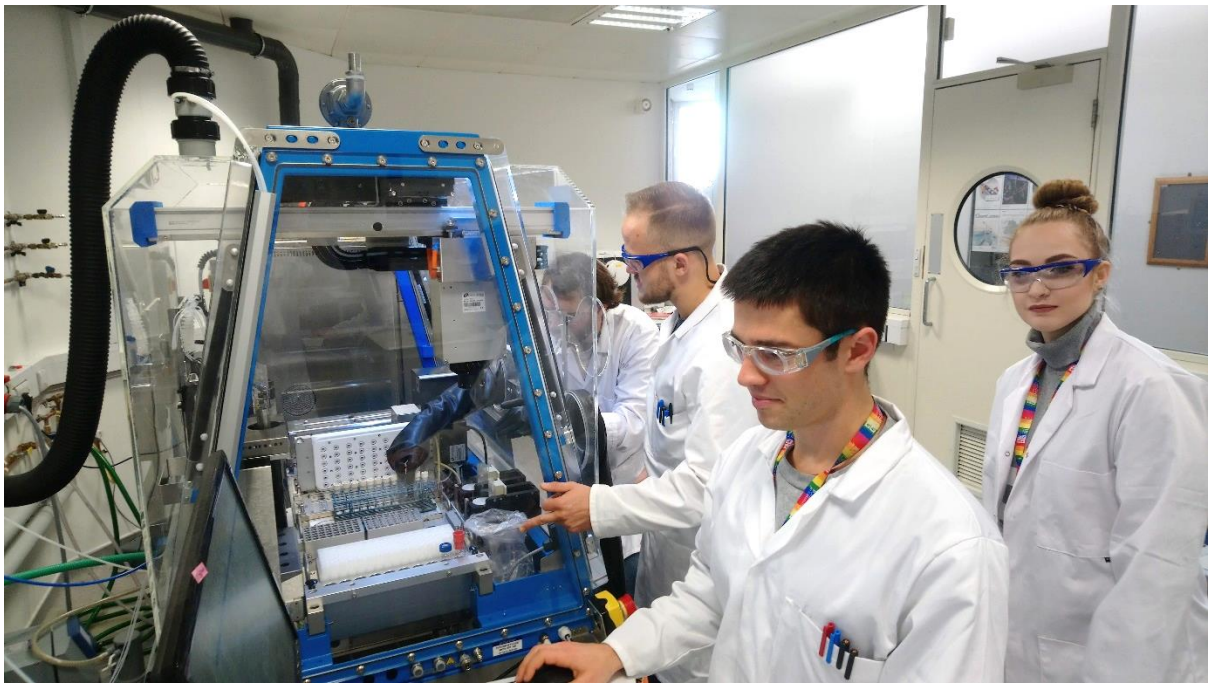
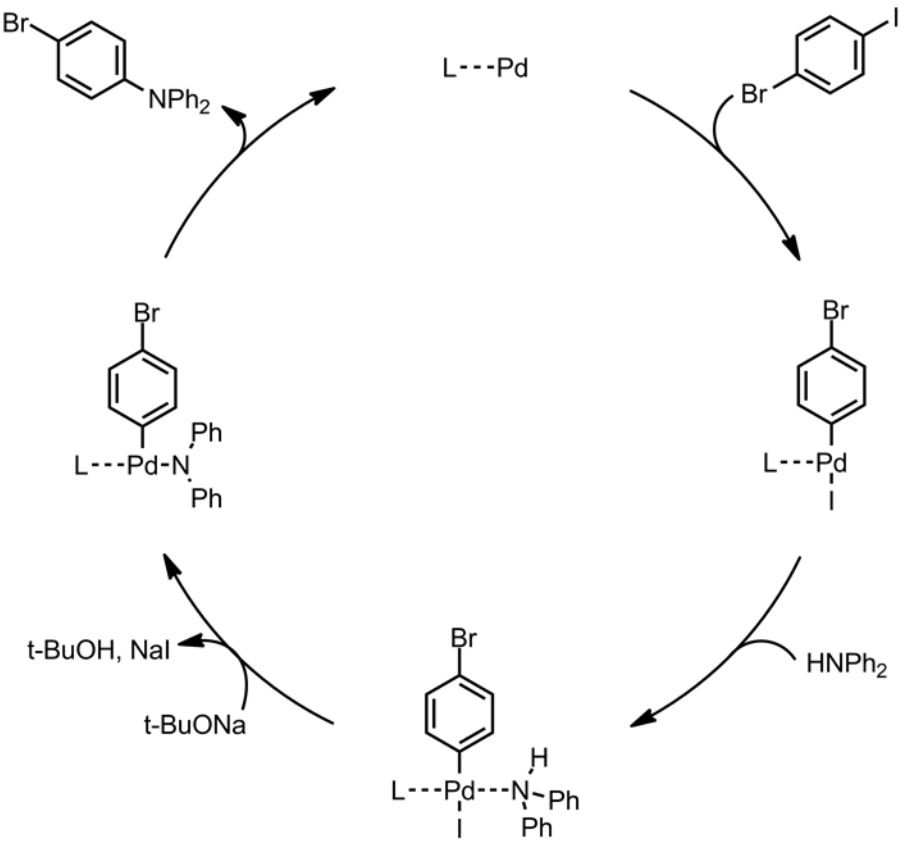
Optimising a Buchwald-Hartwig amination using the ChemSpeed.



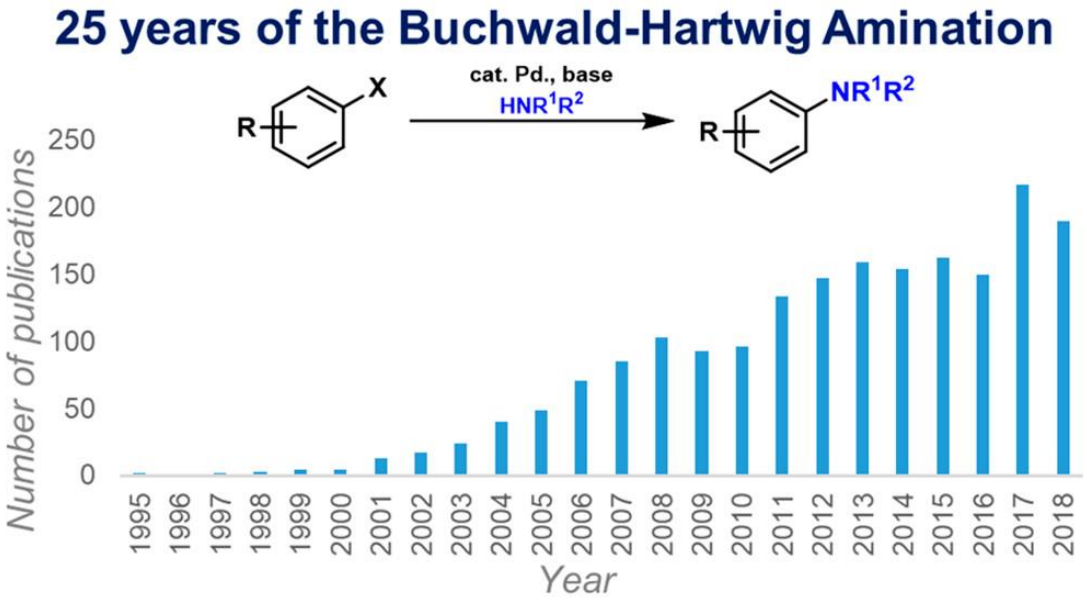
Some of the 2019 cohort using the ChemSpeed automated synthesis platform.

This blog post goes through an example of using the ChemSpeed and design of experiments to optimise a reaction. One of the first groups to use the ChemSpeed automatic synthesis robot was the 2019 Technology Enhanced Chemical Synthesis (TECS) centre for doctoral training (CDT) cohort. They optimised a Buchwald-Hartwig amination.

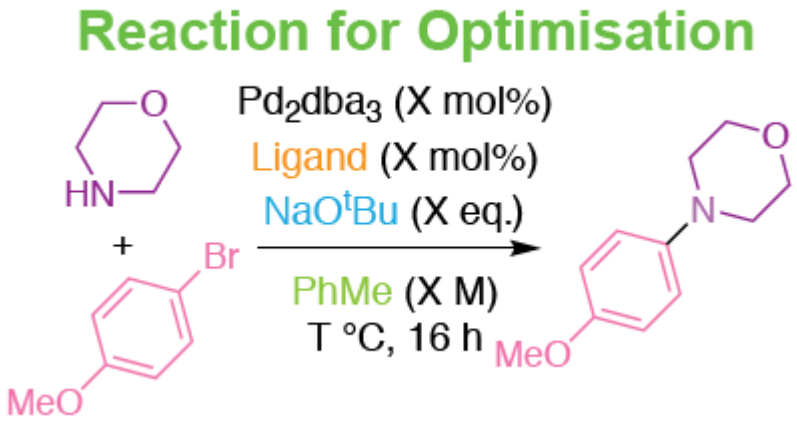


The catalytic cycle of the Buchwald-Hartwig amination. Taken from [2,3].

Aromatic amines are fundamental building blocks of great importance for the pharmaceutical and agrochemical industries, the reaction has been used in medicinal chemistry, materials science, ligand, heterocycle and natural product synthesis. The palladium catalyses the formation of carbon-carbon or carbon-heteroatom bonds that are difficult to make, and due to its usefulness, interest in this reaction is only increasing.



Buchwald-Hartwig aminations are incredibly well-used. Taken from [1].

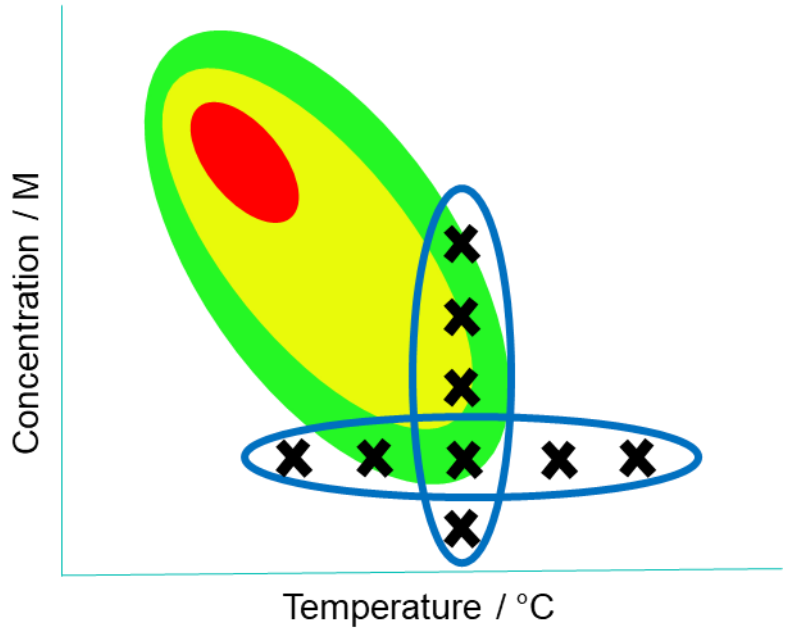


As this reaction is so widely used and useful, we decided to try and optimise it using the ChemSpeed combined with the design of experiments (DoE) methodology.

The reaction to be optimised.

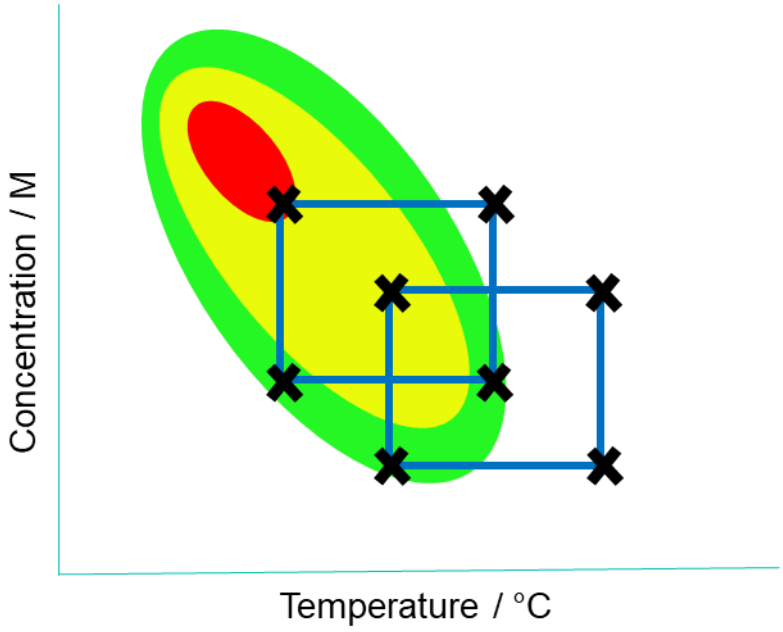
Design of Experiments

Design of experiments is a method commonly used in the chemical industry. At school, budding chemists are taught that the correct way to run experiments is to only change a single thing (variable) at once (called OVAT – one variable at a time). This approach to experimentation gives us information the effects of a variable on a system. A vast amount of scientific progress was made this way. However, what if instead of wanting to describe an effect you want to optimise a system, such as improving the yield of a reaction? Using OVAT, you might run the reaction at different temperatures to see the effect of temperature. You could then optimise the reaction by taking the best temperature and changing the concentration, as shown below. This will optimise the reaction, but it is likely to not find the optimum yield.



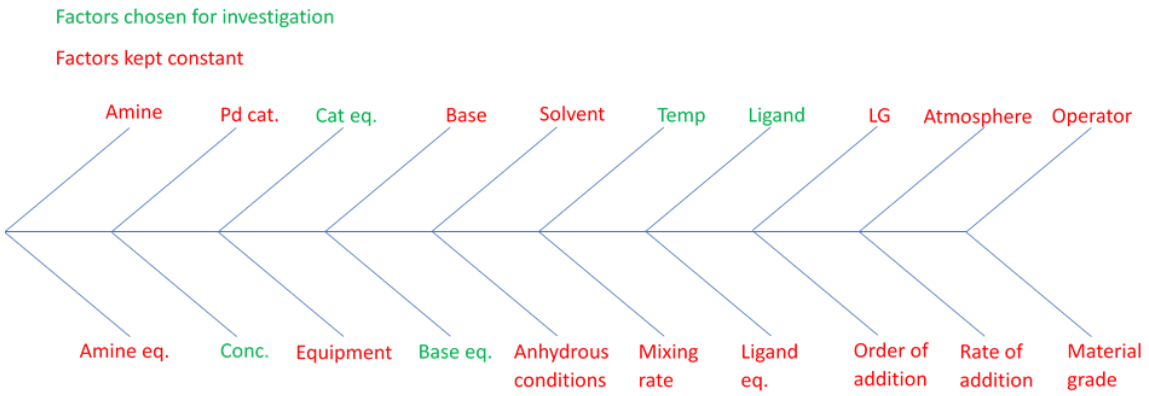
One variable at a time (OVAT) approach to optimisation. First the temperature is changed at a set concentration to find the best temperature, then the concentration is changed. The optimum yield is the red area of the diagram.

One of the most exciting developments in synthetic chemistry in recent years has been the application of statistical methods of experimentation. In the example below, using as many reactions as the OVAT example above, we have managed to cover a larger part of the chemical space, and have hit on the higher yielding part of that chemical space. We change more than one variable at a time, but, and this is the crucial bit, we build statistical models to describe how those variables affect the output. We build models that are tuned towards **predicting** the best yield, rather than just **fitting** the data we have. Then we can use that model to predict where we would find the best yield (and we then test it out).



The design of experiments approach: by changing two variables together, more of the chemical space can be explored, and models can be made to help find the optimum conditions.

The crucial (and often overlooked) first step in a design of experiments methodology is to come up with all possible things (factors) that can affect the experiment. It is important to think beyond the obvious ones (such as those written in the reaction scheme). To help come up with these it can be useful to think of 'man, machine, method'. We have the factors that are obvious from the reaction scheme, for example, concentration of reagents, or ligand choice. 'Machine' is the things we are using to do the reaction, for example, the equipment we are using. Method is how we are doing the reaction, for example, under which atmosphere, in what order are we adding the reactants, at what temperature. Finally, 'man' refers to human effects on the reaction, for example, the 'operator' factor below. We must scope out as many things that could possibly affect our reaction at this point as it helps us to choose the correct factors to change (and make sure that we are asking the correct question) and make sure that we consider how to keep the ones we are not changing constant. Imagine a lab with many large south-facing windows and poor air conditioning that is known to be hotter in the afternoons than the mornings. One way to remove the effect of the factor 'lab temperature' is to randomise the order of the experiments so that factor is random (and not mistaken for any of the factors we are using to optimise the reaction). Fixing the factors of 'equipment' and 'operator' over the experiment can involve using the same equipment and operator, however the ChemSpeed offers an advantage here, as it is one of the operators and should do each reaction exactly the same way.



Factors identified by the students as possibly affecting the reaction. Those is green were investigated and efforts were taken to control those in red.

The optimisation

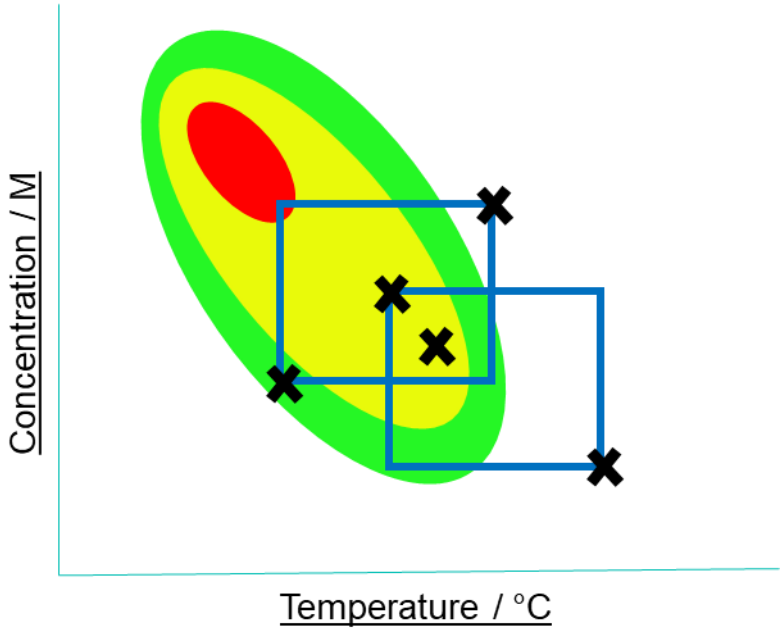
The students chose to optimise the following 5 factors:

Factors:

1. Ligand (XPhos and Xantphos)
2. Temperature (80 – 100 °C)
3. Catalyst Loading (1 – 5 mol%)
4. Base Equivalents (1.2 – 2.0 eq.)
5. Concentration (0.1 – 0.4 M)

And the response variable (the thing we wish to optimise) was the solution yield. An advantage of the ChemSpeed is that it can take sample/aliquots at different time points, so the yield was measured at 1, 4, and 16 hours. These three separate time points were analysed and modelled separately. As the ChemSpeed can be programmed and is willing to work overnight (!) reactions can be timed correctly, avoiding the often seen reactions times of 24 hours or 48 hours.

A DoE fraction factorial model of resolution V+ was used, this does half the points of the hypercube, allowing you to map as large an amount in space but with less detail. This type of model is good for exploring chemical space as it is very efficient, but can miss out higher order interactions between factors. Centre points were also done. The figure below shows what a V+ fractional factorial model would look like for the example experiment discussed above. For 5 factors a resolution V+ model requires 22 experiments. Doing these experiments one by one for 16 hours would require 352 (22x16) hours! The ChemSpeed comes with multi-well plates so the 22 experiments can be done in parallel and the reaction only takes 16 hours. As the ChemSpeed is fully automated, the synthetic chemist does not need to be standing at the bench for those 16 hours either. The program Modde was used to set-up and analyse the results.



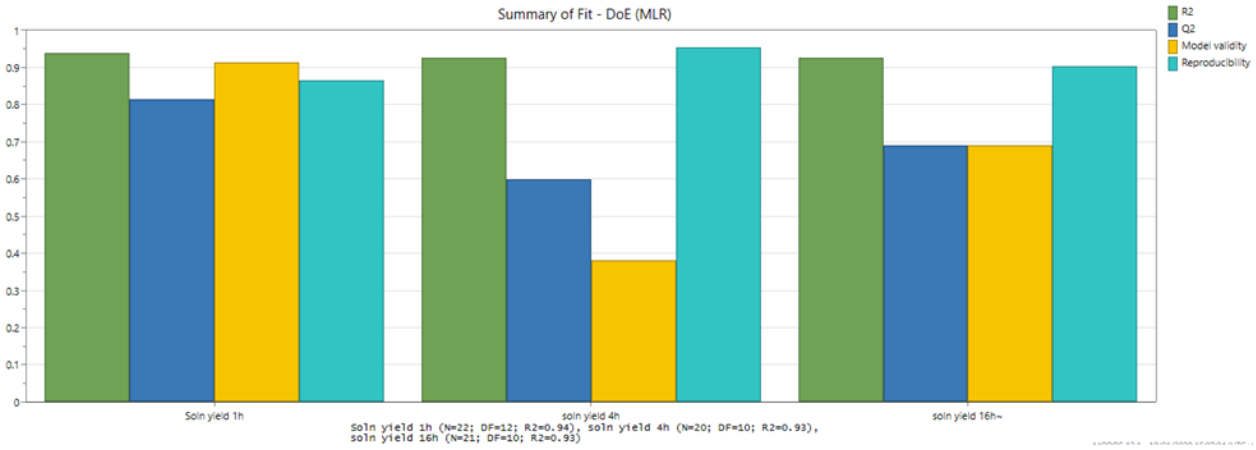
A fractional factorial model applied to the example above. Centre points are also done.

The students made up stock solutions for the ChemSpeed to dispense (to remove human error in dispensing). The Pd catalyst and ligand were stirred together for one hour before the reactions began. The internal standard 4,4'-di-*tert*-butyl-1,1'-biphenyl was used to measure the solution yield and this was added directly to the reactions. Solution yields were analysed by LC-MS (conveniently located next to the ChemSpeed).

Whilst automated synthesis offers a great deal of advantages, it requires some care in setting up and carrying out the experiments, and, as this was one of the first experiments run on the ChemSpeed there were some challenges that needed to be overcome. There were inaccuracies in weighing out the internal standard, so it was dispensed via a pre-made-up stock solution. The Pd/ligand stock solutions showed a discrepancy in between measurements, and the base, NaOtBu, so small glass balls were added to improve the mixing (the ChemSpeed shakes the reaction vials), which required the height of the needle (which takes samples) to be adjusted. For the reactions with a high concentration of base sonication was found to improve matters. It was important to use the final volume of the stock solution in the calculations, due to mixing effects.

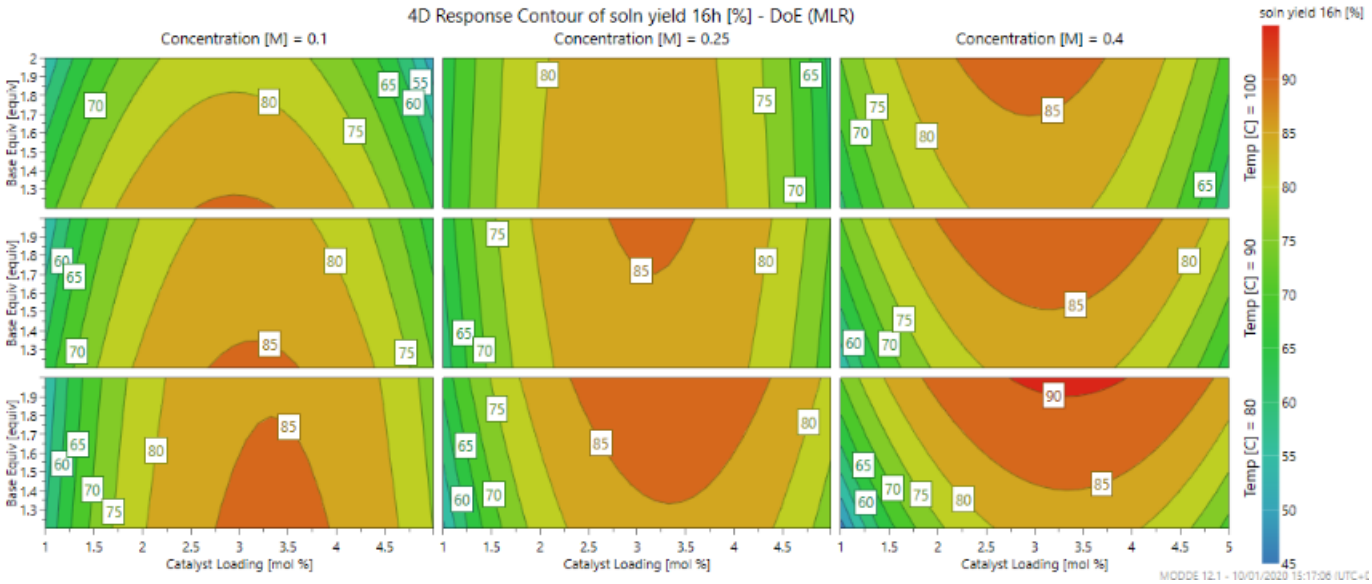
Sonication was used to free up the base stuck at the bottom of the vials.

Modde is a nice and easy to use DoE software package and the students used this. After calculating the yield and inputting it, Modde fitted the model, and the results for the model is shown below, for the yields after 1 hour, 4 hours and 16 hours. The green columns are the Pearson R^2 coefficients for the model, this is a measure of how well the model fits the data. A value of 0 is a bad fit (the model is just guessing the average), a value of 1 is a perfect (and unrealistic!) fit, so we can see all three models are very good at fitting their data. The Q^2 coefficient (dark blue bars) is like the R^2 coefficient but it measures how good the model is at predicting the data. It does this via the appropriately named 'leave one out' methodology, where one of the points is left out as a test point, and the model is built using the other points and used to predict the test point. This is done for every point in the dataset, and the measure of how well the model predicts each point is a good measure of how it would perform on new data. As you can see, the model on the 1 hour data is best, but the 16 hour data is not bad either. Modde calculates a measure called the model validity (yellow bars), so long as it is above 0.25 the model is usable, and all these models are usable. It is assumed that the reproducibility of all the experiments is the same, and thus only one or two points are measured more than once, and these are used to calculate the reproducibility for the entire dataset. As you can see, the reproducibility (cyan bars) is very good for all models, showing that using the ChemSpeed leads to highly reproducible results. Both the models made on the data taken at 1 hour and 16 hours are perfectly good for further use, however the students were keen to get the highest yield possible, so they decided to proceed with the 16 hours model and experiment.

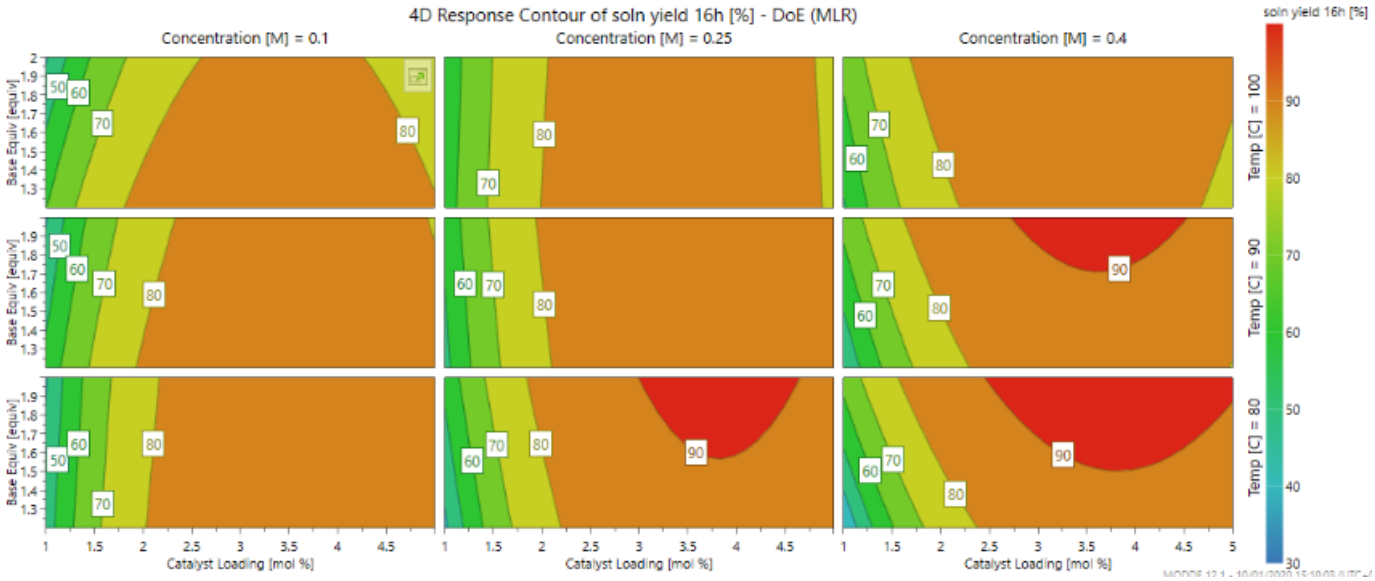


Summary of fit for the 3 models made at 3 time points

The fitted models can be used to predict the yields over chemical space, and this information is invaluable for deciding where to look next for increasing yield. The students had already decided to fix the time factor to 16 hours, so now we look at the predicted response (yield) contours for this time period. As the ligand factor is discrete, we must choose which ligand is the best. Quickly glancing at the two 4D yield response plots below we can see a much larger amount of chemical space coloured red, indicating a predicted yield above 90%, so we can immediately say that XPhos is the better ligand. Now we can examine these plots in more detail. They are called 4D plots as the effect of base equivalent, catalyst loading, concentration and temperature are all displayed. We start by picking the plot with the largest amount of >90% yield on it, which is the bottom right plot in the XPhos contour plot, this fixes the temperature at 80°C and concentration at 0.4M. Then we read that plot to figure out which values of catalyst loading and base equivalent is the best. By eye, it looks like around 3.5—4.5 catalyst loading and 1.7—1.9 base equivalent. However, we can use Modde to pick the values that the model thinks have the highest chance of success.



*4D yield contour plots for the **Xantphos** ligand after 16 hours reaction time.*

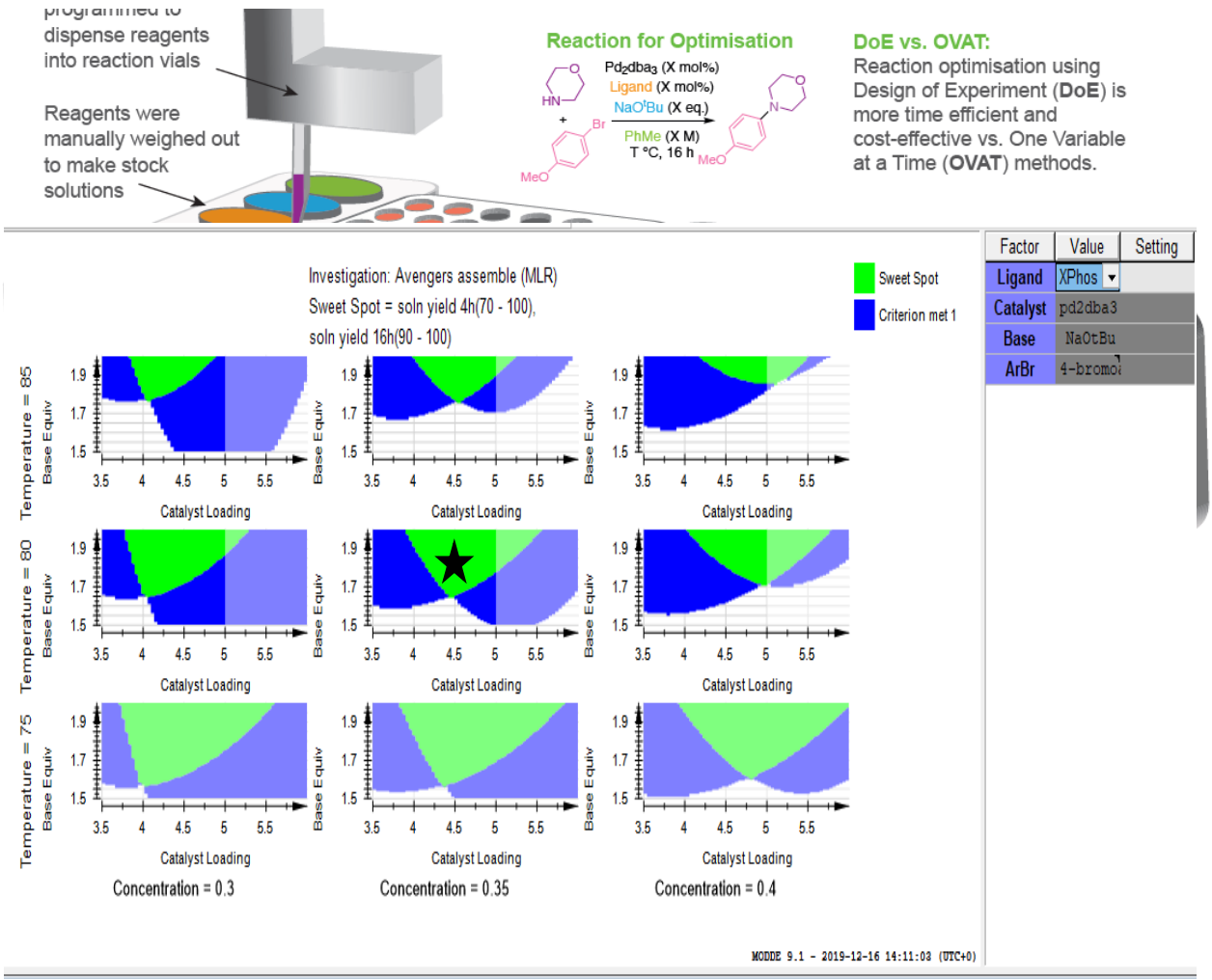


*4D yield contour plots for the **XPhos** ligand after 16 hours reaction time.*

Picking the best values for the next experiment is done using the 'sweet spot' plot. Here you can input any constraints that you might want to add (this is where your chemical knowledge comes in). Here the model picks a different concentration. Note that, once we've settled on a ligand, the model itself is 4 dimensional (temperature, catalyst loading, base equivalents and concentration) and in the 4D plots above, the concentration was discretised for plotting, but concentration can take any value between 0 and 1. Modde can deal with this 4 dimensional model and predict the optimum conditions. This is displayed in the sweet spot plot below. Also, the high concentration experiments were the ones that ran into difficulties, so it's sensible to go with lower concentrations if at all possible.

PROJECT OUTLINE:
The solution yield of a Buchwald-Hartwig amination was

The sweet spot plot shows predictions from the model of where the best yield will be found.



After this, the predicted optimised conditions were:

1. Ligand: XPhos
2. Temperature: 80 °C
3. Catalyst Loading: 4.5 mol%
4. Base Equivalents: 1.8
5. Concentration: 0.35 M

With a prediction that the solution yield will be 92% after 16 h.

The next step is to test it out!

This was done and the mean optimised yield was **86%.** This method has increased the yield.

The students were then tasked with making an infographic to outline the experiment which is shown below.

I hope you enjoyed this blog post and found it useful.

This work was done by A. Dean, C. Cope, C. Harris, F. de Courcy-Ireland, J. Heeb, J. Heeley, M. Jesani, J. Jiang, V. Juba (students) and B. Banecki (specialist technician). Blog post written by E. Gale.

References:

[1] *Org. Process. Res. Dev.,* **2019**, 23, 8, 1478—1483
[2] *J. Am. Chem. Soc.*, **1994**, 116, 13, 5969—5970
[3] *J. Am. Chem. Soc.*, **1994**, 116, 17, 7901—7902

For further information about design of experiments see: Design of Experiments for engineers and scientists, Jiju Antony (Elsevier) or the Modde training materials.