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Concanavalin A layered calcium alginate–starch beads immobilized β galactosidase as a therapeutic agent for lactose intolerant patients

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ABSTRACT

A novel therapeutic agent in the form of β galactosidase immobilized on the surface of concanavalin A layered calcium alginate–starch beads has been developed. Immobilized β galactosidase exhibited significantly very high stability against conditions of digestive system such as pH, salivary amylase, pepsin and trypsin. Soluble and immobilized β galactosidase exhibited same pH-optima. However, the immobilized enzyme retained greater fraction of catalytic activity at higher and lower pH to pH-optima as compared to soluble enzyme. Immobilized enzyme preparation was quite stable under conditions present in mouth, stomach and intestine. Immobilized β galactosidase retained 65% activity even after its sixth repeated use.

1. Introduction

β galactosidases are abundant among micro-organisms, animals and plants (Haider and Husain, 2007a). These enzymes are increasingly employed in the dairy industry. Their capacity as biocatalysts is to hydrolyze lactose in milk and whey which helps in the production of low-lactose dairy products for people intolerant to this sugar and also serves to recycle whey which can be used as an additive for human or cattle feed (Zhou et al., 2003; Phadtare et al., 2004). Lactase is the common name for lactase-phlorizin hydrolase (LPH), an enzyme located in the brush border of small intestinal enterocytes that is necessary for the digestion of lactose. Human LPH is encoded by the lactase gene located on the long arm of chromosome 2 (Kerber et al., 2007). Lactose intolerance occurs in children shortly after weaning, when production of the lactose-digesting enzyme, lactase is down-regulated in the gut (Stefano et al., 2001).

The symptoms of lactose intolerance tend to be primarily gastrointestinal in origin (Wilson, 2005). When lactase is absent or deficient, hydrolysis of lactose is incomplete as it is osmotically active, the undigested sugar will pull fluid into the intestine. Thus these lactose intolerant people are discouraged from consuming milk and may lose a major source of calcium and high quality protein from their diets (Suarez et al., 1995; Kim et al., 1999; Heyman, 2006). Milk is the most important source of energy during the first year of a human's life, providing almost half the total energy requirement of infants (Vesa et al., 2000). In order to prevent lactose intolerance, the need for lactose-free milk and its products arises.

Immobilized enzymes are generally more stable and there are many potential applications that range from chemical synthesis to biotechnology and medicine (Liang et al., 2000). Immobilization of β galactosidase in liposomes may be useful in order to overcome the shortcoming of lactose hydrolysis. β galactosidase microencapsulation in lipid vesicles has been delivered for treating lactose intolerance but there was a problem of contact between the enzyme and substrate (Walde and Ichikawa, 2001; Monnard, 2003; Nogales and Lopez, 2006). Very few other immobilized β galactosidase preparations have shown their potential in targeting lactose present in small intestine. Based on applications, there are many different types of polymers used for enzyme immobilization (Propkop et al., 1998). Chitosan is biocompatible and has been used in many applications including drug delivery systems. The disadvantage of chitosan is its limited solubility in water and the low pH of chitosan solution tends to denature most proteins and cells (Taqieddin et al., 2002). Starch is a high molecular weight polymer and is used as the coating polymer which afforded the probiotic strain survives in the adverse environmental conditions (O'Riordan et al., 2001; Lanthong et al., 2006). Now need has arisen to develop such a preparation where enzyme would be immobilized on large

Abbreviations: Con A, concanavalin A; ONPG, *o*-nitrophenyl β-D-galactopyranoside; SβG, soluble β galactosidase; IβG, immobilized β galactosidase.

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surface area of the support and it could easily hydrolyze lactose deposited in the lumen of the small intestine.

Here an attempt has been made to immobilize β galactosidase on the large surface of concanavalin A (Con A) layered calcium alginate–starch beads. In order to examine the suitability of this immobilized β galactosidase as oral therapeutic agent for the treatment of patients suffering from lactose intolerance, we have studied the stability of immobilized β galactosidase against the conditions of alimentary canal/digestive system, such as varying pH, trypsin, pepsin and salivary α amylase. The reusability of immobilized enzyme in the buffer of varying pH and in the assay buffer was also investigated.

2. Materials and methods

2.1. Materials

Aspergillus oryzae β galactosidase (3.2.1.23), galactose and glucose were obtained from Sigma Chem. Co. (St. Louis, MO) USA. *o*-Nitrophenyl β-D-galactopyranoside (ONPG) and starch were obtained from SRL, Chemicals, Mumbai, India. Sodium alginate was the product of Koch-Light Lab (Colnbrook, UK). Jack bean meal was procured from DIFCO, Detroit, USA. All other chemicals and reagents used were of an analytical grade.

2.2. Preparation of calcium alginate–starch beads

An aqueous mixture of sodium alginate (2.5%) and starch (2.5%) was slowly extruded as droplets through a 5.0-mL syringe with attached gauge needle No. 20 into 0.2 M calcium chloride solution. The formation of calcium alginate–starch beads was instantaneous and the beads were further gently stirred in calcium chloride solution for 2 h (Matto and Husain, 2006). The beads were washed and stored in 0.1 M acetate buffer, pH 4.6 at 4 °C, until further use.

2.3. Binding of Con A on the surface of calcium alginate–starch beads

Jack bean extract (10%, w/v) was prepared by adding 5.0 g of jack bean meal to 50 mL of 0.1 M Tris–HCl buffer, pH 6.2 with slight modification from the earlier used method (Haider and Husain, 2007b). Calcium alginate–starch beads were incubated overnight with jack bean extract (25 mL) containing Con A, at 30 °C with mild stirring. β galactosidase (2100 U) was incubated overnight with Con A layered calcium alginate–starch beads at room temperature (30 °C) with slight stirring. The bound enzyme was separated from the unbound enzyme by repeatedly washing with 0.1 M sodium acetate buffer, pH 4.6.

2.4. Crosslinking of immobilized β galactosidase

Con A layered calcium alginate–starch beads immobilized β galactosidase was crosslinked by 0.5% (v/v) glutaraldehyde 2 h at 4 °C. Ethanolamine was added to a final concentration of 0.01% (v/v) to stop the crosslinking. Crosslinked beads were allowed to stand with ethanolamine for 90 min at 30 °C. The integrity of crosslinking was examined by incubating beads in 1.0 M methyl-α-D-glucopyranoside for 2 h. No enzyme activity was released from the beads; it indicated complete crosslinking of the immobilized enzyme.

2.5. Effect of pH on the activity of soluble and immobilized β galactosidase

The activity of β galactosidase (2.0 U) was measured in buffers of various pH values. The buffers used were glycine–HCl (pH 2.0 and 3.0), sodium acetate (pH 4.0–6.0) and Tris–HCl (pH 7.0–10.0). The molarity of each buffer was 0.1 M. The activity at pH 4.6 was taken as control (100%) for the calculation of percent activity.

2.6. Stability of soluble and immobilized β galactosidase at pH 2.0, 4.6 and 7.0

The stability of soluble and immobilized β galactosidase was monitored by incubating the enzyme in the buffers of different pH (2.0, 4.6 and 7.4) for various time intervals at 37 °C. After incubation in varying buffers, beads were washed with assay buffer and their activity was determined by ONPG.

2.7. Reusability of immobilized β galactosidase in the buffers of varying pH

The activity of immobilized β galactosidase was initially checked in 0.1 M sodium acetate buffer, pH 4.6. β galactosidase bound beads were taken out from the assay tubes and washed and incubated in 0.1 M glycine–HCl buffer, pH 2 for 30 min for assaying the activity with ONPG. These beads were further washed with 0.1 M sodium acetate buffer pH 4.6 and the same beads were taken for determining the activity of β galactosidase in Tris–HCl, pH 7.4. The same procedure was repeated after 1 and 2 h.

2.8. Effect of salivary amylase on the activity of soluble and immobilized β galactosidase

Con A layered calcium alginate–starch beads surface immobilized β galactosidase (2.0 U) was incubated with increasing concentrations of salivary α amylase (20–200 U) in 0.1 M sodium acetate buffer, pH 4.6 for 4 h at 37 °C. The activity of the enzyme without salivary amylase treatment was considered as control (100%) for the calculation of remaining activity.

2.9. Effect of trypsin/pepsin on the activity of soluble and immobilized β galactosidase

Soluble and immobilized preparations of β galactosidase (2.0 U) were incubated with increasing concentrations of trypsin/pepsin (0.025–0.150 mg mL^{-1}) at 37 °C for 1 h. After incubation period the β galactosidase activity was determined as described in the assay procedure.

2.10. Reusability of immobilized β galactosidase

Calcium alginate–starch beads were taken in triplicates and were assayed for the activity of β galactosidase. After each assay, beads were taken out and stored in 0.1 M sodium acetate buffer, pH 4.6 for 6 h. This procedure was repeated for six successive cycles. The activity determined for the first time was considered as control (100%) for the calculation of remaining percent activity after each use.

2.11. Assay of β galactosidase

β galactosidase was determined by measuring the release of *o*-nitrophenol from *o*-nitrophenyl β-D-galactopyranoside at 405 nm (Haider and Husain, 2008).

One unit (1.0 U) of β galactosidase activity is defined as the amount of enzyme that liberates 1.0 μmole of *o*-nitrophenol (ε_m = 4500 L mol^{-1} cm^{-1}) per minute under standard assay conditions.

Table 1
Immobilization of β galactosidase on the surface of Con A layered calcium alginate–starch beads

Enzyme preparation	Enzyme activity loaded, X (U)	Enzyme activity in washes, Y (U)	Activity bound g^{-1} alginate–starch beads		Activity yield $B/A \times 100$
			Theoretical $(X - Y)=A$	Actual $= B$	
Enzyme adsorbed on the surface of beads	2100	250	1850	1406	76
Enzyme adsorbed on the surface of beads and crosslinked by glutaraldehyde	–	–	1850	1314	71

2.12. Protein estimation

Protein concentration was determined by using dye binding method (Bradford, 1976). Bovine serum albumin was used as a standard protein.

2.13. Statistical analysis

Each value represents the mean for three-independent experiments performed in duplicates, with average S.D. <5%. The data expressed in various studies was plotted using Sigma Plot-9 and Origin-6.1 and expressed as mean with standard deviation of error (±). Data was analyzed by one-way ANOVA. P-values <0.05 were considered statistically significant. The diameter of the calcium alginate–starch beads (0.33 cm) was calculated by Vernier Callipers with the least count of 0.01 cm.

3. Results

3.1. Immobilization of β galactosidase on the surface of Con A layered calcium alginate–starch beads

The calcium alginate–starch beads were spherical in shape, the area calculated was found to be 341.94×10^{-3} cm^2. The volume of the spherical beads was 18.80×10^{-3} cm^3. Table 1 demonstrates the immobilization of β galactosidase on Con A layered calcium alginate–starch beads. Calcium alginate–starch beads retained nearly 76% of the original activity. However the crosslinking with glutaraldehyde resulted in a marginal loss of enzyme activity and it showed nearly 71% of the initial activity.

3.2. Effect of pH on the activity of soluble and immobilized β galactosidase

Fig. 1 demonstrates the pH-activity profiles of soluble and immobilized β galactosidase. The soluble and immobilized β galactosidase preparations showed same pH-optima. However, the immobilized enzyme had a significant broadening in the pH-activity profile as compared to the soluble enzyme. Immobilized β galactosidase retained very high activity at acidic and alkaline side of the pH-optima than the soluble enzyme. Immobilized β galactosidase retained 84% and 95% activity at pH 3.0 and 5.0, respectively whereas the soluble enzyme showed 52% and 78% of the original activity under similar conditions, respectively.

The pH stability was monitored by incubating the soluble and immobilized enzyme in the buffers of various pH (2.0, 4.6 and 7.4) for varying times. There was no appreciable loss in the activity of immobilized β galactosidase when it was incubated in the buffer of pH 4.6 for 5 h whereas, soluble enzyme has exhibited a loss of 9% activity under similar experimental conditions (Table 2). Thus soluble β galactosidase denotes significant ($P < 0.05$) change (decrease)

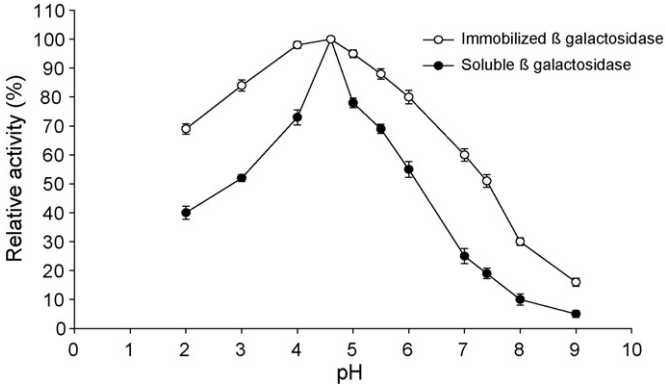

Fig. 1. pH-activity profiles of soluble and immobilized β galactosidase. The enzyme activity of soluble and immobilized β galactosidase (2.0 U) was measured in buffers of various pH. The buffers used were glycine–HCl (pH 2.0 and 3.0), sodium acetate (pH 4.0–6.0), and Tris–HCl (pH 7.0–10.0). The molarity of each buffer was 0.1 M. The activity at pH 4.6 was taken as control (100%) for the calculation of percent activity. The symbols show soluble (●) and immobilized (○) β galactosidase.

Table 2
Effect of time on the stability of soluble and immobilized β galactosidase at varying pH

Time (min)	Remaining activity (%)					
	pH 2.0		pH 4.6		pH 7.4	
	SβG	IβG	SβG	IβG	SβG	IβG
0	39.63 ± 0.75	70.56 ± 0.59	99.58 ± 0.58	99.92 ± 0.08	24.82 ± 1.29	53.66 ± 1.10
30	37.68# ± 1.73	70.53# ± 0.85	99.42# ± 0.54	99.73* ± 0.25	23.79# ± 0.71	52.78# ± 0.85
60	34.77# ± 1.17	69.76# ± 1.49	96.58# ± 0.49	99.67* ± 0.33	20.45# ± 0.84	52.04# ± 0.78
120	30.70# ± 1.32	68.73# ± 0.73	93.93# ± 1.01	99.02* ± 0.18	19.41# ± 1.75	49.52# ± 0.67
180	27.56# ± 0.65	68.22# ± 1.61	92.45# ± 0.98	98.78* ± 0.32	17.02# ± 1.20	48.62# ± 1.32
240	25.27# ± 0.93	68.19# ± 1.43	91.32# ± 1.23	98.60* ± 0.48	14.47# ± 0.96	46.48# ± 0.69
300	22.09# ± 0.97	66.71# ± 0.67	90.69# ± 0.63	98.50* ± 0.46	10.65# ± 0.80	45.04# ± 0.81

The stability of soluble and immobilized β galactosidase was examined by incubating the enzyme in the buffer of different pH (2.0, 4.6 and 7.4) for various times at 37 °C. After incubation in varying buffers for various times the beads were taken out and washed with assay buffer. The activity of enzyme was determined as described in the text. The values of soluble and immobilized β galactosidase at pH 2.0, 4.6 and pH 7.4, were analyzed by one-way ANOVA. #, denotes that the values ($P < 0.05$) were statistically significant. All the values of soluble and immobilized β galactosidase at pH 2.0, pH 4.6 and pH 7.4 at different time intervals were compared with their corresponding pH at 0 times (control); *, denotes that the values of immobilized β galactosidase at pH 4.6 were nearly constant and there was no change in activity till 5 h.

Table 3
Reusability of immobilized β galactosidase in the buffers of varying pH

Time of incubation (min)	Remaining activity (%)		
	pH 2.0	pH 4.6	pH 7.4
0	70.78# ± 1.05	99.57 ± 0.48	53.77# ± 0.83
30	68.88# ± 0.98	99.55 ± 0.31	53.58# ± 0.27
60	66.26# ± 1.19	99.51 ± 0.41	49.58# ± 0.82
120	59.59# ± 1.45	99.50 ± 0.22	46.71# ± 0.61

The activity of immobilized β galactosidase at pH 4.6 was considered as control (100%) for the calculation of percent activity at other pH. Enzyme activity of same beads was also determined in buffers of pH (2.0 and 7.4). The morality of each buffer was 0.1 M. The value of immobilized β galactosidase at pH 2.0 and pH 7.4 was compared with control, i.e. immobilized β galactosidase at pH 4.6 and analyzed by one-way ANOVA. #: denotes that the values ($P < 0.05$) were statistically significant.

in the activity of soluble enzyme whereas, there was no significant change in the activity of immobilized β galactosidase even after 5 h incubation at pH 4.6. The immobilized preparation was highly stable as compared to its soluble form.

The soluble and immobilized β galactosidase showed 22% and 67% activity at pH 2.0 and 11% and 45% activity at pH 7.4 after 5 h incubation, respectively (Table 2). At pH 2.0 and 7.4, in case of both soluble and immobilized β galactosidase ($P < 0.05$) as all the values were statistically significant.

3.3. Reusability of immobilized β galactosidase in the buffers of varying pH

In view of the severe conditions of acidity and bile concentrations found in the gastrointestinal tract, we have studied the activity of the enzyme immobilized on the same beads at different pH. The enzyme activity was first monitored at its optimum-pH then the beads were exposed to pH 2.0 and again the activity of same beads was monitored at pH 7.4. The initial activity of enzyme was found to be 71% and 54% at pH 2.0 and 7.4, respectively whereas after 60 min the activity of enzyme was 66% at pH 2.0 and 50% at pH 7.4. However, immobilized enzyme preparation retained 60% and 47% activity at pH 2.0 and 7.4 after 2 h incubation, respectively. These results thus suggested that prolong incubation of this immobilized enzyme preparation does not affect much enzyme activity. In all the cases ($P < 0.05$) which thus denotes that the values of the immobilized β galactosidase were remarkably significant (Table 3).

3.4. Effect of salivary amylase on the activity of soluble and immobilized β galactosidase

The effect of α amylase activity was examined on the stability of immobilized β galactosidase (Fig. 2). The activity of immobilized β galactosidase was nearly constant on incubating with high concentrations of salivary amylase.

3.5. Effect of pepsin/trypsin on the activity of soluble and immobilized β galactosidase

The effect of increasing concentration of pepsin/trypsin on soluble and immobilized β galactosidase was demonstrated in Table 4. Immobilized β galactosidase showed no change in activity till (0.050 mg mL^{-1}) when treated with pepsin for 1 h. Soluble β galactosidase showed 79% activity after exposure to pepsin (0.150 mg mL^{-1}) for 1 h, while the immobilized β galactosidase retained nearly 95% enzyme activity under similar incubation conditions. When compared with control, in case of both soluble and immobilized β galactosidase ($P < 0.05$) after exposure to pepsin (0.150 mg mL^{-1}) when incubated for 1 h.

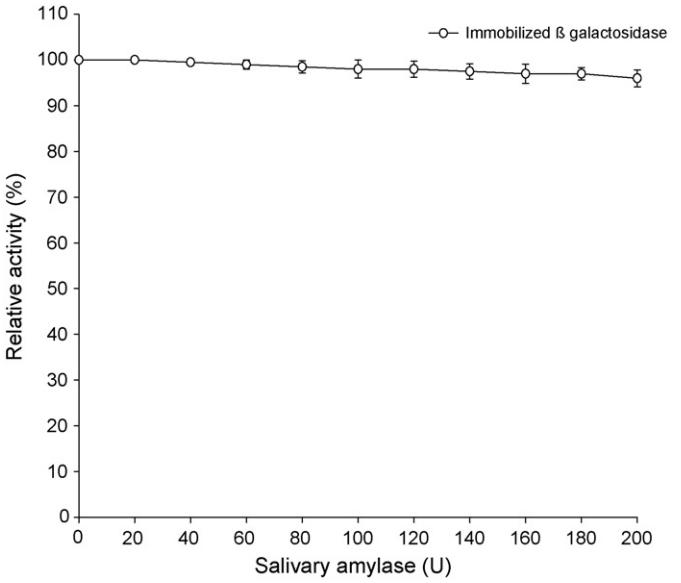

Fig. 2. Effect of salivary amylase on immobilized β galactosidase, Con A layered calcium alginate–starch beads surface immobilized β galactosidase (2.0 U) was incubated with increasing concentrations of salivary amylase (20–200 U) in 0.1 M sodium acetate buffer, pH 4.6 for 4 h at 37 °C. The activity of the enzyme without salivary amylase treatment was considered as control (100%) for the calculation of remaining percent activity after α amylase exposure. The activity of the enzyme was determined as described in the text. The symbols show immobilized (○) β galactosidase.

The activity of soluble enzyme increased to nearly 135% when treated with trypsin (0.075 mg mL^{-1}) for 1 h and its activity became constant on adding increasing concentration of trypsin. On exposure to higher concentration of trypsin (>0.100 mg mL^{-1}) the activity of soluble enzyme started to decrease whereas, the immobilized β galactosidase showed enhanced activity of nearly 145% under identical exposure. In case of both soluble and immobilized β galactosidase ($P < 0.05$) indicating that the values were statistically significant when treated with trypsin (0.150 mg mL^{-1}) for 1 h.

3.6. Reusability of immobilized β galactosidase

Fig. 3 demonstrates the reusability of immobilized β galactosidase. Immobilized enzyme preparation retained 65% of the original activity after sixth repeated use (Fig. 3).

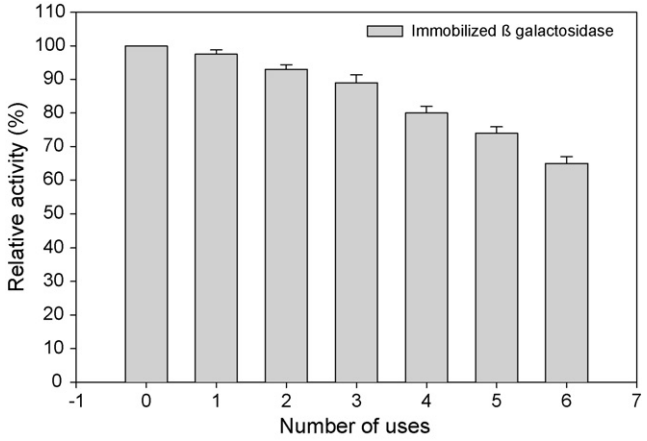

Fig. 3. Reusability of immobilized β galactosidase. The reusability of immobilized β galactosidase was monitored at the gap of 6 h. The beads were taken in triplicates and were assayed for the remaining activity.

Table 4
Effect of trypsin/pepsin on soluble and immobilized β galactosidase

Concentration (mg mL^{-1})	Remaining activity (%)			
	Trypsin		Pepsin	
	SβG	IβG	SβG	IβG
Control	99.61 ± 0.27	99.78 ± 0.23	99.63 ± 0.16	99.90 ± 0.01
0.025	114.62# ± 1.54	123.33# ± 0.68	96.62# ± 0.37	99.78* ± 0.07
0.050	128.67# ± 1.76	139.28# ± 1.38	93.99# ± 1.76	98.90* ± 0.24
0.075	134.37# ± 0.77	145.08# ± 1.29	89.58# ± 1.13	97.82# ± 0.65
0.100	120.85# ± 0.21	143.85# ± 0.33	87.10# ± 1.87	96.62# ± 1.12
0.125	102.12# ± 1.31	141.76# ± 0.23	83.41# ± 1.13	96.19# ± 0.40
0.150	95.52# ± 0.79	141.50# ± 0.52	78.62# ± 0.59	95.11# ± 0.41

Soluble and immobilized β galactosidase (2.0 U) was independently incubated with increasing concentrations of pepsin/trypsin (0.025–0.150 mg mL^{-1}) in 0.1 M sodium acetate buffer, pH 4.6 for 4 h at 37 °C. The activity of the enzyme without pepsin/trypsin treatment was considered as control (100%) for the calculation of remaining percent activity. The activity of the enzyme was determined as described in the text. The values of soluble and immobilized β galactosidase treated with trypsin/pepsin at various concentrations were analyzed by one-way ANOVA. #, denotes that the values ($P < 0.05$) were statistically significant; *, the values of soluble and immobilized β galactosidase treated with trypsin/pepsin which is close to control values are not remarkably significant.

4. Discussions

In order to immobilize enzyme on the large surface area of calcium alginate gel, a hybrid gel of calcium alginate–starch has been developed. These gel beads were layered with Con A by using jack bean extract. In order to make the preparation useful for the human consumption, without any side effects we have immobilized glycosylated β galactosidase from *A. oryzae* on the surface of Con A layered calcium alginate–starch beads. Alginate has been the most widely used polymer for the immobilization and microencapsulation of enzymes and cells (Funduenanu et al., 1999; Velten et al., 1999; Prashanth and Mulimani, 2005). The leaching of the enzyme from support could be prevented by crosslinking the immobilized enzyme with glutaraldehyde (Table 1). Crosslinking of the enzyme with glutaraldehyde improved both binding efficiency and reusability of the immobilized enzymes but the enzyme activity was decreased (Hennink and Nostrum, 2002; Haider and Husain, 2007b).

If immobilized β galactosidase would be given orally to remove the lactose deposited in the lumen of small intestine, it has to pass through mouth, stomach and then to the intestine. Therefore, the examination of stability of immobilized enzyme against α amylase is necessary due to presence of starch in the support. The effect of α amylase showed that immobilized enzyme was not much affected by the presence of α amylase (Fig. 2). Immobilized β galactosidase was significantly more stable on exposure to salivary amylase. Approximately 1 L of saliva is secreted into the human mouth each day by three pairs of salivary glands. Saliva contains many enzymes, including salivary amylase, an enzyme which catalyzes the breakdown of starch into smaller molecules (Hirtz et al., 2005).

Further, the immobilized enzyme has to reach in stomach and normally food stay there for more than 2 h. Due to extreme conditions of acidic media and pepsin there are chances to lose activity by immobilized β galactosidase. Immobilized β galactosidase was found remarkably stable at pH 2.0 and digestion by pepsin (Tables 2 and 4). Pepsin is a nonspecific acidic endopeptidase produced in an inactive precursor form (pepsinogen) in the mucosal lining of the stomach of vertebrates (Sun et al., 2005). Here, immobilized β galactosidase was not affected much by this protease. It showed a marginal loss of nearly 5% activity when exposed to pepsin (0.150 mg mL^{-1}) for 1 h at 37 °C.

Immobilized β galactosidase retained remarkably very high reusable activity at pH 2.0, 7.4 and 4.6. Immobilized enzyme was quite stable over prolong storage in these buffers (Tables 2 and 3).

Furthermore, immobilized β galactosidase will enter into small intestine where it meets the conditions of pancreatic and bile juices. Pancreatic juice contains proteolytic enzymes; chymotrypsin, trypsin, etc. and bile juice make the medium towards slightly alkaline. Immobilized β galactosidase has shown significant stability on long storage in the buffer of pH 7.4 (Table 3) and digestion by trypsin (Table 4). The activity of immobilized β galactosidase was constant to exposure over a wide range of trypsin (Table 4). Trypsin is a proteolytic enzyme present in the intestine (Hinsberger and Sandhu, 2004). The trypsin present in the intestinal fluid can inactivate β galactosidase in strains of *Propionibacterium freudenreichii* but not in *Propionibacterium acidipropionic* (Zarate et al., 2000).

β galactosidase immobilized on the surface of Con A layered calcium alginate–starch beads could be used for the hydrolysis of lactose present in the lumen of small intestine. Kim et al. (1999) showed lipid vesicle assisted lactose hydrolysis in which the entrapped β galactosidase is added to milk and is released into the stomach by the presence of bile salts, allowing an 'in situ' degradation of lactose. Numerous methods of enzyme entrapment in liposomes have already been reported (Walde and Ichikawa, 2001). But the choice of an encapsulation method is a difficult compromise between efficiency and preservation of catalytic activity (Monnard, 2003; Nogales and Lopez, 2006).

Immobilized β galactosidase retained 65% activity after sixth repeated use (Fig. 3). The high cost of some of the enzymes used for industrial purposes and the time necessary for their immobilization and their subsequent use, have led to the increase in interest in the possibility of reusing the immobilized preparation (Melgarejo et al., 2006). Enzyme reuse provides a number of cost advantages that are often an essential prerequisite for establishing an economically viable enzyme catalyzed process (Norouzian, 2003). Thus we have tried to focus on the aspect that if the enzyme immobilized on the surface of beads were taken orally as a drug it will greatly help in reducing the problem of lactose intolerance. The size of these beads is spherical in shape which would remarkably help in the hydrolysis of lactose as they can easily reach near to the lumen of the small intestine and would help in the hydrolysis of lactose.

5. Conclusion

Con A layered calcium alginate–starch beads bound β galactosidase has large surface area and the enzyme present on its surface can make easy contact with more substrate. β galactosidase bound to the surface of calcium alginate–starch beads has no problem of substrate and product diffusion as it has been reported in case of microencapsulated and entrapped enzyme. Immobilized enzyme exhibited significantly higher activity and stability at different pH. This immobilized enzyme preparation was found to be remarkably stable against the inactivation mediated by α amylase, pepsin and trypsin. Immobilized β galactosidase also retained very high

activity on its repeated uses. On the basis of these findings one can suggests that such immobilized enzyme preparation could be simply employed for the hydrolysis of lactose in lactose intolerant patients.

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