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Investigation of the Effect of Beta-Cyclodextrin and Polyvinyl Alcohol on Activity of *Candida Rugosa* Lipase

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Abstract:- Surfactants can affect activity through enzyme-surfactant interactions such as interface adsorption where the substrate is located or competitive binding to the active site. The catalytic activity of lipases is mediated by interface activation. The interfacial activation is a feature that begins with an emulsion of the lipid substrate and thus provides an interface for the enzyme to function. Addition of surfactant reduces the surface tension between the organic and aqueous phase in the reaction medium and increases the emulsification rate. In this study, beta cyclodextrin and polyvinyl alcohol were used as a surfactant to see the effect on the hydrolytic activity of Candida rugosa lipase (CRL). The effects of neutral surfactant concentrations and ring effects on CRL activity were investigated and kinetic parameters were determined.

Keywords:- *Surfactant; Lipase; Activity; Cyclodextrin.*

I. INTRODUCTION

Lipases (E.C.3.1.1.3) are a type of enzyme that has the ability to hydrolyze triglycerides at the water-oil interfaces. Since this reaction is reversible, lipases also catalyze the formation of acylglycerols from glycerol and free fatty acids [1-3].

Surfactants are chemical compounds that affect surface tension when dissolved in water or an aqueous solution. Surfactants consist of two groups of molecules: hydrophilic (hydrophilic) and water-repellent (hydrophobic). Surfactants are of four types. These; anionic, cationic, neutral (nonionic) and zwitter (amphoteric) surfactants. Most of the recent literature on the enzyme activity of surfactants is lipases and phospholipases. The natural substrates of these enzymes are insoluble in water and function at the interface [6-8]. The addition of surfactant reduces the surface tension between the organic and aqueous phases in the reaction medium and increases the emulsification rate [1,5,9,10]. By binding to the enzyme, these can affect the enzyme's activity by affecting the enzyme's secondary, tertiary structure and flexibility [1,4,5].

Beta-cyclodextrins (CDs), which have a macrocyclic structure, have been used successfully to improve the activity of enzymes and increase the reaction rate in enzyme catalyzed reactions [11,12]. Cyclodextrins are of 3 types according to the glycopyranose number in the ring, α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin. α-, β-, and cyclodextrin are formed from the bonding of 6, 7, 8 glucopyranose rings by α- (1,4) glycoside bonds, respectively. β-Cyclodextrin is the cheapest, useful and most available derivative. Cyclodextrins are non-toxic, low cost, high complexing capacity, very suitable compounds for some specific applications. Beta-cyclodextrins have a cone structure with hydrophilic outer surface and hydrophobic cavity in the center, making them ideal for modeling enzyme substrate binding [13]. There is very little literature on the effect of surfactants on enzyme activities [1,5,14-18]. Therefore, in this study, the effect of cyclic and acyclic (beta cyclodextrin and polyvinyl alcohol) surfactants on *Candida rugosa* lipase (CRL) activity was investigated.

II. MATERIALS AND METHODS

A. Materials

Candida rugosa lipase and *p*-nitrophenyl palmitate (p-NPP), were obtained from Sigma-chemical. Beta-cyclodextrin, and polyvinyl alkol were provided from Merck. All other chemicals used were obtained from various commercial sources.

B. Preparation of surfactants

Aqueous solutions (0.625, 1.25, 2.5 and 5 mM) of surfactants of different concentrations were prepared and the pH of each solution was adjusted to 7. Afterwards, 2 mL of CRL (1 mg/mL) was added to the surfactant solution and the mixture was incubated at 30 °C for 15 minutes.

C. Lipase activity

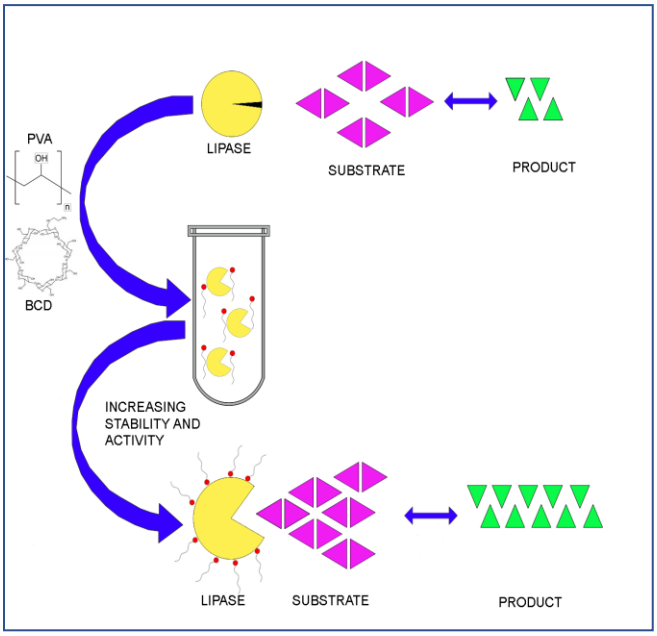
Lipase activity was performed using p-nitrophenyl palmitate (*p*-NPP) as substrate and was determined by measuring *p*-NPP absorption at 405 nm with a UV visible spectrophotometer.

D. Enzyme kinetics

The hydrolytic activity of free lipase at different concentrations of *p*-NPP was investigated. The kinetic parameters of K_m and V_{max} were calculated.

III. RESULTS AND DISCUSSION

Beta-cyclodextrin (CD), and polyvinyl alcohol were used as neutral surfactants (Scheme 1). CD solutions of different concentrations (0.625 mM, 1.25 mM, 2.5 mM, 5 mM) were prepared and used in lipase activity. The lipase enzyme activity was the highest at 2.5 mM concentration of CD surfactant (Figure 1). Enzyme activity was decreased after 2.5 mM.



Scheme 1:- The effect of cyclic and acyclic neutral Surfactants on enzyme activity

Cyclodextrins are non-toxic, low cost, high complexing capacity, very suitable compounds for some specific applications. They can be obtained with high purity and are inert in pharmacological reactions. They are easily biodegradable. Host-guest type complexes can be made with suitable hydrophobic molecules because of its polar hydrophilic outer shell and hydrophobic space [19,22,23]. Cyclodextrin, a non-ionic surfactant, binds to the enzyme through hydrophobic interactions. Cyclodextrin, a non-ionic surfactant, binds to the enzyme through hydrophobic interactions and increased enzyme activity by facilitating micelle formation. In higher concentrations, since the surfactant denatures the protein structure to a large extent, large losses in activity occur [1,4] (Figure 2).

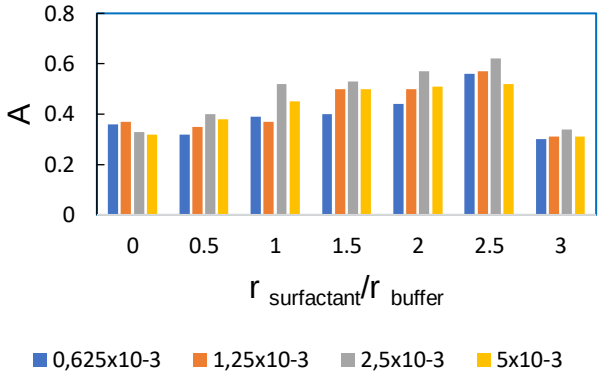


Fig 1:- Effect of beta-CD surfactant concentration on the lipase activity

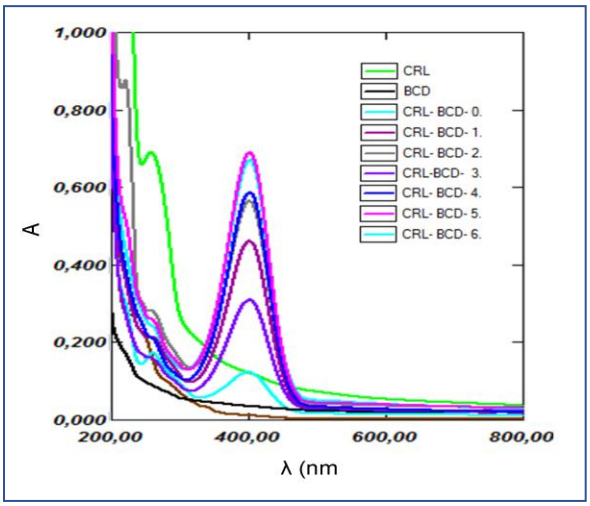


Fig 2:- Enzyme activity results of different concentrations of the cyclic beta CD

In this study, non-cyclic polyvinyl alcohol was chosen for comparison with cyclic beta-CD. (Scheme 1). Figure 3 shows that, when the results of acyclic polyvinyl alcohol surfactants in 0.675 mM concentration were used, high values of lipase activity were observed.

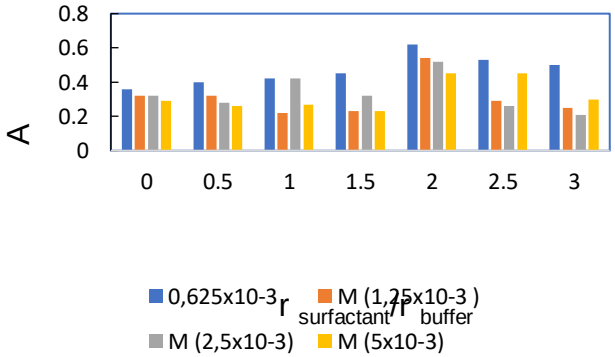


Fig 3:- Effect of polyvinyl alcohol surfactant concentration on the lipase activity

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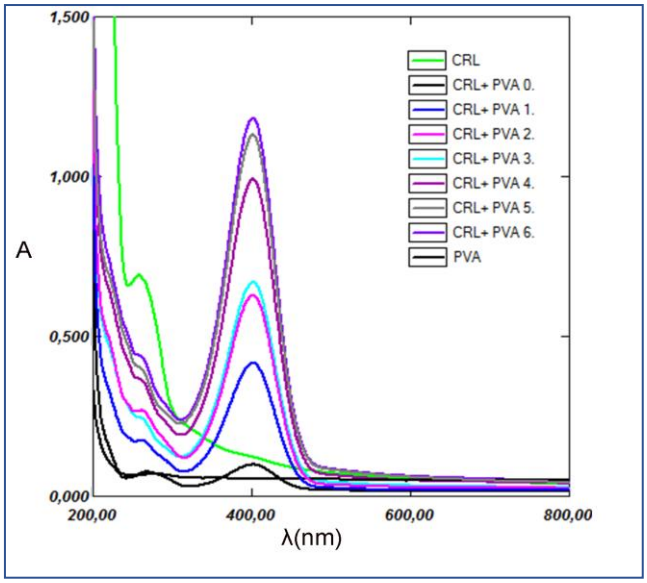

Fig 4:- Enzyme activity results of different concentrations of the cyclic polyvinyl alcohol

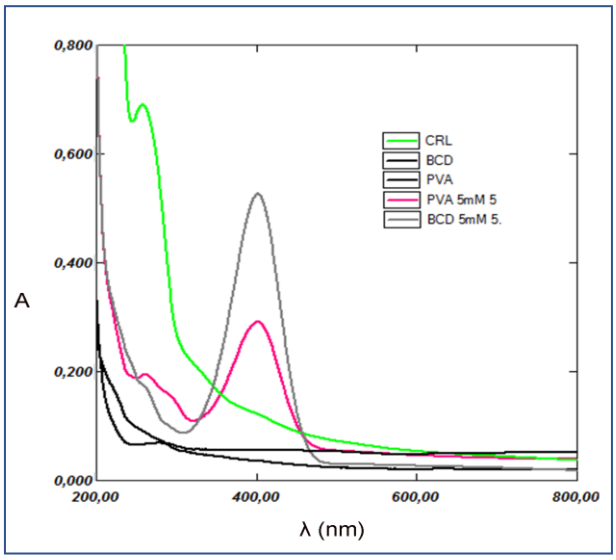

Fig 5:- The effect of different surfactants on lipase activity

Since polyvinyl alcohol could not interact with amino acids with active centers of the enzyme enough, it could not open the enzyme's lid so the increase in enzyme activity was less than another surfactant (Figure 5).

➢ *Enzyme kinetics*

For a deeper understanding of the action of beta-CD and polyvinyl alcohol surfactants, the kinetic parameters of the CRL have been determined. All data points were found to fit the Michaelis-Menten kinetics and kinetic parameters were calculated. Km and Vmax values of lipase enzymes are given in Table 1.

High K_m indicates low affinity of enzyme to substrate. The increase in K_m can be explained by the tendency of surfactants to form different micelles. The highest V_{max} (59.4 U / mg) was seen when beta-CD surfactant was used, and this confirms our study.

	V_m (U/mg)	**K_m (mM)**
Free CRL	115	0.44
Beta-CD	59.4	1.90
PVA	20.5	2.60

Table 1:- The Effect of beta-CD and Polyvinyl Alcohol Surfactants on Kinetic Parameters of CRL

IV. CONCLUSION

In this study, surfactants in neutral structures (cyclic and non-cyclic) were prepared. The effect of these surfactants on lipase activity was investigated. Kinetic parameters (V_{max} and K_m values) were found. Comparing surfactants, the highest enzyme activity was obtained with the addition of beta-cyclodextrin. Besides, the neutral surfactant beta-CD proved to be more suitable because it did not denature the enzyme and acted as a stabilizer for organic/aqueous phase emulsions, promoting substrate hydrolysis.

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