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IMMOBILIZATION OF LACTASE TO PERLOZA CELLULOSE RESINS

This thesis was presented in partial fulfilment of the requirements for the degree of Master of Science in Chemistry at Massey University

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2000

AMENDMENTS

p. ii, line 15 should read '**the** best of these results'
p. iv, paragraph 3 – 'thank Dave Elgar for his **valuable** advice' and 'paragraph 5 – '**heaps** of help?'
p. xi 'NaOH sodium hydrox**ide**'
p. 2, line 1, imid**a**zole, line 3 should be **sulphydryl**
p. 4, Table 1.1, Streptococcus thermoph**i**lus
p. 15, line 6, '**of** the commercial matrices available'
p. 26, line 15 'ECH (0.75**ml**)'
p. 28, line 28, 'the beads **were**'
p. 30, line 24 '**rpm**' not rmp, also p. 32, line 24
p. 31, line 14, '1 mM $MgSO_4$'
p. 32, line 2 ' **were** warmed to'
p. 33, line 10, '**2.11**' , not 1.11
p. 33, line 17 should be acetonitrile and p. 34, line 2
p. 37, line 6 '**Matsumoto**'
p. 65 line 23, 'in other w**o**rds'
p. 72, line 8, change 'Binding Buffer **2 two** tested' to 'Binding Buffer 2 tested'
p. 86, line 5, '**shifted**' not shift.
p. 87, line 11, 'highest activity of immobilised lactase **being** achieved at low ECH activation level**s**'

ABSTRACT

A bead cellulose matrix, Perloza, was chemically modified by two attachment chemistries to prepare inexpensive resins for immobilization of lactase. A commercial product, the base-activated matrix Eupergit C was studied for comparison.

Three types of Perloza (Perloza 100 MT, Perloza 200 MT, Perloza 500 TM) were activated by epichlorohydrin (ECH) to achieve different activation levels. The best result for lactase immobilization was gained at low activation level (activated at 2% NaOH) for two attachment chemistries. The first attachment chemistry studied was that lactase immobilized directly to ECH activated Perloza. The second chemistry again used ECH activation and followed by attachment of the 6-amino caproic acid (ACA) spacer arm and then the lactase.

In the first chemistry, Perloza 100-ECH-Lactase obtained the highest activity 11.4 NLU/g (wet resin) over Perloza 200-ECH-Lactase and Perloza 500-ECH-Lactase (40 hours immobilization). In the second chemistry, Perloza 200-ECH-ACA-Lactase retained the highest activity 30.9 NLU/L (wet resin) over Perloza 100-ECH-ACA-Lactase and Perloza 500-ECH-ACA-Lactase. Overall the best results were obtained for the ECH-ACA resins. This best of these results showed about 3 times better immobilization than without ACA spacer arm.

The activity of immobilized lactase on Eupergit C obtained was 124~131.3 NLU/g (wet resin) for 24 hours immobilization. Although this result is about four times greater than Perloza, Perloza is a much cheaper matrix.

In the storage stability studies, both Perloza and Eupergit C immobilized lactase showed a sharp drop in activity initially within 1 day, then activity loss leveled out. Perloza 200-ECH-ACA-Lactase retained 82% of its original activity after 9 days storage. However, Eupergit-Lactase only retained 39% of its original activity after the same storage period.

This result indicated that Perloza 200-ECH-ACA-Lactase may possess much better storage stability than that of Eupergit-Lactase.

Studies on the inter-relationships between pH, temperature and Perloza immobilized lactase using the substrate (ONPG) indicated that maximum hydrolysis was attained at pH 6.5-7.2 and over a temperature range of 30-42°C. No shift in the pH and temperature optima in comparison to free enzyme was observed as a result of the process of immobilization of lactase on Perloza for both attachment chemistries.

The pH-activity curve of Eupergit-Lactase shifted towards more acidic pH values in the pH optimum in comparison to free lactase. The temperature optimum of Eupergit-Lactase shifted towards higher temperature compared to free lactase.

This study showed that Perloza has potential for the large scale use as a matrix of lactase immobilization.

ACKNOWLEDGEMENTS

First I would like to sincerely thank my supervisor Associate Professor David R.K. Harding for his time, advice, encouragement and especially his friendship to me throughout the two years study.

I would like to thank my supervisor Neill Haggarty for his support and chemicals supplement from NZDRI.

I would like to thank David Elgar for his valueless advice and help.

I would also like to thank Malcolm Chick for his advice on enzyme assays.

Another big thanks to the Centre for Separation Science, for those of you given me hips of help: Kate, Dick, Yishan, Claire, Satha, Rekha and Adrian.

Finally I would like to thank my family, friends for all their support.

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LIST OF ABBREVIATIONS

ACA	6-aminocaproic acid
ADH	alcohol dehydrogenase
BCA	bicinchoninic acid
CDI	1,1'-carbonyldiimidazole
CMC	1-cyclohexyl-3-(2-morpholino-ethyl) carbodiimide
ECH	epichlorohydrin
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
FPLC	fast protein liquid chromatography
GRAS	generally recognized as safe
HCl	hydrochloric acid
HL	hydrolyzed lactose
K-phos	potassium phosphate buffer
NaOH	sodium hydroxyl
NaCl	sodium chloride
NHS	N-hydroxysuccinimide
ONP	o-nitrophenyl
ONPG	o-nitrophenyl-β-D-galactopyranoside
UF	ultrafiltration