Application Note

Continuous Flow Process Development

Sampling Reactions Over Extended Periods

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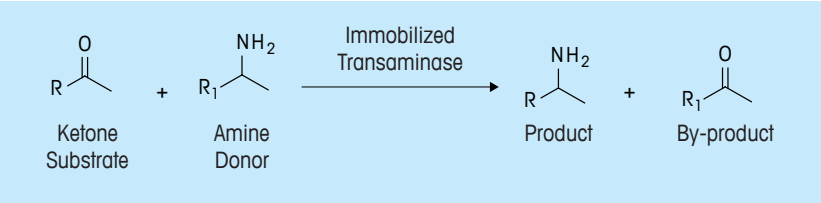
Automated and unattended sampling of continuous biocatalytic reactions allows reliable monitoring of catalyst performance over extended periods of time to enable rapid process development.

Biocatalytic reactions are of increasing interest in the application of API manufacture due to excellent selectivities as well as their potential to access faster and more efficient synthetic routes. Recent examples have shown that flow chemistry can significantly increase the product throughput for biocatalytic processes as well as addressing traditional limitations including unfavourable reaction equilibria, substrate, and product inhibition. As part of the ReMediES (Re-configuring Medicines End-to-end Supply) initiative, GSK was working to develop technologies to improve the utility of continuous biocatalytic reactions in pharmaceutical supply.

Achieving a high enzyme lifetime is essential to the development of an economical continuous biocatalytic process. In our case study, accurate monitoring of reaction performance over extended periods of time (100+ hours) was necessary to provide a reasonable estimate of enzyme half-life during a continuous transamination and thus allow assessment of the viability of the prototype process. Analysis of these reactions was complicated by the minimal structural differences between substrate and product. As a result, differentiation between reaction components using online techniques (e.g. IR, Raman, NMR) proved difficult, necessitating the use of offline GC analysis.



Scheme 1. Biocatalytic transamination reaction

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Manual sampling of this continuous biocatalytic reaction was a labour-intensive process, requiring collection of reactor output two to three times a day, followed by manual pipetting and dilution to provide samples suitable for GC analysis. Each sample took approximately 10 minutes to collect, directly consuming 30 to 40 minutes per day. Also, the need for constant physical interaction with the reaction throughout the day significantly reduced productivity by limiting the amount of work which could be conducted in parallel. In addition to the time-consuming process, no samples could be collected overnight or through the week-end, resulting in substantial gaps in reaction data-sets. Samples were generally duplicated to ensure accuracy and minimize the impact of sampling gaps on data analysis.

It was anticipated that EasySampler™ would provide a flexible and robust solution to many of these problems, providing frequent and consistent sampling over the whole course of the reaction, with minimal interaction needed. To use EasySampler in a flow context, a 3/8″ Swagelok T-piece was modified to allow the EasySampler probe to be connected to a flow reactor via 1/8″ HPLC tubing (Figure 1). The probe tip was positioned in the T-piece with the sample pocket perpendicular to the direction of flow, then sealed and fixed in place using a nut and PTFE ferrule (Figure 2). A sampling sequence could then be set and run, allowing flexible sampling of flow reactions over timescales from minutes to days.

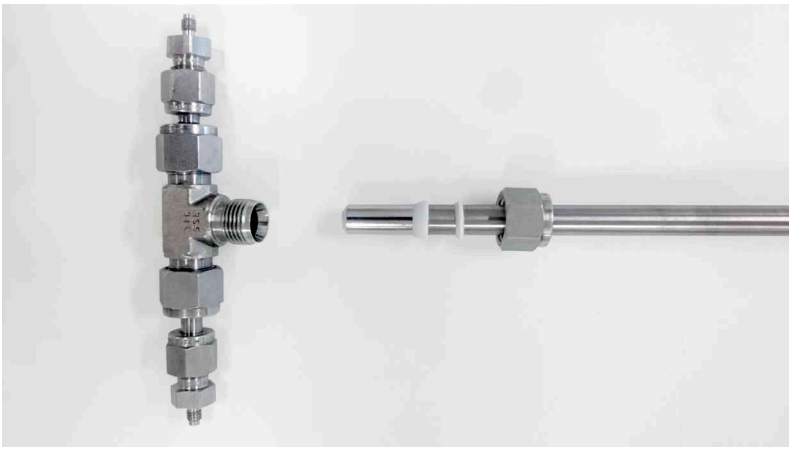

Figure 1: T-piece mounting for EasySampler probe

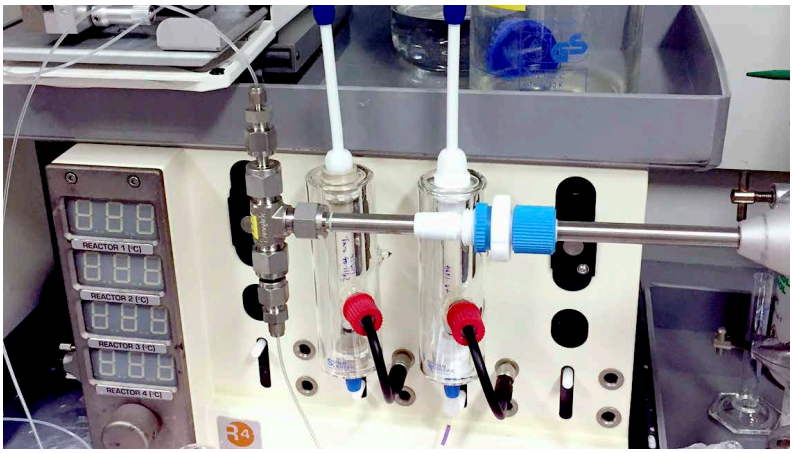

Figure 2: EasySampler connected to a flow reactor

Results

To test the consistency and accuracy of the T-piece mounted EasySampler, a 1.0 M solution of transamination substrate was pumped directly through the T-piece, sampled in parallel manually and using the EasySampler at several flow rates and the concentration measured by GC. Comparison of the EasySampler and manual samples showed that automated sampling provided accurate and consistent sampling for flow rates down to 0.1 mL/min (Figure 3).

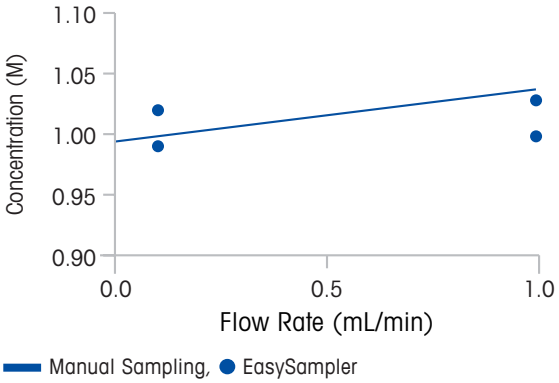

Figure 3: Comparison of 20 µL sampling from 1.0 M solution using EasySampler and manual pipette

Once the proof of concept had been demonstrated, EasySampler was used to monitor a transamination reaction in flow over 120 hours. Manual samples were taken periodically to provide an additional validation and comparison with the automated sampling. A custom sampling sequence was used to provide an increased sample density as the reaction equilibrated, followed by sampling every 8 hours for the duration of the experiment. As shown in Figure 4, this automated sampling regime allowed for a very consistent reaction profile to be obtained over a long timescale, with the entire experiment operating almost completely unattended for a week of continuous operation. This detailed reaction profile could then be used to provide a confident estimate of biocatalyst stability under these reaction conditions.

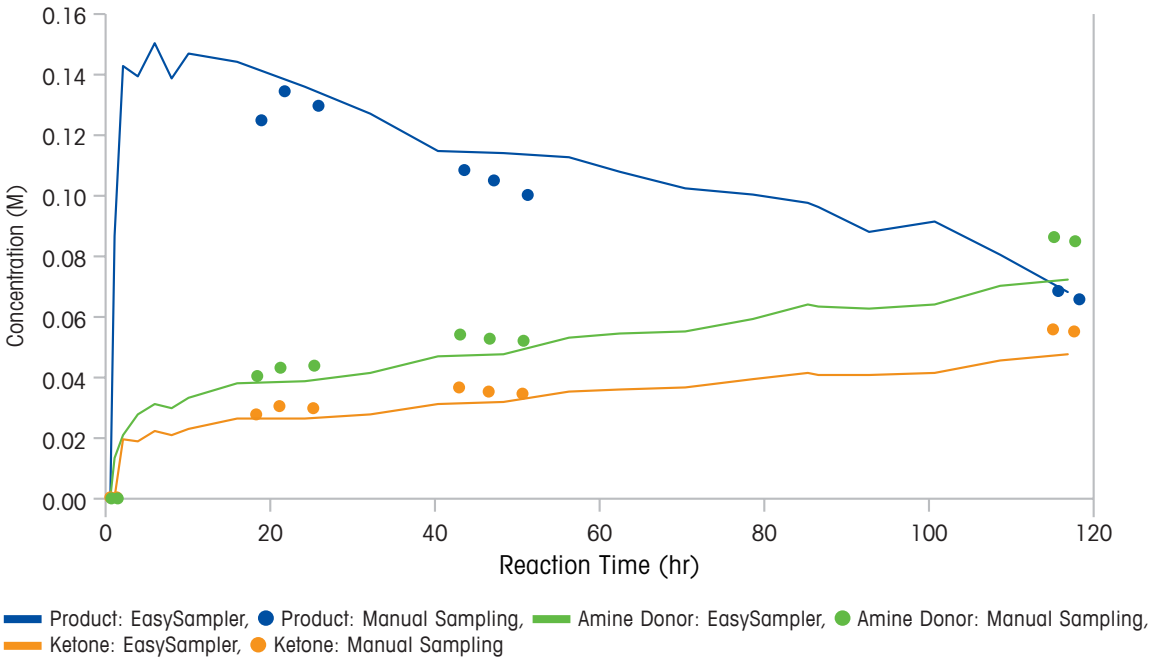

Figure 4: Flow transamination operating at 0.1mL/min

Conclusions

It has been demonstrated that EasySampler readily provides robust automated sampling of flow reactions. This fills a notable analytical gap where the nature of the reaction or substrate make online monitoring techniques difficult or impractical. The use of EasySampler under these circumstances eliminates laborious and time-consuming manual sampling procedures, directly saving 4 to 5 hours during a week-long experiment. In addition, EasySampler allows consistent and reliable data to be collected overnight and through week-ends, this increases productivity by increasing the number of reactions which can be run per week and freeing chemists' schedules from the burden of frequent sample collection. The availability of flexible automated sampling for long-running flow reactions has helped achieve one of the goals of the ReMediES initiative by streamlining the workflow for rapidly developing continuous biocatalytic processes.

EasySampler™

EasySampler: Unattended, Representative Sampling

Difficult to Sample Reactions
The in situ sampling probe eliminates tedious manual sampling and sample preparation steps of continuous reactions.

Representative and Reproducible
Sample reactions in the same way, from the same point in the reactor, and into a pocket of fixed volume for highly reproducible samples.

Automated and Unattended
Samples are taken as scheduled by the user to provide high quality samples for complete reaction information and understanding.

Application of EasySampler in other reactions that are difficult to sample:

- Reactions at elevated pressure
- Sub-ambient temperature reactions
- Heterogeneous reactions
- Multi-phase reactions
- Toxic reactions

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