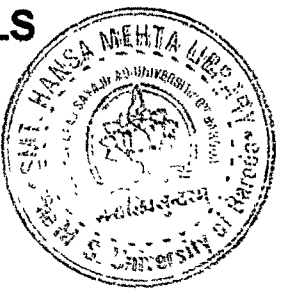
IMMOBILIZATION OF ENZYMES ON K-CARRAGEENAN AND OTHER POLYMERIC MATERIALS



Summary of the
Thesis Submitted to
The Maharaja Sayajirao University of Baroda.
For the Degree of

Doctor of Philosophy

IN
CHEMISTRY

BY
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Enzymes have been utilized by human beings since ancient times , well before their nature was understood. The use of enzymes has gradually extended into a variety of fields such as brewing , food production , textile , tanning and medicine . The enzymes are not effective in free form and get denatured under adverse conditions hence need immobilization. The immobilization of enzymes can be carried out either by physical or chemical means.

The present thesis has been divided into four chapters. In the first chapter a brief introduction about enzymes , its classification and different methods of immobilization are discussed . The second chapter includes immobilization of horseradish peroxidase on both natural support K-carrageenan and synthetic support copolymer of AAm-HEMA . Here all the conditions for entrapment and covalent coupling were optimized. The entrapped enzyme was further used for hydroquinone oxidation by batch process while the covalently bound enzyme was used for phenol oxidation by fixed bed reactor. In the third chapter details of pepsin immobilization on K-carrageenan and AAm-HEMA copolymer is discussed . Optimization of various conditions has been discussed in details. Use of entrapped enzyme for milk clotting by using fluidized bed reactor and use of covalenlty bound enzyme for casein hydrolysis in fixed bed reactor has been explored . Chapter 4 gives details of immobilization of β-galactosidase on these two supports , optimization of various conditions and use of immobilized enzyme in fixed bed reactor for lactose hydrolysis

The entrapment in K-carrageenan was carried out by preparing K-carrageenan beads. While the covalent coupling to the copolymer of AAm-HEMA was done by activating both OH and NH_2 groups. Simultaneous activation of both the groups was also carried out and a synergistic effect was observed . The activity was found to increase more than two fold on activation of amino group followed by activation of hydroxyl group . But on reversing the order of activation the activity of the enzymes decreased .

On comparison of performance of free , entrapped and covalently bound enzyme it was observed that the immobilized enzyme shows superior behavior to free enzyme .Tables 1,2,3 show comparative results for the enzymes used in the study.

Table 1 .Comparison of properties of Free and immobilized Horseradish peroxidase.

Properties	Free enzyme	Entrapped enzyme	Covalently bound enzyme
Optimum pH	8	7	7
Optimum Temperature (° C)	35	35	35
Storage stability at 35°C till 50% loss in activity .(days)	7	40	60
Durability (Cycles)	-	4	8
Thermodeactivation constant (K_d) at 45 °C.	0 811 x 10^{3}	0 510 x 10^{3}	0.510 x 10^{3}
Michaelis constant (K_m) (mM)	8 x 10^{-5}	8.3 x 10^{-5}	8.14 x 10^{-5}
Maximum velocity (V_{max}) (mM/min)	1.53	2 18	2.0
Turnover number	-	51.6 x 10^{6}	10 3 x 10^{7}

Table 2. Comparison of properties of Free and immobilized Pepsin.

Properties	Free enzyme	Entrapped enzyme	Covalently bound enzyme
Optimum pH	2	2	2
Optimum Temperature (° C)	35	35	35
Storage stability at 35°C after one month. (%)	-	80	60
Durability (Cycles)	-	5	6
Thermodeactivation constant (K_d) at 45 °C.	1.66×10^{2}	0.551×10^{2}	1.12×10^{2}
Michaelis constant (K_m) (mM)	$1\ 1 \times 10^{-6}$	$1\ 3 \times 10^{-6}$	$1\ 2 \times 10^{-6}$
Maximum velocity (V_{max}) (mM/min)	0.88	1.03	0.98
Turnover number	-	500	650

Table 3. Comparison of properties of Free and immobilized β-galactosidase.

Properties	Free enzyme	Entrapped enzyme	Covalently bound enzyme
Optimum pH	4	4	4
Optimum Temperature (° C)	40	40	40
Storage stability at room temperature till 50 % loss in enzyme activity (days).	7	37	35
Durability (Cycles)	-	5	7
Michaelis constant (K_m) (mM)	$0\ 22 \times 10^{-5}$	$0\ 38 \times 10^{-5}$	$0\ 27 \times 10^{-5}$
Maximum velocity (V_{max}) (mM / min)	0 16	0 27	0 20
Turnover number	-	$1\ 7 \times 10^{4}$	1.14×10^{4}

From our work following observation were made.

1. The enzymes have been successfully entrapped in K-carrageenan beads using polyethyleneimine as hardening agent while covalent coupling of enzyme showed that both the groups of the support can be activated simultaneously and the activity was found to increase more than two folds.
2. Immobilized enzymes were observed to show better stability than free enzyme under all tested conditions like pH , temperature and storage stability.
3. The kinetic parameters did not show much change when enzyme concentration was kept constant and substrate concentration was varied and vice-versa indicating no conformational change during immobilization.
4. Oxidation of hydroquinone was carried out using both free and entrapped horseradish peroxidase in batch process and it was observed that about 90 % oxidation is achieved in 1 h.
5. Phenol Oxidation was carried out using covalently bound Horseradish peroxidase in fixed bed reactor and it was observed that maximum 80 % oxidation takes place at 0.5 cm ^{3}/ min flow rate , 45 $^{\circ}$C temperature and l/d ratio 6.
6. Milk clotting studies were carried out using entrapped pepsin in K-carrageenan in a fluidized bed reactor and it was observed that clotting efficiency increases in acidic pH. The maximum clotting was observed using 50 μg of enzyme at pH 2 at 20 $^{\circ}$C and 1 cm^3 flow rate.
7. Casein hydrolysis in a fixed bed reactor showed 90 % of hydrolysis at 2 cm^3/min flow rate at 35°C and 1mM casein concentration.

8..Lactose hydrolysis carried out using both entrapped and covalently bound β-galactosidase showed 94 % and 91 % hydrolysis in fixed bed reactor at optimum conditions : 1 cm^3/min flowrate at 50°C and l/d ratio of 8 at 1 mM lactose concentration.