CALB Immobilized On Octyl-Agarose- An Efficient Pharmaceutical Biocatalyst For Transesterification In Organic Medium.

Authors : Vedant Chimote,Vidya Karhale.

Abstract :

The growing need for better and safer methods to separate enantiomers is driven by the presence of chiral molecules, especially those with medicinal benefits. Biocatalysis is seen as a promising approach in pharmaceutical research. However, free enzymes often have limitations when it comes to their activity and stability, which restricts their wide use. To overcome these issues, new technologies are being developed. In this study, we present improved ways to immobilize enzymes.

Candida antarctica lipase B (CALB) is attached to an octyl-agarose support to ensure high enantioselectivity in an organic reaction environment.

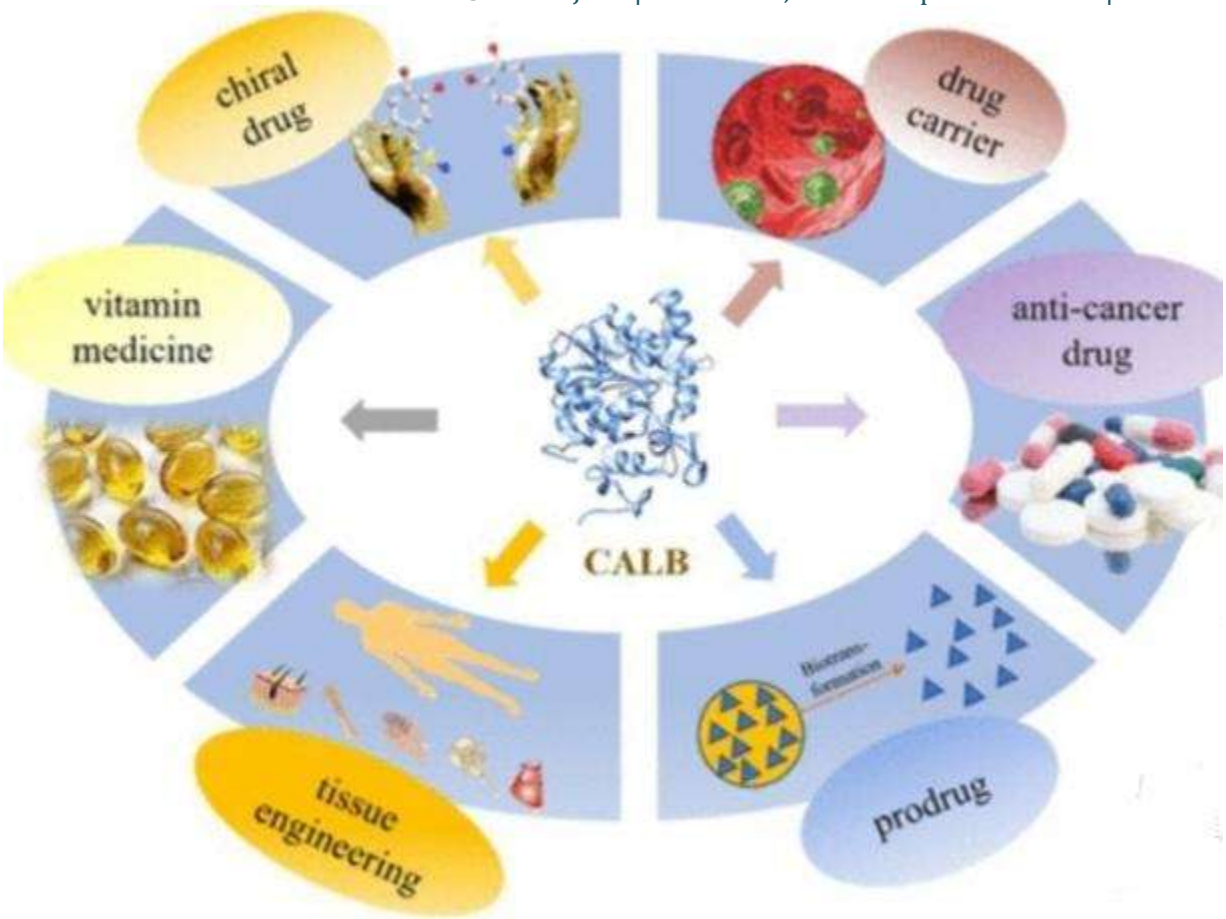
The immobilization process involves several steps, including drying and testing different buffer conditions. The effects of temperature and immobilization time were studied. The best results were achieved using a citrate buffer with a pH of 4 and a concentration of 300 mM. The CALB immobilized on the octyl-agarose showed strong catalytic performance.

This was tested using enantioselective transesterification of (R,S)-1-phenylethanol with isopropenyl acetate as a model reaction in 1,2-dichloropropane (DCP).

Monitoring was done on a small scale, and HPLC analysis confirmed the production of (R)-1-phenylethyl acetate. The conversion was about 40%, with an enantiomeric excess (ee) greater than 99%. The enantiomeric ratio was more than 200, and the yield was 40%. Thermal and storage stability were tested.

After seven days, the immobilized CALB on octyl-agarose showed excellent stability.

When tested at 65°C in a climate chamber, the (R)-1-phenylethyl acetate still had an ee over 99% at a conversion of around 40%, which is similar to the performance of a non-stored lipase. The CALB-octyl-agarose supports high catalytic activity and stability, making it a useful tool for enantioselective transesterification in organic conditions.



Keywords :

Octyl-Sepharose CL-4B, Octyl-Agarose, Lipase B From Candida Antarctica, Immobilization , Climatic Chamber, Thermal Stability, Storage Stability, Kinetic Resolution.

Introduction :

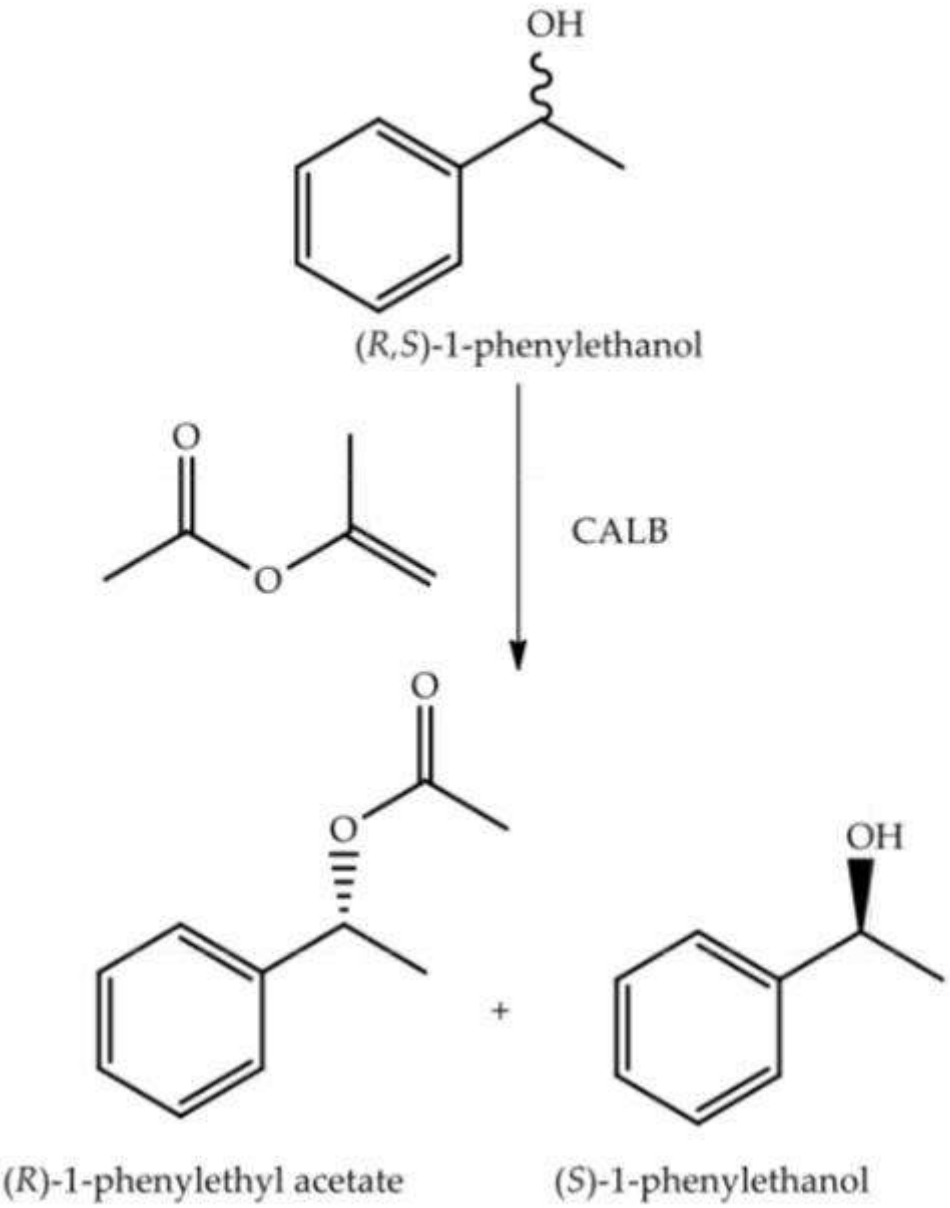
The differences in side effects and how the two optical isomers of a specific drug molecule work in the body have been well studied [1,2]. Because of this, there has been growing interest in developing new ways to get and study optically pure molecules, which is now a major area in pharmaceutical research [3,4]. It's important to remember that these methods are aimed at being more environmentally friendly and not using harmful chemicals [5]. Modern enzyme-based methods are a good solution to these issues, which are mainly caused by the wide use of synthetic materials, among other things. These enzymes act as natural replacements for traditional synthetic methods or help in breaking down racemic mixtures [6].

Lipases are the most commonly used enzymes in biocatalysis.
They have an active site that is more accessible, allowing solvents and substrates to enter. This process is known as "interfacial activation" [7,8].

It's also important to note that lipases are stable in organic solvents and don't need extra chemicals to work. Because of their ability to choose between different chemical groups, positions, and enantiomers, they are useful for many reactions that are important in pharmaceuticals [9,10].

One of the most commonly used biocatalysts is lipase B from Candida Antarctica, called CALB.
It's often used to make enantiomerically pure chemicals [11,12] . The reason why the lid of CALB remains open is still unclear. It's a topic that scientists keep studying. Some research suggests that CALB uses a "lid scaffold" structure to start the reaction on its own [13,14] In the scientific literature, there are many reports about using CALB in different chemical reactions. Chen et al.[15] used CALB to help make a specific type of acetylation on phloridzin, which has several useful properties like fighting cancer, reducing inflammation, and acting as an antioxidant. Pérez-Venegas et al. [16] described a technique that uses CALB to create enantiomers [23] of (R,S)-ketorolac with high purity. Zappaterra et al. [17] used an immobilized version of CALB (called Novozym-435) in a setup without solvents to help make esters of ursodeoxycholic acid, which is used to treat certain liver diseases and cholesterol gallstones [17,18]

This compound is also used in cosmetics and pharmaceuticals as a chiral building block. (R,S)-1-phenylethanol [19-22] is one such compound that can be used as a fragrance ingredient. This method is simple, cheap, and effective for cleaning, fixing, stabilizing, and controlling the biocatalytic process [24,25,26] It also helps to prevent lipases from being overactivated [27] Importantly, lipases tend to stick to various hydrophobic surfaces, such as other hydrophobic proteins or open surfaces of other lipases [28] .



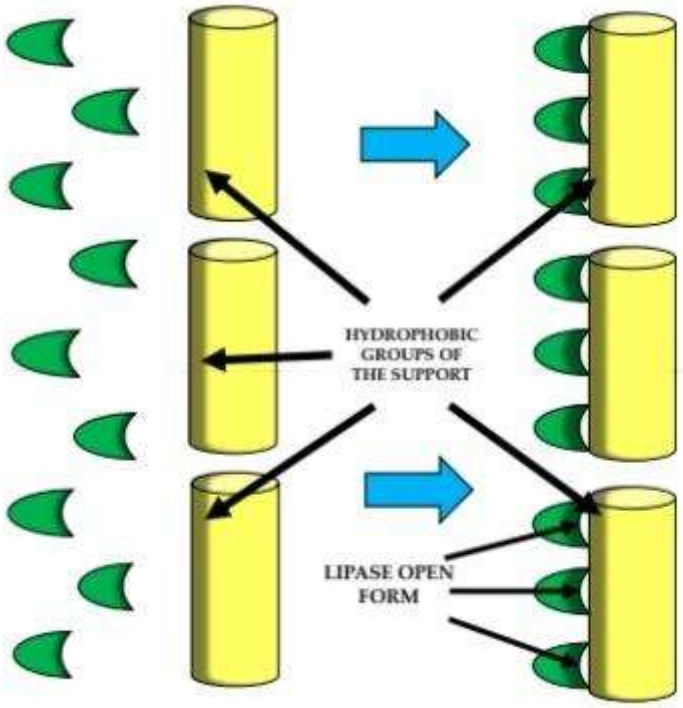
Scheme 1 : The kinetic resolution of (R,S)-1-phenylethanol via transesterification using isopropenyl acetate as the acyl donor.

One of the key factors that allow the biocatalyst to be immobilized is the use of octyl-agarose beads.
Interfacial activation is the foundation of the lipase process, which this method relies on. Lipases that are immobilized on hydrophobic supports through interfacial activation tend to be more stable. However, there are some disadvantages to using them, such as the possibility of the enzyme protein being released from the support in the presence of detergents or high concentrations of hydrophobic organic cosolvents. It's interesting to note that octyl-agarose beads can be very useful because they can undergo various modifications, making it possible to create heterofunctional supports [29] . An example of a commercially available support is Octyl-Sepharose CL-4B, which is made up of octyl groups attached to a cross-linked 4% agarose matrix. The positive effect of the lipolytic activity of immobilized CALB on Octyl-Sepharose CL-4B has been reported in studies [30,31].

Scheme 2 :

A simpler method for lipase immobilization using interfacial activation.

Given that there is not much research on this topic, we decided to use the CALB-octyl-agarose support in reactions taking place in an organic solvent.
This choice allows for further study in this area. The results from this study add value to the conversation about how effective the tested immobilized biocatalyst is in organic chemistry.
The transesterification process in an organic solvent, specifically 1,2-dichloropropane (DCP), is examined in this study.
The product formed is (R)-1-phenylethyl acetate, which is created through the reaction of CALB immobilized on an octyl-agarose support with (R,S)-1-phenylethanol.
The performance of the immobilized CALB is evaluated based on the enantiomeric excess, enantiomeric ratio, and conversion rates.



Scheme 2 : The Immobilization Of Lipase Via Interfacial Activation On Hydrophobic Support.

Results and Discussion

1 . Enhancing the Immobilization of Candida antarctica Lipase B (CALB)

The Impact of Buffer pH In the early stages of this study, we examined the effect of different organic solvents as a reaction medium, including n-hexane, and evaluated the catalytic parameters of immobilized CALB in n-heptane, dichloromethane (DCM), 1,2-dichloroethane (DCE), 1,2-dichloropropane (DCP), diisopropyl ether (DIPE), and t-butyl methyl ether (MTBE).
As the pH decreases, the information in Table 1 and the buffer used for lipase immobilization has a significant effect, and the value of this changes. The citrate buffer (pH 4) used throughout the immobilization process enabled the transformation to occur.This resulted in the highest conversion values (C = 40.8 ± 0.8%) after 24 hours of kinetic activity.

For the enantioselective transesterification with isopropenyl to resolve (R,S)-1-phenylethanol,
acetate was used as the acylating agent.
Over 99% of ee was achieved, and the transesterification showed excellent enantioselectivity (E > 200).

Compared to lipase immobilization in the buffer at pH 4, there was more than a twofold improvement compared to the buffer at pH 9.
The immobilization yield (Iy) was determined.
The approach using citrate buffer (pH 4; 100 mM) for immobilization achieved the best results.

The phosphate buffer (pH 7; 100 mM) gave an immobilization yield of Iy = 32.4 ± 0.4%.

With the Tris base buffer (pH 9; 100 mM),
Iy = 15.3 ± 0.3%, while Iy = 21.1 ± 0.7% when using the Tris base buffer.

From these results, the pH value and nature (chemical composition) of the buffer
can be inferred. These factors play a crucial role in achieving ideal immobilization conditions
for the tested lipase (ideal structural configuration). Immobilization of CALB on octyl-agarose beads may stabilize the active site of the biocatalyst using a citrate buffer with a pH of 4. It is hypothesized that the buffer's influence on the network can alter the catalytic activity of the lipase. This affects the interaction, the ionization condition of the catalytic triad, and the enzyme charge between the support [33,34] and CALB. The loading of CALB on the octyl-agarose support could be another key factor influencing the lipase activity.
As mentioned in our previous publication [32]. The amount of immobilized lipase was higher under acidic conditions compared to basic conditions. Based on these findings, it was highlighted that the pH was better buffered.
Inactivation tests carried out in various buffers revealed the composition of the buffer.
It was found that the buffer interferes with the stability of interfacial activation-immobilized lipase [35]. Another study suggested that the buffer and the enzyme loading presumably via intermolecular interaction were responsible for the enzyme's activity.

The enzyme's performance, like its stability, ability to target specific reactions, or how well it speeds up chemical reactions, can be influenced by its environment [36] . The relationship between the reaction setting whether it's water or an organic solvent and the activity of the lipase enzyme can be seen through the substrates used and the types of buffers with specific pH levels applied. As previously mentioned, the enantioselective transesterification of (R,S)-1-citrate with isopropenyl acetate in 1,2-dichloropropane produced a significant amount of phenylethanol. For CALB (Candida antarctica lipase B) immobilization, a buffer with a pH of 4 was used. In a similar way, as noted in [31], the best lipolytic activity (measured as activity recovery) for CALB was achieved. A citrate buffer at pH 4 was used for immobilization. On the other hand, as shown in [32] , the Tris base buffer (pH 9) was most effective for the methanol esterification of (R,S)-flurbiprofen. Based on these findings, it seems that the key elements in setting up the right conditions for CALB immobilization are the type of reaction, including the reaction medium, and the chemical makeup of the substrate.

Lipase	Immobilization Conditions	Lipase Loading (mg/g Support) *	ee_s (%)	ee_p (%)	C (%)	E
CALB	pH 4; 100 mM	64.8 ± 0.9	68.8 ± 2.1	99.8 ± 0.2	40.8 ± 0.8	>200
	pH 7; 100 mM	42.1 ± 1.4	47.4 ± 1.8	99.8 ± 0.2	32.2 ± 0.8	>200
	pH 9; 100 mM	30.5 ± 0.6	21.9 ± 1.1	99.8 ± 0.1	18.0 ± 0.7	>200

Table 1 : The Influence Of Buffer PH On The Values For The Conversion ,Enantiomeric Excess And Enantiomeric Ratio.

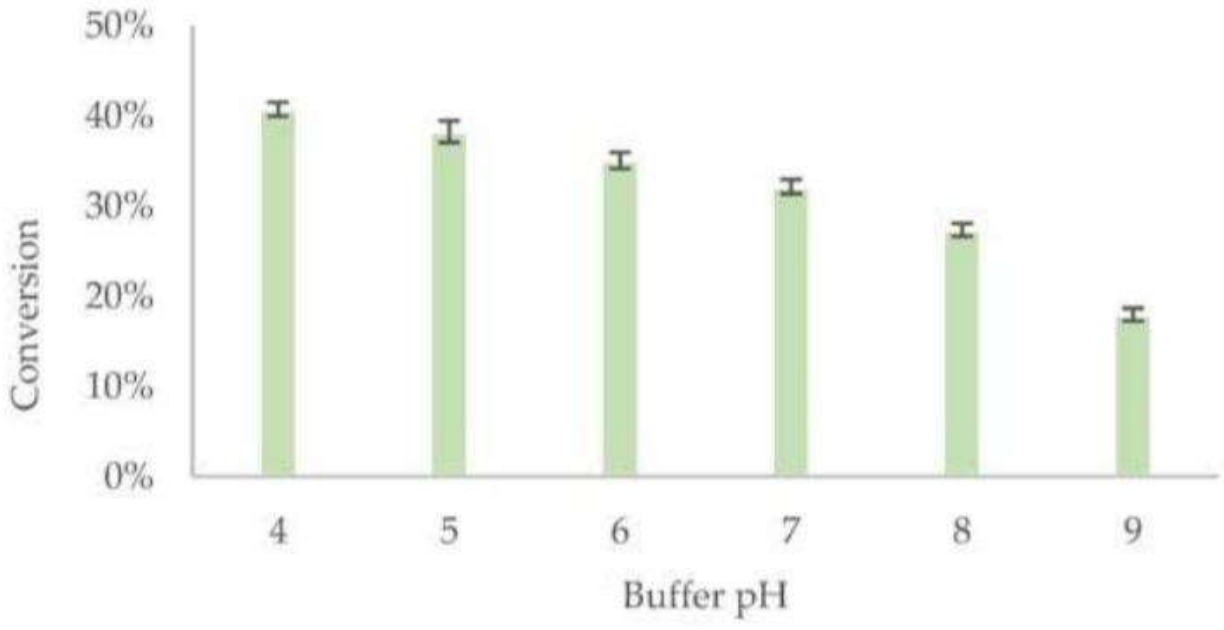


Fig.no 1 : the buffer pH used during immobilization affects conversion rates. The pH values tested were 4, 5, 6, 7, 8, and 9. All experiments were done at a concentration of 100 mM. The reaction conditions included immobilized (R,S)-1-CALB (50 mg of support), isopropenyl acetate (0.3 mM), and phenylethanol (0.08 mM). The medium used was DCP (410 microliters), with molecular sieve 4 Å. The reaction temperature was 37°C, with shaking at 550 rpm in a polypropylene reactor. The kinetic resolution time was 24 hours, and the conversion of C was measured. The data is shown as the average ± standard deviation from three separate experiments (n = 3). The standard deviations are shown as error bars. This represents the average of the results.

2 . The effect of temperature, the time spent immobilizing, and the concentration of the buffer used.

Citrate buffers of different pH levels (pH 4) were used to immobilize CALB.
The buffer concentrations were 50, 100, 300, and 500 mM. The reaction containers were made of polypropylene. The transesterification process was carried out in 1,2-dichloropropane (DCP) as the reaction medium. The concentration of the buffer used for immobilizing CALB impacted its enantioselectivity in the kinetic resolution of (R,S)-1-phenylethanol with isopropenyl acetate. High levels of catalytic performance were consistently achieved at all tested buffer concentrations (50 mM, 100 mM, 300 mM, and 500 mM). Our findings support the idea that there isn't a one-size-fits-all for lipase activity, and the conditions for immobilizing CALB on the tested support vary. When looking at the reaction process, it's important to consider the immobilization conditions, the substrate molecules, the reaction medium, and the method used for immobilizing CALB. It was found that CALB activity was impacted by the concentration of the buffer used during immobilization. This effect could change depending on the materials used in the reaction. It's also important to note that ions in the solution can influence factors like solubility, stability, surface charge, as well as viscosity and surface tension of the liquid used.

Lipase	Immobilization Conditions	ee_s (%)	ee_p (%)	C (%)	E
CALB	pH 4; 50 mM	61.3 ± 2.4	99.8 ± 0.2	38.1 ± 1.0	>200
	pH 4; 100 mM	68.8 ± 2.1	99.8 ± 0.2	40.8 ± 0.8	>200
	pH 4; 300 mM	75.6 ± 1.6	99.8 ± 0.2	43.1 ± 0.5	>200
	pH 4; 500 mM	69.7 ± 1.8	99.9 ± 0.1	41.1 ± 0.6	>200

Table 2 : The Influence Of Buffer Concentration On The Values For The Conversion , Enantiomeric Excess, Enantiomeric Ratio.

The immobilization time was identified through initial experiments carried out in our lab and was additionally optimized.
The samples were mixed for five minutes at room temperature in a citrate buffer (pH 4; 300 mM) and then stored at 4°C for 2, 8, 10, 14, and 20 hours.
The results of the model reaction (transesterification of (R,S)-1-phenylethanol) indicated that the best performance in terms of conversion was achieved after 14 hours of immobilization because it produced the highest conversion values. Extending the incubation period beyond 14 hours didn't lead to significant increases in conversion and enantioselectivity. After 2 hours of immobilization, the conversion was 14.3 ± 0.2%; after 8 hours, 30.3 ± 1.1%; after 10 hours, 38.4 ± 1.3%; after 14 hours, 43.1 ± 0.5%; and after 20 hours, 43.6 ± 1.9%.

Immobilization temperature was another factor that was optimized.

The samples were prepared at room temperature, mixed for five minutes in a 300 mM buffer at pH 4, and then incubated with CALB for 14 hours at 4°C, 22°C, and 37°C. The results showed that the highest conversion values were obtained when the immobilization was done at 4°C. The conversion values at other temperatures were slightly lower. The conversion values for lipase immobilized at 4°C were lower: 41.1 ± 1.0% at 22°C and 40.4 ± 0.8% at 37°C.

Choosing the right lipase, providing the correct substrates (with their modifications), and considering the reaction type, medium, and method used for activity assessment are important.
It's also worth noting that the literature highlights the use of lipases immobilized on agarose. However, there's not much information about using them in organic solvents [37] . In contrast, our research points to using CALB immobilized on octyl-agarose for reactions in organic solvents. Using CALB immobilized on octyl-agarose makes it possible to carry out bio-catalysis through esterification or transesterification. Our method for producing an octyl-agarose support with CALB for biocatalysis in an organic reaction medium, including the immobilization process, offers excellent enantioselectivity. CALB immobilized on octyl-agarose in an acidic pH buffer citrate buffer (pH 4; 300 mM) was used, followed by air-drying. To our knowledge, air-drying has not been widely discussed in the literature. This approach makes it easier to introduce the immobilized CALB into organic media and can be used for the kinetic resolution of (R,S)-1-phenylethanol through transesterification in DCP (logP value 1.98), with excellent enantioselectivity. We also suggest that by adjusting the pH of the immobilization buffer, the activity of CALB can be controlled.

A Research on the Thermal, Storage, and Operational Stability of CALB.

A thermal stability test was performed to assess how stable the catalytic protein is when exposed to heat.
A climatic chamber was used to test the CALB in its dry form. These tests were carried out in a controlled environment.
The tests were conducted for a week.
The effect of a temperature of 65 Â°C was examined in the climatic chamber.
The temperature was chosen, and the study also looked at the impact of visible light (wavelength).
The range of 400 to 800 nm was also tested.
To compare it with very high temperatures,
A parallel experiment was conducted in a refrigerator at 4 degrees Celsius without any visible light exposure.

As a control, the effect of high temperature (65 Â°C) and the effect of light (400- 800 nm) on the thermal stability of the immobilized enzyme was studied. The impact of light on thermal stability was evaluated after 7 days.
(R,S)-1-phenylethanol was kinetically resolved using immobilized biocatalysts that had undergone transesterification of isopropenyl acetate in DCP (reaction time was 24 hours).
The catalytic data were compared with the results from biocatalysts used in the experiment but not stored.
Kinetic resolution was carried out after drying and immobilization. The immobilization took place in a 300 mM citrate buffer at pH 4.
Polypropylene reactors were used for both immobilization and the experiment itself.
This was done along with kinetic resolution.

The data collected for the catalytic parameters clearly show that the manufactured immobilized biocatalysts have excellent thermal stability. The data on lipase enantioselectivity during storage are similar to those from preparations that were not kept in a refrigerator or climatic chamber.

After being stored in a climatic chamber at a high temperature (65 Â°C) for seven days in closed polypropylene containers, the immobilized lipase showed catalytic activity comparable to that of CALB that was not stored in the climatic chamber (C = 42.5 Â± 0.8%, eep = 99.9 Â± 0.1%).
The activity was also similar for samples stored at different conditions: C = 42.9 Â± 0.4%, eep = 99.7 Â± 0.3%; and C = 43.1 Â± 0.5%, eep = 99.8 Â± 0.2%, respectively. (Figure 2). Biocatalysts stored in the refrigerator in sealed vials also showed protection. These samples showed comparable catalytic activity to those not stored, even under light exposure. The values were (C = 39.4 Â± 0.8%, eep = 99.7 Â± 0.2%; and C = 43.1 Â± 0.5%, eep = 99.8 Â± 0.2%, respectively).

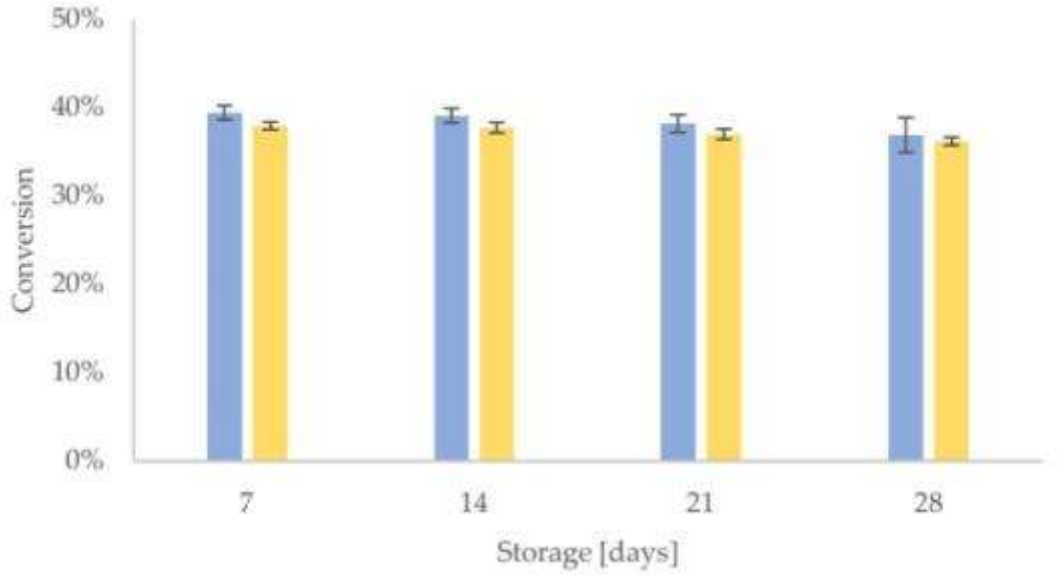


Fig.no 2 : Storage stability : The conditions used to store something affect its ability to convert. The reaction uses (R,S)-1-phenylethanol at 0.08 mM, isopropenyl acetate at 0.3 mM, and immobilized CALB. The temperature is 37 degrees Celsius, with a molecular sieve of 4 angstroms. The medium is DCP, 410 microliters, and there is 50 mg of support. The reactor is made of polypropylene, takes 24 hours for kinetic resolution, and is shaken at 550 rpm. Immobilization is done in a citrate buffer with a pH of 4 and 300 mM. The data is presented as C - conversion. The results show the average with standard deviations from three tests (n = 3), and the standard deviations are shown as error bars. The blue bar graph represents 4°C, and the yellow bar graph represents 22°C.

These results suggest that the immobilization conditions may have a beneficial effect on the thermal stability and catalytic activity of the kinetic resolution.
We believe that the immobilized CALB can maintain its open structure when stored in a refrigerator or climatic chamber.

The findings of this study should be highlighted because they match the patterns seen in our previous publications [32], where we observed that after storage, the CALB immobilized on octyl-agarose showed strong stability, often with an increase in activity during catalysis.
However, in this study, there was no evidence of increased activity, but the results still show outstanding thermal stability and catalytic activity even after storage.

The stability of the immobilized biocatalyst has been studied in a limited number of researches.
These studies are based on the kinetic catalytic parameters of the CALB immobilized on octyl-agarose using resolution in organic solvents.

The next step was to study the storage stability of CALB.

Using the same immobilization method as in the thermal stability study, CALB was stored in two dry environments: one at 4 Â°C in a refrigerator (no visible light), and the other at room temperature (22 Â°C, without visible light). The storage stability was assessed at four time points over a 28-day period: after 7, 14, 21, and 28 days. The same reaction was used, under the same conditions as in the thermal stability experiment. The results suggest that under all storage conditions, the catalytic system remained stable over the 28-day period.

The biocatalyst maintained its performance in subsequent catalytic cycles of the transesterification of (R,S)-1-phenylethanol.
After the third cycle, the reaction conversion was C = 10 Â± 0.3%, which indicates a noticeable drop from the original value (C = 43.1 Â± 0.5%). We think that the results from the first cycle were affected by technical challenges. The small scale of the experiments (50 mg of support), the nature of the interactions between CALB and the support (suggesting the support can be recovered from the reaction medium), and the nature of the interactions between CALB and the support all point to the possibility that the support can be recovered from the reaction medium, which may allow for enzyme leakage. The lipase was immobilized in a citrate buffer with a pH of 4 and a concentration of 300 mM.

Material And Techniques.

1 . Resources

(R,S)-1-Phenylethanol, octyl-sepharose CL-4B (GE Healthcare, Uppsala, Sweden), (R)-1-phenylethanol, (S)-1-phenylethanol, diisopropyl ether (DIPE), n-hexane, n-heptane, trifluoroacetic acid, 1,2-dichloropropane (DCP), 2-propanol, t-butyl methyl ether (MTBE), isopropenyl acetate, hydrochloric acid, Tris base reagent, dichloromethane (DCM), 1,2-dichloroethane (DCE), citric acid, and more were obtained from Sigma-Aldrich (Steinheim, Germany). Molecular sieve 4 Å, disodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate monohydrate, and acid monohydrate were purchased from POCH in Gliwice, Poland. Trisodium citrate was sourced from Chempur (Piekary Śląskie, Poland). Lipase B came from *Candida antarctica* (CALB, made in yeast) from ChiralVision—Leiden, The Netherlands.

2 . Chromatographic conditions and instrumentation:

The Milli-Q Water Purification System (Millipore, Bedford, MA, USA) was used to purify the water used in this study.

The thermal stability of the lipase in the water was then tested. The fixed form was done in a climatic chamber KBF P240 (Tuttlingen, Germany). Buffers were prepared using a SevenMulti pH meter from Mettler-Toledo (Switzerland, Schwerzenbach). The Octyl-Sepharose CL-4B support was made by an unknown source. The Velp Scientifica vortex and the Eppendorf MiniSpin Plus centrifuge (Hamburg, Germany) were used. The ZX4 mixer is made in Italy by Usmate. A Thermomixer was used for incubating the samples. Eppendorf AG (Hamburg, Germany) was used for kinetic resolution. HPLC was used to analyze (R,S)-1-phenylethanol. The Shimadzu HPLC system from Kyoto, Japan, included a UV–VIS detector and a pump (LC-20 AD), a column oven, an autosampler (SIL-20ACHT), a degasser (DGU-20A5R), and an SPD-20A (CTO-10ASVP). A Lux Cellulose-3 (LC-3) (4.6 mm × 250 mm) column was used as a chiral separator, containing cellulose tris(4-methylbenzoate) and a pre-column (Guard Cartridge System, KJO-4282). The experiment used a column with a 5 µm particle size. The best chromatographic conditions for (R)- and (S)-1-phenylethanol and their esters were determined using an n-heptane/2-propanol/trifluoroacetic acid (98.7/1.3/0.15, v/v/v) mobile phase at a flow rate of 1 ml/min. The UV detector's wavelength was set to 254 nm. The chromatographic procedure was conducted at 15°C.

The enantiomeric excesses of the substrate (ees) and the product (eep), the enantiomeric ratio (E) and conversion (C) were calculated using the following formulas .

The ees and eep values were stated as follows: The highest areas represent the enantiomers of the substrate ((R,S)-1-phenylethanol), Rs and Ss. The maximum for the R- and S-enantiomers, respectively. The maximum is represented by the Rp, Sp enantiomers of the product ((R,S)-1-phenylethyl acetate). These regions correspond to the R- and S-enantiomers, respectively.

Technique :

Octyl-Agarose Support Preparation

The method for preparing the support was taken from manufacturer papers and data [30-32].

A polypropylene tube was filled with 110 µL of octyl-agarose beads suspension.

The next step was to inject 1 mL of filtered water into the tube that contained the support suspension.

The mixture was then centrifuged for 3 minutes while being mixed using a vortex.

After the support was separated from the supernatant, it was subjected to 15 minutes of centrifugation at 9000 rpm, resulting in a weight of 50 mg. Octyl-Agarose Support for CALB Immobilization In our lab, we used a slightly modified version of the immobilization procedure

Scheme 3 shows the simple approach.

A quantity of 10.0 mg of CALB was suspended in 1.0 mL of an appropriate buffer and placed in a 2.0 mL Eppendorf tube. The sample was kept at room temperature for 15 minutes.

The CALB was then added to a polypropylene vial (2.0 mL) containing 50 mg of the produced octyl-agarose support. After mixing for 5 minutes, the mixture was kept at 4°C for 14 hours. The supports with the remaining supernatant were then removed.

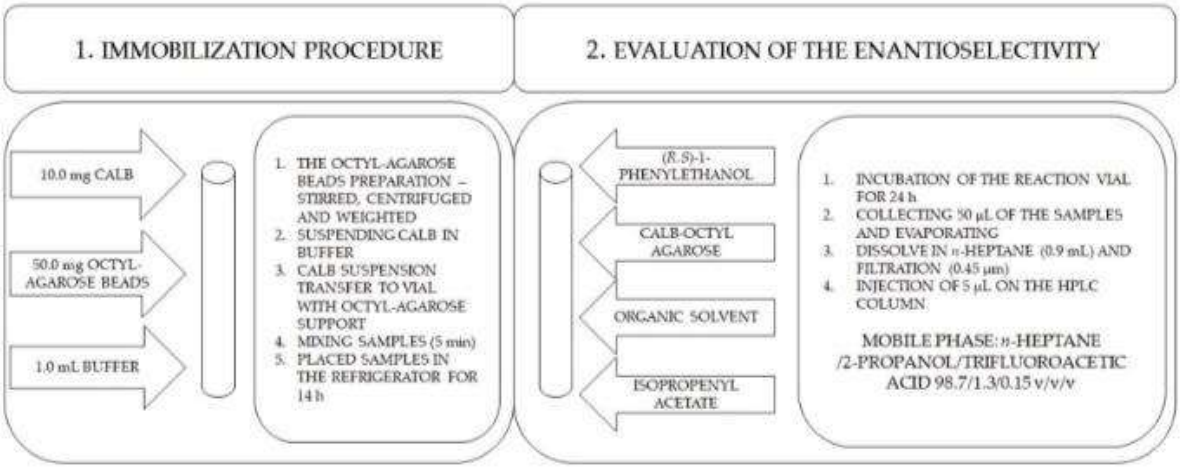
The immobilized CALB was air-dried for 48 hours.The procedures were repeated three times.

The following buffers were used for immobilization: citric buffer with pH 4 (50 mM, 100 mM, 200 mM, 500 mM, 1 M), pH 5 (100 mM), pH 6 (100 mM, 300 mM, and 500 mM); phosphate buffer—pH 7 (100 mM); Tris base buffer, pH 9 (100 mM); and pH 8 (100 mM).

The time required for immobilization was recorded.

At room temperature, the samples were mixed for 5 minutes in a citrate buffer (300 mM, pH 4) and then kept at 4°C for 2, 8, 10, 14, and 20 hours.

Temperature optimization was also carried out. The samples were combined in a citrate buffer - pH 4; 300 mM- for 5 minutes at room temperature before being incubated for 14 hours at three different temperatures-4°C, 22°C, and 37°C.



Scheme 3 : The Simplified Procedure Of The Immobilization Of CALB Onto Octyl Agarose Beads And The Evaluation Of The Enantioselectivity Of Immobilized CALB In The Kinetic Resolution Of (R,S)-1-Phenylethanol.

The following formula was used to calculate the immobilization yield (Iy):

$$(\%)ee_s = \frac{|R_s - S_s|}{R_s + S_s} \times 100$$

$$(\%)ee_p = \frac{|R_p - S_p|}{R_p + S_p} \times 100$$

$$(\%)C = \frac{ee_s}{ee_p + ee_s} \times 100$$

$$E = \frac{\ln[(1 - C)(1 - ee_s)]}{\ln[(1 - C)(1 + ee_s)]}$$

$$I_y = \frac{LA_B}{LA_{10}} \times 100\%$$

with Iy being the immobilization yield and LAB being the difference in the lipase amount between the initial quantity of CALB and the amount left in the supernatant after immobilization.
The initial quantity of lipase is 10 mg (LA10), and 50 mg of support is used for immobilization (data from lipase loading).

$$I_y = \frac{LA_B}{LA_{10}} \times 100\%$$

(R,S)-1-Phenylethanol Kinetic Resolution using CALB as a Catalyst

The kinetic resolution of (R,S)-1-phenylethanol was performed following a method described in the literature [19,20,49], which was adjusted and simplified, as shown in Scheme 3.

The reaction mixture included

(R,S)-1-phenylethanol (0.08 mM), molecular sieve 4 Å, isopropenyl acetate (0.3 mM), and DCP (410 µL). These components were added to the octyl-agarose support with the immobilized lipase (CALB) in a polypropylene vial. The sample vials were sealed and protected using thermal insulation tape. The mixture was then incubated while stirring at 37°C and 550 rpm in a Thermomixer. The reaction took 24 hours to complete.

After the reaction, 50 µL of the sample was collected and evaporated at room temperature.
It was then dissolved in n-heptane (0.9 mL) and filtered through a 0.45 µm filter before being injected into the HPLC (5 µL) for analysis.

CALB Stability Tests :

The thermal stability of the CALB was tested following a procedure outlined in the literature [30-32] . The supernatant was immobilized in a citrate buffer (300 mM, pH 4). The supports containing the immobilized lipase were air-dried for 48 hours. The octyl-agarose beads with the immobilized enzyme were then stored in polypropylene vials either in a refrigerator at 4°C or in a climatic chamber at 65°C using a KBF P240 model. The visible spectrum range in the climate chamber was kept constant at 65°C between 400–800 nm. The immobilized lipase was kept for seven days before its enantioselectivity was measured. Section 3 of the immobilized lipase was tested, and the reaction time was 24 hours.

a . Used after immobilization, but without a storage method;

b . Climatic chamber : storage temperature of 65°C, no light in the visible spectrum range

c . Climatic chamber (65°C, visible spectrum 400–800 nm): storage temperature of 65°C, with light in the visible spectrum range

d . Refrigerator (4°C, no light): storage temperature of 4°C, with no light in the visible spectrum range.

Conclusion :

The research aimed to find out if the CALB-octyl-agarose support could be used in the future. The kinetic resolution of (R,S)-1-phenylethanol was used as a method to test transesterification. Isopropenyl acetate was used as the acylating agent in DCP as the reaction medium. The reactions showed very high enantioselectivity (E > 200). The (R)-1-phenylethyl acetate reached a conversion of around 40%, with an enantiomeric excess (ee) over 99%. The paper explains how CALB was immobilized on an octyl-agarose support, which was the main focus of the study. Immobilized biocatalysts were created and showed good catalytic activity in enantioselective transesterification. The study also looked into thermal stability (tested in a climate chamber), storage stability, and operational stability. It was found that the pH and concentration of the buffer used were important factors. Since CALB can be attached to an octyl-agarose support, it could be a key factor in determining the enantioselectivity of the immobilized biocatalyst. Using a 300 mM citrate buffer at pH 4 gave the best conditions for achieving high lipase enantioselectivity. The immobilized CALB showed excellent stability during the tests. After 7 days of storage at 65°C, the (R)-1-phenylethyl acetate still had an enantiomeric excess over 99% at a conversion rate of around 40%—results similar to those achieved with a non-immobilized lipase. The high catalytic activity and stability of the created lipase, as shown by the data, are significant. The fact that CALB is immobilized makes the octyl-agarose support a very useful tool for enantioselective transesterification in organic media.

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