SUMMARY AND CONCLUSION

Enzymes are power houses in generating energy as well as performing various reactions. Enzymatic reactions are not only important in biological activities but they are equally important in various chemical reactions used in industries. They can catalyse a reaction under very mild conditions in aqueous solutions at normal temperature and pressure and with very high specificity. Purification and extraction of enzymes is not an easy task and hence their use in various reactions is governed stringently. In olden times free enzymes were used for the chemical reactions. Since the recovery yield and reusability of the free enzymes as industrial catalysts is quite limited, gradually they were replaced by immobilized enzymes. Enzyme immobilization offers advantages over free enzymes such as use in batch, continuous processes, rapid termination of reactions, controlled product formation, ease of enzyme removal from the reaction mixture and adaptability to various engineering designs. Furthermore the interest in the immobilized enzymes and their applications to bioprocessing, analytical systems and enzyme therapy have steadily grown in the past few decades. Covalent coupling of the enzyme to the support is popular due to high accessibility and reusability of the bound enzyme. In addition no leaching of enzyme takes place during repeated uses; Binding forces are stronger than those in adsorption or ionic binding and diffusion problems and protein loading can be controlled. Hence in the present study we have prepared covalently coupled enzyme-polymeric support systems through various activation processes and their properties are compared with free enzymes.

Synthetic and natural polymeric supports were used for the immobilization of enzymes. Salicylic acid - resorcinol-formaldehyde (SRF), glucosamine polysaccharide chitosan, Amberlite IRA-400, and crosslinked poly(vinyl alcohol) were used for the immobilization of enzymes.

Enzymes from hydrolases class, such as ß-galactosidase (lactase), urease, pepsin, trypsin and chymotrypsin were selected for the present study.

Immobilization of enzymes was carried out through different hydroxy and amino group activation methods. Albumination techniques were also used for the immobilization of the enzymes. Various conditions for the preparation of immobilized systems such as type and concentration of crosslinking reagent, enzyme concentration, pH of the coupling medium and immobilization time were optimized for all the enzymes to achieve maximum loading and retention of their activity. It was observed that the coupling reaction is influenced by enzyme/support, activator/support ratios, pH and coupling time. Protein coupled was determined by Lowry method and the activity of the coupled protein was estimated by using appropriate substrates.

Important results obtained from the optimized conditions for selected immobilized systems are summarized in Table 1.

Table 1. Optimized conditions for selected immobilized enzyme systems.

Conditions	ß-Galactosidase	Urease	Pepsin	Trypsin	Chymotrypsin
Substrate	ONPG	Urea	Hemoglobin	BAPNA	BTEE
Support (0.2 g)	Chitosan	PVA-F	SRF	SRF	SRF
Crosslinking Reagent	Glutaraldehyde	Cynuric chloride	p- tolyl sulphonyl chloride	Cynuric chloride	p- benzo quinone
Concentration of crosslinking reagent (g)	6 % (w/w)	1.0	0.1	0.4	0.4
pH of coupling medium	4.5-6.5	7-9	2-4	7-8	4-7
Coupling Time (h)	1-2	2	1-2	4	2
Enzyme Loaded (mg g^{-1})	3.5	8.2	8.5	50.4	20
Retention of Activity (%)	100	70-80	100	18 5	- 2

All immobilized enzymes were thoroughly characterized through their pH, temperature, kinetics, storage, solvent stabilities and their performance was compared with free enzymes. The selected immobilized systems and their properties are summarized in Table 2, 3 and 4.

Table 2. Comparison of properties of free and immobilized ß-Galactosidase

Properties		ß-Gal-Chitosan	ß-Gal-PVA-F	ß-Gal-SRF	ß-Galactosidase
Optimum pH		4.5-6.5	3.5-4.5	6-7	4 5
Optimum Temperature (°C)		40-60	40-60	40-60	40-50
Michaeli's Constant (mM)		6.25	5.0	n. d.	6.25
Maximum Velocity (mM min^{-1})		3.0×10^{-2}	4.0×10^{-2}	n. d.	$5\ 7 \times 10^{-2}$
Storage Stability (%) [after 3 months]		100	70	50	50
Durability (cycles)		> 10	> 7-8	> 4	-
Thermal Stability (%) [at 65 °C]		95	93	27	40
Solvent Stability (%)	Methanol	82	70	18	60
	Ethanol	72	93	18	79
	Propanol	75	92	17	65

n. d. not determined.

Table 3. Comparison of properties of free and immobilized Urease

Properties		Urease-PVA-F	Urease
Optimum pH		8-9	8
Optimum Temperature (°C)		40-60	50
Michaeli's Constant (mM)		0.71	2.60
Maximum Velocity (mM min^{-1})		0.24 x 10^{-4}	0.38 x 10^{-4}
Storage Stability (%) [after 3 months]		45	nil
Durability (cycles)		> 6	-
Thermal Stability (%) [at 60 °C]		75	10
Solvent Stability (%)	Methanol	80	80
	Ethanol	70	73
	Propanol	55	67

Table 4. Comparison of properties of free and immobilized Pepsin

Properties	Pepsin-SRF	Pepsin
Optimum pH	2-4	2
Optimum Temperature (°C)	30-60	30-37
Michaeli's Constant (µg)	0.76	0.76
Maximum Velocity (µg min^{-1})	2.38	1 36
Storage Stability (%) [after 3 months]	75	40
Durability (cycles)	> 6	-
Thermal Stability (%) [at 45 °C]	70	30

Immobilized ß-galactosidase systems were further used for the study of hydrolysis of lactose in a fixed bed reactor and ~ 94% of the lactose hydrolysis at 50-60°C was achieved.

Immobilized urease was further used in a fixed bed reactor for the continuous hydrolysis of urea. Different reactor parameters were optimized and 90% of urea hydrolysis was achieved.

From the study of immobilization of proteolytic enzymes, trypsin and chymotrypsin exhibited comparatively inferior properties in terms of retention of activity hence these systems were not used for further characterization.

Immobilized pepsin was used for the study of secondary phase milk clotting. Various conditions for the coagulation of milk were optimized and maximum clotting activity was observed to be at 40-50°C and pH 6-6.2.

From the study the following observations have been made :

- Among the various polymeric supports studied, SRF, PVA-F and Chitosan were very effective and useful supports for the immobilization of pepsin, urease and ß-galactosidase.
- Extent of immobilization of protein and retention of enzyme activity depend on the enzyme/support and activator/support ratios, pH and coupling time.
- Improved thermal, solvent and storage stability and excellent durability was achieved for pepsin-SRF, urease-(PVA-F) and ß-galactosidase-chitosan immobilized systems.
- Immobilized ß-galactosidase system was successful in the recovery of glucose and galactose from milk whey through continuous reactor studies.
- Immobilized urease was useful for the hydrolysis of urea in fixed bed reactor studies.
- Improved clotting activity of milk was achieved through immobilized pepsin treatment in a fluidized bed reactor.