Batch-to-continuous process intensification for small-molecule pharmaceutical manufacturing

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ABSTRACT

The pharmaceutical industry has been moving towards using continuous processes because they are cheaper, easier to maintain and operate, and more environmentally friendly than their batch counterparts. However, the research of continuous pharmaceutical systems is limited, which makes many industry professionals hesitant about this transition. To help establish successful pharmaceutical-based reactions in continuous systems, this MQP studied the production of *R*-phenylethylacetate via the dynamic kinetic resolution of (*R*,*S*)-1-phenylethanol with H-Beta zeolite and *candida antarctica lipase B*. Kinetic resolution, racemization, and dynamic kinetic resolution reactions were validated in batch and examined across a range of flow rates in packed bed reactors. The dynamic kinetic resolution reactor increased reaction yield by 23% over the analogous kinetic resolution system. This progress suggests that an industrially viable zeolite driven DKR system could be developed with the use of a higher temperature, greater zeolite load, or an acyl donor with more steric bulk in the reactor.

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CHAPTER 1 Introduction

1.1 Current Outlook

In recent years, the pharmaceutical and fine chemical industry has been moving away from the traditional batch processing and towards the more efficient method of continuous processing.[1–3] Continuous processing is the preferred method of production for many other industries due to its cost-effectiveness, smaller equipment usage, and high sustainability.[4] The fine chemical and pharmaceutical industries have trailed behind in this switch, largely due to the unique requirements for product regulation.[5] However, with the development of new technology, automation, process controls, and regulatory support, the industry has been more open to the change.[4] Survey results from 10 pharmaceutical companies and 15 commercial manufacturing organizations (CMO) showed that industry members expect to see a paradigm shift in the coming years.[6]

1.2 Batch versus. Continuous Processing

Currently, most companies in this industry primarily depend on batch processing.[6] In batch processing, an operation reaches completion before proceeding onto the next step. Processing steps are split into separate units with the product entering a holding time before continuing onto the next operation. This method allows for versatility as units are less specialized and can be recycled between different processes.[3] For pharmaceutical companies, flexibility is very important due to the nature of the quickly changing market and the high failure rate of drugs at the drug discovery level.[4] As this production method has been in use for many decades, there is an explicit body of knowledge surrounding usage, controls, and regulations. Well-established quality checks and cleaning protocols are in place to meet governing body approval.[3,4,7]

However, these batch processes reach a price ceiling as large equipment sizes and more raw materials are needed to reach adequate yields.[4,8] Intermediates must be stored and regulated at all points in between unit operations, adding to the cost and the waste of energy as large factory systems are needed to ensure the safety and efficacy during the hold times.[9] Batch processing also heavily relies on manual operation due to the handling steps in between unit operations.[5] Scaling-up batch processes requires multiple steps as the system can demonstrate very hydrodynamic properties at differently sized reactors.[9] Simply due to the "stop and start" nature of separate unit operations, companies can only optimize batch processes to a certain extent, reaching a maximum efficiency and time limit.[4,8,10]

Continuous processing offers a solution to the downsides of batch processing. In continuous operation, reactants are continuously fed through a connected series of unit operations that are operating in unison at steady state. This method reduces the equipment footprint in comparison to batch processes as the continuous method produces higher yield for the same amount of raw materials.[3] The process itself is more efficient, for multiple reasons. Due to the geometry of the reactor, there is higher mixing and heat transfer, leading to higher yield and a shorter reaction time.[9] It is streamlined into one operation, eliminating the holding time between operations and thus, the costly and high energy usage needed to store intermediates in a batch methodology.[4] The high yield and low capital investment cost make continuous processing the more cost-effective method of production.[3,4,8,10]

When comparing a stainless steel batch downstream processing model to a model with continuous methods, the annual cost at all titers and volumes was 1.5 times more expensive across all scale, from benchtop scale to commercial manufacturing.[8] In addition, continuous processes rely less on manual labor as all operations are interconnected. Instead, they use automation to

perform the necessary steps for running the equipment and making necessary corrections in real-time.[5,11] Lastly, the scale-up procedure for continuous processes may require less steps than the batch processes as one way to produce more material is to just run the reactor for a longer time rather than switch to a bigger reactor.[12] Overall, continuous processing is significantly more efficient and cost-effective method in comparison to batch processing.

There are some drawbacks to continuous processing that have made companies hesitant to leave the familiar ground of batch processing. Due to the interconnectedness inherent to the continuous method, this process lacks the flexibility found in batch as continuous processes are usually intended for one specific process.[8-9] Continuous processes must also have a very different system for quality checks. In batch processes, quality checks are performed on each batch after leaving a unit operation. Continuous processes lack the clear boundaries separating each batch so process control technology that delivers information in real-time about the status of the process must be developed synchronously with continuous equipment.[4,13] Lack of clarity in regulations surrounding continuous processing has also made companies unwilling to risk switching processes.[5,7] The slight distrust of continuous processing also results from the inexperience with the methods itself. Most current process chemists, engineers, etc. were trained in batch processing methods, so it is difficult for the culture as a whole to feel comfortable making a paradigm shift from what is familiar and well-established.[4,6] Pharmaceutical companies are known to focus on short-term goals. Transition of a whole production system takes time to develop, and this time costs money that they might spend on drug discovery.[14]

1.3 Addressing the Roadblocks to Continuous Processing

Recent advancements and collaborative efforts have begun to address some concerns associated with moving to continuous processing. While exact regulations remain slightly unclear, regulators in general support the move to continuous processing.[2,4,5] The use of automation appeals to regulators as this will limit the impact of human error in operations.[2,3] Continuous processing offers a more robust quality system as continuous measurements are taken throughout the entire process rather than at the start and end of a batch.[7,10] As early as 2004, the FDA encouraged the use of Process Analytical Technology, the type of sensor control and data analysis tool that is intertwined with continuous manufacturing.[15] The Emerging Technology Program, created in 2014, supports continuous manufacturing to make sure companies are still meeting FDA approval. Further progress was made in 2019 when the FDA released "Quality Considerations for Continuous Manufacturing" and clarified regulations for a small subsection for drugs.[4]

To meet regulatory standards, quality checks need to be performed in such a way that contamination and deviations can be detected early, and feed forward loops can make a change to correct the process.[10] Process Analytical Technology and the corresponding sensor tools have made great headway in the past years to deliver real time information about the quality status of the process.[11,13] In-process sensors give constant feedback about the status of the problem. A combination of chemometrics, image analysis, spectroscopy, and machine learning then analyze the collected data and decide on the appropriate response for deviations to the system.[4,5,7] Based on the analysis, the system automatically corrects the deviation later in the system.[11,13] This type of technology and data analysis is essential to the success of continuous manufacturing as the pharmaceutical industry has very strict quality standards.

Even though technological advancements and corresponding regulatory support have improved the outlook for continuous manufacturing, as of 2021, there are still only a few drugs that were produced in industry using a continuous flow setup or a partially continuous flow set-up.[9,14] The lack of professional expertise and established case studies must also be addressed in order to accelerate this move towards continuous processing.[6] Professionals in these industries have been trained for batch processes, not continuous ones.[6] Inexperience in operation and troubleshooting increases the risk of failure during production.[4,5,7] Batch processes are well-studied and well-tested with countless success stories that newer processes can pull information from.[14] Continuous processes are a risk as they are novel technologies.[14] This body of research continues to grow as more studies are published proving that multi-step active pharmaceutical ingredients, such as Ibuprofen, Diazepam, and Clozapine, can be successfully synthesized in flow processes.[9,12]

1.4 Enantiospecific synthesis of active pharmaceutical ingredients in continuous processing

Among the current areas of interest is the synthesis of chiral molecules in continuous flow systems.[12] Chiral molecules play a large role in many active pharmaceutical ingredients as a vast number of bodily functions are stereospecific.[16] Enantioselective processing is particularly suited for continuous processing as the addition of complex steps needed in batch processing to purify enantiomers create large amounts of waste.[16] One specific of enantioselective synthesis used to create purely R-enantiomers is dynamic kinetic resolution.[17] This method uses a biocatalyst to convert only the R-enantiomer of the racemic reagent into the corresponding R-enantiomer product.[18,19] A second reaction, racemization, uses a metal catalyst to convert the leftover S-enantiomer back into the racemic mixture to improve the yield.[17,19,20] These two reactions have been performed simultaneously in batch[19,20] and studied before in a continuous flow setup, but

more research is needed to establish the success of this method in continuous flow.[17] Adding to this area of research would combine the benefits of enantioselective catalysis and continuous flow methodology.

CHAPTER 2 BACKGROUND AND LITERATURE REVIEW

2.1 Dynamic Kinetic Resolution Overview

Kinetic resolution (KR) refers to the use of a stereospecific enzyme catalyst to selectively transform one enantiomer of a compound into a desired product while the other enantiomer remains largely unreacted. This selective conversion allows the desired chiral configuration to be separated from the undesired enantiomer by physical or chemical methods that are simpler and more cost-effective than chiral separation techniques. As such, kinetic resolution is a common operation in the production of small-molecule active pharmaceutical ingredients (APIs) that require enantiopurity. However, the effectiveness of kinetic resolution is limited by the abundance of the desired in enantiomer; in a racemic mixture, no higher than a 50% conversion can be achieved. This limitation can be overcome by employing dynamic kinetic resolution (DKR), where the undesired enantiomer is continuously racemized simultaneously with the kinetic resolution process. The constant conversion of the unwanted enantiomers increases the theoretical yield of the kinetic resolution to 100%. However, significant challenges exist in developing systems where the racemization catalyst and the kinetic resolution catalyst can operate simultaneously.[17]

2.2 1-Phenylethanol

One compound of interest to the pharmaceutical industry with relevance to DKR is 1-phenylethanol. 1-phenylethanol is a secondary phenyl alcohol with a chiral center on its *sec* carbon. 1-phenylethanol is frequently used as a starting reagent in asymmetrical organic syntheses, and fluorinated derivatives of it are commonly used as active pharmaceutical ingredients (APIs). Highly enantioselective kinetic resolution of (*R)*-1-phenylethanol can be achieved using lipase enzymes, particularly the *candida antarctica* lipase B (CALB), in hydrophobic environments.

Meanwhile, the alcohol racemization can be achieved by using transition metal catalysts or zeolites.[18]

Solubility limits of 1-phenylethanol in solvents used for racemization and kinetic resolution are important to note as precipitation of 1-phenylethanol out of solution can occur when this solubility is reached. In water, a solvent used in racemization with zeolite catalyst,[16] solubility limits are reached at approximately 15.5 mg/mL at 20°C in water.[17] 1-phenylethanol is much more soluble in organic solvents.[18] Its solubility in toluene, a common organic solvent used in dynamic kinetic resolution[16,19,20] of 1-phenylethanol with lipase and zeolite catalysts is >1000mg/mL at 20°C.[21]

2.3 Kinetic resolution of racemic 1-phenylethanol

Kinetic resolution refers to the technique of isolating one enantiomer of a chiral compound via a stereospecific chemical reaction. This method is a critical step for manufacturing small-molecule chemicals used in food, agriculture, and pharmaceuticals, where enantiopurity is critical for ensuring the product's safety and efficacy. Biocatalysts are used increasingly often in kinetic resolution processes because of their greatly reduced environmental risks they pose when compared to traditional chemical catalysts. Lipases are one of the most popular enzymes in industry due to their low cost, easy availability, high thermal stability, and compatibility with a wide range of solvents and substrates. They are often used when immobilized to inert resins to improve their recyclability and separability from product media. *Candida antarctica lipase B* is one of the most commonly employed lipases due to its unusually high activity, and its interaction with (*R*, *S*)-1-phenylethanol is a commonly used and deeply studied case study in the technique of

kinetic resolution.[22] This subsection will review *candida antarctica lipase B* and its interaction with 1-phenylethanol.

2.3.1 Origin and function of *candida antarctica lipase B*

Candida antarctica lipase B (CALB) is a lipase enzyme expressed naturally by *candida antarctica*, a yeast native to Antarctica. For industrial applications, CALB is usually extracted from recombinant *Aspergillus oryzae*, which is capable of generating higher yields of the protein than natural *candida antarctica*.[22] The enzyme is expressed by the LIPB gene, and its primary amino acid sequence is well-studied and known to be 317 amino acids long.[23] The enzyme has two main catalytic functions that are of industrial interest: hydrolysis and transesterification. The hydrolysis reaction is the main natural function CALB, and it decomposes esters into their corresponding carboxylic acids and alcohols. The hydrolysis reaction dominates in aqueous media due to a conformational change where the hydrophobic α5 domain of CALB (a series of hydrophobic residues at positions 141-147) obscures the main active site of the enzyme.[24]

That active site is the source of CALB's other main catalytic function: transesterification. In this reaction, a secondary alcohol is combined with an acyl group donor to form an ester. The balanced equation of this reaction with (*R*, *S*)-1-phenylethanol as the alcohol substrate is pictured below:

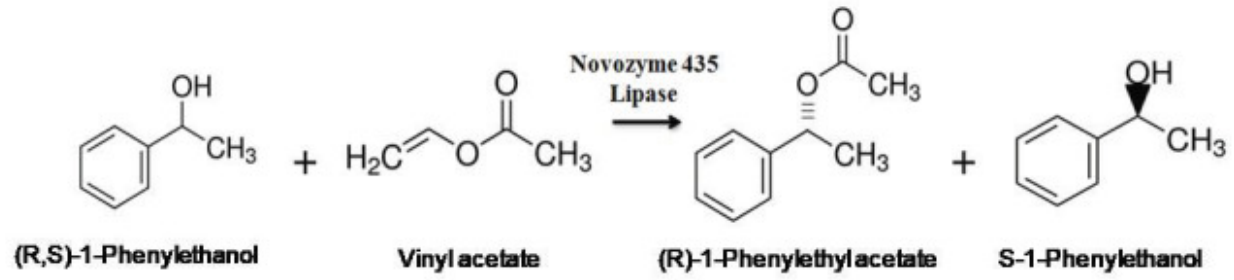


Figure 1. Balanced reaction for kinetic resolution of (R,S)-1-phenylethanol with CALB.[22]

The catalytic mechanism for this reaction has been attributed to three residues in CALB: Ser105, His224, and Asp187.[22] His224 is the most important catalytic residue, using its basic properties to abstract the proton from (*R*)-1-PE's hydroxyl group. This hydrogen abstraction makes the hydroxyl group a very strong nucleophile, allowing it to perform Sn1 substitution on the electrophilic carbon in the first position of the acyl donor's alkyloxy chain. The acid Asp187 uses its hydrogen bonding capabilities to fix His224 in a favorable position for this abstraction, while the polar Ser105 serves to stabilize the alkoxy anion intermediate that forms as a result of the substitution.[24] The role of the residues in the mechanism is summarized in the figure below:

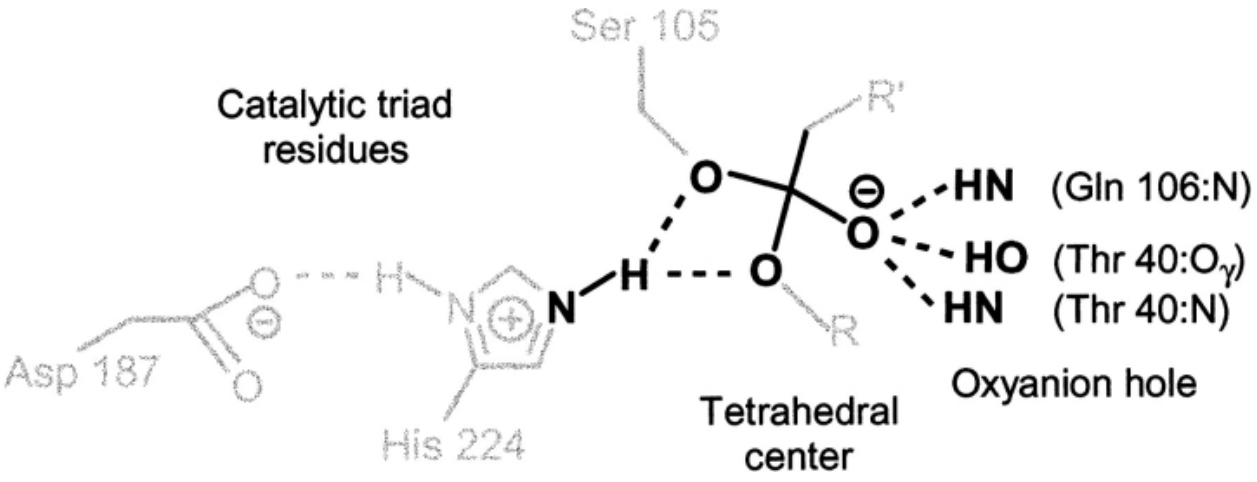


Figure 2. Diagram of residues active in CALB's alcohol transesterification mechanism.[24]

The specificity of His224 and Ser105's positioning in this mechanism is responsible for CALB's high selectivity to the *R* enantiomer when catalyzing transesterification.[24] Substrate access to this

reactive site is only possible when the hydrophobic α5 lid is in its open configuration, so CALB can only support this transesterification reaction efficiently in nonpolar organic media.[23]

2.4 Zeolite racemization of 1-phenylethanol

Zeolite catalysis has historically proven to be a more effective method of racemizing 1-phenyl ethanol than comparable transition metal or biochemical methods, and zeolites hold the advantage of being heterogeneous catalysts, which makes them easier to separate from the product medium. This section discusses the structure of zeolites and their specific effects on 1-phenylethanol racemization.[16]

2.4.1 Zeolite structure and function

"Zeolites" are conventionally defined as crystalline ionic structures composed of alternating AlO_4 and SiO_4 formula units. Zeolite salts can form naturally or be synthesized by heating aluminosilicate gel to high temperature in very alkaline aqueous solution. Natural zeolites are generally comprised of approximately five times as much SiO_4 as AlO_4, but synthetic zeolites can have Si:Al ratios that are much higher (however, all zeolites must have more SiO_4 than AlO_4 in their framework). The aluminum substituents are what gives zeolites their catalytic activity. The presence of an aluminum ion in place of a silicon atom creates a small local negative charge within the zeolite crystal structure. This partial charge is balanced by alkali metal cations trapped in the framework, which can be replaced by H^+ ions in aqueous solution. The presence of H^+ ions at aluminum sites gives zeolites acidic properties, which lets them facilitate acid catalysis mechanisms. Thus, zeolite activity is increased when there is a higher abundance of aluminum in the framework. Zeolites have a functional advantage over homogenous acid catalysts due to their

pores, which rarely exceed 0.8 nm in diameter. These very fine pores highly restrict the chemical availability of the zeolite acid sites, improving the selectivity of the catalyst. [19]

2.4.2 Zeolite catalysis of 1-phenylethanol racemization

Acidic zeolites catalyze the racemization of 1-phenylethanol through the acid-catalyzed dehydration and S1 substitution of the alcohol's hydroxyl group. When the alcohol comes into contact with the zeolite's catalytic site, it abstracts the acidic hydrogen from its region of partial negative charge. This transforms the relatively stable HO- group on the 1-PE molecule into H_2O^+, a very effective leaving group. This ionic structure detaches from the 1-PE as H_2O, creating a trigonal planar carbocation. Both sides of the carbocation are equally sterically favorable, so if a H_2O nucleophile performs a S1 substitution on the intermediate, 1-phenylethanol will be returned as a product with both enantiomers present in roughly equal quantities. There is significant opportunity for product loss in this mechanism; in the presence of excess acid, the hydration substitution become less favorable, and the carbocation undergoes E1 elimination to form a styrene byproduct instead.[20]

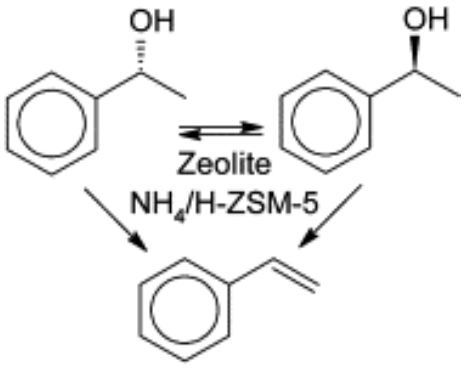


Figure 3. Equilibrium of 1-phenylethanol with styrene in the presence of a zeolite catalyst.

In addition to consuming 1-phenylethanol and decreasing the theoretical yield of dynamic kinetic resolution, the styrene generated from this side reaction can polymerize and accumulate

within the pores of the zeolite. This process, known as "coking", diminishes the catalytic effectiveness of the zeolite. As a result, styrene conversion is one of the primary obstacles in racemizing 1-phenylethanol with zeolites. This issue can be mitigated by reducing the aluminum content (and thus, the acidity) of the catalyst.[16] Experimentation performed by Zhu et. Al demonstrated that reducing the Si:Al ratio of an Al-Beta zeolite catalyst from 150 to 300 reduced the selectivity of styrene formation from almost 60% to essentially 0%.[21] In this same study, higher temperatures (>60ºC) resulted in more byproducts during racemization even though reaction rates would be faster. Higher temperatures (>100 ºC) are used to dehydrate phenyl-ethanol to styrene with zeolite catalysis in some applications.[22]

2.4.3 Optimal zeolite composition for 1-phenylethanol racemization

Screening experiments performed by Costa et. al determined the optimal combination of zeolite type and solvent. The group tested four different zeolites – H-Beta Si:Al 12.5, H-Beta Si:Al 75, H-LZY-82, and HY – by racemizing (*R*)-1-PE in five different solvents: water, ethyl acetate, vinyl acetate, isooctane, and *tert*-butyl methyl ether. The performance of the catalysts was evaluated using the "enantiomeric excess" of the reaction mixture after a sampling period of 24 hours. Enantiomeric excess is defined as the difference in concentration of the enantiomers divided by the total 1-PE concentration. A lower enantiomeric excess indicates a more complete racemization. The calculation of enantiomeric excess is shown below:

$$\%ee = \frac{|(R)PE - (S)PE|}{(R)PE + (S)PE} \times 100\%$$

Of the combinations studied, the best enantiomeric efficiencies with the lowest rate of byproduct conversion were observed in the following combinations: H-Beta Si:Al 75 in ethyl

acetate, H-Beta 12.5 in isooctane, H-Beta 12.5 in water, and H-Beta 12.5 in vinyl acetate. The H-Beta zeolite family was concluded to be the most promising catalyst for the aqueous racemization of 1-PE. However, the group noted that kinetic resolution operations would not be compatible with an aqueous medium due to inactivation of the lipase catalyst.[16]

2.5 Dynamic Kinetic Resolution of 1-Phenylethanol

The dynamic kinetic resolution of 1-phenylethanol combines the core kinetic resolution reaction with the supplementary racemization step. This is accomplished by cycling the 1-PE through two reactors in series or combining the catalysts into a single mixture. In both configurations, the system works by first esterifying the (*R*)-1-PE into (*R*)-1-phenylethylacetate. The racemization step then converts the excess *S* enantiomer back into a racemic mixture, continuously refreshing the supply of *R*-alcohol. If enough of these cycles occur, the theoretical yield of the *R*-ester eventually becomes 100%. This process is visualized by Figure 4:

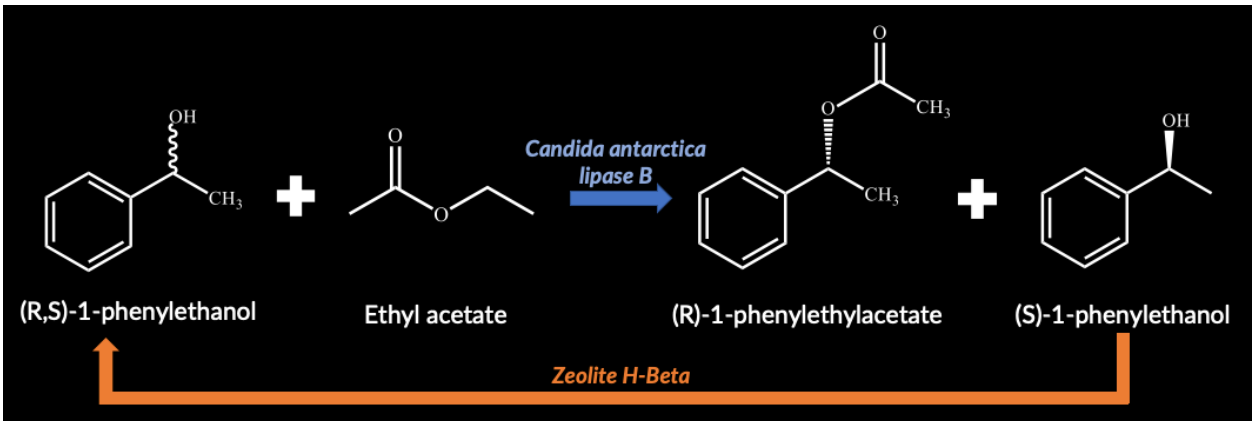


Figure 4. Visualization of 1-phenylethanol DKR.

DKR of 1-phenylethanol with this reaction scheme has been successfully realized in a batch system. However, there are no peer-reviewed instances of it being employed in a flow reactor; the

only continuous flow 1-PE DKR reactors have used ruthenium catalysts, which are significantly more expensive toxic, and sensitive to changes in reactor conditions than zeolites.

2.6 Analytical methods for 1-phenylethanol dynamic kinetic resolution

Costa et. al used a HP5890A gas chromatography with a Varian CP-Chirasil-Dex CB (25 m) and a flame ionization detector (FID) to measure the concentration of each 1-PE stereoisomer in their experiments.[21] Gas chromatography (GC) is a popular method of separating enantiomers and determining their concentrations, as it is a simpler method than alternatives such as liquid chromatography that require the management of factors such as mobile phases and pH. Like all GC systems, chiral chromatographs consist of a column affixed with a stationary phase that applies differential intermolecular forces to components in the analyte that cause them to elute at different rates. Chiral columns use one of three main types of stationary phases: non-racemic chiral amino acids that interact with analytes using hydrogen bonds; non-racemic chiral metal coordination compounds that form complexes with the analyte; and derivatives of cyclodextrin. The column used by Costa et al. utilizes a cyclodextrin derivative, permethylated β-cyclodextrin, as its stationary phase. This structure is fixed to the polydimethylsiloxane wall of the column using a polyethylene chain, and the cyclodextrin itself forms a conical structure with a hydrophilic exterior and a hydrophobic interior. The molecular structure of the column's stationary phase is displayed in Figure 5:

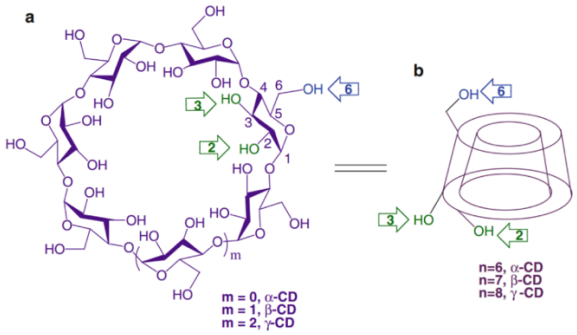


Figure 5. Structure of cyclodextrin stationary phase in chiral column.[25]

This variation in hydrophobicity and Van der Waals forces along the surface of the cyclodextrin network are what cause the cyclodextrin's differential interactions with stereoisomers.[25]

2.6.1 Gas Chromatography Parameter Optimization

For use with the Agilent CP-Chirasil-Dex CB Column, Agilent suggests the following parameters: H_2 carrier gas at 14.5 psi, oven temperature of 100-130 C (ramp 2 °C per minute), and split inlet with an FID (Appendix A). Following these parameters, an elution time of approximately 5 minutes is expected. To further optimize GC resolution, several parameters can be altered: Split ratio, injection volume, carrier gas, and temperature. High concentrations or injection volumes can result in baseline drift of a sample. Increasing the split ratio and decreasing the injection volume are both ways to combat this issue. The split ratio describes how much of the carrier gas and sample are entering the GC versus how much is being released through the split vent (**Figure 6**).[26] Agilent suggests ratios between 20:1 and 100:1, with the lowest ratio of 1:10 for a column of inner diameter 0.25mm.

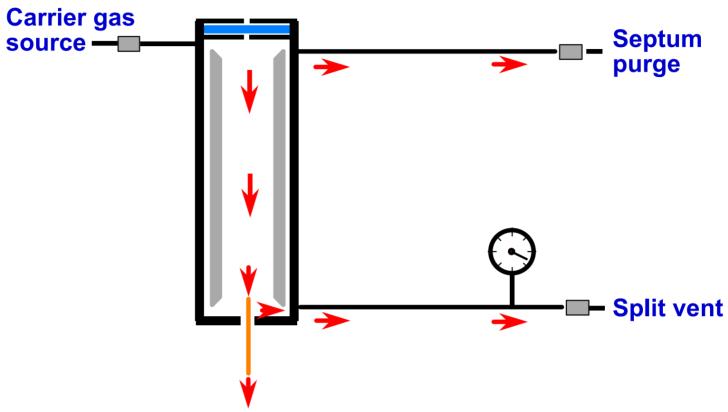


Figure 6. Demonstration of the split ratio.[26]

Oven temperature impacts the speed at which samples elute. Higher oven temperatures offer faster elution but can also decrease the separation between samples eluted closely together. For samples with many peaks eluting over a prolonged period of time, ramping the temperature with a constant rate over a prolonged period of time will maintain the same peak width for all samples eluted while peak width is altered for later eluted peaks if the temperature is kept isothermal.

2.7 Fundamentals of Packed Bed Reactor Design

The general method of examining continuous flow systems in the laboratory is to continuously pump reagent solutions into a reactor over an extended period of time. The most generic plug flow laboratory-scale reactors consist of a length of flexible tubing placed in temperature conditions that promote reaction progress. This tubing is often coiled multiple times to increase the volume of the reactor. While this equipment scheme is suitable for many homogenous reaction not requiring packing materials, it is ill-suited for heterogeneous catalytic reactions. For this purpose, packed bed reactors are the standard design option. The packed bed

reactors are generally stainless steel, straight tubing, with diameters wide enough to fit the specified packing material.

2.7.1 Reactor volume and packing design considerations

These small reactors have the same general operating mode as homogeneous reactors, but the reactor coil is replaced with a "packed bed", a section of stainless steel tubing packed with solid catalyst and static mixing agents. High performance liquid chromatography (HPLC) columns are a common choice for this tubing. Meanwhile, packing material varies significantly for every design. In very small volume reactors with sufficiently low heat effects to avoid hots spots, inert material may even be omitted entirely from the reactor. Regardless of the character of the packing, a common heuristic is that the packing particle diameter must be 10% or less of the bed diameter.[27] This rule of thumb ensures that catalyst particles must be small enough to support sufficient contact between the reactor feed, and the catalyst is sustained to promote mass transfer and reaction. Particles that are too large can introduce mass transfer limitations, have poor mixing/channeling due to wall effect, and can be difficult to physically pack into the bed. However, caution must also be taken to ensure that the particle size not so small as to cause pressure drop across the reactor that overwhelms the system's pump.

Residence time is another consideration needed in a packed-bed reactor setup. Residence time describes the length of time it takes for one molecule of the reactant to move from the inlet of the reactor to the outlet. It is mathematically defined as the reactor dead volume divided by the flowrate. Thus, flowrate is inversely proportional to residence time. Typically, higher yield occurs with longer residence times as the reagent has more time to be in contact with the catalyst particles contained inside the bed.[27]

To predict the pressure drop caused in a packed bed by a particular particle size, the Ergun equation. The Ergun equation is an empirical correlation that predicts pressure drop as a function of fluid properties, the relative geometries of the reactor and packing material, and the flow rate of the fluid. The complete Ergun equation is expressed by equation 2 below:[28]

$$\frac{\Delta P}{L} = \frac{150\mu(1-\epsilon)^2 u_o}{\epsilon^3 d_p^2} + \frac{1.75(1-\epsilon)^2 \rho u_o^2}{\epsilon^3 d_p}$$

In this expression, ΔP is the pressure drop across the bed, μ is the velocity of the fluid, d_p is the particle diameter of the packing material, u_o is the superficial velocity of the fluid, ρ is the density of the fluid, L is the length of the packed bed, and ϵ is the void fraction of the bed. The Ergun equation is not expected to deliver highly accurate results, but it is capable of generating order of magnitude predictions suitable to guide the selection of packing particle size, in addition to other flow process components.[28]

2.7.2 Auxiliary components of packed bed reactor design

The reactor bed is the key component of a micro-packed bed continuous flow systems, but there are several other critical components to the successful design of this type of reactor. The first are pumping devices. Per Jamison et. all, syringe pumps are the most suitable pressure source for micro-packed bed reactors.[27] These devices allow syringes loaded with reagent solution to be discharged at very precise volumetric flow rates, with precision at the nanoliters per minute magnitude. Other components common to laboratory-scale continuous flow reactor systems are static mixers and back-pressure regulators. Static mixers are stationary obstructions in the flow path of a fluid system that disrupt flow layers and mitigate radial concentration and temperature

variations. Static mixers can be sections of tubing packed with irregularly-folded plastic rods, specially-made, geometrically-complicated flow devices, or simple layers of inert material (e.g. silica beads) in reactor packing. These components are necessary to install after tubing junctions to ensure the complete mixing of combined process streams. Backpressure regulators are devices that apply pressure to the flow system in the opposite direction of flow. They are needed to mediate the system pressure when large fluctuations occur due to small packing particles, reaction phase changes, or heat effects in the reactor. A generic diagram of a laboratory-scale plug flow reactor can be viewed in Figure 7:

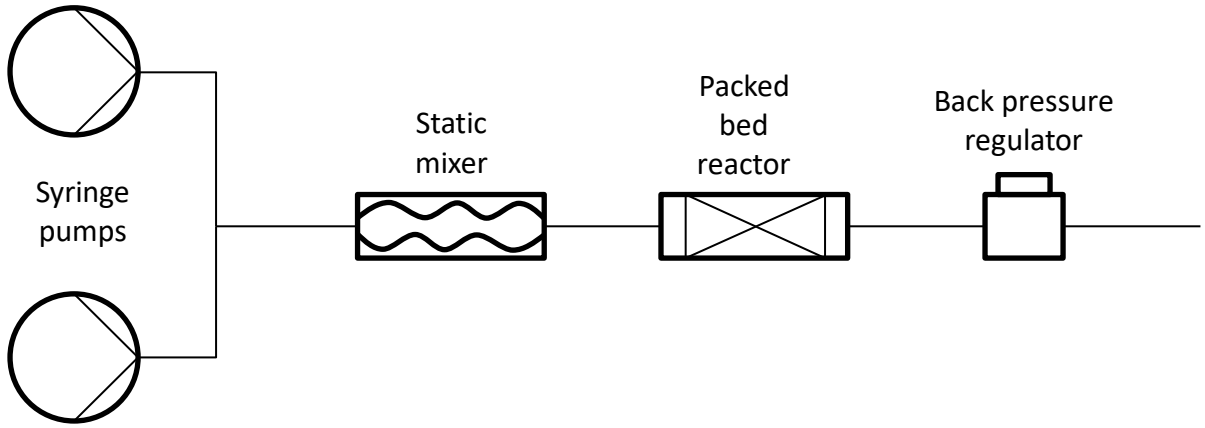


Figure 7. Generic packed bed reactor schematic.

CHAPTER 3 Experimental Methods

This chapter discusses the series of experiments that were performed to develop the zeolite-catalyzed DKR reactor. Instrumental analysis of all experimental results was performed on a Agilent gas chromatographs (GC) (6890 and 7820A) with an Agilent CP-Chirasil-DEX CB 0.25 mm x 25 m chiral column.

3.1 Determine operating parameters of Agilent 7820 gas chromatograph for 1-phenylethanol separation

The first work completed to analyze the zeolite racemization of 1-phenylethanol was the determination of the operating parameters of the utilized HP5890 CG that provided the best peak resolution for (*R*)-1-phenylethanol and (*S*)-1-phenylethanol. To obtain optimal resolution of the enantiomer separation, several different parameters were altered: carrier gas, split ratio, temperature settings, inlet, and injection volume. The column used to achieve separation of the 1-phenylethanol enantiomers was Agilent CP-Chirasil-Dex CB Column, a fused silica capillary column of 0.25 mm inner diameter and 25 m long. Initial parameters were suggested by Agilent (Appendix A) but a variety of settings were tested on two different GC systems to identify best resolution at multiple concentrations ranging from 5-25 mg/mL of 1-phenylethanol in water. Column width was adjusted to 1mm after seeing poor resolution on the 7820A GC. Baking for 8 hours at 130 °C was performed after Trials 1-2 in the 6890A and before Trials 3-8 in the 7820A. All samples were analyzed with FID. Key trials are listed in **Table 1**.

Table 1. GC settings for the optimization of parameters for the chiral separation of 1-PE.

Trial	GC	Concentration (mg/mL)	Inlet	Carrier Gas	Temp Setting	`Time (s)	Injection Volume (uL)	Split Ratio
1	6890A	15	Purge-Packed	N_2	100-130	15	5	N/A
2	6890A	15	Purge-Packed	H_2	100-130	15	5	N/A

3	6890A	5	Purge-Packed	H_2	100-130	15	5	N/A
4	6890A	5	Purge-Packed	H_2	100-130	15	1	N/A
5	6890A	5	Purge-Packed	H_2	100-130	15	0.2	N/A
6	7820A	25	Split/Splitless	H_2	100	20	0.2	1:10
7	7820A	25	Split/Splitless	H_2	100	20	0.2	1:100
8	7820A	5	Split/Splitless	H_2	100	20	0.2	1:100

3.2 Chromatogram Peak Identification

To identify peaks present in the chromatograms, solutions were prepared that isolated key components of both the reagent and the product. The chromatograms of all listed solutions (**Table 2**) were compared to determine peak identities. Solutions 1-5 were prepared in a volumetric flask on an analytical balance to ensure accurate concentrations. Sample products (solutions 6-8) were chosen from the racemization, kinetic resolution, and dynamic kinetic resolution flow experiments. These sample products chosen were the samples eluted at the sixth reactor volume for the 60 min residence times as these particular products demonstrated the most successful completion of the reaction. All samples were analyzed on the GC on the same day as solution preparation.

Table 2. Solutions used to identify peaks in chromatograms.

Solution Number	Solution	Peaks Identified
1	Pure Toluene	Toluene
2	Toluene with 30 mg/mL ethyl acetate	Toluene, ethyl acetate
3	Toluene with ~30mg/mL ethanol	Toluene, ethanol
4	Racemization Reagent (15 mg/mL 1-(R)-phenylethanol, 30 mg/mL ethyl acetate)	1-(R)-phenylethanol, toluene, ethyl acetate
5	KR/DKR Reagent (15 mg/mL 1-(R,S)-phenylethanol, 30 mg/mL ethyl acetate)	1-(R,S)-phenylethanol, toluene, ethyl acetate
6	Racemization reaction product	1-(R,S)-phenylethanol, toluene, ethyl acetate, possibly styrene
7	Kinetic resolution product	1-(R,S)-phenylethanol, toluene, ethyl acetate, possibly ester

8	Dynamic kinetic resolution	1-(R,S)-phenylethanol, toluene, ethyl acetate, possibly styrene and ester

3.3 Validation in Batch Process

Once the separating parameters for the GC were determined and a calibration curve for 1-phenylethanol was constructed, experiments were conducted to verify the catalytic performances of H-Beta zeolite in batch racemization of 1-phenylethanol and Lipase B in the kinetic resolution of 1-phenlethanol. These experiments were not intended to be a one-to-one comparison of batch to continuous flow systems but rather a validation of the success of catalytic performances as established in the reviewed papers. Success was determined based on similarity to previously established %ee in literature. The batch validation set-up for both racemization and kinetic resolution were performed in a beaker on a hot plate, with a thermometer monitoring temperature (**Figure 8**). The beaker was covered with aluminum foil to retain heat.

Figure 8. Reactor setup for batch validation experiments.

3.3.1 Racemization reaction in batch

For all experiments, H-Beta Si:Al 300 was used as the racemization catalyst, (*R*)-1-phenylethanol (99% pure, 97% ee) was used as the substrate, and water was used as the reaction medium. Initial batch experimentation was run using two different concentrations of 1-(R)-phenylethanol: 15mg/mL and 18 mg/mL. Reagent solutions were created in a volumetric flask on an analytical balance to avoid losing reagent. Reaction volumes used were 1 mL of reagent. The ratio of Zeolite-Beta catalyst to reagent was 50mg catalyst: 10mL reagent, so approximately 50 mg of catalyst was used for all reagent concentrations. Catalyst aggregate particle size was 150-250 um with individual particle size around 700 nm. Catalyst was massed inside reaction vessels then 2 mL of reagent was pipetted into vessel. The reaction was allowed to run for 90 minutes with constant stirring and the cap closed. 0.3 mL aliquots were withdrawn from the reaction vessel at time points of 60 minutes and 90 minutes. Catalyst was filtered out of the reaction vessel to quench the reaction with a 0.22 um syringe filter. The solutions were then filtered a second time to remove the remaining catalyst particles. Solutions were then closed with caps and wrapped with parafilm to avoid evaporation. Concentrations were then analyzed using the HP 7820A Agilent Technologies Gas chromatograph with a Cp-7502 Chirasil Dex-CB Column. Parameters used for operation were the same as Trial 7 in **Table 1**.

To further validate the feasibility of the 1-PE racemization study in water, an identical reagent solution was added to 50 mg of zeolite catalyst in a sealed vial and placed in a water bath over a hot plate. The temperature of the vial was maintained at approximately 45°C by wrapping the water bath in tin foil and adjusting the hot plate output until the temperature remained roughly stable, indicating a thermodynamic steady state. The reaction was allowed to continue for 24 hours,

with 0.3 mL aliquots being collected at 12 hours and 24 hours. 0.2 uL of each aliquot were injected to the GC at the established parameters to monitor the progress of the racemization.

3.3.1.1 Effect of catalyst concentration

The first series of batch experiments were performed to characterize the effect of zeolite concentration on racemization. Five runs as were conducted using a 1 mL reaction solution with a 1-PE concentration of 15 mg/mL. The zeolite concentration was varied in each run from 12 mg/mL to 18 mg/mL. The least concentrated run was conducted first and the zeolite concentration was increased by increments of 3 mg/mL for each successive run. Each run was permitted to react for 35 minutes, after which a 0.2 µL aliquot was run through the GC for 20 minutes at the previously determined optimal parameters. The area beneath the peaks corresponding to the *R* and *S* enantiomers was recorded and compared to the calibration curve to ascertain the concentration and enantiomeric excess of the solution.

3.3.1.2 Effect of reactant concentration

The next set of batch experiments sought to identify the effect of 1-phenylethanol concentration on racemization performance. Runs were conducted using water as a solvent, H-Beta Si:Al 300 zeolite at the best-performing concentration identified in previous experiments, and three different starting concentrations of (*R*)-1-phenylethanol. All tests were completed using a 1 mL reaction volume and were allowed to proceed for 2 hours before analysis. The substrate concentrations ranged from 12 mg/mL to 15 mg/mL, increasing in increments of 3 mg/mL. After the reaction period, a 1 µL aliquot of each solution was run in the GC for 15 minutes at the selected optimal conditions, and the peak area of each enantiomer was used to determine the enantiomeric efficiency.

3.3.2 Kinetic resolution in batch

A 2 mL solution containing 15 mg/mL (*R*, *S*)-1-phenylethanol and 30 mg/mL ethyl acetate in toluene was mixed and placed in a vial with 100 mg/mL of immobilized lipase beads. The system was placed under low stirring and allowed to sit for 24 hours at room temperature (approximately 20 °C). After 24 hours passed, a 0.2 μL aliquot of the reaction solution was analyzed in the GC to examine the change in enantiomeric excess compared to the starting material.

3.4 Characterization of Continuous Flow Systems

After verifying the success of the kinetic resolution and racemization reactions in batch with the chosen catalysts, different packed-bed reactors of the same 4mm inner diameter were constructed to test the characterize the three reactions in flow. As initial proof-of-concept-work purely for the racemization reaction, various temperatures and residence times were studied for the racemization reaction using water as a solvent in a 38 cm PBR. To characterize all three reactions in a one-to-one manner, identical residence time studies were performed for the racemization, kinetic resolution, and dynamic kinetic resolution reactions using toluene as the solvent, at a temperature of 40ºC in separate 10 cm PBR (**Table 3**). All samples were analyzed by injecting 0.2uL into the GC, with GC operating parameters set to those established in section **3.1** .

Table 3. Specifications for continuous flow reactor experiments.

Reaction	PBR Length (cm)	Catalyst	Solvent	Residence Time (min)	Temperature (ºC)
Racemization	38	H-Beta Zeolite	Water	60, 120, 180	20, 45, 70
Racemization	10	H-Beta Zeolite	Toluene	5, 15, 30, 60	40

Kinetic Resolution	10	Lipase B	Toluene	5, 15, 30, 60	40
Dynamic Kinetic Resolution	10	5:3 ratio of H-Beta Zeolite to Lipase B	Toluene	5, 15, 30, 60	40

3.4.1 Packed-bed reactor construction

All reactor beds were comprised of a stainless-steel Agilent liquid chromatography column of 4mm inner diameter. Each column was fit with 1/4" to 1/16" Swagelock unions and ¼" stainless unused steel frits with a particle exclusion size of 10 μm. Reagent was held in a polypropylene syringe and delivered using a Harvard Apparatus PhD syringe pump. All connections were built with DuPont 1/16" PFA tubing and the appropriately sized connections. Only one feed stream was connected to the system, so no static mixer was utilized. To comply with the heuristic established in Jamison et. al, the catalyst particle size for the zeolite was chosen to be 150-250 μm. Pressure drop was calculated for each reactor using the Ergun Equation (Appendix C, **Calculation 1**). Because of these low pressure drops, a back pressure regulator was deemed unnecessary for the system and not installed (**Table 4**).

Table 4. Reactor design and sizing.

Reaction	PBR Length (cm)	Catalyst	Catalyst Particle Size (um)	Pressure Drop (psi)
Racemization	38	H-Beta Zeolite	150-250	0.023
Racemization	10	H-Beta Zeolite	150-250	0.00084
Kinetic Resolution	10	Lipase B	150-500	0.00084
Dynamic Kinetic Resolution	10	5:3 ratio of H-Beta Zeolite to Lipase B	150-500	0.00084

Heating tape and an Omega Type-K Controller were used to heat each column. A thermocouple was placed at the T-junction shortly after the exit of the reactor to measure the outlet temperature of the solution leaving the column. The column, connecting piping, and T-junction were wrapped in one layer of heating tape, then covered with cloth insulation to prevent heat from escaping. A conceptual diagram and picture of the reaction system can be viewed in **Figure 9** and **Figure 10**.

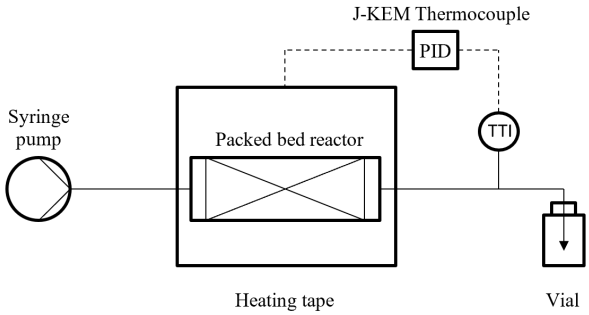


Figure 9. Conceptual diagram of the continuous reactor system.

Figure 10. Lab set-up of the continuous reactor system.

3.4.2 Catalyst preparation and loading

Each catalyst required slightly different methods of insertion into the column due to their difference in physical properties. The completed reactor bodies were all massed prior to packing. After packing, the top of each reactor was sealed with the appropriate frit and fittings, and the filled reactor was weighed to determine the catalyst load.

3.4.2.1 Racemization: Zeolite catalyst

The H-Beta Si:Al 300 zeolite was packaged as a very fine powder with a primary particle size of 700 nm. To increase the secondary particle size to the 150-250 μm value established in section 3.2, the catalyst was loaded into a 2-inch pelletizing die. This loaded die was placed on hydraulic press and pressurized to the maximum possible value. The press was allowed to rest for approximately 30 seconds before the die was pressed again at the same pressure and duration. The resulting catalyst cake was reduced to a coarse powder in a mortar and pestle. The power was fed into a 250 μm sieve that was secured above a 150 μm sieve. The sieves were shaken vigorously for 5 minutes. Coarse particles that remained above the 250 μm sieve were placed back into the mortar and pestle to be further refined, while fine particles that fell below the 150 μm sieve were collected to be re-pelletized. The catalyst trapped between the two sieves was collected as the material to be packed into the reactor.

To load the granularized zeolite into the reactor bed, the 150-250 μm catalyst was loaded into a 12 mL syringe. This syringe was fitted with a pipette cap with a hole cut in its tip to match the diameter of the selected LC column's opening. One cap and frit was removed from the selected LC column to create an opening for the catalyst. The syringe was pressed to deposit catalyst into the bed until it became full. Once the column was completed full, a pipette tip was used to pressure the catalyst down to ensure complete packing.

3.4.2.2 Kinetic resolution: Lipase B catalyst

Candida antarctica lipase B immobilized on Immobead 150 resin beads was purchased with a specific activity of ≥2000 U/g. The completed reactor body was massed prior to packing. The resin beads were observed to exert strong electrostatic forces on one another, making dry transfer

into the reactor bed difficult. To minimize this problem, the reactor body, transfer funnel, transfer spatula, and lipase storage bottle were rubbed with dryer sheets to remove excess charge. The lipase beads were added to the packed bed via a funnel until they reached the top of the column and could not be impacted into it further.

3.4.2.3 Dynamic kinetic resolution: Zeolite and Lipase B catalyst

Due to the faster kinetics of the kinetic resolution reaction in comparison to the racemization reaction, H-Beta Zeolite and Lipase B catalyst were packed into the reactor at a 5:3 mass ratio.[20] Dryer sheets were used to wipe down all contact surfaces before adding the lipase B. Using an analytical balance to measure mass, both catalysts were added to a dram vial. The catalysts were mixed by slowly turning the vial until the mixture looked sufficiently homogenous. Only 3 g of catalyst mixture were created to reduce the possibility of adding improper ratios due to poor mixing. Using a small pipette funnel, approximately 10 mg of the catalyst mixture was added to the reactor. Toluene was then pipetted into the column to wet the mixture. Air was pumped into the column to clear out the remaining toluene. A small metal instrument was used to gently pack down the mixture. This process was repeated until the catalyst mixture reached the top.

3.4.3 Residence time and temperature studies in water

Initially, racemization reactions were studied using water as a solvent. Multiple trials were conducted at multiple different temperatures and residence times in the 38 cm packed-bed reactor. **Table 5** organizes all trials performed with water as the solvent in chronological order (i.e. trial 1 was performed first, with Trial 7 being performed last) for the sake of clarity later in this section.

Table 5. Trials for racemization temperature and residence time study with water as solvent, organized in chronological order by trial number.

Trial Number	Replicate	Temperature (ºC)	Residence Time (hr)
1	1	45 ± 5	1
2	1	70 ± 15	1
3	1	20 ± 2	1
4	1	45 ± 5	3
5	2	70 ± 15	1
6	1	60 ± 5	1
7	2	45 ± 5	2

For the temperature study, the racemization reaction was run at four temperatures ranging between room temperature (20 ºC) and 70 ºC with a 1hr residence time (**Table 6** and **Table 7**). For the residence time study, the racemization reaction was run at 45 ºC with three different residence times selected:1 hour, 2 hours, and 24 hours.

Table 6. Trials for racemization temperature study with water as solvent.

Trial Number	Replicates	Temperature (ºC)	Residence Time (hr)
1	1	45 ± 5	1
2, 5	2	70 ± 15	1
3	1	20 ± 2	1
6	1	60 ± 5	1

Table 7. Trials for Racemization Residence Time Study with Water as Solvent.

Trial Number	Replicates	Temperature (ºC)	Residence Time (hr)
1	1	45 ± 5	1
4	1	45 ± 5	3
8	1	45 ± 5	2

Reagent was prepared at 15 mg/mL of 1-(*R*)-phenylethanol in water. To minimize loss of solution, solution was prepared in a volumetric flask on an analytical balance by first massing the 1-(*R*)-phenylethanol, then adding solvent to fill the remainder of the volumetric flask. Reagent was then heated and stirred to ensure complete homogeneity. Reagent was then analyzed on the GC periodically prior to some runs to validate reagent condition.

Each trial was conducted for the length of four residence times. The reactor was considered to have reached steady state after three residence times. Product was collected after the reactor had reached steady state. Trials 1-5 were performed in the first packed-bed reactor assembled. Contamination was discovered after trial 5, so trials 6-8 were performed in a new, identically assembled packed bed reactor system. Samples were capped, wrapped with parafilm, and stored at room temperature after each trial.

Trial 1 was analyzed on the GC on the day the experiment was performed. Trials 2-8 were analyzed 3-7 days after their samples were collected. Samples from trials 2-8 were heated and stirred for approximately 20 minutes before GC analysis to melt any precipitates that had formed.

3.4.4 Residence time studies in toluene

As kinetic resolution is incompatible with a water solvent, the racemization, kinetic resolution, and dynamic kinetic resolution reactions were then studied in toluene under identical conditions. Reactions were run in separate packed-bed reactors at four different flowrates corresponding to four residence times: 5 min, 15 min, 30 min, and 60 min. All reactions were operated at a temperature of 40 ºC, the optimal operating temperature for CALB.[29] Ethyl acetate and 1-phenylethanol were input at a 3:1 molar ratio. Parameters for the residence time studies are summarized in **Table 8**:

Table 8. Operating parameters for continuous flow residence time studies in toluene.

Reaction	Residence Times Studied (min)	Operating Temperature (°C)	Catalyst	Reactor length (cm)	Reagent
Racemization	5, 15, 30, 60	40	H-Beta Zeolite	10	15 mg/mL 1-(R)-phenylethanol, 30 mg/mL ethyl acetate in toluene

Kinetic Resolution	5, 15, 30, 60	40	Lipase B	10	15 mg/mL 1-phenylethanol, 30 mg/mL ethyl acetate in toluene
Dynamic Kinetic Resolution	5, 15, 30, 60	40	H-Beta Zeolite & Lipase B in 5:3 ratio	10	15 mg/mL 1-phenylethanol, 30 mg/mL ethyl acetate in toluene

To minimize loss of solution, solution was prepared in a volumetric flask on an analytical balance by first massing the 1-phenylethanol and ethyl acetate, then adding toluene to fill the remainder of the volumetric flask. The reagent was then stirred for complete homogeneity. After beginning the reaction, samples were collected for one full reactor volume. Each reaction was conducted for 6-8 residence times, the equivalent of 6-8 full reactor volume elutions. 6-8 samples were collected per run. Each sample was analyzed on the GC on the day of the reaction or the following day. Only one full trial for each reaction was included in the analysis. More trials were performed, but there were problems with establishing accurate flowrate, so they were excluded from analysis.

CHAPTER 4 Results and Discussion

This chapter reviews the results of the kinetic study of each component of the dynamic kinetic resolution system and the relevance of each study to complete system.

4.1 Agilent 7820 GC Method Development

Parameters yielding the best results were using the split/splitless inlet with H_2 as the carrier gas at a 1:100 split ratio, an injection volume of 0.2 µL and an isothermal temperature of 100 °C, held for 20 minutes. Trials 1-2, pre-baking, revealed a large baseline drift, although separation was observed at 15 mg/mL when H_2 was used as carrier gas (**Figure 11**). Little separation was seen when using N_2 as a carrier gas.

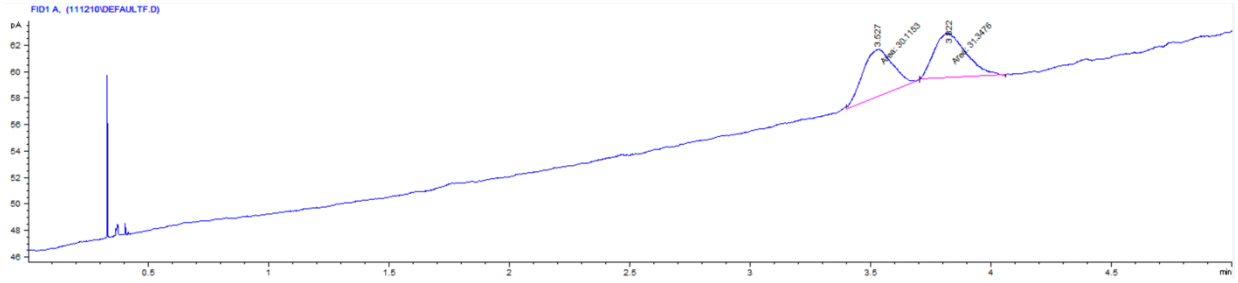


Figure 11. Chromatogram of Trial 2 (15 mg/mL, H2 gas, 6890, pre-baking).

Post-baking, large amounts of tailing were seen at injection volumes of 5 µL even when reducing the concentration to 5 mg/mL (**Figure 12**). Tailing was reduced when decreasing injection volume to 1 µL (**Figure 13**) and further reduced at 0.2 µL (**Figure 14**), but peaks were still not completely separated. The peaks eluted very closely together. Using higher concentrations of 1-phenylethanol even at 0.2 µL resulted in significant amounts of tailing.

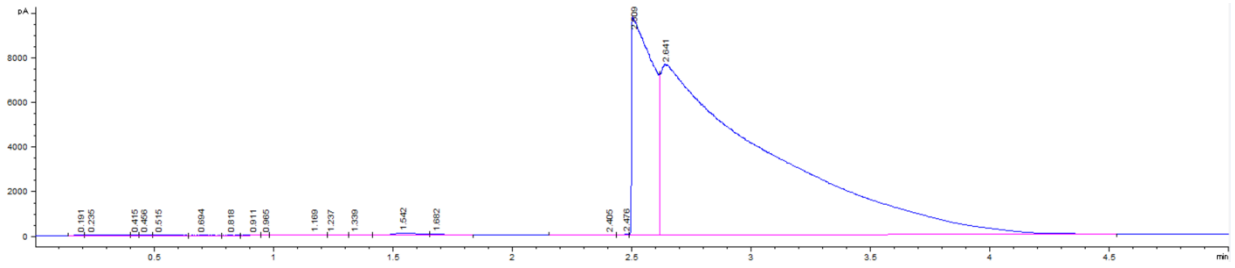


Figure 12. Chromatogram of Trial 3 (5 µL, 5 mg, H2 gas, 6890, post baking).

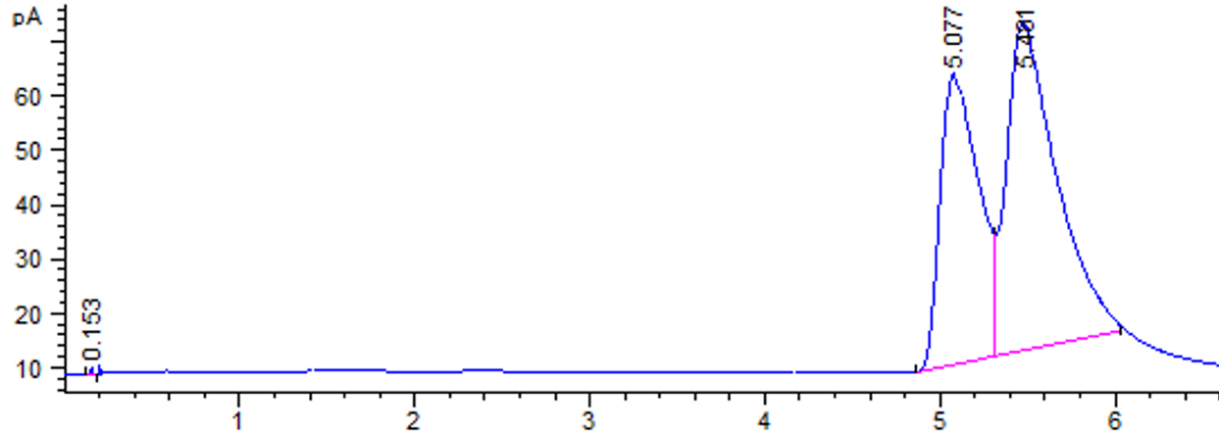


Figure 13. Chromatogram of Trial 3 (1 µL, 5 mg, H2 gas, 6890, post baking).

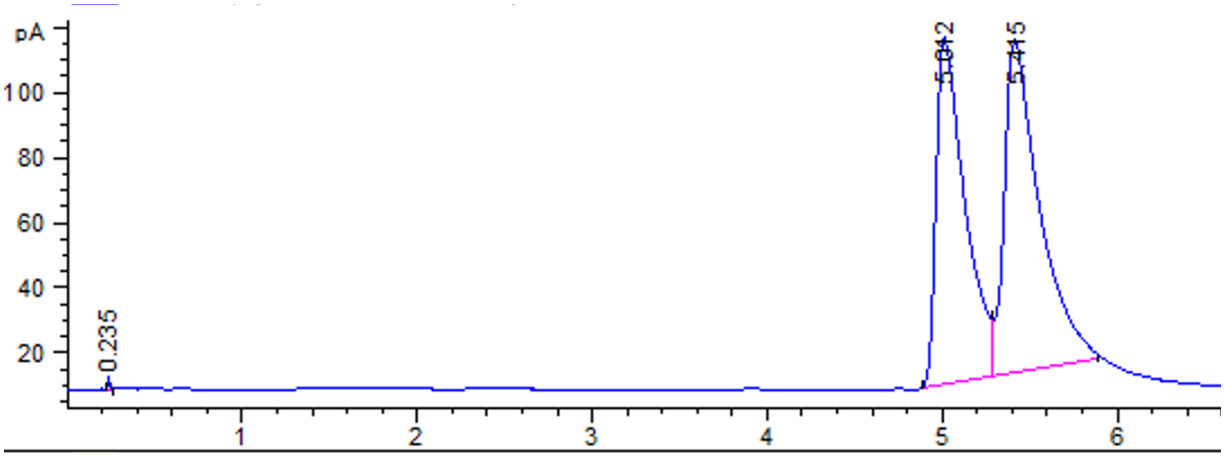


Figure 14. Chromatogram of Trial 5 (0.2 µL, 5 mg/mL, H2, 6890, post baking, 100-130C).

Operations on the 7890A GC on the split/splitless inlet revealed better separation than the previously discussed trials on the 6890A GC on the purged-packed inlet. Keeping the temperature isothermal at 100 C, rather than a continuous ramp 15 minutes from 100-130 °C resulted in a

slower elution of the S enantiomer, allowed more noticeable separation between the two enantiomers. A split ratio of 1:10 (Trial 6) displayed separation, but a split ratio of 1:100 further increased resolution (**Figure 15**) at a sample concentration of 25 mg/mL. Running these same conditions (split ratio of 1:100 and isothermal temperature of 100 C) at 5 mg/mL (**Figure 16**) produced similar resolution with a signal of around 20 pA. When run at an isothermal oven temperature of 100 °C, the split ratio of 1:100 at an injection volume of 0.2 µL displayed high resolution even at high concentrations and produced high signal even for the lowest concentrations.

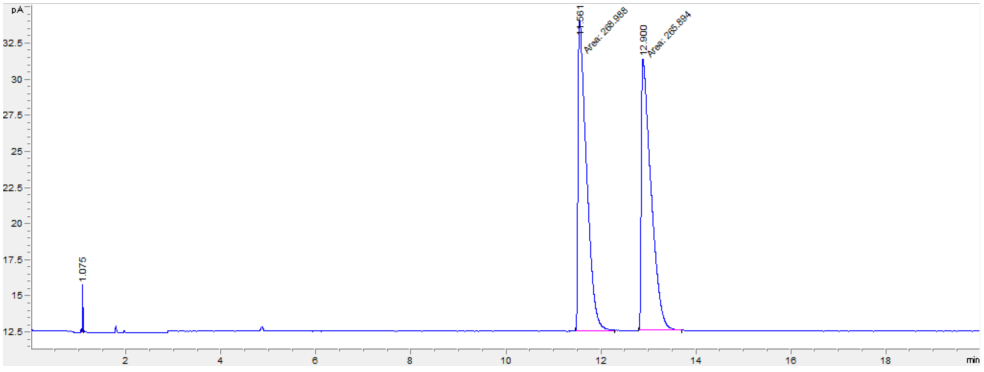


Figure 15. Chromatogram of Trial 7 (7820A, 0.2 µL, isothermal, 25 mg/mL, 1:100 split ratio).

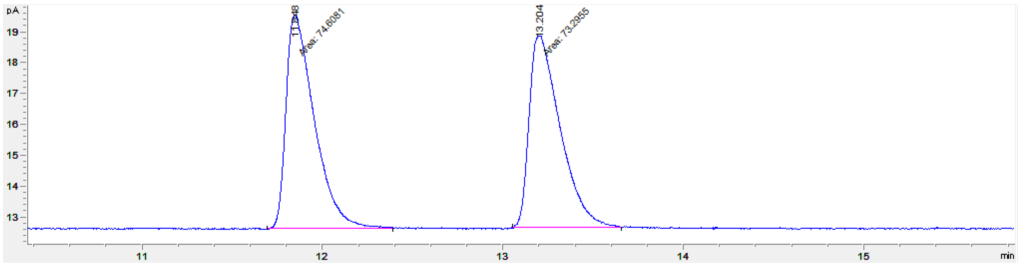


Figure 16. Chromatogram of Trial 8 (7820A, 0.2 µL, isothermal, 5 mg/mL, 1:100 split ratio).

4.2 Chromatogram peak identification

All GC data was normalized to unit area to account for magnitude differences due to slightly different input volumes, as the overall shape was the item of importance, as opposed to

the magnitude. Input times and instrument calibration caused slight time shifts between different samples. If peaks were of similar shape, position, and magnitude and within a minute of each other, they were identified as the same peak.

When pure toluene was run on the GC, a peak was seen around 0.9 min. Toluene was identified as the large peak around 0.9 minutes (**Figure 17**, **Figure 36**). The smaller peaks to the right of toluene are most likely contaminants in the toluene solution. The ethyl acetate peaks occur around the same timeframe as the toluene, illustrated below. The peak directly to the left, around 0.8 min is the main ethyl acetate peak with two smaller peaks occurring at 0.5-0.7 min (**Figure 18**).

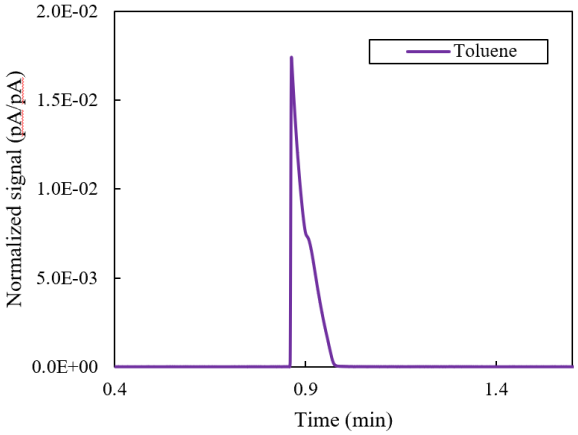


Figure 17. Toluene sample, scaled-out.

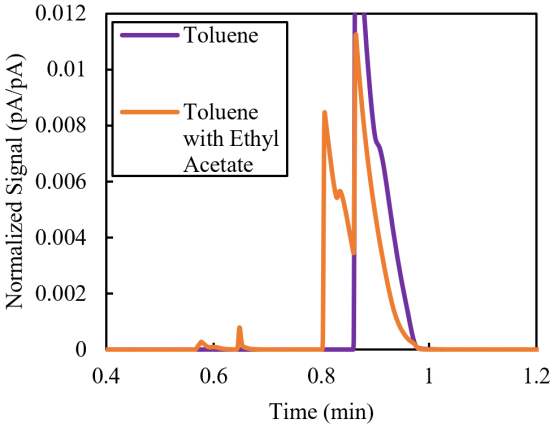


Figure 18. Toluene with ethyl acetate peaks overlaid.

The (*R*,*S*)-1-phenylethanol peaks eluted in the 11-13 minute range (**Figure 19**). All solutions with (*R*,*S*)-1-phenylethanol—the KR/DKR reagent, the KR product, and the racemization product—showed these two peaks. The racemization reagent, containing only 1-(*R*)-phenylethanol, not the racemic mixture eluted just the left peak. The peaks were not present in the remaining solutions that did not contain 1-phenylethanol. This suggests that the left peak occurring around 10.5-11.5 min was 1-(*R*)-phenylethanol, while the right peak, occurring around 12-13 min, was 1-(*S*)-phenylethanol. This identification makes sense as the cyclodextrin column favors the S enantiomer, retaining it longer than the R enantiomer.

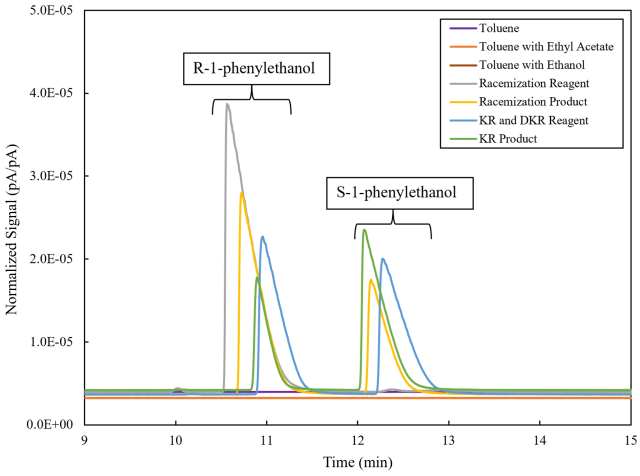


Figure 19. 1-(*R*,*S*)-phenylethanol peaks identified.

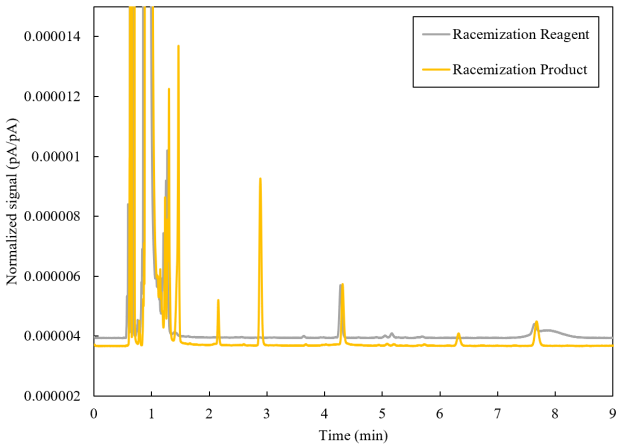


Figure 20. Racemization reagent v. product eluted between 0 and 9 minutes.

With the three components comprising the reagent solutions identified, racemization and kinetic resolution products could now be identified. The primary product of the racemization reaction was the racemic mixture of 1-phenylethanol, which as previously stated, occurs in two peaks eluting at 11-13 minutes. However, multiple peaks eluted in the racemization product that were not present in the racemization reagent. The two largest peaks occur at an elution time of 3 minutes

and 1.45 minutes (**Figure 20**, **Figure 37**). As the dominant side reaction in racemization is the production of styrene, it is likely that one of these two peaks is styrene.

The kinetic resolution product peaks were then identified by comparing the kinetic resolution reagent to a product sample (**Figure 21**). The largest product peak for the kinetic resolution reaction occurred around 8 minutes, with roughly the same magnitude of the 1-phenylethanol peaks. Another small peak occurred around 6.5 minutes. Based on the size of the 8-minute peak and its proximity to the 1-phenylethanol, it was concluded that this peak was the 1-phenylethylacetate.

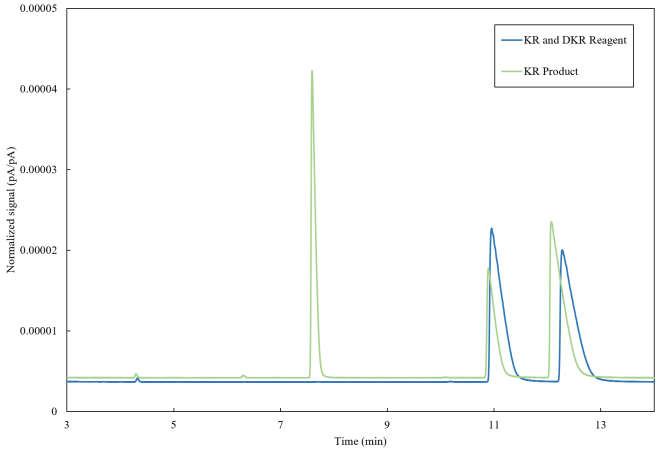


Figure 21. KR/DKR Reagent v. KR product eluted.

The DKR product sample contained peaks seen in both the kinetic resolution and the racemization product samples (**Figure 38**, **Figure 39**). The following table (**Table 9**) contains a summary of the information presented in this section. As not all peaks were fully identified, some peaks are named as "unidentified product.

Table 9: Chemicals present in experiment solutions and their respective elution times.

Chemical	Approximate Elution Time (min)	Solutions Containing Chemical
Toluene	0.8-1.2	Racemization reagent and product, KR/DKR reagent and product, DKR product
Ethyl Acetate	0.58-6.2, 0.7-1	Racemization reagent and product, KR/DKR reagent and product, DKR product
1-(R)-phenylethanol	10.5-11.5	Racemization reagent and product, KR/DKR reagent and product, DKR product
1-(S)-phenylethanol	12-13	Racemization product, KR/DKR reagent and product, DKR product

Styrene	1.45 or 3	Racemization and DKR product
(R)-phenylethylacetate	8	KR and DKR product
Unidentified KR Product	6.5	KR and DKR product

4.3 Examination of the effect of concentration in batch configuration

With confident calibration curves established for both 1-PE enantiomers, the batch reactions run at 12 mg/mL, 15 mg/mL, and 18 mg/mL starting (*R*)-1-PE concentration over 35 minutes were analyzed. The quantitative results of these tests are displayed below:

Table 10. Quantitative results of initial batch racemization experiment.

Init. (*R*)-1-PE [mg/mL]	Catalyst load [mg]	*R* response [pA]	*S* response [pA]	*R* conc. [mg/mL]	*S* conc. [mg/mL]	%ee
11.53	5.2	5558.0	-	-	108497.5	100%
14.51	6.2	6418.3	84.99	84.99	125288.4	97%
18.14	5.7	8537.3	-	-	166646.3	100%

Both the 12 mg/mL and the 18 mg/mL solutions demonstrated no signal response from the *S* enantiomer and enantiomeric excess of 100%. This indicates that the racemization reaction was not able to proceed at the reaction conditions utilized for these trials. The 15 mg/mL trial did demonstrate a small response signal from the *S* enantiomer, yielding an enantiomeric excess of 97%. While well below the objective of the experiment, this small level of conversion verified that the racemization reaction could happen. A possible explanation for this is the elevated catalyst load in the 15 mg/mL trial; the mass of catalyst used for that run was almost 10% higher than any other run, indicating that a larger load of catalyst may be necessary to achieve a more complete degree of conversion. However, the minimal conversion observed in each of these runs suggests that, even with a larger catalyst load, elevating the temperature of the reaction may be necessary to achieve racemization on significant magnitude.

In addition to the high enantiomeric excess, each batch trial chromatogram demonstrated much higher peak area responses than those observed in the calibration process. This led to the calculation of concentrations several orders of magnitudes higher than the starting solution for every trial. This implication is impossible and is likely because an unrecorded excess of solution was injected into the GC during analysis of the batch results. This explanation is verified by the chromatogram generated by the product solution of the 15 mg/mL trial (**Figure 22**).

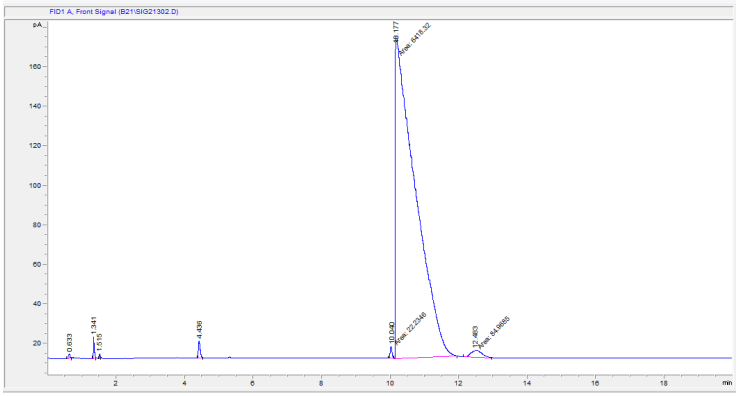


Figure 22. Chromatogram generated from product solution of batch reaction trial with initial (R)-1-PE concentration of 15 mg/mL.

The pictured signal demonstrates a similar peak height to that observed in the 15 mg/mL standard solution chromatograph, but it also demonstrates significant signal tailing that was not previously observed. This tailing accounts for the excess peak area observed in all analyses of batch experiments, and it supports the explanation that excessive sample volume was added during said analysis.

4.4 Validation of racemization reaction in batch

Trial 1 exceeded the enantiomeric excess levels seen at 24 hours in Costa et al (**Table 11**). Trial 1 used operating conditions nearly identical to Costa et al. apart from the reaction temperature (45 ºC v. Costa et al.'s 30ºC) and Si:Al ratio (300 v. Costa et al.'s 75). Trial 1 reached 64%ee at

12h and 44% at 24h. Costa et al. reported a 65% ee at 24 hours. Trial 2 was run at conditions that mimicked the amount of catalyst present in the 38cm packed-bed reactor but at 70 ºC.

Table 11. Results of racemization reaction in batch reactor.

Trial Number	Operating Conditions	Temperature (ºC)	Residence Time (h)	%ee
1	2mL solvent, 50 mg catalyst	45 ± 5	12	64
			24	44
2	6mL solvent, 1.8g catalyst	70 ± 15	1	No 1-PE detected

No significant 1-phenylethanol was detected in trial 2 but another peak that was not present in the reagent appeared with a strong signal at 1.2 minutes (**Figure 28**). The 1-phenylethanol peaks are visible when scrolling in but they are approximately 400 times smaller than the 1.2min peak. The 1-phenylethanol signal was not strong enough to be integrated. This 1.2min peak may have been styrene, a side reaction that occurs when an E1 elimination is performed. A small peak was observed in the 45ºC around the same time point but was approximately 10 times smaller than the 1-phenylethanol peaks (**Figure 29**).

When the reaction temperature is raised for the racemization reaction, the rate of reaction increases but side reactions also increase, leading to a decreased 1-phenylethanol production. These results suggest that the racemization reaction can successfully produce 1-phenylethanol when run at 45ºC but due to possible side reactions, fails to produce 1-phenylethanol product at 70ºC.

4.5 Racemization residence time and temperature studies in flow with water as solvent

Few trials of the racemization studies in water yielded any type of enantiomeric excess results (**Table 12**). Out of the 8 trials performed at varying residence times and temperatures, only

Trial 1 (45ºC with 1hr residence time and 80%ee) seen in , Trial 3 (20ºC with 1hr residence time and 37%ee), and Trial 8 (45ºC with 2hr residence time and 1%ee) yielded chromatograms where enantiomeric excess could be calculated. The 1% ee seen in trial 8 may be due to a carryover effect from a previous syringe containing 1-phenylethanol, so this result is unreliable. In all other trials, the concentration of 1-phenylethanol appeared to be insufficient to analyze (on the scale of 0.1pA) even when injected at higher volumes. As no enantiomeric excess was calculated for most trials, it was not possible to identify trends in the data based on residence time or temperature.

Table 12. Results for racemization studies with water as solvent

Trial Number	Replicate	Temperature (ºC)	Residence Time (h)	%ee
1	1	45 ± 5	1	80
2	1	70 ± 15	1	No 1-PE detected
3	1	`20 ± 2	1	37
4	1	45 ± 5	3	No 1-PE detected
5	2	70 ± 15	1	No 1-PE detected
6	1	60 ± 5	1	No 1-PE detected
7	2	45 ± 5	2	1

In the chromatograms for all trials, a strong peak at ~1.8 min with a magnitude of nearly 100-1000 times the size of the 1-phenylethanol peaks appeared (**Figure 23**, **Figure 24**). Strong signal of the 1-(*R*)-phenylethanol peak and lack of signal of the peak at 1.8 minutes were confirmed by GC analysis of the reagent solution used in all trials (**Figure 37**). The lack of the 1.8 min peak in the reagent and the strong presence of the 1.8 min peak in the product samples, particularly those with extremely weak 1-phenylethanol signals, suggests that this peak may be a byproduct of the reaction. While samples would need to be run on the GC-MS to confirm identity, this peak may be styrene, a byproduct of the racemization reaction that occurs when an E1 elimination is performed on the carbocation rather than an S1 substitution.

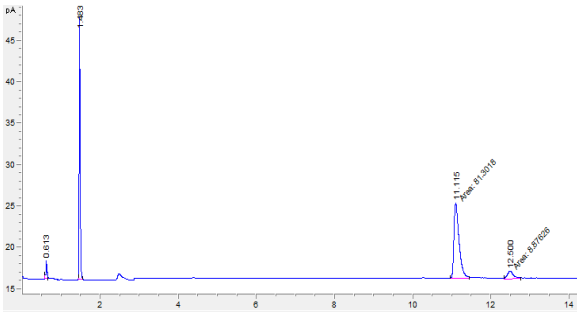


Figure 23. Racemization Study at 45°C and 1hr RT with an 80% EE.

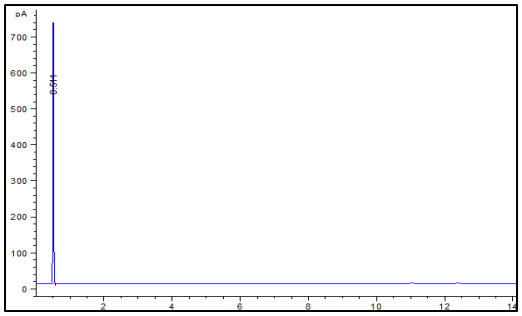


Figure 24. Racemization study at 45°C and 3hr RT with no discernible 1-phenylethanol peaks.

Decreased production of 1-phenylethanol are particularly likely at higher temperatures (>60 ºC) as increased side reactions and enzyme denaturation take place under these conditions. The chromatograms of the trials at higher temperatures and longer residence times suggest the occurrence of side reactions, most likely styrene, that consumed the reagent and minimized the amount of 1-phenylethanol produced.

A second possible reason for the lack of 1-phenylethanol may be due to precipitation of the reaction at some point, either in the column or in the syringe. The trials were operating very close to the solubility limit of (*R*)-1-phenylethanol in water, at 15 mg/mL. Precipitation of crystals in the collection vials was observed after they had been sitting out for two days. Solutions were heated before GC analysis to melt the crystals, but precipitation may have occurred while in the syringe for longer residence times.

Contamination was also observed in the reactor. This may have impacted the results of some trials, particularly trials 3-5, as a new reactor was packed after trial 5.

Due to possible side reactions, precipitation, and/or contamination, the racemization reaction did not show promising results using water as a solvent as there was a minimal concentration of 1-phenylethanol left in the product streams, for operating conditions run at higher

temperatures and longer residence times. The enantiomeric excess of the racemization when run at 20C and 45ºC for a 1hr residence time are similar to the results seen when performed in batch at 45 ºC for 12 hours, suggesting that batch and flow operations for this reaction are comparable.

4.6 Validation of CALB-catalyzed kinetic resolution reaction in batch.

With the racemization reaction well-characterized, work was begun to study CALB's transesterification of (*R*)-1-PE. Reagent solution was prepared according to the procedure outlined in section **3.3.2** and peaks were verified on the GC (**Figure 34**).

Based on the observed peak areas, the 1-PE had a %ee of 1.1%. In the 1 hour batch product sample, (*R*)-1-phenylethanol displayed a peak area of 166.3 pA•S, while (*S*)-1-phenylethanol had a response of 349.8 pA•S, resulting in a %ee of 35.5% (**Figure 35**). This dramatic increase in the prominence of the *S* enantiomer demonstrated the successful completion of the transesterification reaction. This rate of conversion was decided to be sufficient proof of concept to begin examination of the kinetic resolution reaction in flow.

4.7 Residence time study of kinetic resolution in flow.

With the transesterification reaction verified to proceed satisfactorily in a batch regime, work was begun to understand its performance in a flow reactor. Toluene solutions containing racemic 1-PE and ethyl acetate were run through the immobilized CALB-packed reactor using the methodology described in section 3.4. The success of the experiment was measured in terms of "effective yield" – the ratio of *R*-ester peak area to the sum of the peak areas of the ester, *R*-alcohol, and *S*-alcohol. The effective yield as a function of reactor volumes eluted is plotted below for the four tested residence times.

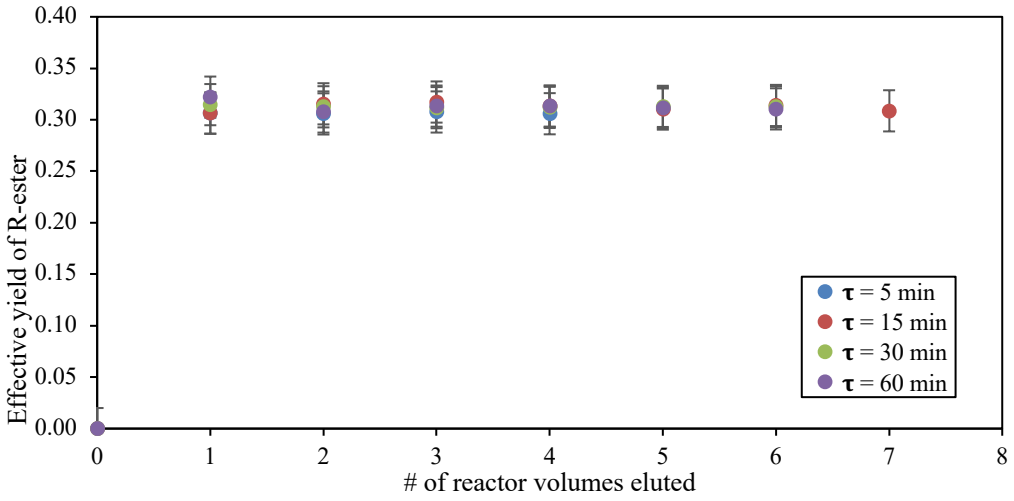


Figure 25. Effective yield of R-ester vs. reactor volume eluted for all residence times.

The effective yield of every reactor volume collected across all residence times was approximately 31%. The rapid attainment of steady state and independence of yield on flow rate imply that transesterification reaction's kinetics are very fast. The limitation of the reaction's yield at 31% suggests that there is some external limitation on reaction progress in the KR system. Ethyl acetate's molar excess in the reagent solution and strong presence in all product samples suggest that an equilibrium condition is the cause of this limitation.

4.8 Residence time study of racemization reaction in flow.

Slower kinetics were observed in the racemization reaction in comparison to the kinetic resolution reaction. Trials were performed at the same four residence times — 5 min, 15 min, 30 min, and 60 min. For the 5 min and 15 min residence times, the reaction did not reach steady state even after the 6th reactor volume had eluted (**Figure 26**). In contrast, the %ee began to plateau around the 4th and 5th reactor volumes eluted for the 30 min and the 60 min residence times. The inability to reach steady state at shorter residence times suggests that the racemization has either

slower kinetics and/or the residence time of the binding of the catalyst to the 1-(R)-phenylethanol is greater than required of the binding of the lipase catalyst to the racemic 1-phenylethanol.

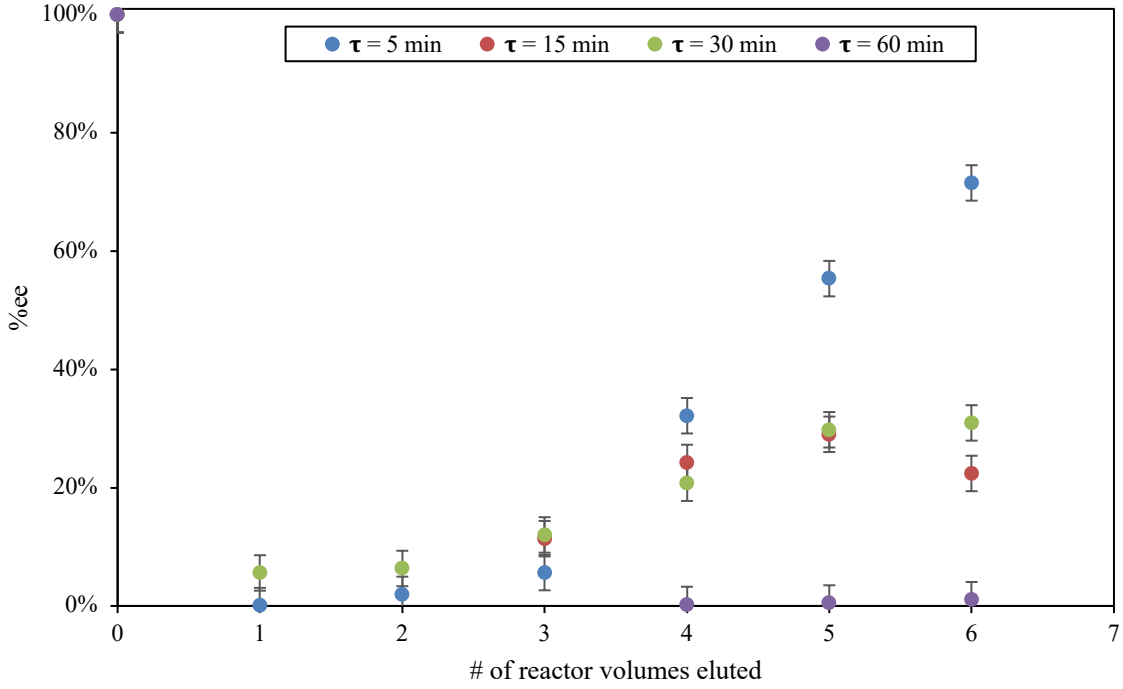


Figure 26. Racemization reaction %ee vs. reactor volume eluted for all residence times.

Reagent solution for all trials demonstrated 100%ee, a completely racemic solution, verifying that change in %ee was due to flow through the reactor. As neither the 5 min nor the 15 min residence time trials reached steady-state, final %ee was not found (**Table 13**). At 30 min and 60 min, the %ee reached 31.0±1% and 0.7±1%. With a measurement error of 1% due to variability in manual integration methods, it is possible to conclude that optical purity is reached at a residence time of 60 mins.

Table 13. %ee for each racemization residence time.

Trial	Residence time (min)	Steady-state %ee
1	5	Steady state not reached
2	15	Steady state not reached

3	30	31.0 ± 1
4	60	0.7 ± 1

Side products, likely styrene, were visible in the chromatograms of the racemization reaction, but the magnitudes of the 1-phenylethanol peaks were 10-150x larger than any of these products.

4.9 Residence time study for dynamic kinetic resolution reaction.

The impact of the racemization reaction was evident in the kinetic studies of dynamic kinetic resolution (DKR) reactor. Six reactor volumes were collected for residence times of 5 minutes, 15 minutes, 30 minutes, and 60 minutes. The effective yield of *R*-ester as a function of reactor volume is plotted for each residence time in **Figure 27**.

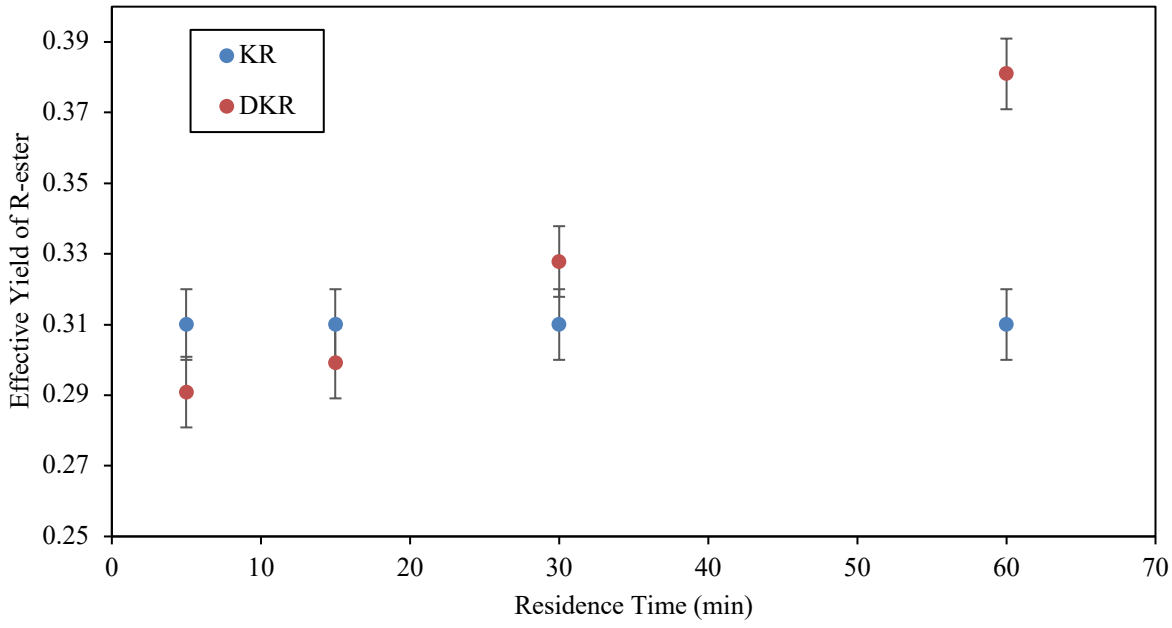


Figure 27. Effective yield vs. residence time for KR and DKR reactor systems.

At residence times of 5 minutes and 15 minutes, the DKR reactor resulted in a slightly lower yield of *R*-ester compared to the KR system. This is expected, as the DKR reactor contained a smaller

load of CALB than the KR reactor, and the racemization reaction was not observed to be effective at those fast residence times. For the 30 minute residence time, the DKR reactor produced a slightly higher ester yield than the KR, reflecting the progress observed in the corresponding racemization experiment. The 60 minute residence time in the DKR experiment resulted in an *R*-ester yield of 0.37%, representing a 23% increase over the analogous KR trial. The trend of the DKR results suggest that the doubled yield that is theoretically possible with DKR could be attainable with further elongation of the system's residence time.

CHAPTER 5 Conclusions and Recommendations

5.1 Conclusions

This project sought to develop a flow reactor system for the dynamic kinetic resolution of (*R*, *S*)-1-phenylethanol using catalysts H-beta zeolite and *candida antarctica lipase B*. To accomplish this task, a gas chromatography method was developed for the chiral separation of *R* and *S*-1-phenylethanol. That analytical method allowed for the zeolite-catalyzed racemization reaction and the lipase-driven kinetic resolution reaction to each be studied in a batch reactor. Both batch experiments demonstrated substantial conversion of the desired enantiomer, and it was concluded that they were sufficient to validate the subject reactions' viability in the target flow reactor system.

With the reactions confirmed to work, they were each analyzed examined in a packed bed reactor across four different flow rates. The kinetic resolution reaction reached steady-state very quickly in all trials, and it resulted in the same yield of *R*-ester across all tested flow rates. These results indicated that the reaction was kinetically fast and limited by an equilibrium condition. The racemization flow experiments, meanwhile, showed that the reaction proceeded much slower, with the two fastest flow rates failing to reach a conclusive steady state. However, significant racemization was achieved at higher flow rates, with the highest tested flow rate demonstrating complete racemization.

The dynamic kinetic resolution experiments reflected these differing kinetic behaviors. At faster residence times, little difference was observed between the DKR flow reactor and the KR. However, at a residence time of sixty minutes, the DKR product demonstrated at 38% yield of *R*-ester compared to the KR's 31%. This 23% increase between the two systems strongly exceeds

any experimental error and demonstrates that the project was successful at developing a viable continuous flow DKR reactor using a lipase catalyst.

This accomplishment is significant. Continuous flow reactor systems are seldom used in pharmaceutical production, and the only flow reactors studied in the case of 1-phenylethanol DKR use transition metal catalysts, which are unstable, toxic, and expensive.[18] A zeolite-catalyzed 1-phenylethanol DKR reactor combines the cheapness, safety, and robustness of zeolite catalysts will all the advantages afforded by continuous flow systems: easier quality control, less process waste, and lower operating costs.

5.2 Recommendations

One immediate area of future work that should be explored is the improvement of the yield of *R*-phenylethylacetate from the reactor. The most promising way to improve the reactor's yield lies in its kinetic resolution component. In this project, ethyl acetate was used as the KR acyl donor because of its low cost and immediate availability. However, this choice was not optimal for the yield of the system. The KR reaction's maximum yield of 31% fell far below the 50% yields achieved in literature studies of the reaction. One explanation for this is equilibrium. Lipase enzymes do not only catalyze transesterification; they also facilitate the reverse reaction, which is the hydrolysis of the ester back into its foundational alcohol. Steric bulk in the acyl donor reduces the favorability of this reverse reaction, so future work on this system should use a much bulkier acyl donor like vinyl acetate or vinyl butyrate.[23] Another way to possibly improve the yield of ester would be to increase the reagent's contact time with the racemization catalyst, which was found to be the slow step in the system. This improved contact could be facilitated by increasing the reactor's residence time than this project or increasing the load of zeolite in the packed bed.

Increasing temperature is another possible way to improve ester yield; the lipase operates optimally at 40 °C, but the zeolite will be more active at higher temperatures. Because the zeolite is the slower catalyst, testing higher temperatures may result in higher yield.

Another salient area for the future development of this project is the treatment of byproducts in the product solution. While both enantiomers of 1-phenylethanol, toluene, ethyl acetate, and the *R*-phenylethylacetate product were all confidently identified and analyzed with the recorded gas chromatograms, numerous small peaks appeared in the product solution that were not characterized. Future iterations of this project should use a combined gas chromatography-mass spectroscopy instrument to identify these impurities, allowing for their quantification and separation. The analytical component of this project could also be expanded to include an inert internal standard in the reagent solution. The presence of this standard will allow the molar concentration of each species in the product to be calculated, which will establish the rate at which 1-phenylethanol is converted into waste products instead of the desired *R*-esters. The currently employed calculations using relative peak area are not able to account for those losses.

Understanding these additional operating parameters and side reactions will allow this reactor to be intensified to an industrial scale. The impact of a production-sized zeolite DKR system would be monumental on the pharmaceutical industry. Isolating the *R*-enantiomer of 1-phenylethanol will become cheaper, less environmentally wasteful, more controllable, and more reliable. The numerous therapeutics that depend on 1-phenylethanol derivates will become more plentiful and affordable, allowing more people to receive life-saving care. Moreover, the successful development of this process from a batch system to a continuous one will blaze a trail for similar intensification in other processes, closing the knowledge gap that has become so prominent in the pharmaceutical industry.

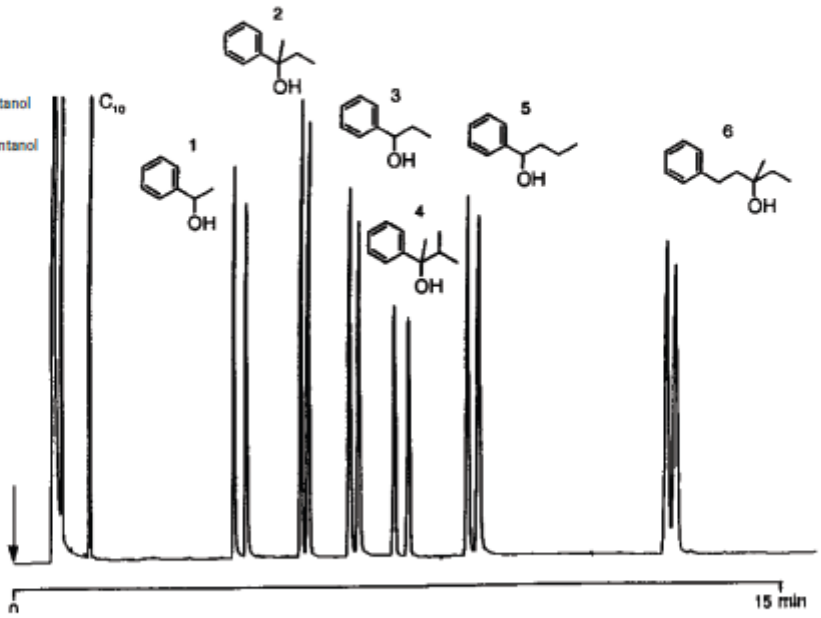
APPENDIX A – AGILENT SUGGESTED GC PARAMETERS

Conditions

Technique	: GC-capillary
Column	: Agilent CP-Chirasil-DEX CB, 0.25 mm x 25 m fused silica WCOT CP-Chirasil-DEX CB (df = 0.25 µm) (Part no. CP7502)
Temperature	: 100 °C → 130 °C, 2 °C/min
Carrier Gas	: H_2, 100 kPa (1 bar, 14.5 psi)
Injector	: Split
Detector	: FID
Courtesy	: Prof. V Schurig, Universität Tübingen, Tübingen, Germany

Peak identification

1. (±)-α-methylbenzyl alcohol
2. (±)-2-phenyl-2-butanol
3. (±)-1-phenyl-1-propanol
4. (±)-2-phenyl-3-methyl-2-butanol
5. (±)-1-phenyl-1-butanol
6. (±)-3-methyl-5-phenyl-3-pentanol



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APPENDIX B – Tables

Table 14. Raw data for continuous racemization studies.

Date	Res Time (min)	Res Time (hr)	Interval	Peak 10 (R)	Peak 11 (S)	%ee
3/25/22	5	0.083333	0	20400.8	0	100%
3/27/22	5	0.083333	1	248.026	248.338	0%
3/27/22	5	0.083333	2	221.2	212.7	2%
3/27/22	5	0.083	3	211.515	188.909	6%
3/27/22	5	0.083333	4	280.084	143.718	32%
3/27/22	5	0.083333	5	1029.41	295.702	55%
3/27/22	5	0.083333	6	1422.74	235.877	71.6%
3/25/22	30	0.5	0	1782.7	0	100.0%
3/28/22	30	0.5	1	174.433	155.988	5.6%
3/28/22	30	0.5	2	222.811	252.959	6.3%
3/28/22	30	0.5	3	540.643	424.659	12.0%
3/28/22	30	0.5	4	887.203	582.129	20.8%
3/28/22	30	0.5	5	1072.58	580.081	29.8%
3/28/22	30	0.5	6	1067.74	562.879	31.0%
3/25/22	15	0.25	0	20400.8	0	100.0%
3/28/22	15	0.25	3	9789.92	7790.06	11.4%
3/28/22	15	0.25	4	11102	6765	24.3%
3/28/22	15	0.25	5	10110	5560	29.0%
3/28/22	15	0.25	6	1238.46	785	22.4%
3/29/22	60	1	4	525.626	523.001	0.3%
3/29/22	60	1	5	4571.13	4525.71	0.5%
3/29/22	60	1	6	4534.11	4439.68	1.1%
3/29/22	60	1	7	5672.73	5591.1	0.7%

Table 15. Raw data for continuous KR experiments.

Res Time (min)	Res Time (hr)	Interval	peak 8 (ester)	peak 9 (R)	peak 10 (S)	%ee	Effective Yield
60	1	0	0	900	898.72	0.08%	0.00
60	1	1	529.7	358	757.3	35.78%	0.32
60	1	2	427.65	301	661.94	37.51%	0.31
60	1	3	562.8	390	843.4	36.73%	0.31
60	1	4	418.9	284	634	38.10%	0.31
60	1	5	521.084	362	788.5	37.05%	0.31

60	1	6	537.287	372	821.19	37.69%	0.31
15	0.25	1	507.554	363	786.9	36.90%	0.31
15	0.25	2	507.847	348	754.3	36.92%	0.32
15	0.25	3	500.974	334	744.6	38.11%	0.32
15	0.25	4	541.2	370	817.5	37.70%	0.31
15	0.25	5	510.551	352	781.9	37.85%	0.31
15	0.25	6	515.46	348	778.4	38.18%	0.31
15	0.25	7	483.975	340	744.0	37.28%	0.31
30	0.5	1	547.849	381	812.3	36.16%	0.31
30	0.5	2	579.487	405	869.3	36.43%	0.31
30	0.5	3	599.956	416	908.3	37.13%	0.31
30	0.5	4	558.847	379	853.0	38.43%	0.31
30	0.5	5	528.857	360	800.4	37.92%	0.31
30	0.5	6	582.441	398	883.0	37.87%	0.31
5	0.08	1	562.885	402	871.4	36.90%	0.31
5	0.08	2	507.854	363	790.7	37.10%	0.31
5	0.08	3	534.908	377	827.7	37.47%	0.31
5	0.08	4	517.3	371	802.9	36.78%	0.31

Table 16. Continuous flow dynamic kinetic resolution results.

Date	Residence Time (mins)	Interval	Ester Peak	R Peak	S Peak	%ee	Effective Yield
4/4/22	30	2	39.06	37.14	17.45	36.01%	0.417
4/4/22	30	3	382.4	314.2	452.8	18.07%	0.333
4/4/22	30	4	477.3	424.3	738.6	27.03%	0.291
4/4/22	30	5	502.5	484.1	826.4	26.12%	0.277
4/4/22	30	6	520.5	505.3	871.1	26.57%	0.274
4/4/22	30	7	521.3	491.2	835.5	25.94%	0.282
4/4/22	30	8	530.4	478.4	814.8	26.02%	0.291
4/4/22	5	4	469.8	359.4	715.5	33.12%	0.304
4/4/22	5	5	520.2	410.0	818.1	33.22%	0.298
4/4/22	5	6	499.1	397.8	796.0	33.36%	0.295
4/4/22	5	7	491.4	382.9	772.6	33.72%	0.298
4/4/22	5	8	478.2	369.7	751.0	34.02%	0.299
4/4/22	15	1	492.0	378.0	719.4	31.11%	0.310
4/4/22	15	2	533.2	401.4	641.6	23.03%	0.338
4/4/22	15	3	508.4	352.1	652.6	29.91%	0.336
4/4/22	15	4	503.1	365.9	693.5	31.00%	0.322
4/4/22	15	5	494.3	349.2	680.7	32.18%	0.324
4/4/22	15	6	516.9	367.3	711.4	31.90%	0.324

4/4/22	15	7	497.5	342.8	670.8	32.36%	0.329
4/4/22	15	8	469.0	324.9	636.6	32.41%	0.328
4/4/22	60	1	594.5	410.8	764.7	30.11%	0.336
4/4/22	60	2	608.5	409.1	612.9	19.94%	0.373
4/4/22	60	3	651.7	425.7	585.6	15.81%	0.392
4/4/22	60	4	658.2	435.5	597.7	15.71%	0.389
4/4/22	60	5	626.8	412.7	571.7	16.15%	0.389
4/4/22	60	6	608.1	406.1	553.5	15.36%	0.388
4/4/22	60	7	611.5	404.3	575.0	17.40%	0.385
4/4/22	60	8	566.3	383.5	536.7	16.65%	0.381

APPENDIX C – Calculations

Calculation 1. Calculation of reactor volume (V) and flow rate (F) for 30 minute residence time.

$$\mathrm{L} = 20\ \mathrm{cm}, \mathrm{ID} = 2.1\ \mathrm{mm}, \epsilon = 0.5$$

$$\mathrm{V} = \mathrm{L}\left(\frac{\mathrm{ID}}{2}\right)^2 \pi(1-\epsilon)$$

$$\mathrm{V} = 20\ \mathrm{cm}\left(\frac{2.1\ \mathrm{mm}\left(\frac{1\ \mathrm{cm}}{10\ \mathrm{mm}}\right)}{2}\right)^2 \pi(1-0.5)$$

$$\mathbf{V = 0.346\ cm^3 = 0.346\ mL}$$

$$\tau = 30\ \mathrm{min}$$

$$\tau = \frac{F}{V}$$

$$30\ \mathrm{min} = \frac{F}{0.346\ \mathrm{mL}}$$

$$\mathbf{F = 0.0115\ \frac{mL}{min}}$$

Calculation 2. Calculation of pressure drop for the packed bed reactor.

Determine fluid velocity from the surface area and flow rate:

$$u_o = \frac{A_s}{\dot{V}} = \frac{3.46 * 10^{-6}\ m^2}{3.84 * 10^{-10}\ \frac{m^3}{s}} = 0.00011\ \frac{m}{s}$$

Determine Pressure Drop Across Pipe, assuming properties of pure water:

$$\frac{\Delta p}{L} = \frac{150\mu(1-\epsilon)^2 u_o}{\epsilon^3 d_p^2} + \frac{1.75(1-\epsilon)\rho u_o^2}{\epsilon^3 d_p}$$

$$\frac{\Delta p}{0.2m} = \frac{150(0.001052\ Pa*s)(1-0.5)^2\left(0.00011\frac{m}{s}\right)}{(0.5^3)(0.002m)^2} + \frac{1.75(1-0.5)\left(997\frac{kg}{m^3}\right)\left(0.00011\frac{m}{s}\right)}{(0.5^3)(0.002m)}$$

$$\Delta p = 175\ Pa = 0.03\ psi$$

Nomenclature:

$$\Delta p = pressure\ drop$$

$$L = length\ of\ reactor$$

$$\mu = fluid\ viscosity$$

$$\epsilon = void\ space\ of\ the\ bed$$

$$u_o = fluid\ superficial\ velocity$$

$$d_p = aggregate\ particle\ diameter$$

Calculation 3. Computation of percent enantiomeric excess.

$$Area\ (R) = 1067.74\ pA$$

$$Area\ (S) = 562.879\ pA$$

$$\%ee = \frac{|A_R - A_S|}{A_R + A_S}$$

$$\%ee = \frac{|1067.74\ \ pA - 562.879\ pA|}{1067.74\ \ pA - 562.879\ pA} = 31.0\%$$

Calculation 4. Calculation of effective yield of R-ester.

$$Area\ (R) - ester = 566.633\ pA$$

$$Area\ (R) - alcohol = 383.547\ pA$$

$$Area\ (S) - alcohol = 536.743\ pA$$

$$Effective\ yield = \frac{A_{ester}}{A_{ester} + A_R + A_S}$$

$$Effective\ yield = \frac{566.633\ pA}{566.633\ pA + 383.547\ pA + 536.743\ pA} = 0.381$$

Appendix D—Chromatograms

Validation in Racemization Batch Chromatograms

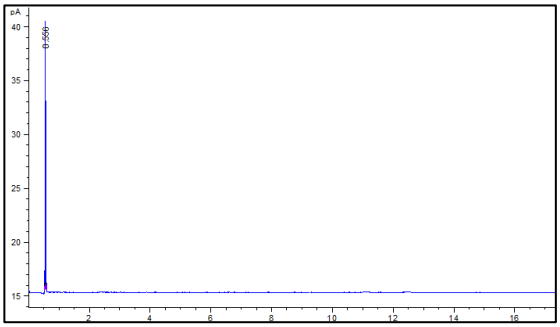

Figure 28: Trial 2, racemization reaction in batch at 70 °C.

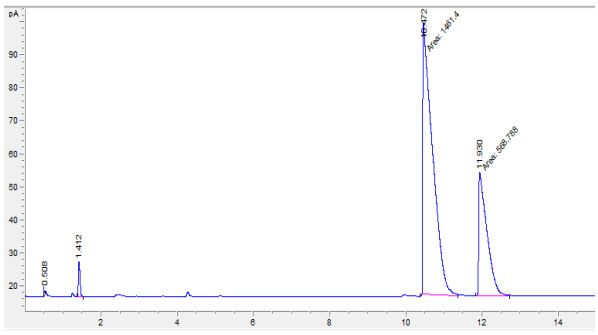

Figure 29. Trial 1, racemization reaction in batch at 45 ºC.

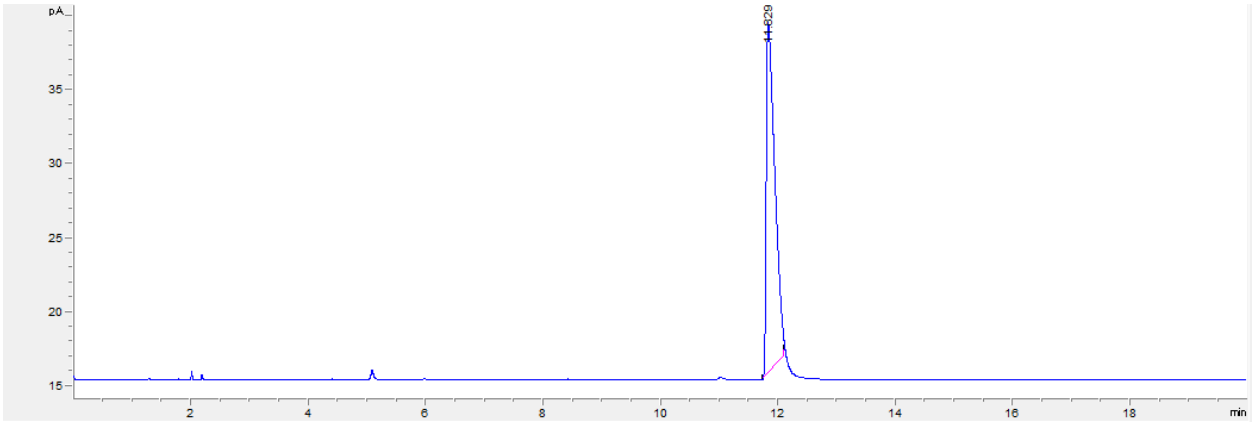

Figure 30. Batch racemization reagent diagram.

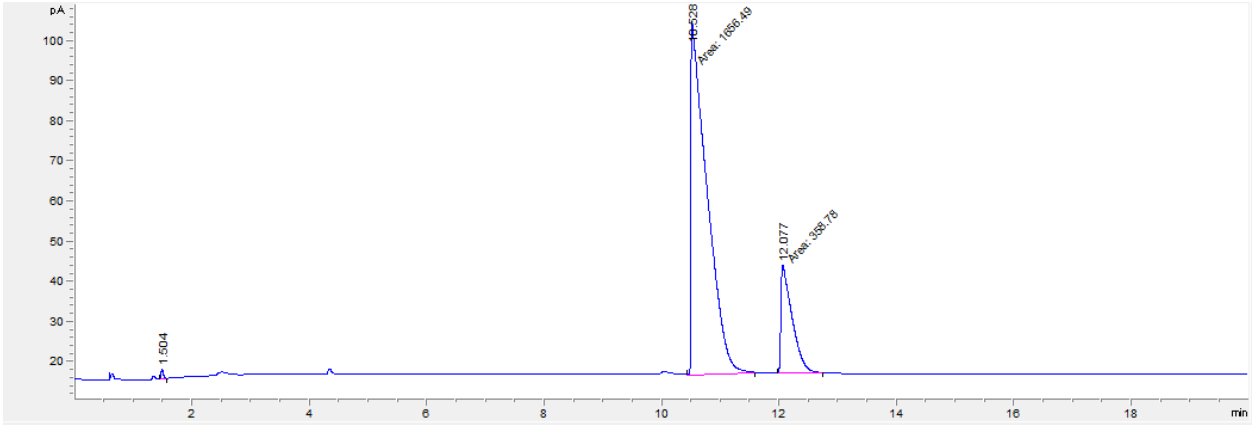

Figure 31. Racemization reaction in batch (1/20/22), 12h residence time, 50mg catalyst, 2mL solvent, 15mg/mL concentration.

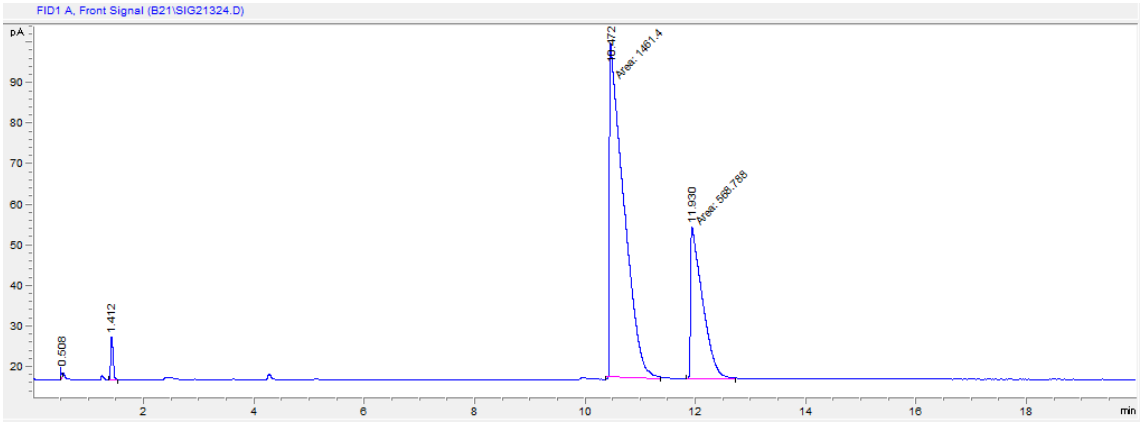

Figure 32. Racemization reaction in batch (1/20/22) with a 24h residence time, 50mg catalyst, 2mL solvent, and 15mg/mL concentration at 45 °C.

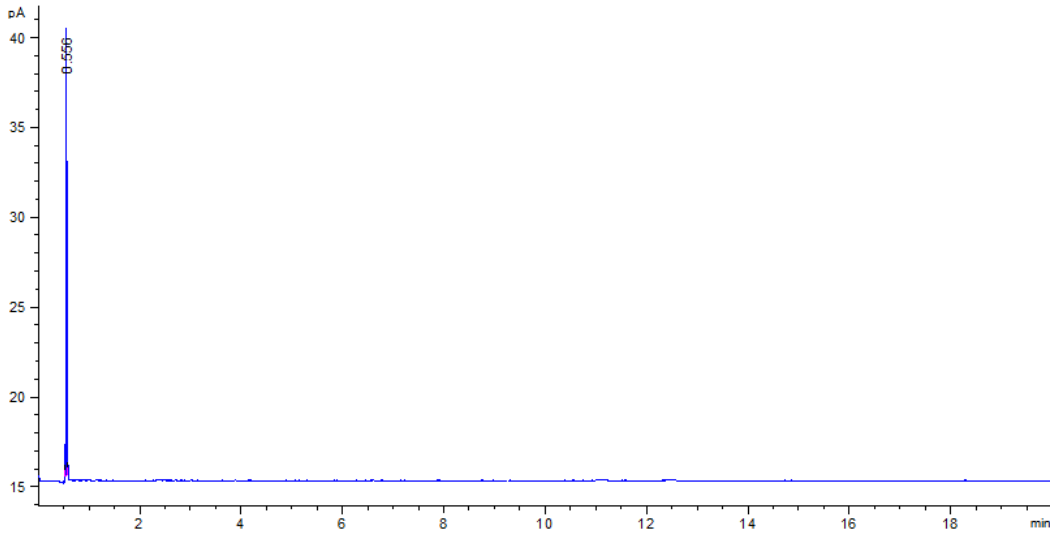

Figure 33. Racemization reaction in batch with a 1hr residence time, 1.8g catalyst, 6mL solvent, 15mg/mL concentration at 70°C

Validation of Kinetic Resolution in Batch

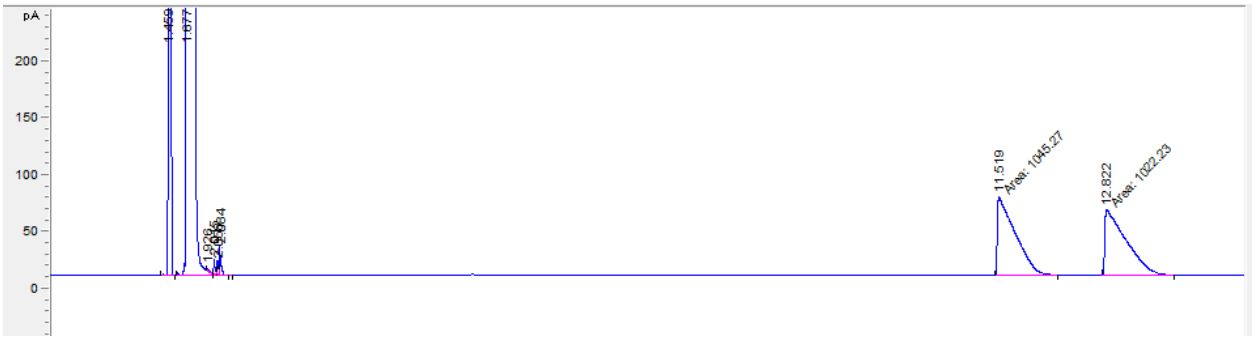

Figure 34. Chromatogram of toluene solution containing 15 mg/mL racemic 1-phenylethanol and 30 mg/mL ethylacetate.

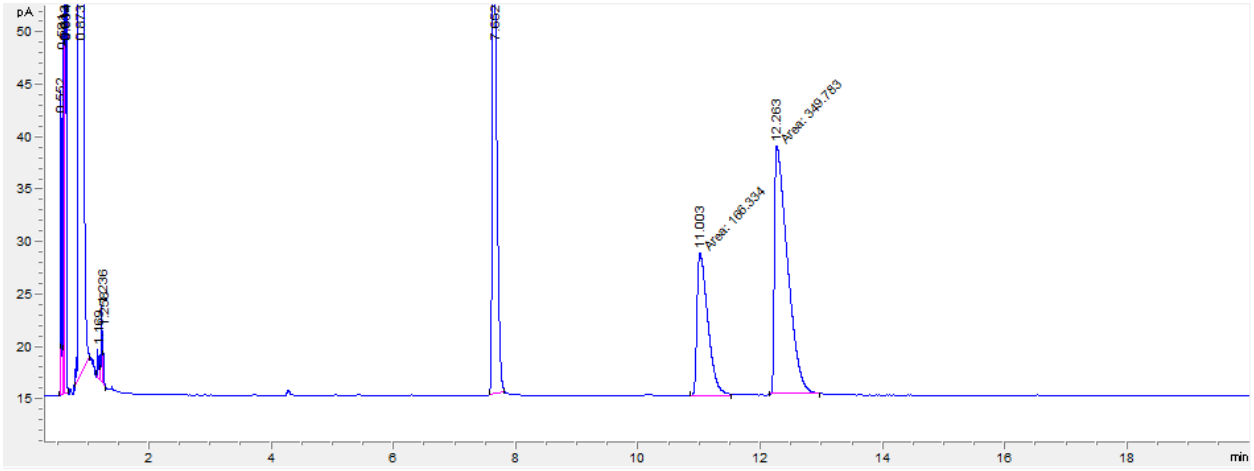

Figure 35. Batch validation of kinetic resolution in toluene after 1 hour.

Peak Identification Chromatograms

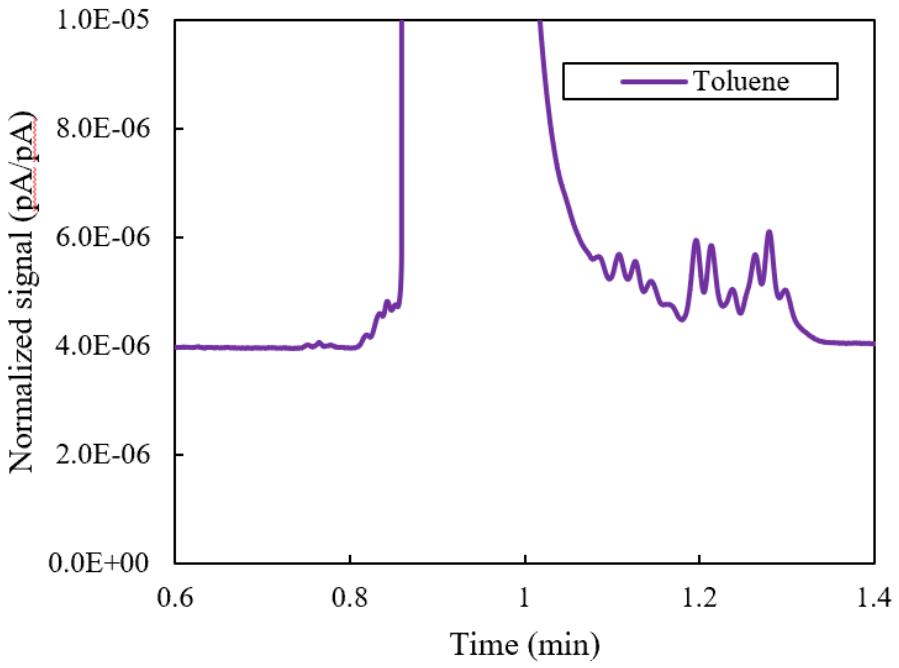

Figure 36. Toluene sample, scaled-in.

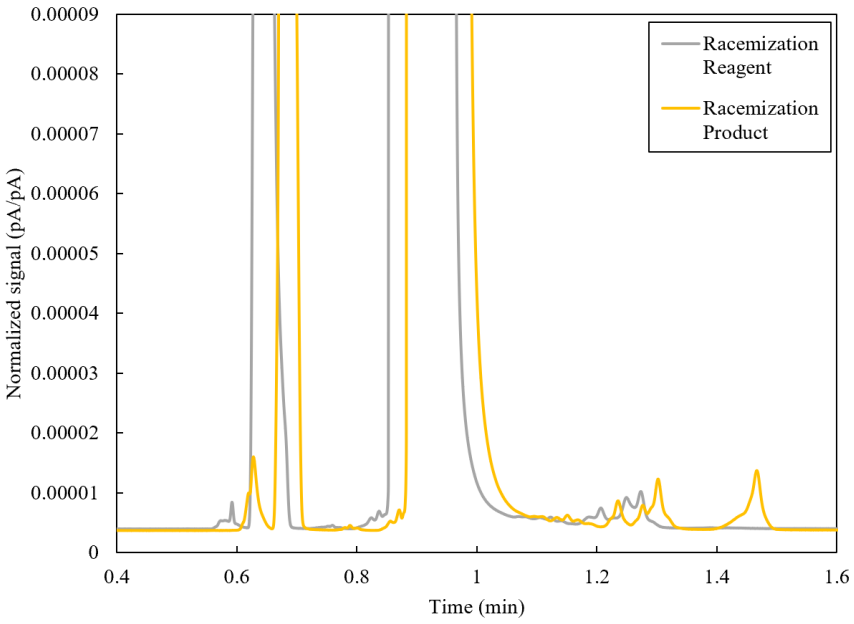

Figure 37. Racemization reagent and product eluted between 0.4 and 1.6 minutes.

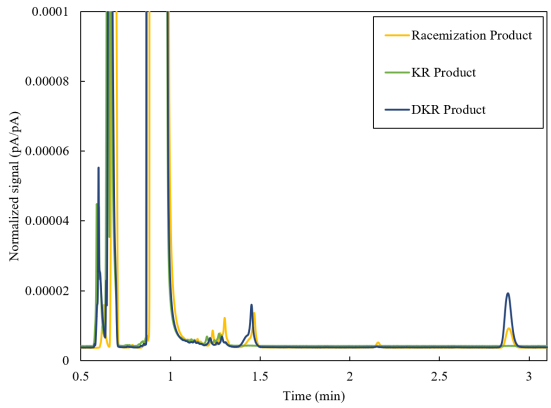

Figure 38. Racemization, KR, and DKR comparison for peaks eluted between 0.5 and 3 minutes.

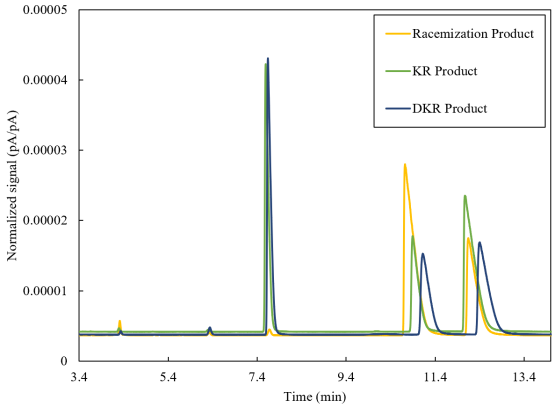

Figure 39. Racemization, KR, and DKR comparison for peaks eluted between 3.4 and 13.4 minutes.

Racemization residence time and temperature studies in flow

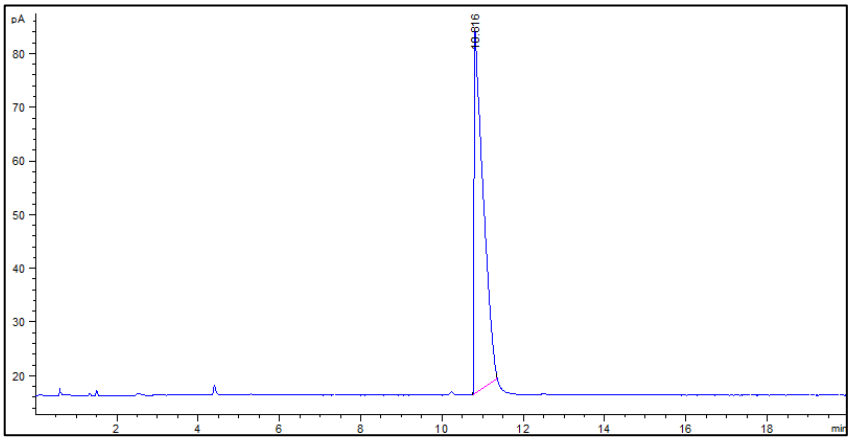

Figure 40. Reagent for aqueous racemization study.

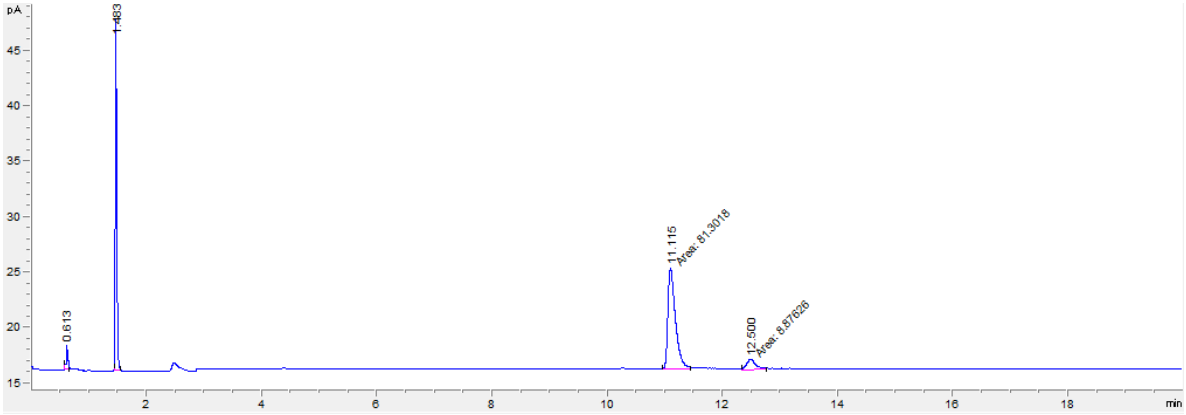

Figure 41. Racemization Study at 45°C and 1hr RT with an 80% EE. Experiment performed on 1/28/22 and analyzed on 1/29/22.

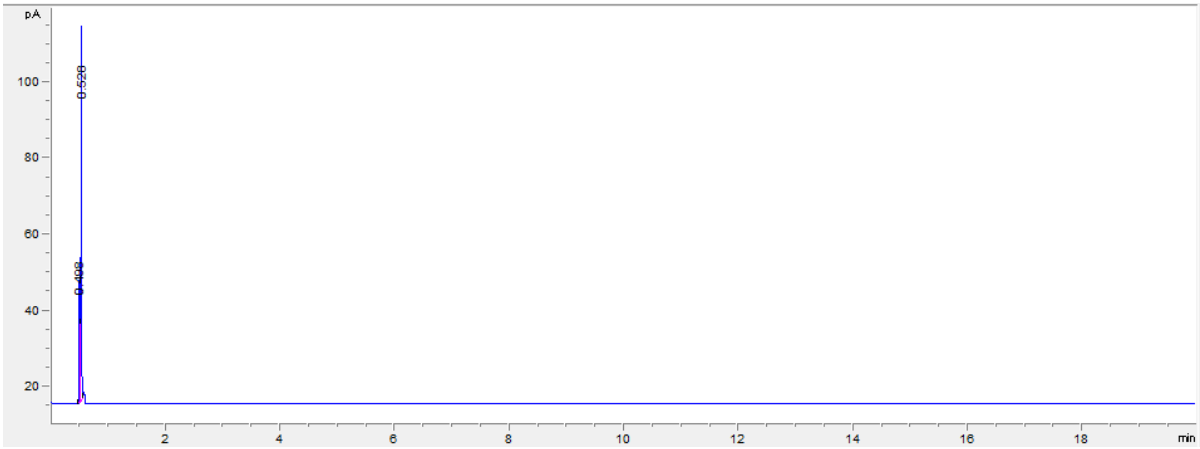

Figure 42. Racemization Study at 45°C and 1hr RT with an 80% EE. Experiment performed on 1/28/22 and analyzed on 2/11/22.

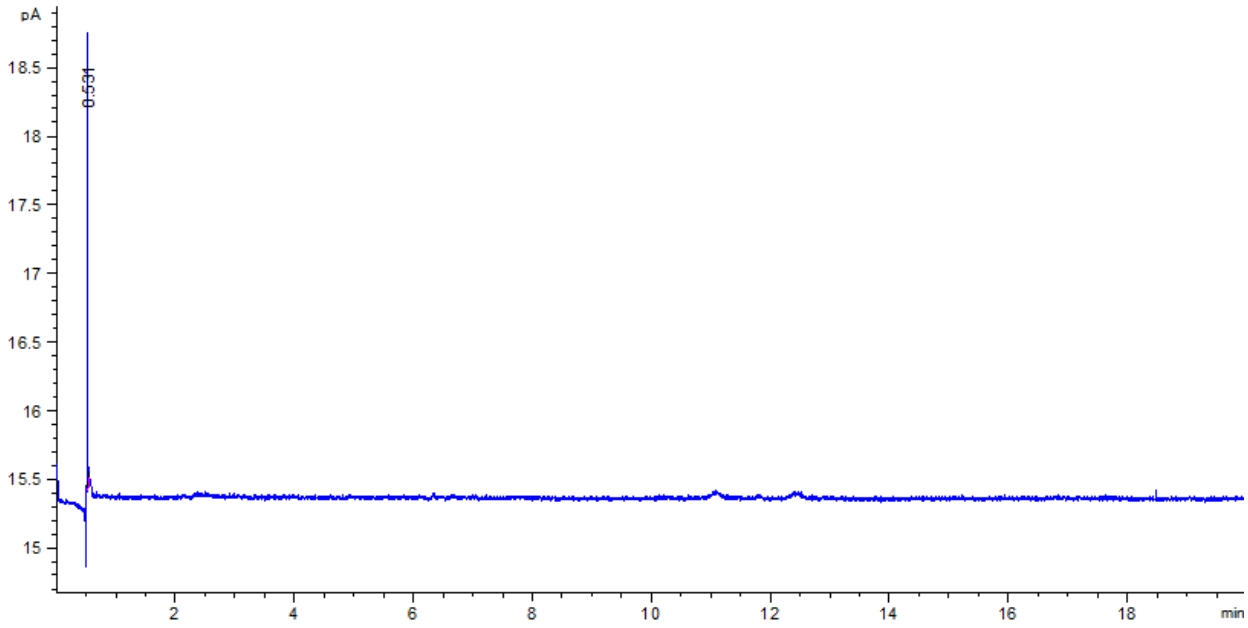

Figure 43. Racemization Study at 70°C and 1hr RT. No discernible %EE. Experiment performed on 2/1/22 and analyzed on 2/11/22.

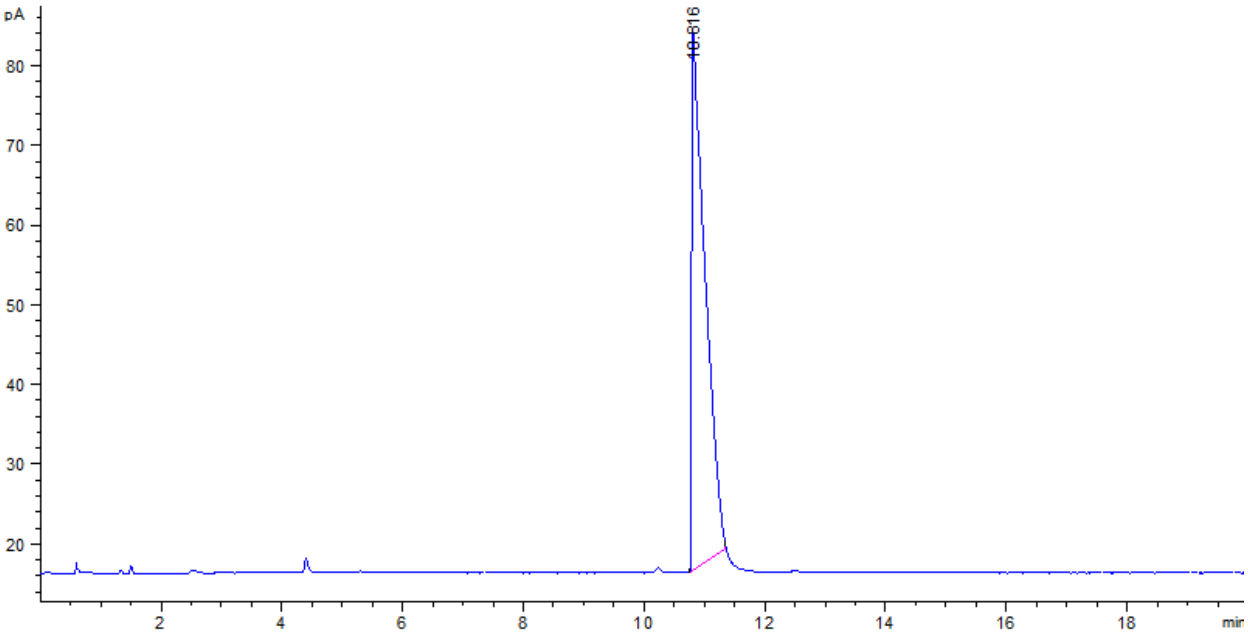

Figure 44. Reagent for racemization study at 70°C and 1hr RT on 2/1/22. Reagent analyzed before experiment.

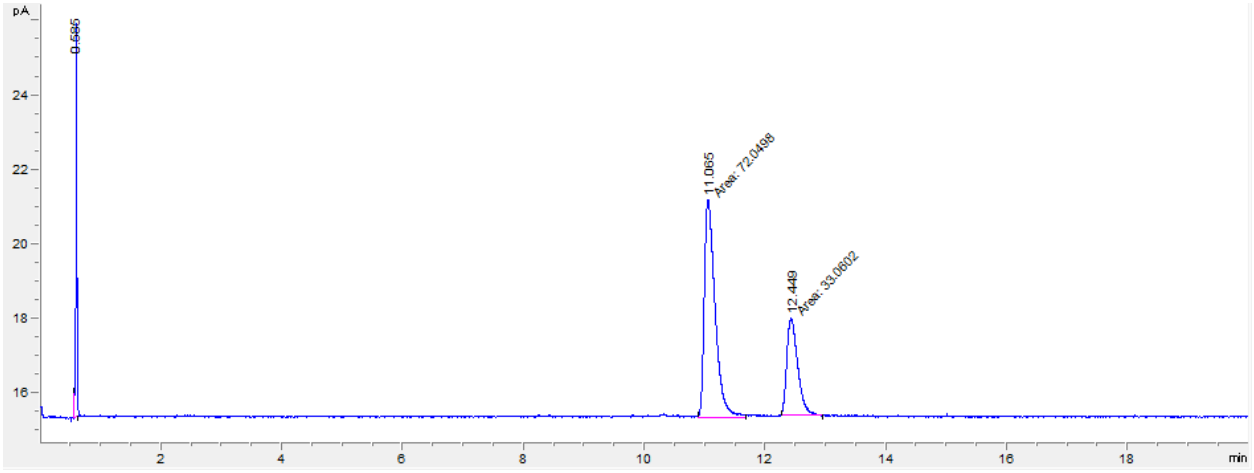

Figure 45. Racemization Study at 20°C and 1hr RT with 37%EE. Experiment performed on 2/2/22 and analyzed on 2/11/22.

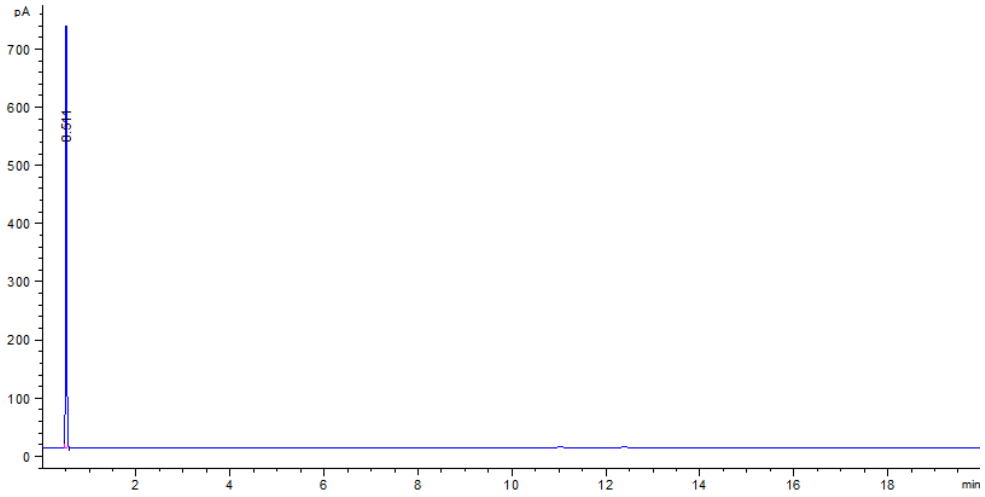

Figure 46. Racemization Study at 45°C and 3hr RT with no discernible 1-phenylethanol peaks. Experiment performed on 2/10/22 and analyzed on 2/11/22.

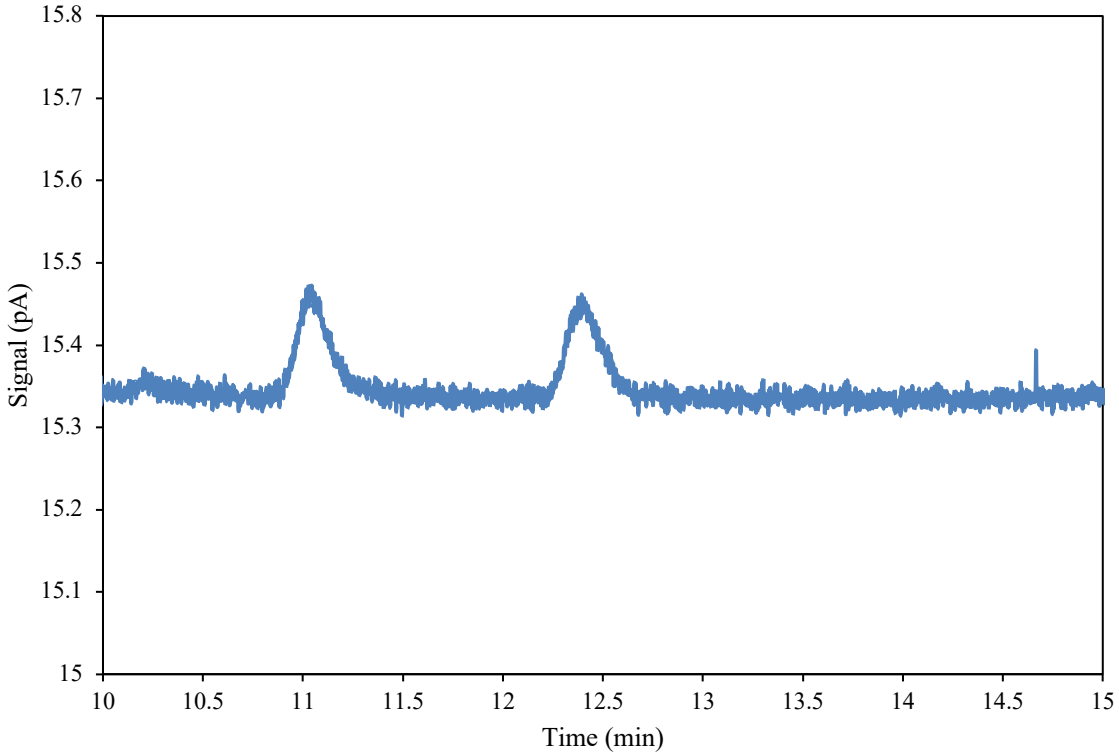

Figure 47. Racemization Study at 45°C and 3hr RT, zoomed-in. Experiment performed on 2/10/22 and analyzed on 2/11/22.

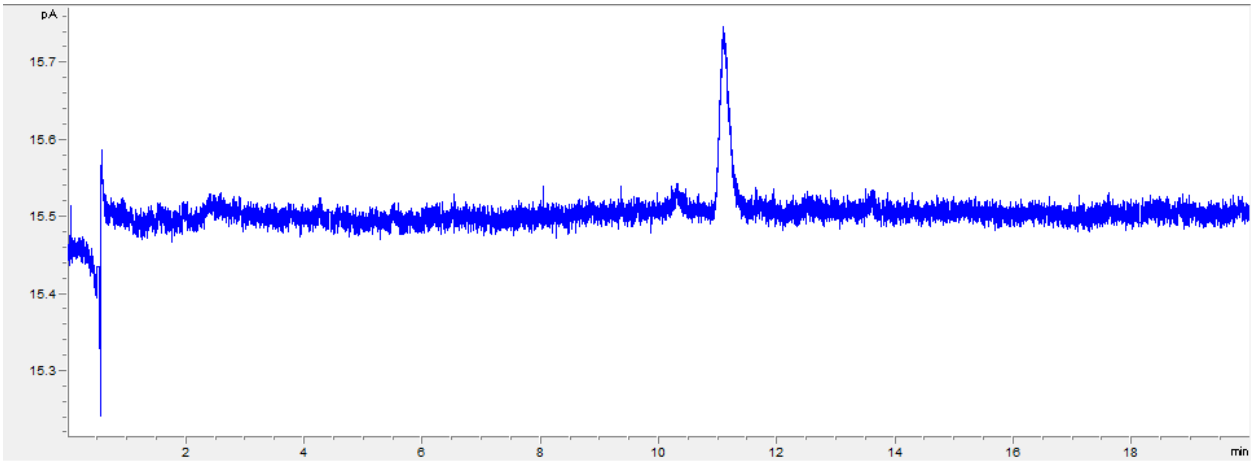

Figure 48. Racemization Study at 70°C and 1hr RT with no discernible 1-phenylethanol peaks. Experiment performed on 2/14/22 and analyzed on 2/14/22.

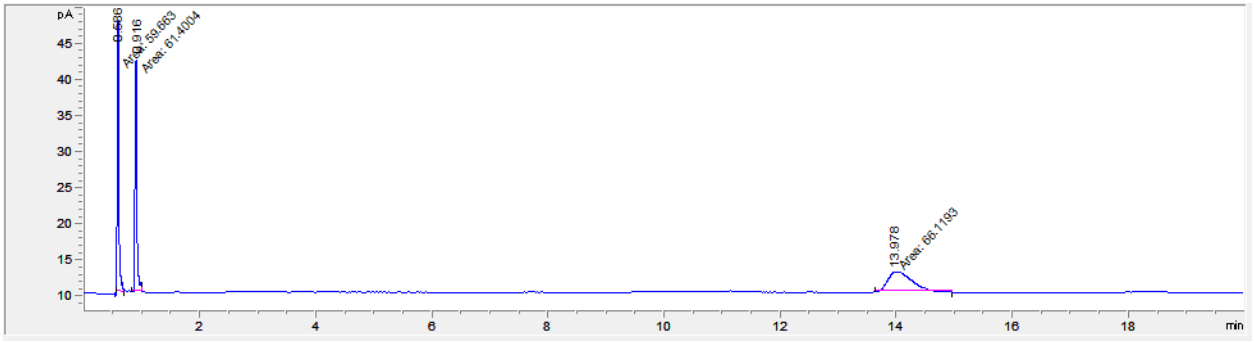

Figure 49. Racemization Study at 60°C and 1hr RT with only 1-(R)-phenylethanol visible. Experiment performed on 2/17/22 and analyzed on 2/17/22.

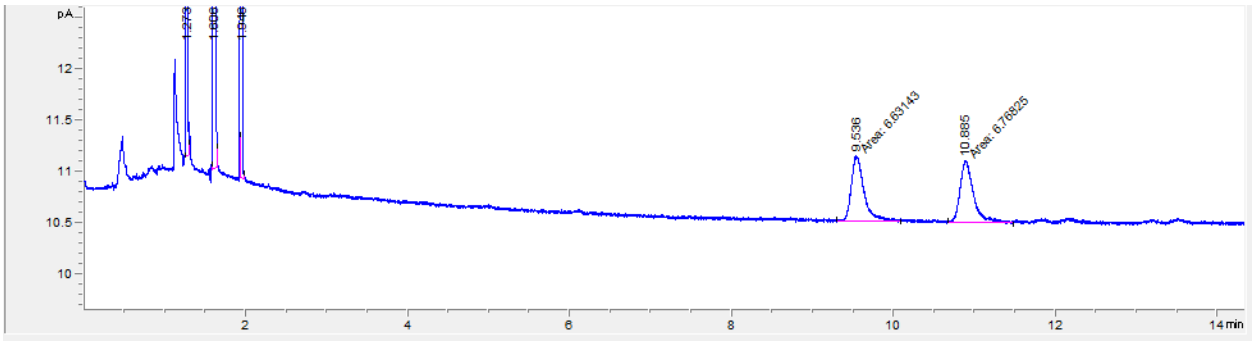

Figure 50. Racemization Study at 45°C and 2hr RT with 1%EE. Experiment performed on 2/20/22 and analyzed on 2/20/22.

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