.8	•
3	0.0	2.5	0.0	2.5	5.0	•
4	0.0	5.0	0.0	0.0	5.0	•
5	0.0	0.0	2.9	0.0	2.9	•
6	0.0	0.0	5.0	0.0	5.0	•
7	2.5	0.0	0.0	2.5	5.0	•
8	0.0	0.0	0.0	2.6	2.6	•
9	0.0	0.0	0.0	5.0	5.0	•
10	0.0	0.0	2.9	0.0	2.9	•
11	2.5	0.0	0.0	2.5	5.0	•
12	0.0	2.5	2.5	0.0	5.0	•
13	5.0	0.0	0.0	0.0	5.0	•
14	0.9	1.3	1.1	0.4	3.8	•
15	0.0	0.0	2.5	2.5	5.0	•
16	0.0	5.0	0.0	0.0	5.0	•
17	2.5	2.5	0.0	0.0	5.0	•
18	1.1	0.5	1.0	1.3	3.9	•
19	0.0	0.0	0.0	2.6	2.6	•
20	0.3	0.2	0.4	0.0	1.0	•
21	0.0	2.7	0.0	0.0	2.7	•
22	2.5	0.0	2.5	0.0	5.0	•
23	1.1	0.5	1.0	1.3	3.9	•
24	0.0	0.0	2.5	2.5	5.0	•
25	5.0	0.0	0.0	0.0	5.0	•

Table 2 – An experiment design with linear constraints. The "Total Dye" column has been added to check that the constraints have been obeyed.

Non-Linear Constraints

Human beings prefer linear constraints because they are easier to deal with. Nature does not share this preference. The real world has many nonlinear constraints.

An example of a nonlinear constraint would be when factor levels are multiplied. For example, suppose you were studying current and resistance, among other factors, and you had a requirement to keep the voltage between two levels, E_{low} and E_{high}. The constraints would look like this:

$$I \times R > E_{low}$$

$$I \times R < E_{high}$$

The remainder of this paper will examine three examples of nonlinear constraints and how JMP's Custom Designer can be used to create experiment designs to obey them.

A Simple example: Laser Drilling[2]

A medical equipment manufacturer needs to drill holes in polymer parts with a pulsed laser. They want to use a designed experiment to study the Number of Pulses and the Laser Power to optimize the quality of the holes drilled. They are interested in 13 to 200 pulses and power from 100 to 800 mW.

Holes cannot be drilled if the product of pulses and power is less than 10,000–the material is not penetrated. Thus, this experiment has a nonlinear constraint:

$$Power \times Pulses > 10{,}000$$

One way to create the necessary design would be to generate the unconstrained design and adjust it to obey the constraint. With this simple situation that would be easy–you would only need to adjust the low, low trial (100, 13), possibly to (100, 100) as in Figure 2.

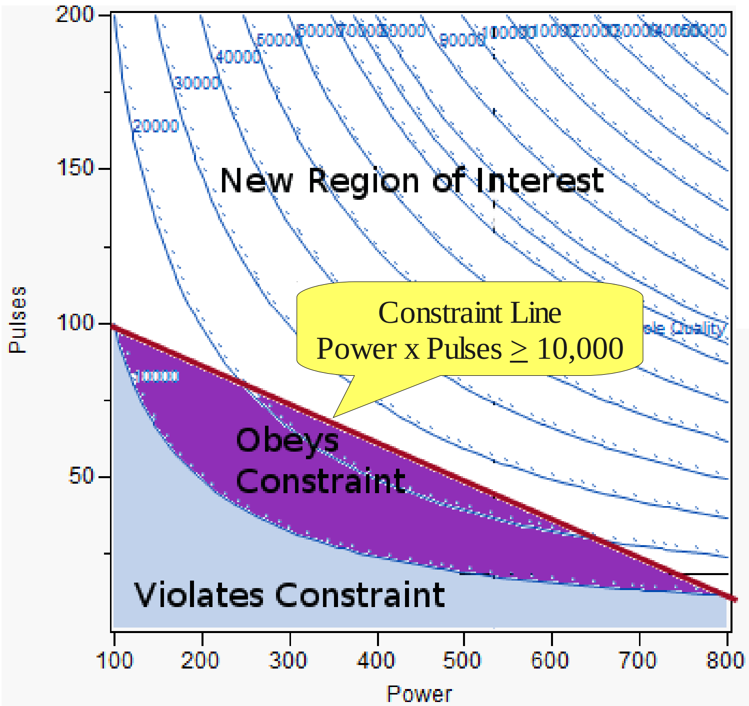

Figure 2 – The red line shows the new region of interest created by adjusting the low, low corner to obey the constraint. Some legitimate combinations are not included.

[2]See Appendix 1 for a better way.

Notice that many combinations of Power and Pulses that obey the constraint are outside the new region of interest.

In order to include more legitimate combinations in the region of interest, we can approximate the nonlinear constraint with several linear constraints, as shown in Figure 3.

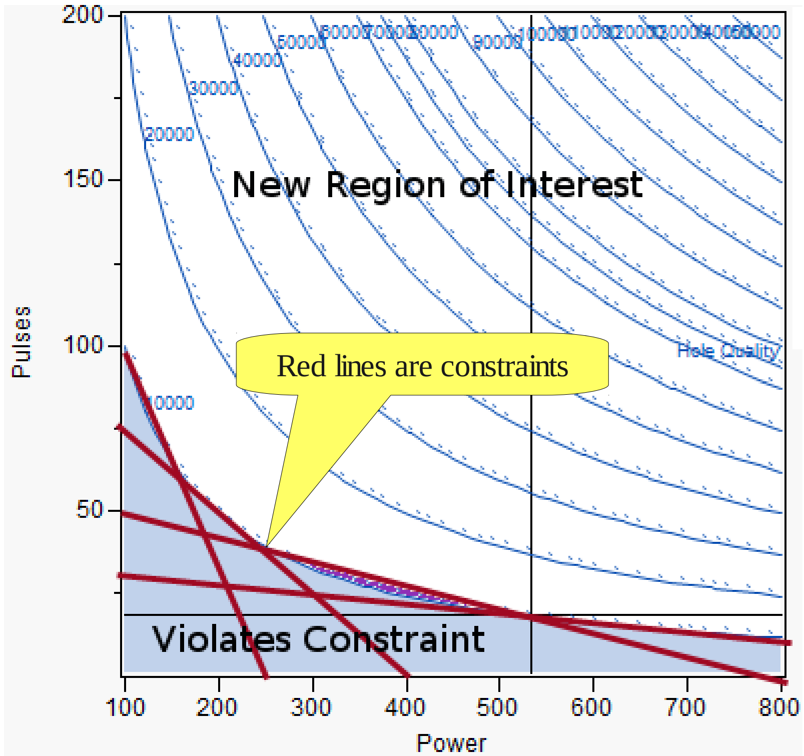

Figure 3 – Using multiple linear constraints to approximate a nonlinear constraint.

Notice that with 4 linear constraints we include nearly all of the legitimate Power-Pulse combinations. Specifically, the constraints are:

$$\text{Constraint 1: } \frac{2}{3}\times Power + Pulses \geq 166.7$$

$$\text{Constraint 2: } \frac{1}{4}\times Power + Pulses \geq 105$$

$$\text{Constraint 3: } 0.07\times Power + Pulses \geq 56$$

$$\text{Constraint 4: } 0.028\times Power + Pulses \geq 35.4$$

These are specified in JMP as shown in Figure 5.

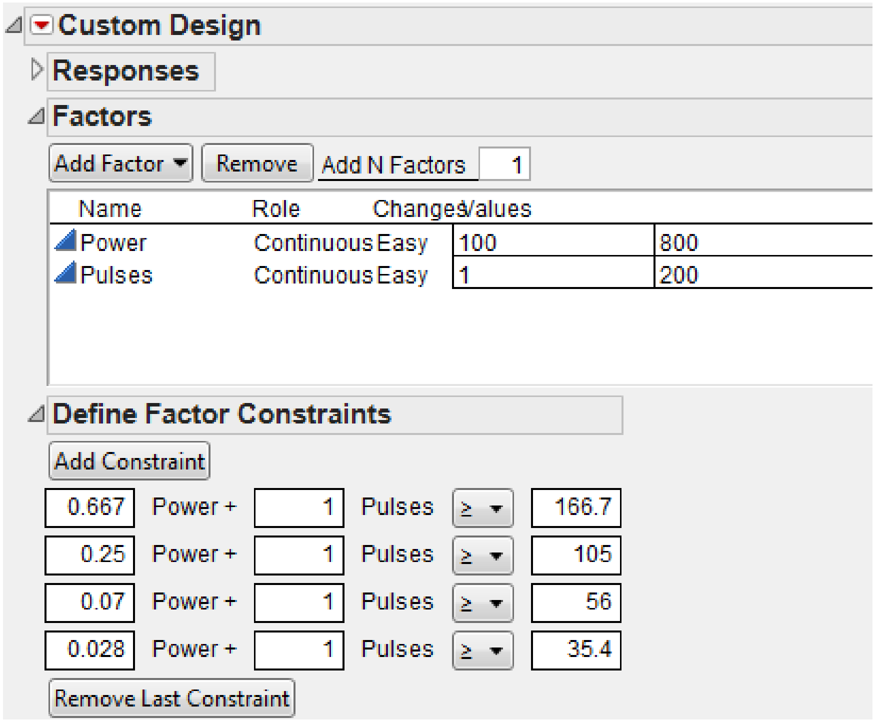

Figure 5 – Specifying Laser Drilling constraints in JMP.

An experiment design created with these constraints is shown in Table 3.

	Power	Pulses	Power x Pulses	Y
1	100	200	20000	•
2	272	37	10057	•
3	445	127	56419	•
4	537	112	60141	•
5	450	200	90000	•
6	800	13	10400	•
7	537	112	60141	•
8	800	13	10400	•
9	800	107	85200	•
10	800	200	160000	•
11	100	200	20000	•
12	800	200	160000	•
13	272	37	10057	•
14	800	107	85200	•
15	490	22	10627	•
16	465	127	59096	•
17	100	100	10000	•
18	450	200	90000	•
19	521	114	59274	•
20	100	100	10000	•

Table 3 – Constrained design for Laser Drilling. The Power X Pulses column is for checking the constraint.

A More Complex Example: A Known Constraint–Osmolality

Igenica needs to to optimize an injectable formulation for shelf-life. The factors are,

Factor	Low Level	High Level
An Amino Acid	10	80
A Sugar	20	150
A Salt	20	150
pH	5	7

The optimal formulation must have an osmolality[3] between 270 and 330 mOsmo. A formulation outside this range will cause pain when injected. Ideally our formulation will be as close to 300 mOsmo as possible, so the goal will be to stay between 295 and 305 mOsmo.

$$Osmolality = A.A.\ Multiplier \times [Amino\ Acid] + [Sugar] + 1.86 \times [Salt]$$

Here is the mathematical expression of this constraint:

$$A.A.\ Multiplier \times [Amino\ Acid] + [Sugar] + 1.86 \times [Salt] > 295$$

$$A.A.\ Multiplier \times [Amino\ Acid] + [Sugar] + 1.86 \times [Salt] < 305$$

Now for ... s with pH. Paul ha... le below show ex... erent pH values.

pH	A.A. Multiplier
7.0	1.0
6.5	1.2
6.0	1.5
5.5	1.7
5.0	1.8

A Least Squares fit to these data yields,

$$AA\,Multiplier = 3.808 - 0.396 \times pH$$

Substituting this in our constraints gives,

$$3.808\,x\,[Amino\ Acid] - 0.396 \times pH \times [Amino\ Acid] + [Sugar] + 1.86 \times [Salt] > 295$$

$$3.808\,x\,[Amino\ Acid] - 0.396 \times pH \times [Amino\ Acid] + [Sugar] + 1.86 \times [Salt] < 305$$

The first thing to try might be creating an unconstrained experiment design and adjusting the levels that violate the constraints. Unfortunately, this only results in tremendous tedium and frustration[4].

[3] Osmolarity measures electrolyte-water balance.
[4] Give this a try – you will quickly understand how difficult this is.

Maybe approximating the constraint with several linear constraints would work, as it did for the Laser Drilling example. Of course this problem has more factors, so constraint planes (or more accurately, constraint *hyper*planes) would be needed instead of lines. Isaac Newton may have found this relaxing, but it is completely impractical for most of us.

Fortunately there is a trick that simplifies this constraint without pain. Instead of using pH as a factor, use pH x [Amino Acid] as the factor. This instantly converts our constraints into linear constraints! This new factor will now require two linear constraints of its own to keep the pH in range, namely,

$$7 \times \mathit{Amino\ Acid} - pH \times \mathit{Amino\ Acid} \geq 0$$

$$5 \times \mathit{Amino\ Acid} - pH \times \mathit{Amino\ Acid} \leq 0$$

Figure 6 shows how to specify these constraints in JMP.

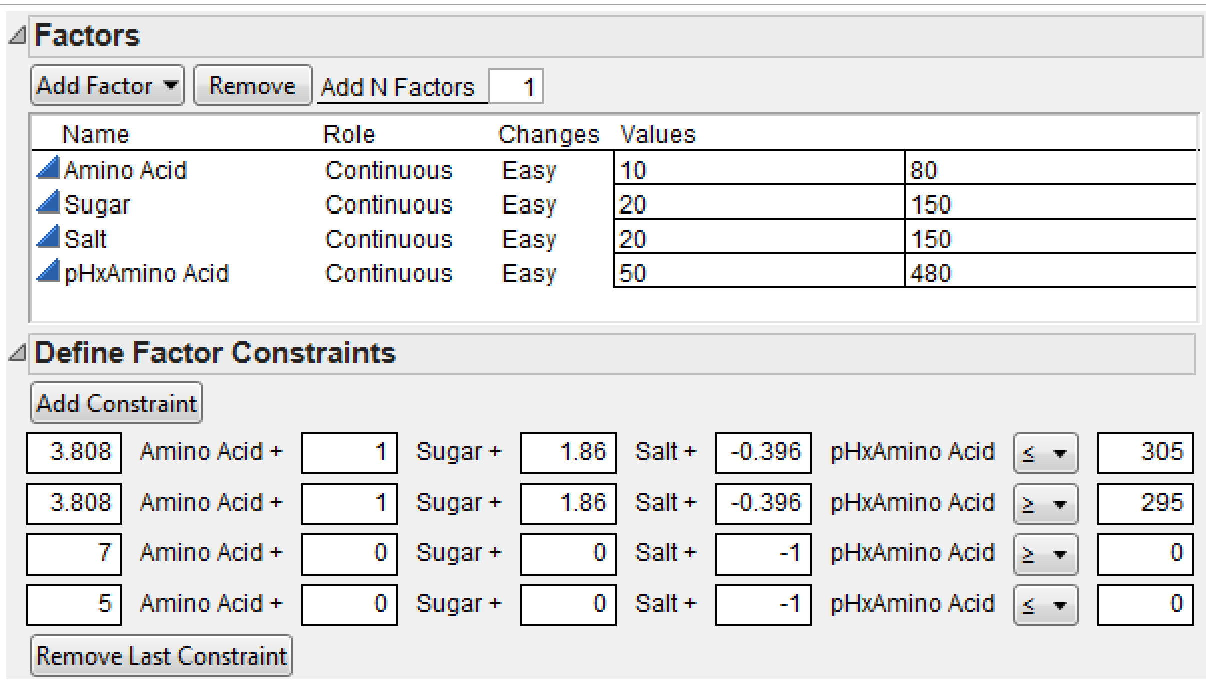

Figure 6 – Specifying the constraints for the osmolality experiment in JMP.

	Amino Acid	Sugar	Salt	pHxAmino Acid	pH	Osmolality w/o Amino Acid	Osmolality	Y
1	69	124	59	480	7.0	234	303	•
2	55	150	30	273	5.0	205	303	•
3	10	85	108	50	5.0	287	305	•
4	48	84	80	301	6.3	233	•	•
5	72	150	32	480	6.6	209	•	•
6	80	112	20	400	5.0	149	291	•
7	80	47	77	480	6.0	190	308	•
8	20	150	70	141	7.0	280	300	•
9	20	150	70	141	7.0	280	300	•
10	76	20	84	429	5.7	176	•	•
11	50	61	101	348	7.0	248	298	•
12	36	20	133	252	7.0	268	304	•
13	10	20	142	70	7.0	285	295	•
14	41	93	71	205	5.0	225	298	•
15	78	119	32	444	5.7	179	•	•
16	80	50	58	400	5.0	159	301	•
17	69	124	59	480	7.0	234	303	•
18	80	47	77	480	6.0	190	308	•
19	49	20	102	247	5.0	210	297	•
20	78	119	32	444	5.7	179	•	•
21	15	150	63	74	5.0	268	295	•
22	15	150	63	74	5.0	268	295	•
23	49	20	102	247	5.0	210	297	•
24	41	93	71	205	5.0	225	298	•
25	50	61	101	348	7.0	248	298	•
26	69	20	111	480	7.0	226	295	•

Table 4 – Experiment Design for the osmolality constraint. Some osmolality values are missing because no experimental value for A.A. Multiplier was available at these pH values.

An experiment design created with these constraints is shown in Table 4. A column for pH has been added to make this easier to run in a lab.

Notice that the calculated osmolality deviates slightly from the constraint, but is well within an acceptable range with no adjustment.

An Even More Complex Example: An Unknown Constraint–Solubility

Alder Biopharmaceuticals produces antibodies[5] for pharmaceutical use. These antibodies are produced by yeast cells that live and grow in a fermentation medium.

Patti needed to optimize this fermentation medium. The formulation had 5 salts (factors) that needed to be optimized, namely A, B, C, D, and E. It was

[5] Antibodies can target a variety of maladies from arthritis to cancer.

important that every formulation in the experiment be soluble, i.e. form no precipitate.

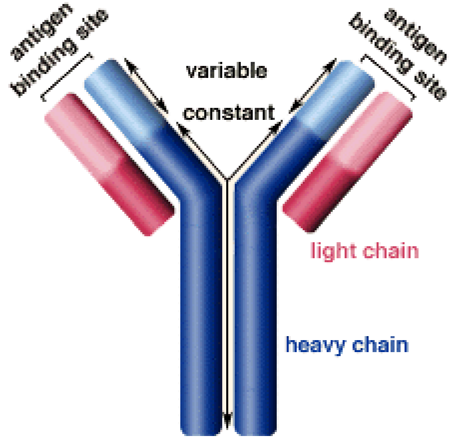

Figure 7 – A typical antibody.

In the last example, Paul had a table expressing the osmolality constraint. In this example, no such table existed for the solubility constraint. Even worse, there was no theory strong enough to formulate a mathematical constraint.

What to do?

Patti came up with a great idea–develop an empirical model of the solubility to define the constraint. This approach required two experiment designs: 1) the first experiment design was needed to build a solubility model which could be used to define the constraint needed for the fermentation medium formulation design and 2) a second design was needed, incorporating the constraint defined with the first design, to optimize the fermentation medium formulation.

Defining the Constraint: The Empirical Model for Solubility

The first design was very straightforward. All of the ingredients were relatively small additions to a bulk medium, so an unconstrained I-Optimal design was used (see Table 7).

Three samples were prepared for each run in the design. The response was the number of samples forming a precipitate (ppt). The standard transformation for proportions was used to meet the assumption of constant Standard Deviation:

$$2 * \sin^{-1}\sqrt{\frac{Y}{3}}$$

	A	B	C	D	E	Precipitation (x/3 with ppt)	Transformed
1	-0.1	-0.1	-0.1	1	1	3	3.1415926536
2	0.5	1	0.6	-0.8	1	3	3.1415926536
3	1	0	1	1	1	3	3.1415926536
4	0.1	-1	-1	-1	-1	0	0
5	-0.6	1	-1	-1	0.9	0	0
6	-0.9	-0.6	0.1	-0.9	-0.3	0	0
7	-1	-1	-1	0	1	0	0
8	-0.9	-0.6	0.1	-0.9	-0.3	0	0
9	0.7	0.1	-1	-0.2	0.3	0	0
10	0.7	0.1	-1	-0.2	0.3	1	1.2309594173
11	-1	1	0.6	0.7	0.2	3	3.1415926536
12	1	-1	-0.6	-1	1	0	0
13	0.9	-1	-1	1	0	0	0
14	-1	1	0.6	0.7	0.2	3	3.1415926536
15	0.9	-1	-1	1	0	0	0
16	-1	0	1	-1	1	3	3.1415926536
17	-1	-1	1	1	-1	0	0
18	1	1	-1	1	1	0	0
19	-0.1	0.2	-0.1	-0.1	-0.2	0	0
20	1	-0.1	1	-1	-0.6	0	0
21	1	1	-0.8	-0.8	-0.8	0	0
22	0.5	0.9	0.9	1	-1	0	0
23	1	1	-0.8	-0.8	-0.8	0	0
24	0.1	-1	1	-0.3	0.4	0	0
25	0.1	-1	1	-0.3	0.4	0	0
26	-1	1	1	-0.7	-1	3	3.1415926536
27	-0.9	0.7	-1	0.8	-1	3	3.1415926536
28	1	-0.8	0	0.3	-1	0	0
29	-0.9	0.7	-1	0.8	-1	3	3.1415926536
30	-1	1	1	-0.7	-1	3	3.1415926536
31	-1	-1	-1	0	1	0	0

Table 7 – Solubility design with data. X/3 is the number of samples out of 3 that formed a precipitate.

The empirical solubility model is,

$$
\begin{aligned}
\mathit{Coded\ PPT} = {} & 0.942411218690698 \\
& -0.629348238862599 \times A \\
& +0.720309186974184 \times B \\
& +0.379636479301734 \times C \\
& +0.3565526596062 8 \times D \\
& +0.538222225976829 \times E \\
& +0.138445327146842 \times A^2 \\
& -0.762409181473293 \times B^2 \\
& +0.0346780045292666 \times C^2 \\
& -0.222065898405 \times D^2 \\
& +1.00270307065298 \times E^2 \\
& -0.492647028464282 \times A \times B \\
& -0.162874007369297 \times A \times C \\
& -0.392240800477207 \times A \times D \\
& +0.358564450912612 \times A \times E \\
& +0.492222067869991 \times B \times C \\
& -0.300376000027045 \times B \times D \\
& -0.0114152224882214 \times B \times E \\
& -0.218483644913467 \times C \times D \\
& +0.865335630348752 \times C \times E \\
& +0.268569512772555 \times D \times E
\end{aligned}
$$

This is very helpful, but extremely cumbersome to work with directly! Where would we be without JMP? Fortunately JMP makes it easy to plot a variety of different views of the data, simplifying the job of working with this highly nonlinear constraint.

Optimizing the Fermentation Medium: The Second Design

The second design needed to optimize the salt concentration to make the best yeast fermentation medium. The first requirement is that the design ask for no salt combination that would form a precipitate, so Predicted PPT must be 0.

Patti decided to hold E constant at -1 for the second experiment[6]. To keep things manageable, a new design with just the 4 salts of interest (A–D) was created. A column was added for E with -1 for every run because it is needed in the formula. A column was created for the response "Coded PPT" by pasting the column properties from the original experiment (nicely

[6]Her reasons had nothing to do with the results seen so far.

bringing the formula along for the ride!). Finally, a column for the uncoded prediction of PPT was created by using a formula to reverse the transformation. Table 8 was the result.

	A	B	C	D	E	Coded PPT	Predicted PPT
1	-1	1	-1	0.5	-1	3.00	3
2	-1	1	-0.1	-1	-1	2.55	3
3	0.6	1	1	-1	-1	0.93	1
4	-0.1	-1	-0.3	0	-1	0.26	0
5	1	-1	-0.7	-1	-1	0.00	0
6	-1	1	-1	0.5	-1	3.00	3
7	-1	0	1	-1	-1	1.76	2
8	0.6	1	1	-1	-1	0.93	1
9	1	-0.2	1	-0.4	-1	-0.08	0
10	-0.1	0	-0.1	1	-1	1.48	1
11	-1	1	1	0.2	-1	3.18	3
12	1	0.7	0.2	0.2	-1	0.17	0
13	1	0.7	0.2	0.2	-1	0.17	0
14	1	1	-1	-1	-1	0.39	0
15	0	0	0.1	-0.1	-1	1.35	1
16	1	1	-1	1	-1	-0.38	0
17	0	0	-1	-0.4	-1	1.77	2
18	-1	-1	1	0.2	-1	-0.13	0
19	-1	0	0.1	1	-1	2.74	3
20	-1	0	0.1	1	-1	2.74	3
21	-1	-1	1	0.2	-1	-0.13	0
22	-1	-1	-1	1	-1	2.17	2
23	0	0	-1	-0.4	-1	1.77	2
24	0	1	-0.2	-0.2	-1	1.40	1
25	0.1	-0.2	-0.1	0	-1	1.20	1
26	0.1	-1	0.6	-1	-1	-1.16	1
27	1	-1	1	1	-1	-1.99	2
28	1	-1	-1	1	-1	0.72	0
29	-1	-1	-1	-1	-1	0.18	0
30	0	1	1	1	-1	0.76	0
31	0.1	-1	0.6	-1	-1	-1.16	1
32	0.1	-0.2	-0.1	0	-1	1.20	1

Table 8 – This table was created to make it easier to work with the empirical solubility model while creating linear constraints.

We are seeking 0 Predicted PPT so, as you can see in Table 8, an unconstrained design is impossible – too many runs show predicted precipitation of 1, 2, or 3 and we must have 0.

Bill needed to find linear constraints to approximate the model for Coded PPT on page 13. To simplify this procedure he decided to look for linear constraints on 2 factors at a time. This made it possible to use lines for the approximation, just as was done in the Laser Drilling example.

Initially, JMP's Graph Builder was used to decide which 2-factor pairs might make sense to work with. After many tries, the graph in Figure 7 was chosen.[7] This graph shows that the right combination of B and D is critical for success. B and D were chosen for the initial factor pair.

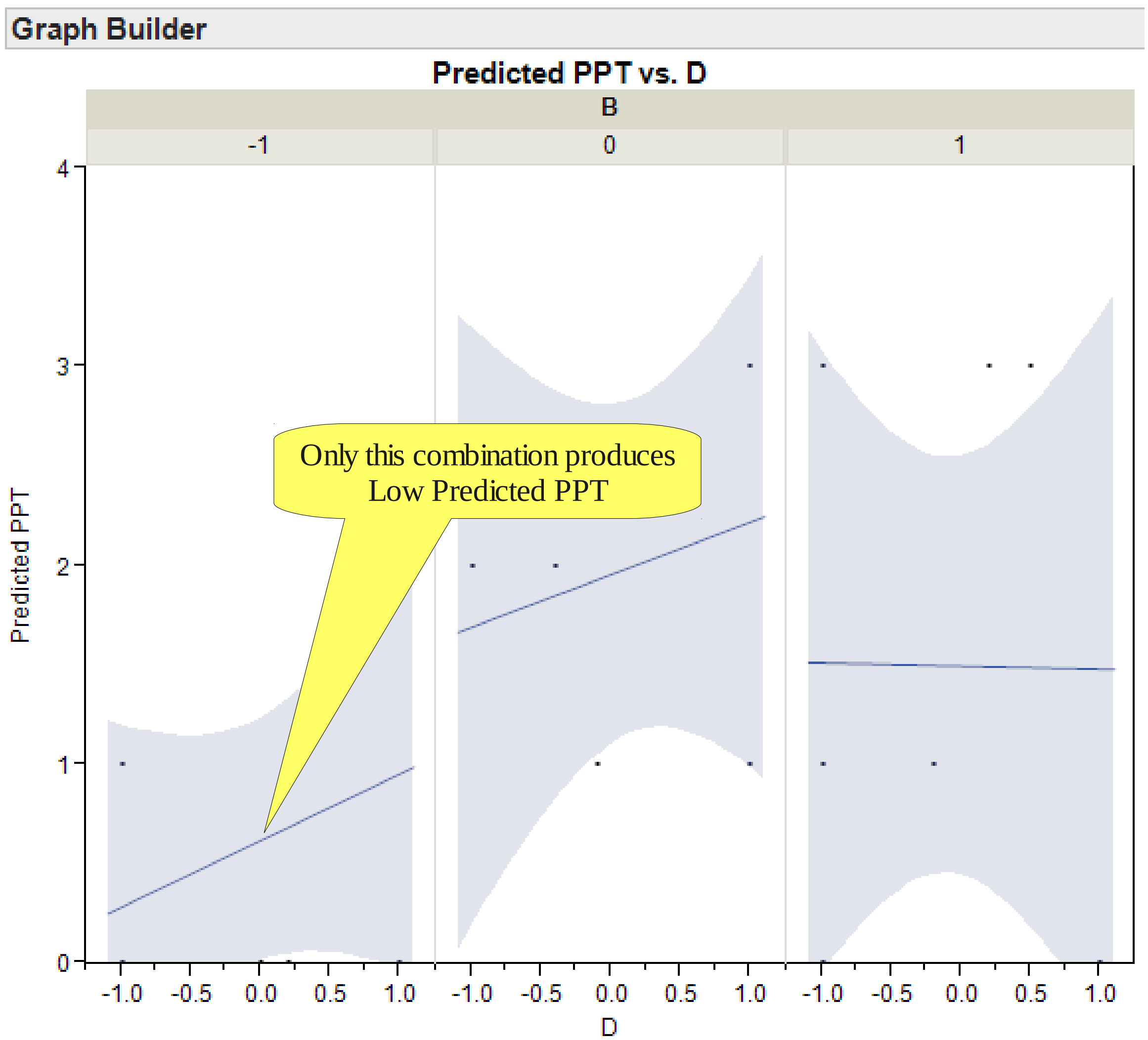

Figure 8 – Graphical relationship of factors B and D to Predicted PPT.

[7]You may be wondering why Bill chose this graph. Many of the graphs suggested interactions. This one is shown as an example because it is relatively easy to read: The slope of the D vs Predicted PPT line changes with the level of B.

Next, this model was fitted:

$$Y = b_0 + b_1 \times B + b_2 \times D + b_{12} \times B \times D$$

A contour plot of B vs D is shown in Figure 9. The red line is a linear constraint to keep the Coded PPT less than 0.8 (equivalent to 0.5 ppt out of 3). A design created with only this constraint was still unacceptable because it had many trials with Predicted PPT of 1 or greater.

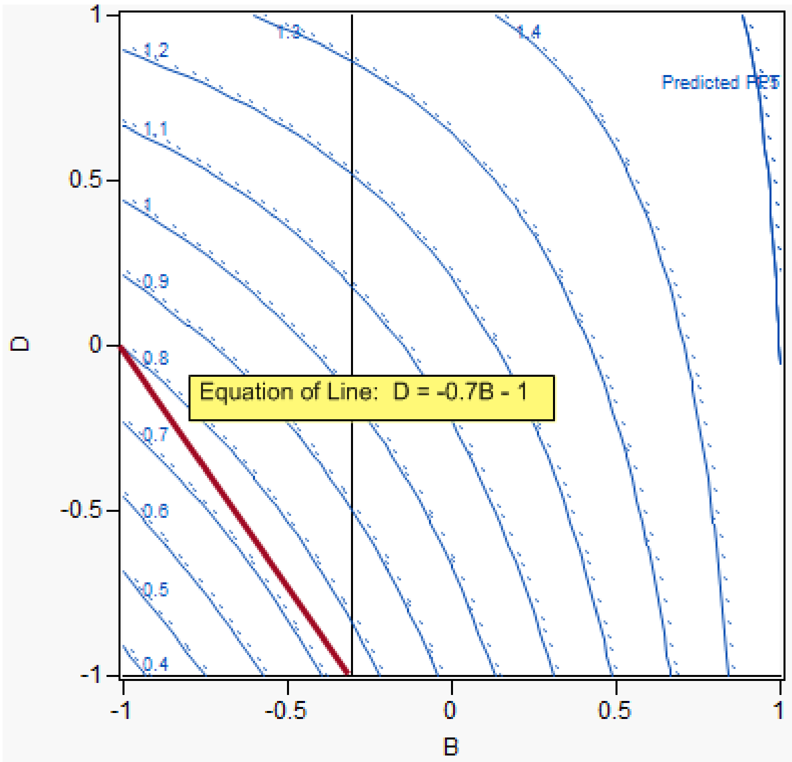

Figure 9 – Contour plot of Coded PPT, A vs B. The red line is a linear constraint.

Next Bill used JMP's Stepwise Regression to give further clues as to the appropriate factor pairs to consider for the next constraint line. The result is shown in Figure 10. This regression indicated the model,

$$Y = b_0 + b_1 \times A + b_2 \times B + b_{12} \times A \times B$$

A contour plot of A vs B is shown in Figure 11. The red line is a linear constraint as before.

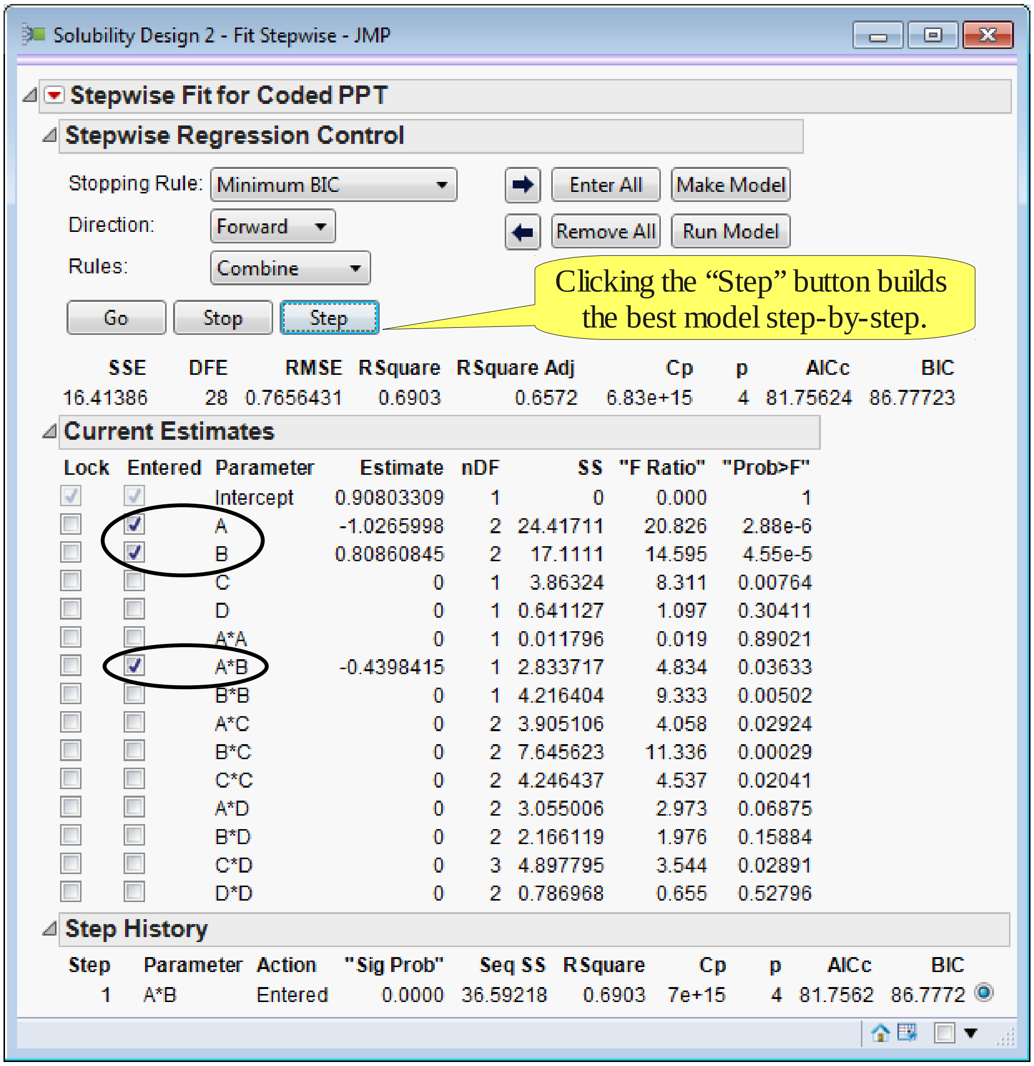

Figure 10 – Stepwise Regression.

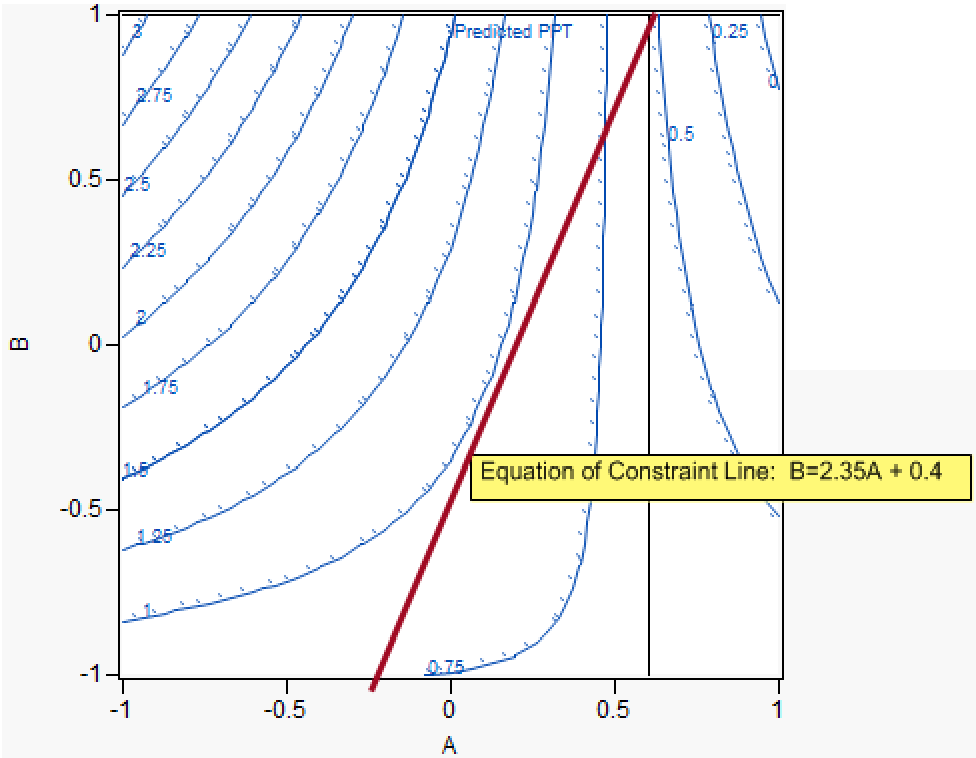

Figure 11 – Contour plot of Predicted PPT, A vs B. The red line is a linear constraint.

An acceptable design was created using these constraints:

$$-2.35 \times A + B \leq 0.4$$

$$0.7 \times B + D \leq -1$$

This design is shown in Table 9. Some hand adjustment was required. The adjusted design is shown in Table 10.

	Random Block	A	B	C	D	E	pH	X	Y	Coded PPT	Predicted PPT
1	1	-0.437	-0.627	0	-0.605	-1	7	-1	•	0.6199	0
2	1	0.361	-1	0	-0.65	-1	6.5	1	•	-0.502	0
3	1	-0.117	-1	1	-1	-1	7	1	•	-1.389	0
4	1	-0.596	-1	1	-0.3	-1	6	-1	•	-0.73	0
5	1	0.361	-1	0	-0.65	-1	6.5	1	•	-0.502	0
6	1	-0.596	-1	0	-1	-1	6	1	•	-0.587	0
7	1	-0.596	-1	0	-1	-1	6	1	•	-0.587	0
8	1	-0.373	-0.881	-1	-0.383	-1	6	1	•	0.9822	1
9	2	1	-1	0	-1	-1	7	-1	•	-0.662	0
10	2	0.526	-0.958	0	-0.329	-1	7	1	•	-0.307	0
11	2	0.680	-1	1	-0.51	-1	7	-1	•	-1.423	0
12	2	0.727	-0.454	-1	-0.682	-1	7	0	•	1.0862	1
13	2	0.526	-0.958	0	-0.329	-1	7	1	•	-0.307	0
14	2	-0.117	-1	-1	-0.3	-1	6.5	-1	•	0.7503	0
15	2	1	-0.357	0	-0.85	-1	7	1	•	0.3818	0
16	2	-0.17	-0.1	1	-1	-1	6	1	•	0.829	0
17	3	0.567	-0.639	0	-0.552	-1	6	-1	•	0.1905	0
18	3	-0.596	-1	-1	-0.72	-1	7	0	•	0.4522	0
19	3	-0.337	-0.392	-1	-1	-1	7	1	•	1.1669	1
20	3	0.244	-0.8	-1	-1	-1	6.5	-1	•	0.4777	0
21	3	0.253	-0.65	0	-0.818	-1	6	1	•	0.1498	0
22	3	0.253	-0.65	0	-0.818	-1	6	1	•	0.1498	0
23	3	0.244	-0.8	-1	-1	-1	6.5	-1	•	0.4777	0
24	3	0.872	-0.256	1	-0.821	-1	6.5	-1	•	-0.021	0
25	4	-0.434	-0.62	1	-1	-1	6.5	-1	•	-0.151	0
26	4	-0.36	-0.446	1	-0.688	-1	7	1	•	0.407	0
27	4	1	-0.182	-1	-0.873	-1	6	1	•	1.1196	1
28	4	-0.275	-0.247	-1	-0.827	-1	6	-1	•	1.4697	1
29	4	1	-1	-1	-1	-1	6.5	1	•	0.2947	0
30	4	0.063	0	0	-1	-1	7	0	•	1.0594	1
31	4	0.063	0	0	-1	-1	7	0	•	1.0594	1
32	4	1	-0.4	0	-1	-1	6	-1	•	0.3018	0
33	5	1	-1	1	-1	-1	6	0	•	-1.55	0
34	5	0.071	-1	0	-0.79	-1	6	-1	•	-0.545	0
35	5	1	-1	-1	-0.51	-1	6	-1	•	0.5642	0
36	5	1	-1	-1	-0.51	-1	6	-1	•	0.5642	0
37	5	1	-1	1	-0.3	-1	6	1	•	-1.503	0
38	5	1	-0.5	1	-1	-1	7	1	•	-0.462	0
39	5	1	-0.3	-1	-1	-1	7	-1	•	1.0216	1
40	5	1	-1	1	-1	-1	6	0	•	-1.55	0

Table 9 – Fermentation Medium Optimization Design before adjustment. Runs requiring adjustment are highlighted in yellow.

	Random Block	A	B	C	D	E	pH	X	Y	Coded PPT	Predicted PPT
1	1	-0.437	-0.627	0	-0.605	-1	7	-1	•	0.620	0
2	1	0.362	-1.000	0	-0.650	-1	6.5	1	•	-0.502	0
3	1	-0.117	-1.000	1	-1.000	-1	7	1	•	-1.389	0
4	1	-0.596	-1.000	1	-0.300	-1	6	-1	•	-0.730	0
5	1	0.362	-1.000	0	-0.650	-1	6.5	1	•	-0.502	0
6	1	-0.596	-1.000	0	-1.000	-1	6	1	•	-0.587	0
7	1	-0.596	-1.000	0	-1.000	-1	6	1	•	-0.587	0
8	1	-0.373	-1.000	-1	-0.383	-1	6	1	•	0.747	0
9	2	1.000	-1.000	0	-1.000	-1	7	-1	•	-0.662	0
10	2	0.527	-0.958	0	-0.329	-1	7	1	•	-0.307	0
11	2	0.681	-1.000	1	-0.510	-1	7	-1	•	-1.423	0
12	2	0.727	-0.600	-0.8	-0.682	-1	7	0	•	0.794	0
13	2	0.527	-0.958	0	-0.329	-1	7	1	•	-0.307	0
14	2	-0.117	-1.000	-1	-0.300	-1	6.5	-1	•	0.750	0
15	2	1.000	-0.357	0	-0.850	-1	7	1	•	0.382	0
16	2	-0.170	-0.100	1	-1.000	-1	6	1	•	0.829	0
17	3	0.567	-0.639	0	-0.552	-1	6	-1	•	0.191	0
18	3	-0.596	-1.000	-1	-0.720	-1	7	0	•	0.452	0
19	3	-0.200	-0.550	-0.75	-1.000	-1	7	1	•	0.768	0
20	3	0.245	-0.800	-1	-1.000	-1	6.5	-1	•	0.478	0
21	3	0.253	-0.650	0	-0.818	-1	6	1	•	0.150	0
22	3	0.253	-0.650	0	-0.818	-1	6	1	•	0.150	0
23	3	0.245	-0.800	-1	-1.000	-1	6.5	-1	•	0.478	0
24	3	0.872	-0.256	1	-0.821	-1	6.5	-1	•	-0.021	0
25	4	-0.434	-0.620	1	-1.000	-1	6.5	-1	•	-0.151	0
26	4	-0.360	-0.446	1	-0.688	-1	7	1	•	0.407	0
27	4	1.000	-0.400	-0.8	-1.000	-1	6	1	•	0.826	0
28	4	0.250	-0.350	-0.5	-1.000	-1	6	-1	•	0.794	0
29	4	1.000	-1.000	-1	-1.000	-1	6.5	1	•	0.295	0
30	4	0.300	0.000	0.3	-1.000	-1	7	0	•	0.839	0
31	4	0.064	-0.200	0	-1.000	-1	7	0	•	0.829	0
32	4	1.000	-0.400	0	-1.000	-1	6	-1	•	0.302	0
33	5	1.000	-1.000	1	-1.000	-1	6	0	•	-1.550	0
34	5	0.071	-1.000	0	-0.790	-1	6	-1	•	-0.545	0
35	5	1.000	-1.000	-1	-0.510	-1	6	-1	•	0.564	0
36	5	1.000	-1.000	-1	-0.510	-1	6	-1	•	0.564	0
37	5	1.000	-1.000	1	-0.300	-1	6	1	•	-1.503	0
38	5	1.000	-0.500	1	-1.000	-1	7	1	•	-0.462	0
39	5	1.000	-0.300	-0.7	-1.000	-1	7	-1	•	0.831	0
40	5	1.000	-1.000	1	-1.000	-1	6	0	•	-1.550	0

Table 10 – Fermentation Medium Optimization Design after adjustment. Adjusted runs are highlighted in yellow. Adjusted levels are highlighted in orange.

Conclusion

Nature is complex. Some experimental situations have physical limitations, or *constraints*. JMP's Custom Designer provides for the inclusion of *linear constraints* in experiment designs. Some experiments have *non-linear constraints*, and these are more difficult to work with. If a constraint requires more than just the simple sum of scaled factors it is non-linear and must be linearized to use the JMP Custom Designer.[8] Linearizing simplifies the constraint. In this paper you have seen how to simplify Nature's complexity without losing your grasp on the real world.

Looking for More?

Here are some interesting references for further information:

Box, Hunter, and Hunter, *Statistics for Experimenters,* J. Wiley and Sons, 1978, ISBN 0-471-09315-7, pp. 307-319.

http://www.ObDOE.com (for information about Design of Experiments)

http://www.jmp.com/ (for information about JMP software)

http://en.wikipedia.org/wiki/Plasma_osmolality (for a nice explanation of osmolality)

http://en.wikipedia.org/wiki/Monoclonal_antibodies (for an explanation of how Monoclonal Antibodies can act as drugs)

About the Authors

Paul Sauer is Vice President of Process Sciences & Manufacturing at Igenica. He is responsible for the development and manufacture of monoclonal antibodies for use in pre-clinical and clinical oncology studies.

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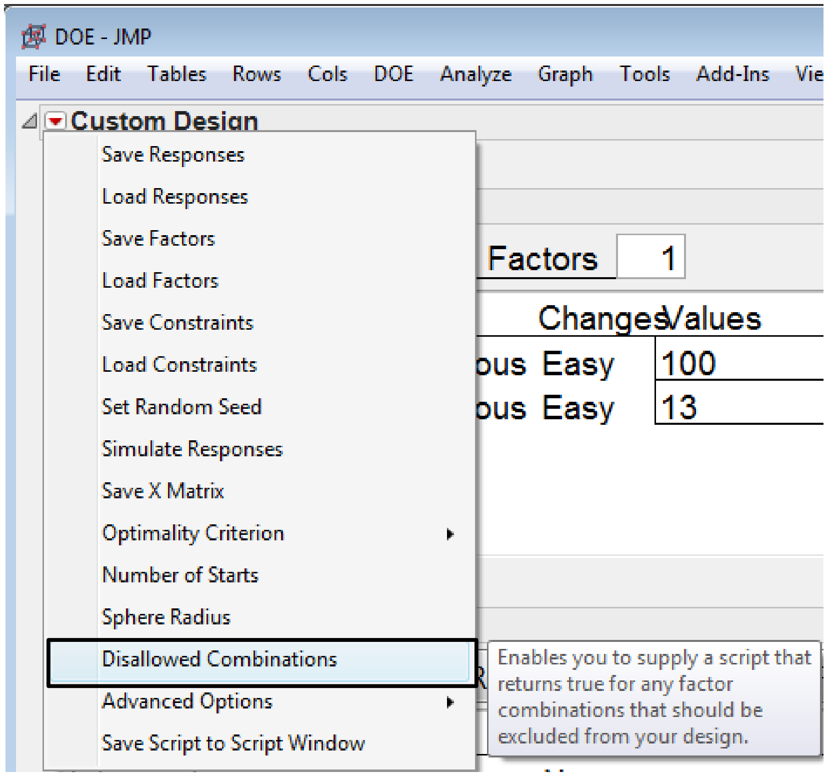
Bill Kappele is President of ObDOE. He is responsible for course delivery and development. Paul and Patti both learned Design of Experiments from Bill.

[8] This statement turned out not to be true, but the constraints for the two case studies were too severe to be used directly, so the techniques in this paper are still applicable.

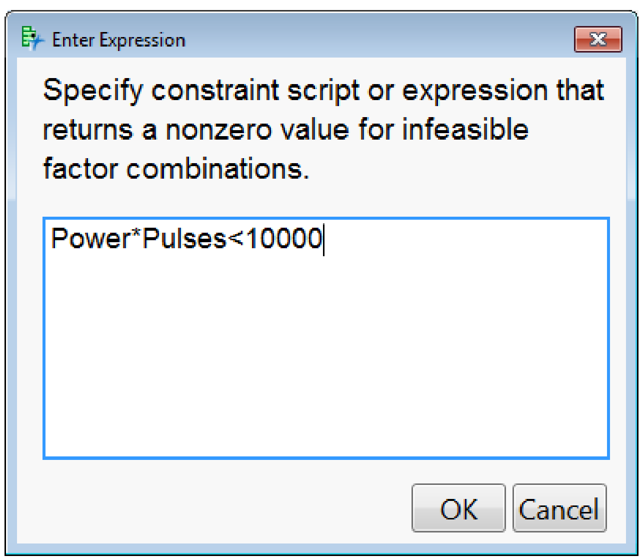
Appendix

JMP *can* handle non-linear constraints. My friend Brad Jones showed me how.

Here is how to tackle the laser problem directly:



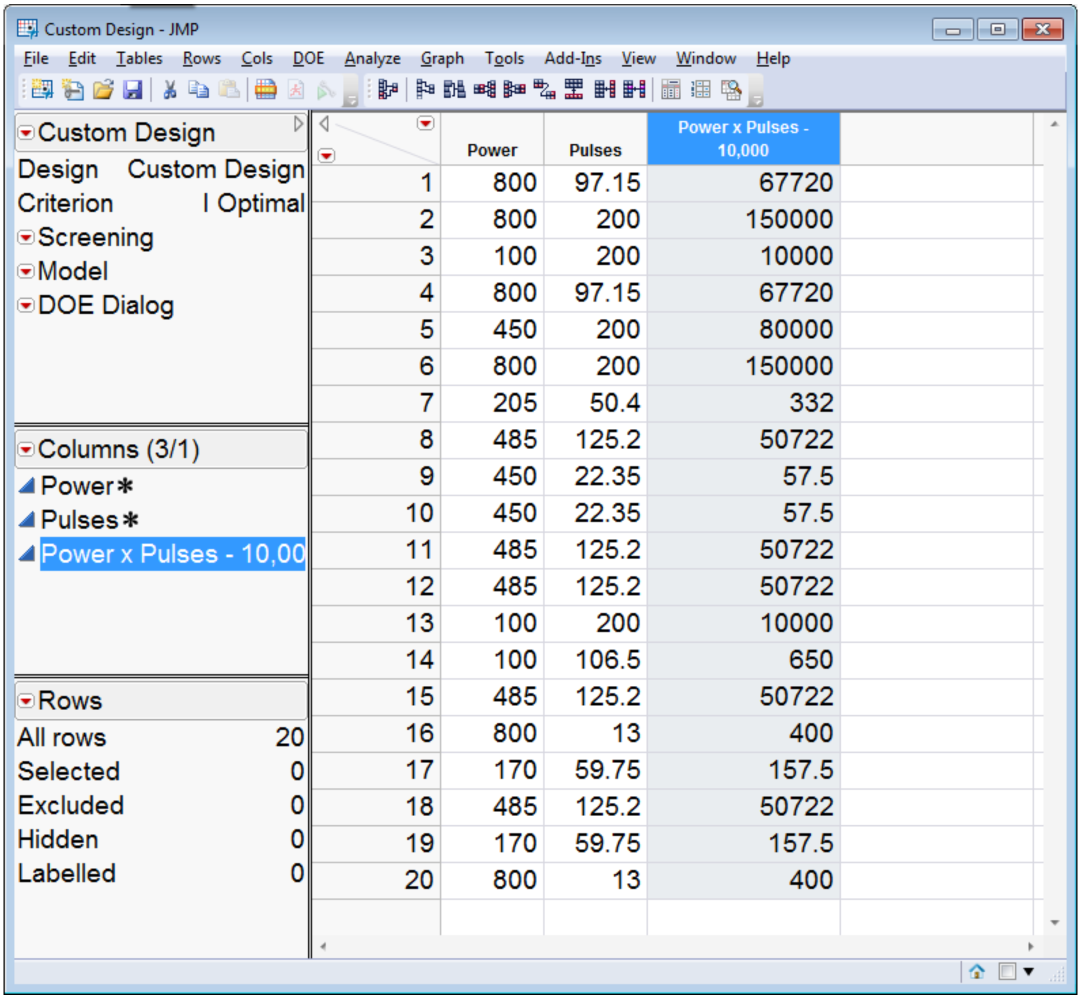
1. In the Custom Design module, select "Disallowed Combinations" from the red triangle drop down menu.



2. Enter the non-linear constraint in the "Enter Expression" window. Please notice that this must be entered differently from a linear constraint. Linear constraints are allowed – these are "Disallowed

Combinations."

3. OK.



	Power	Pulses	Power x Pulses - 10,000
1	800	97.15	67720
2	800	200	150000
3	100	200	10000
4	800	97.15	67720
5	450	200	80000
6	800	200	150000
7	205	50.4	332
8	485	125.2	50722
9	450	22.35	57.5
10	450	22.35	57.5
11	485	125.2	50722
12	485	125.2	50722
13	100	200	10000
14	100	106.5	650
15	485	125.2	50722
16	800	13	400
17	170	59.75	157.5
18	485	125.2	50722
19	170	59.75	157.5
20	800	13	400

4. Voila!