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Streamlining Chemical Synthesis DoE for Successful Process Development

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radleys innovations for chemistry

Contents

Abbreviations

ANOVA	Analysis of variance
BSA	Bovine serum albumin
DBC	Dynamic binding capacity
DoE	Design of experiments
FPLC	Fast protein liquid chromatography
GMAC	Glycidyl trimethylammonium chloride
HPLC	High performance liquid chromatography
IEX	Ion exchange
RBF	Round bottom flask
R&D	Research and development
QbD	Quality by design
Vol cap	Volume capacity

1. Introduction

Process development chemistry involves the development, scale-up and optimization of a chemical synthetic route in order to turn a compound into a commercial product. The goal of the process chemist is to develop synthetic routes that are safe, cost-effective, reproducible and efficient. For this, the process chemist uses route scouting (i.e. identifying the best synthetic route) and then needs to identify the critical factors (e.g. temperature or reactant concentration) of the chemical reaction chosen. This is in order to increase the reliability, efficiency, and safety of the chemical reaction, as well as the purity and yield of the product, and ultimately lead to significant reductions in both time and cost.

Determining the critical factors for a particular process has traditionally been focused on varying one factor at a time. This practice has several drawbacks. Firstly, reaction outputs are highly dependent on the initial starting factors, which are estimated by process development scientists and therefore affected by researcher bias. Secondly, the interactions between different factors are difficult to determine due to the nature of only varying one factor at a time. Finally, it is challenging to identify actual improvements from inherent run-to-run variation unless a large number of reactions are performed.

Once the critical factors have been determined using small-scale reactions, the process is then translated to large-scale reaction vessels used for manufacturing. However, the shortcomings of the “one factor at a time” approach often lead to the realization that the critical factors identified from the small-scale reactions produce products that are out of specification at the manufacturing level. Additional optimization studies at larger scales are then required, ultimately resulting in a lengthy and expensive development process.

In more recent years, process development and optimization has shifted towards statistical methods. By introducing statistics into the planning, conducting, and data analysis of a reaction, more systematic and effective decisions can be made. This methodical approach can achieve fast, efficient, and accurate process development, resulting in products with reduced variability and defects as well as dramatically reducing the translation time from small-scale chemistry to large-scale reactions at manufacturing plants.

One of the crucial factors for delivering high-quality process development chemistry is the experimental setup. Traditional systems use a round bottom flask (RBF) and a hot plate/water bath but they suffer from poor temperature control, which can often lead to inaccurate results and can affect scale-up cost and timeline. This paper discusses approaches to improve the speed and quality of process development chemistry using parallel synthesis and an alternative synthetic chemistry set up and is a case study from the Process Development group at Purolite in Llantrisant (Wales, UK).

2. Design of Experiments (DoE) - A Statistical Approach to Process Development

One statistical approach for process development is design of experiments (DoE), which identifies the critical factors required to achieve optimal conditions for a product through a multivariate approach. In comparison to the traditional one factor at a time approach, DoE has considerable advantages:

- It maximizes knowledge while using the minimum amount of resources; it provides accurate and efficient information.
- Interactions between factors can be determined.
- The significance of each factor can be characterized.
- The process behavior can be predicted before experimentation.
- Cause and effect relationships between critical factors and critical responses can be identified.
- A proven acceptable range of the critical responses can be established.
- Simultaneous optimization of multiple responses can be attained.
- Outliers or anomalous data can be easily recognized.
- A robust production process can be achieved.

There are several steps that need to be followed sequentially to achieve a successful, high-quality DoE study. Firstly, the objectives should be clearly defined. Here, the product profile is created using scientific literature and technical knowledge of the process development scientist. Secondly, a range of input factors that are thought to have an impact on the reaction process are identified. Common factors include raw material concentration, addition rate, stirring speed, temperature, catalyst type or amount, and pH. In this step, the critical responses are also identified, such as yield, selectively, and impurity level (Figure 1).

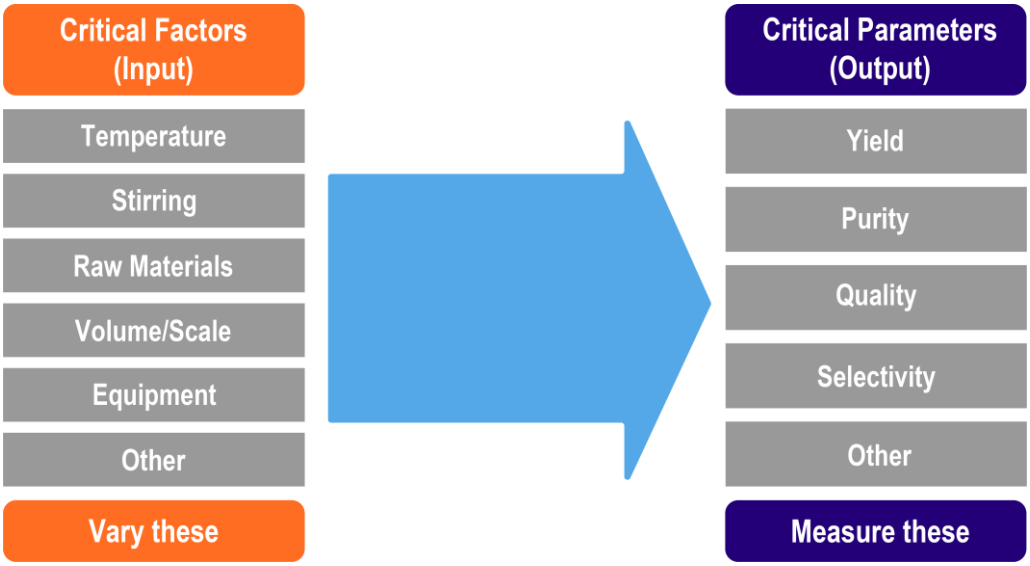

Figure 1. *Schematic example of a DoE process.*

Next, the type of DoE should be determined. There are three main categories: mixture designs, factorial (process) designs, and mixture-process designs. The most commonly used type of DoE are factorial designs, where the factors are deliberately and simultaneously varied, and all combinations are performed in the same DoE study. A three-level factorial design is one of the most popular DoE factorial design options. For these studies, the different factors can be represented in a cube, where each corner is an extreme value (high or low), and the center is the midpoint of all variables (Figure 2). Additional experiments are often carried out at the central point of the space to determine the intrinsic variability of the process.

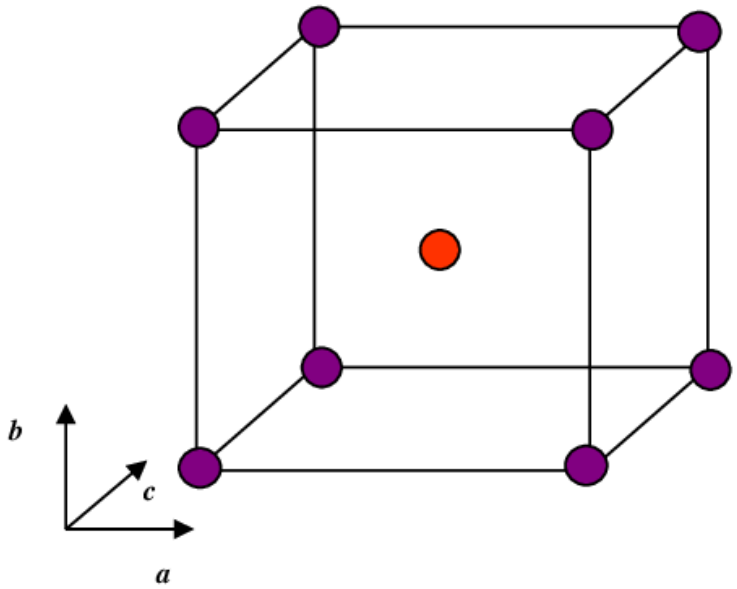

Figure 2. *A three-factorial design representation. The purple dots represent the extreme values and the red dot represents the midpoint of all variables.*

The set of experiments determined by the type of DoE are then performed. Only the critical factors identified are varied, all other non-critical factors are kept constant throughout all experiments. Once the experiments have been completed, the data will be analyzed using analysis of variance (ANOVA) or other mathematical models, which are often used to generate contour plots to graphically represent the relationship between the critical factors and critical responses. Finally, the data can be evaluated, any outliers discovered, and the optimal conditions identified.

Due to the DoE approach, multiple experiments are performed during this process to identify the optimal conditions and it is not unusual for 20 reactions to be required. Therefore, it is critical that the experimental set up, non-critical factors, and execution of each experiment is reproducible in order to perform a successful, high-quality DoE study. In addition, the variation of each factor must be accurately controlled to avoid "noisy" responses, which can result in the effects of the different factors not being seen. Without this precision, the data produced from these studies will be less accurate, making the determination of the optimal process conditions difficult.

Repetition of the experiments may be required to try and obtain accurate data, leading to an increase in time and cost and reducing the advantages of DoE compared to the traditional “one factor at a time” approach. Therefore, the experimental set up used for these studies is key to achieving a successful, high-quality DoE study.

3. Practical Aspects of DoE Studies

Typically, DoE studies are performed on a small-scale (≤ 500 mL) to reduce time and minimize cost. Once all experiments have been performed, the data analyzed, and the optimal conditions identified, the reaction is scaled up. This process development is performed in several stages. Firstly, the reaction is scaled up to 1 to 10 L and any issues at this level are identified. The reaction conditions are then adjusted to resolve these problems before the process is scaled up again for the large-scale reactors (≥ 25 L) of the manufacturing plant.

3.1. Traditional Method: Round Bottom Flask (RBF) Set Up

The DoE reactions' experimental set up traditionally consists of round bottom flasks (RBF) with a hot plate, heating mantle, or water bath as the temperature control device. This equipment is found in every chemical laboratory and is routinely used by chemists, making these types of DoE systems low-cost, straightforward and easy to use.

Despite the advantages, these systems come with many downsides, with the most significant drawback being their poor temperature control. Temperature is often identified as a critical factor and therefore controlling this factor in a reaction is of high importance in the initial DoE studies to obtain accurate information. However, hot plates, heating mantles, and water baths can only heat, they have no ability to cool, and this heating is often erratic, delayed, or sluggish, resulting in an unpredictable and uncontrolled temperature regimen.

Additionally, since these heating methods do not have any active cooling abilities, if a reaction is exothermic, chemists typically lift the reaction vessel from the heat source or place the vessel into a cold bath to adjust for this temperature increase and monitor the reaction temperature until it reaches an acceptable level. This adjustment requires user intervention and introduces bias, decreasing the reproducibility of the reaction and the accuracy of this factor in the DoE study. This manual temperature control also means that hot plates, heating mantles, and water baths cannot perform temperature ramping, so a controlled temperature change in a reaction is not possible.

At the large, manufacturing scale, a fluid is heated or cooled and circulated around the outside of the process vessel to accurately control the reaction temperature. This arrangement enables precise temperature control such as temperature ramping, active cooling for exotherms, and active heating for endotherms. Therefore, in addition to the poor temperature control of RBFs and hot plates, heating mantles, or water baths, these small-scale systems are not representative of the conditions that the final reaction will be performed under at the manufacturing plant.

Another issue with using an RBF and a hot plate, heating mantle, or water bath is their lack of data logging. To monitor the temperature of the reaction, it must be manually noted at regular intervals. This increases the chance of observational errors in the reporting of the data, as well as reducing the cost-effectiveness of the DoE study as the user is required to spend a longer time with the experiments.

With this set up, stirring is commonly performed by use of a magnetic stirrer in contrast to the overhead mixers that are used in large-scale vessels at the manufacturing plant. Overhead stirrers can be used with RBFs, however, they can be difficult to set up and the type of stirrer impeller will be different to those in large-scale reactors as they need to fit through the small neck of an RBF.

These factors often lead to suboptimal conditions being determined from DoE studies with this set up, leading to problems when the reaction is scaled up to jacketed reaction vessels or the large-scale reactors used in the manufacturing plant. If the conditions have not been accurately determined when the reaction is scaled up, the DoE studies may need to be performed again, but this time on a bigger scale, wasting valuable time, money, and raw material.

3.2. Traditional Method: Jacketed Reactors

Jacketed reactors are also used for DoE studies as they are much more representative of the large-scale reactors used in the manufacturing plants compared to RBF set ups. They are also commonly used in the second stage of process development, to determine whether the reaction conditions and critical factors identified from the initial DoE study still meet the critical responses at a larger scale, around 10 L.

These vessels operate with overhead stirring, have a similar geometry to large-scale reactors, and the temperature is accurately controlled in the same way, by heating and cooling a fluid that circulates around the reactor. Software, such as AVA Lab Control Software from Radleys, is also available which enables users to monitor and control reaction factors, including temperature, stirring speed, pH, and reactant additions. This control allows for a completely automated reaction and all the data can be logged.

There are, however, significant drawbacks to using this system for DoE. Jacketed reactors are much larger than the traditional heating-only RBF set up. The instrument footprint is much bigger, increasing the amount of space required for each experiment as well as the quantities of reaction components such as the raw material and solvent. As multiple experiments are performed for a DoE study, multiple jacketed reactors and a thermoregulator for each of these reactors are required, further increasing the amount of space needed and the cost.

The use of partially automated small-scale reaction stations can overcome the issues identified with using traditional set ups for DoE studies, and enable a much smoother, faster, and lower cost process development.

3.3. Alternative Method: Radleys Mya 4 Reaction Station

One such reaction station is Radleys Mya 4 (Figure 3). With the ability to heat as well as actively cool four reactor-style vessels independently, the Mya 4 Reaction Station allows for small-scale DoE studies. The accurate temperature control enables the study of this factor – often the most critical in a reaction – but also the variation of other factors with a reliable constant temperature. The set-up also allows for easy overhead stirring on a small scale to mimic the stirring at larger scale.

Figure 3. *Radleys Mya 4 Reaction Station.*

The compact size and easy set-up of the Mya 4 allows space and time to be saved when performing DoE studies compared to traditional set ups.

The Mya 4 Reaction Station is software controlled to enable data logging and automation. This reduces the probability of manual errors and increases the repeatability of the reaction.

The data from all experiments of a DoE study can then be analyzed to determine the optimum conditions for the reaction process. The Mya 4 is much more representative of large-scale reactors than heating-only RBF set ups. If specific conditions cannot be met at this small-scale (e.g. the desired cooling rate of a reaction cannot be achieved), it provides substantial evidence that these conditions will not be met in the manufacturing plant either and may suggest that the desired factors would be too expensive to make the reaction feasible.

Furthermore, once the process has been scaled-up, the data obtained from these small-scale studies can be compared with the large-scale reactors' data to ensure the reaction processes are similar or to easily identify any areas of difference that may be the cause of variation between the reaction scales.

4. Case Study: Scale Up of Purolite's Agarose Resin

A case study demonstrating the benefits of a partially automated reaction station for DoE analysis is the work the research and development (R&D) team at Purolite performed to optimize a coupling process for their agarose resin.

Purolite is a global manufacturer of resins for separation, purification, and extraction technology for a wide range of markets, including pharmaceutical, biopharmaceutical, food, cosmetics, and fine chemical. One of their largest product categories is agarose resins, which are considered the gold standard for protein purification by chromatography.

Proteins are the largest group of therapeutics in the biopharmaceutical industry and are used for the treatment of a wide range of diseases, including cancer and autoimmune disorders. A critical stage in the development of these therapeutics is purification in order to minimize the risk of side effects from other components in the initial media. Therefore, it is crucial that Purolite manufacture agarose resins that meet specific criteria for effective separation.

Agarose resin is typically supplied as transparent, spherical beads, which can be uncharged or chemically modified, depending on their chromatographic application. One of their agarose products for ion exchange (IEX) chromatography is a strong anion resin due to the attachment of quaternary ammonium groups on the resin. This product is produced by the coupling of glycidyl trimethylammonium chloride (GMAC) with the primary hydroxyl groups on the agarose chain under basic conditions (Figure 4).

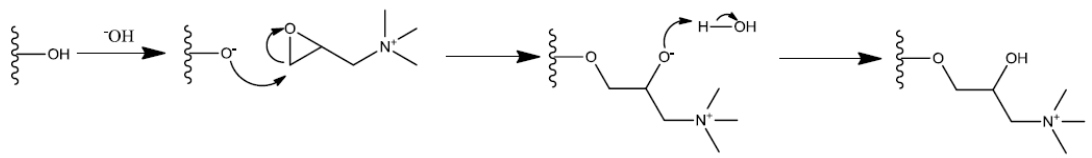

Figure 4. *Addition of a quaternary ammonium group to the agarose resin by coupling glycidyl trimethylammonium chloride (GMAC) to the resin's primary hydroxyl groups under basic conditions.*

To determine the success of this reaction, two critical responses are measured:

- The quantification of quaternary ammonium groups on the resin, also referred to as volume capacity (vol cap), determined by a potentiometric titration with silver nitrate
- The dynamic binding capacity (DBC) of the resin, calculated by the residence time of bovine serum albumin (BSA) in a column of the agarose resin run on a fast protein liquid chromatography (FPLC) system.

In order to produce an agarose resin that meets the three critical responses, precise temperature control is essential, as the reaction is exothermic. If the reaction reaches a critical temperature the raw materials decompose, leading to a loss of product as well as additional time and expense to repeat the process.

4.1. Initial DoE Study Using an RBF Set Up

To determine the critical factors and tolerances of this coupling reaction, a full three-factorial DoE study was performed. The factors were identified as temperature, GMAC volume, and NaOH volume, so all other factors were kept constant, including the stirrer speed, addition rate of NaOH and the concentrations of the raw materials. The DoE planning determined that eight reactions were required to gather sufficient data, with three additional reactions at the center point (the mid-range value for all variable factors).

The experiments were initially performed in 250–500 mL RBFs immersed in a circulating open water bath with overhead stirring provided by a centrifugal impeller and the data is shown in Table 1. A high level of variation (10%) between the three center point values (experiments 9, 10 and 11) was observed, indicating substantial variability of the DoE process. In addition, the vol cap or Ion exchange capacity for the center point data was out of specification (< 0.14 mmol/ml).

Table 1. *Data set of the initial DoE set up. Experiments 9,10,11 represent the center point data. Green samples indicate results are in specification, red samples indicate results are out of specification.*

Exp no.	**Critical factor #1** **GMAC** (ml)	**Critical factor #2** **Temp** (°C)	**Critical factor #3** **NaOH** (ml)	**Critical response** **vol cap** (mmol/ml)
1	80	25	1.2	0.085
2	140	25	1.8	0.106
3	80	35	1.2	0.102
4	140	35	1.8	0.121
5	80	25	2	0.104
6	140	25	3	0.13
7	80	35	2	0.104
8	140	35	3	0.142
9	110	30	2	0.103
10	110	30	2	0.093
11	110	30	2	0.116

Assessment of the data suggested that the factors in experiment 8 were the most promising as it gave the highest IEX capacity value. Therefore, these factors were translated to a 10 L jacketed reactor. However, at this larger scale, the critical degradation temperature of GMAC (59 ºC) was reached, leading to a loss of product (Figure 5). The circulator could not cope with the exotherm and the ΔT between the reaction and the jacket went up to about 90 ºC.

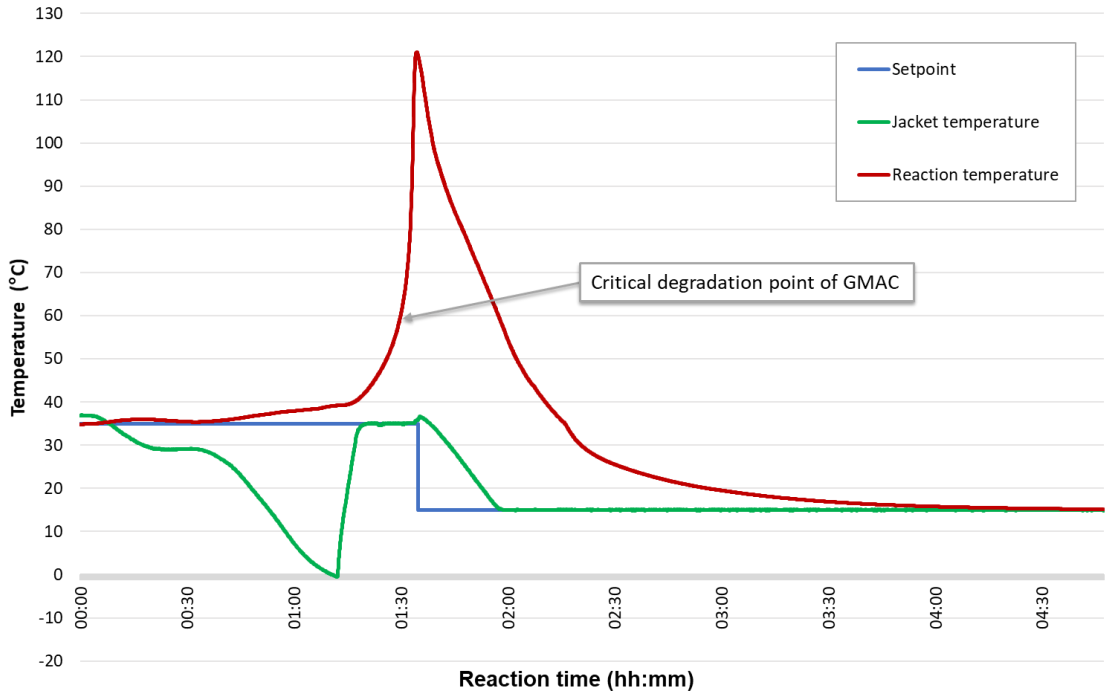

Figure 5. *Results of the reaction in the 10 L jacketed reactor when using factors identified from experiment 8 of the initial DoE.*

To achieve a successful reaction at this scale the Purolite R&D team had to vary multiple factors, causing a three-week scale-up phase, and a significant quantity of raw material being used. Once the most suitable factors for the 10 L jacketed reactor were identified, the process was successfully upscaled to a 100 L jacketed reactor (25 L batch size) without reformulation.
The difficulty of scaling the reaction from 250–500 mL to 10 L identified multiple issues with the DoE study:

- the water bath gave poor temperature control and so multiple temperature loggers were required to monitor the reactions,
- the stirrer was susceptible to slipping, resulting in grinding of the resin,
- RBFs have a different geometry and are not representative of the larger reaction vessels.

4.2. Small-Scale DoE Study with Radleys Mya 4

With the aim to overcome these problems, and using the improvements from the 10 L scale reformulation, the Purolite R&D team performed a second small-scale DoE study with the Radleys Mya 4 Reaction Station. The Mya 4 enabled the use of 250–400 mL process vessels with representative geometries of larger reaction vessels to aid translation. Overhead stirring was easy to set up and remained in position throughout the reactions, minimizing bead grinding. Finally, the precise temperature control and data logging ensured the temperature could be monitored throughout the reaction and automatically recorded.

For this DoE study, the Ion Exchange (IEX) capacity was measured as per the initial DoE but the Dynamic Binding Capacity (DBC) was also tested as it is a more accurate measurement of the success of this coupling reaction. From the data in Table 2, an important improvement in the robustness of the model was observed: the center point data showed only a 5% variation for the vol cap and DBC assessments, which was within the standard deviation for analysis test methods.

Table 2. *DoE factors using Radleys Mya 4. Experiments 9,10,11 represent the center point data. Green samples indicate results are in specification, red samples indicate results are out of specification.*

Exp no.	GMAC (ml)	Temp (°C)	NaOH (ml)	vol cap (mmol/ml)	DBC at 10% BT 2.4 min (mg/ml)	DBC at 10% BT 6 min (mg/ml)
1	200	35	7.5	0.131	20.90	63.80
2	200	25	7.5	0.161	19.60	61.50
3	200	45	2.5	0.113	22.00	47.30
4	200	25	2.5	0.043	35.10	45.60
5	100	45	7.5	0.113	21.95	50.59
6	100	35	7.5	0.138	14.21	32.43
7	100	35	2.5	0.124	13.88	29.91
8	100	25	2.5	0.097	16.79	36.35
9	150	35	5	0.159	37.20	68.90
10	150	35	5	0.150	36.60	71.40
11	150	35	5	0.157	38.60	73.70

A slight overshoot of 2 °C was observed for center points after the addition of NaOH, but this value is in line with production scale exotherm and using 15 °C circulation the temperature was quickly stabilized (Figure 6). Even being able to measure this overshoot in temperature was a massive advantage in comparison to the initial set up with a water bath.

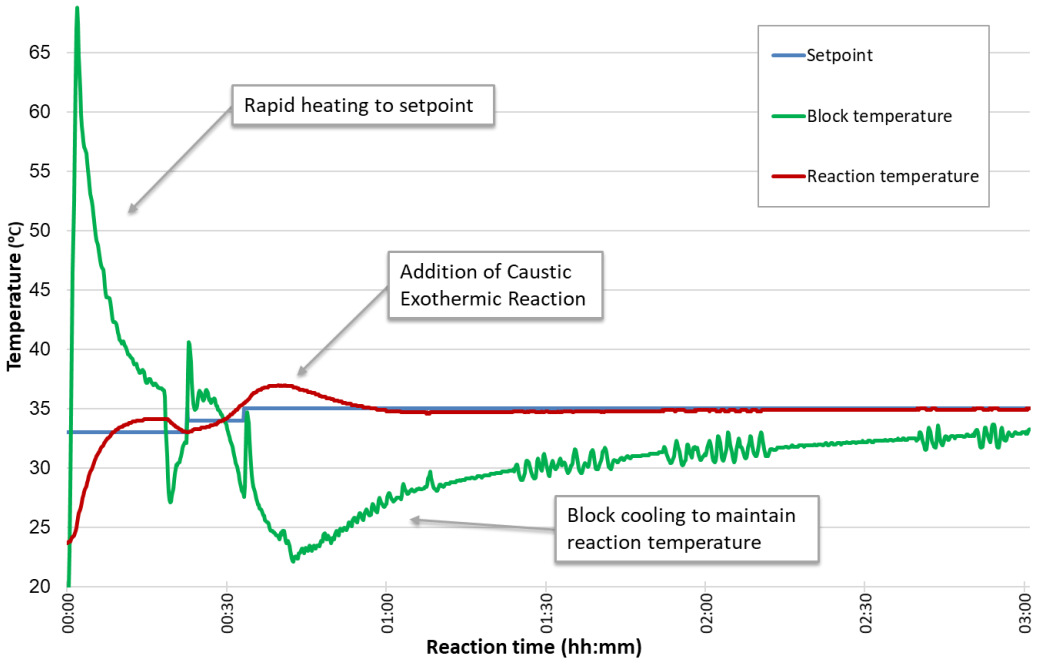

Figure 6. *First 3 hours of center point experiment using the Radleys Mya 4 Reaction Station. Temperature increase of 2 °C occurred after addition of NaOH, but the temperature was easily stabilized.*

According to the DoE data, the critical factors are not at the center point, ideally a higher temperature and a larger volume of NaOH would be used. However, using these factors, the reaction temperature increases to 57 °C, which is very close to the decomposition temperature of GMAC (Figure 7). The Mya 4 circulation can be set to a lower temperature to improve the rate of cooling, however, upon analysis the Purolite R&D team calculated that substantial cooling would be required at the manufacturing scale, which would be expensive and not cost effective. Therefore, a compromise between the critical factors and the practicalities of the manufacturing process had to be reached.

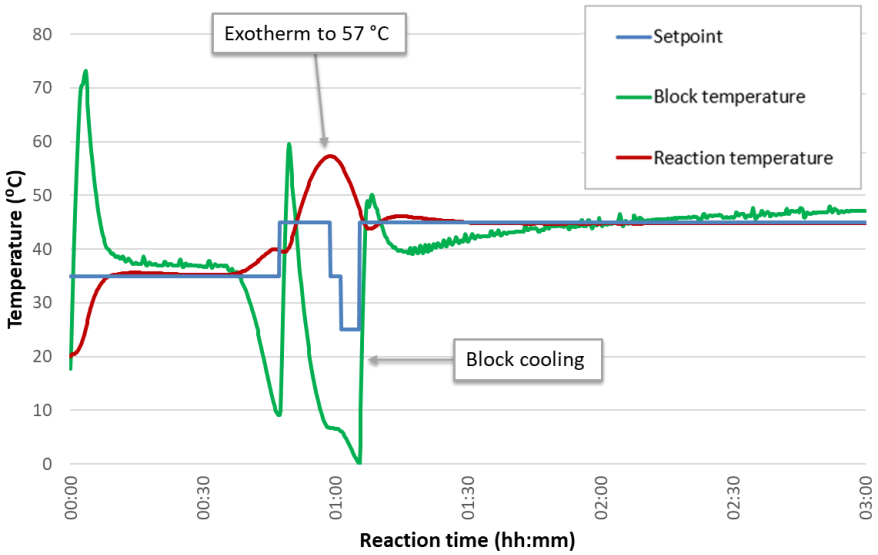

Figure 7. *First 3 hours of experiment 5 (low GMAC, high temp and high NaOH) using the Radleys Mya 4 Reaction Station. Exotherm of 12 °C observed.*

The center point results were not far off those of the experiments performed at a higher temperature and NaOH volume. Therefore, the center point factors were translated for scale-up. This reaction was easily upscaled to manufacturing size batches with no reformulation necessary (Figure 8), demonstrating the Radleys Mya 4 is an effective model for small-scale Process Development.

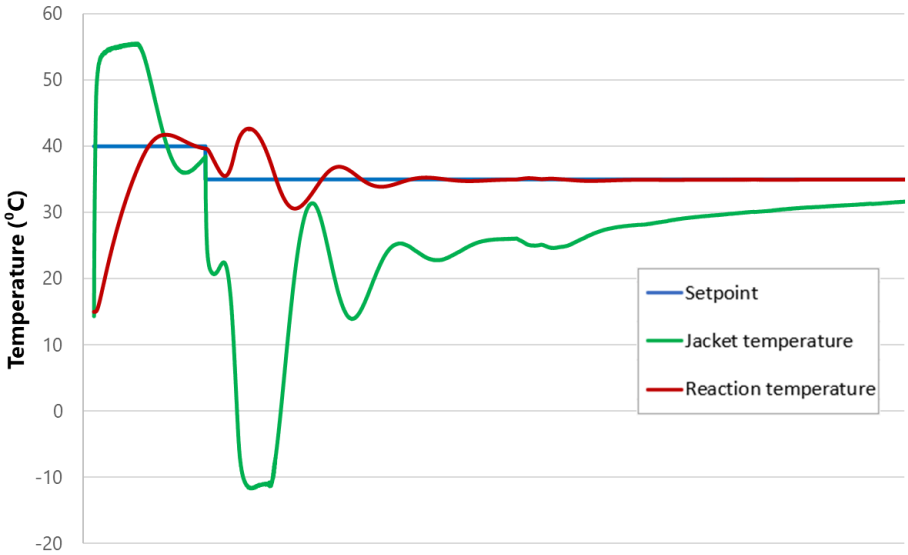

Figure 8. *Centre point conditions applied to a 100 L jacketed reactor with a Julabo W50 circulator. A 7 °C exotherm was observed for a short period, which is acceptable.*

4.3. Purolite Transition to the Radleys Mya 4

Although the Radleys Mya 4 Reaction Station is more expensive than the water bath system the R&D team initially used, the overall process from DoE to manufacturing plant scale reduced Purolite's waste, cost, and project time as the experiments were directly scalable. All factors, including temperature, stirring speed, and NaOH addition rate were recorded using the Mya 4 software, meaning factors could be easily repeated for scale-up. Furthermore, minimal training was required with the Radleys Mya 4 system and a BSc microbiologist with only 6 months' experience was able to run the DoE study, compared with a Ph.D. chemist with 3 years' experience when using the water bath system. When the BSc microbiologist tried to use the water bath system, some issues were encountered, such as slipping of the overhead stirrers, causing grinding of the resin beads.

Dr. Patrick Gilbert who led the study, stated that "I would have loved one two years ago, so I could have used it as the workhorse for most of the project." Purolite is now using the Radleys Mya 4 Reaction Station regularly for their agarose R&D work.

5. Conclusion

By implementing the systematic QbD approach and performing DoE studies to analyze the impact of multiple reaction factors simultaneously, the initial stages of process development can be performed quickly, efficiently, and accurately. In comparison to traditional “one factor at a time” studies, this methodology dramatically reduces the length of time needed to identify the critical factors of a particular reaction.

A fundamental requirement for executing effective DoE studies is the experimental setup. Conventional systems that use an RBF with heating-only equipment suffer from many drawbacks in the context of process development, most significantly poor temperature control. These systems can lead to inaccurate results and therefore, despite the accurate design of a DoE study, the critical factors may not be identified. These errors can then substantially impact the process development timeline, and resulting in further R&D at larger scales to determine the critical factors correctly. This impact ultimately increases the time and expense to translate small-scale reactions to the manufacturing plant.

To minimize these errors, it is crucial that DoE studies are performed using systems with accurate temperature control and are as representative of the large-scale reactors of the final manufacturing step as possible. Automated reaction stations such as the Radleys Mya 4 Reaction Station can offer the solution. They are software controlled to ensure the desired temperate regimen is maintained throughout the experiment; all data is logged so analysis can be quickly performed, and any outliers easily identified. Also, small-scale equipment with similar geometries as the vessels at the manufacturing level can be used to replicate the final environment as effectively as possible.

By correctly identifying the critical factors of a reaction at the small scale, minimal changes will need to be made during the translation of this process to the large-scale reactors of the manufacturing plant. This practice ultimately reduces the time and expense involved in the development of a new product from inception to manufacturing and release.

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