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Immobilisation and Charaterisation of Glucose Dehydrogenase Immobilised on ReSyn™: A Proprietary Polyethyleneimine Support Matrix

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AIM

To characterise and compare the activity of free and immobilised Glucose Dehydrogenase on a proprietary polymer matrix.

INTRODUCTION

Enzyme immobilisation is of considerable interest due to the advantages over soluble enzymes, including improved stability and recovery. Glucose Dehydrogenase (GDH) is an important biocatalytic enzyme due to its ability to recycle the biological co-factor NAD(P)H[1] using inexpensive glucose as a substrate. Co-factors are expensive and not readily recoverable from biocatalytic systems[2] ,thus, there is a great need to recycle them. Co-factor recycling aims to reduce the quantity of co-factors required in these systems. Immobilisation of GDH can result in enhanced stability of the enzyme and when co-immobilised with other co-factor utilising enzymes will result in a system which will recycle the co-factors allowing recovery and continuous reuse[3], thus reducing process costs.

Immobilisation of GDH was performed on a proprietary polymer matrix. Any improvements in the physical properties were quantified.

METHODS

ReSyn™: Particle Preparation

ReSyn™ was manufactured using a bi-emulsion-based method. The first emulsion consisted of 50 µl nonoxynol-4 (NP-4) mixed with 5 ml mineral oil and 200 µl of 10% polyethyleneimine solution. The second emulsion consisted of the former components but however, the PEI was replaced with 20% glutaraldehyde (grade II). Each solution was emulsified at maximum speed for 10 s using a vortex. The polymeric particles were formed by mixing the two emulsions; the glutaraldehyde emulsion was added into the PEI emulsion. The reaction was allowed to take place for 60 min while mixing end over end at 60 rpm.

GDH Immobilisation

An aqueous solution suspension of GDH (1mg/ml: pH 8.0) was immobilised onto the particles for 60 min at 8°C with end over end mixing at 30 rpm. Bound protein was quantified using the Bradford method with pure GDH (Codexis) as a standard. To quantify covalent binding the particles with bound protein were washed with 2 M NaCl and the binding was quantified as above.

Assaying of Free and Immobilised GDH

Activity of free and immobilised GDH was determined by following the kinetic reduction ofNAD+ to NADH at 340 nm with concomitant conversion of glucose to glucono-δ-lactone. One GDH Unit (U) was defined as the amount of enzyme required to reduce 1 µmol of NAD+ per minute at 37°C. The assay reagents consisted of 1 mM NAD+, 100 mM D(+) Glucose in 50 mM Tris-Cl pH 8.0. Maintenance in activity; pH profiling and temperature stability at 55 and 65 °C of the free and immobilised GDH was determined.

RESULTS

Binding Capacity of GDH onto the ReSyn™

PEI was adjusted to four different pHs, 5, 7, 9 and 11, subsequently named Preparation A, B, C and D respectively; this was done to prepare polymer matrices with different degrees of cross-linking ,thus, altering the porosity of the matrix. Reduced cross-linking is achieved with PEI of low pH and the opposite with PEI of a high pH. This characteristic has been shown to affect the amount protein binding[4].

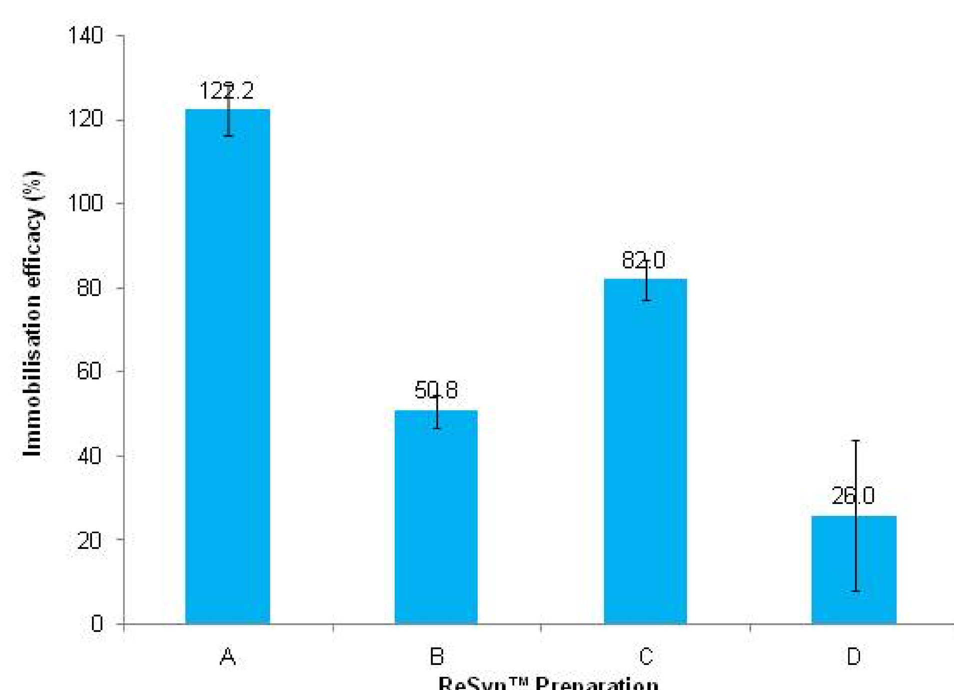

Figure.1: Binding efficacy of Glucose Dehydrogenase on different ReSyn™ matrices using distilled water at pH 8.0

Preparation A displayed the highest binding capacity of 122.2% (m/m), this was followed by Preparation C with 82%. Preparation D displayed the lowest binding capacity with 26% and 50.8% was obtained using Preparation B. These differences can be attributed to the degree of porosity within the polymer particles. Baron and colleagues (1997) immobilised GDH onto controlled-pore silica (CPS) with average pore sizes of 170 and 500 Å (50-100 mg) and they obtained a low immobilisation yield of 0.73 and 1.18%[3].

Enzyme Activity Maintenance

Maintenance in activity was determined by comparing specific activity of immobilised GDH to that of the starting enzyme preparation.

GDH immobilised on preparation D matrix showed the best maintenance in activity with 42.3% compared to GDH immobilised on the other matrices, this was followed closely by GDH immobilised on preparation B matrix with 41.5% and the preparation A matrix displayed the lowest maintenance of activity with 29.1%.

Table.1: Comparison of the covalent immobilisation of GDH on different immobilisation supports.

Support Type	Maintenance of Activity (%)	Immobiisation Yield (%)
ReSyn™ Prep B	41.5 ± 6.1	50.8
CPS-170 (50 mg)[3]	0.72	0.73
CPS-500 (50 mg)[3]	1.15	1.18

CPS = controlled-pore silica

In comparison to GDH covalently immobilised on CPS-170 and 500[3], ReSyn™ Preparation B displayed the highest maintenance in activity as shown in Table.1.

pH profiling

Changes in the pH profile of enzymes occur due to immobilisation[4], thus it was considered important to evaluate this parameter.

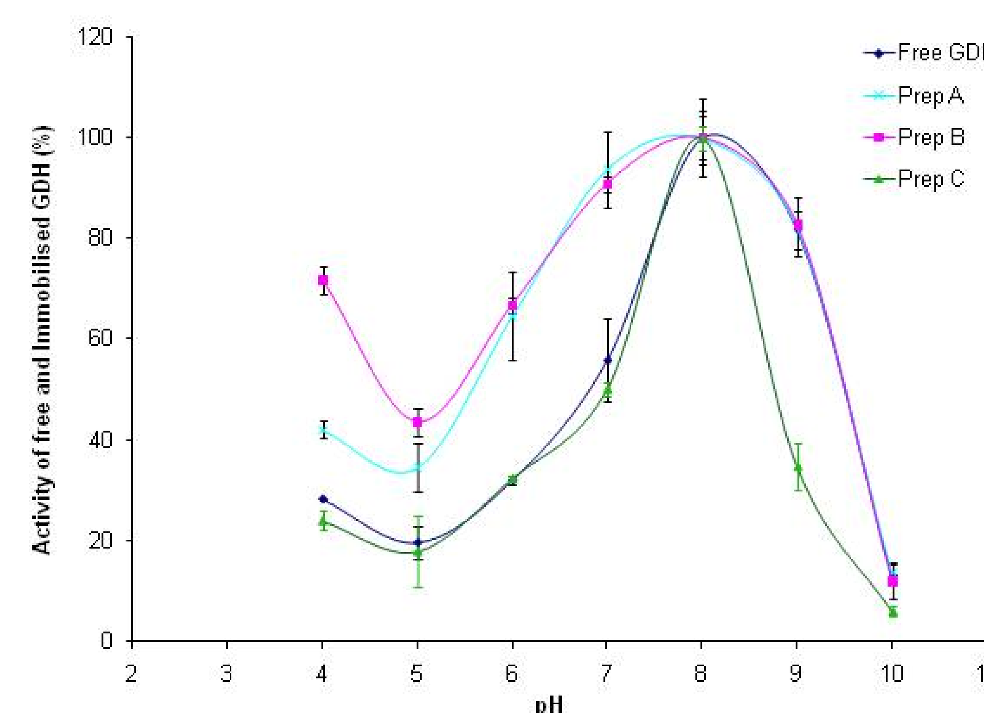

Figure.3: pH profiling of free and immobilised GDH on various ReSyn™ matrices.

The optimum pH for activity was 8.0. GDH immobilised on Preparation A and B ReSyn™ matrices saw an increase in activity in the acidic range, thus broadening the pH profile. These results can potentially enable applications of GDH that require acidic conditions.

Temperature Stability

One of the main aims for immobilisation of enzymes is to confer stability, such as thermal stability. Thermal stability of enzymes is an important parameter in biocatalytic processes as it determines the limits for use and reuse of the enzyme and can therefore impact process costs[5].

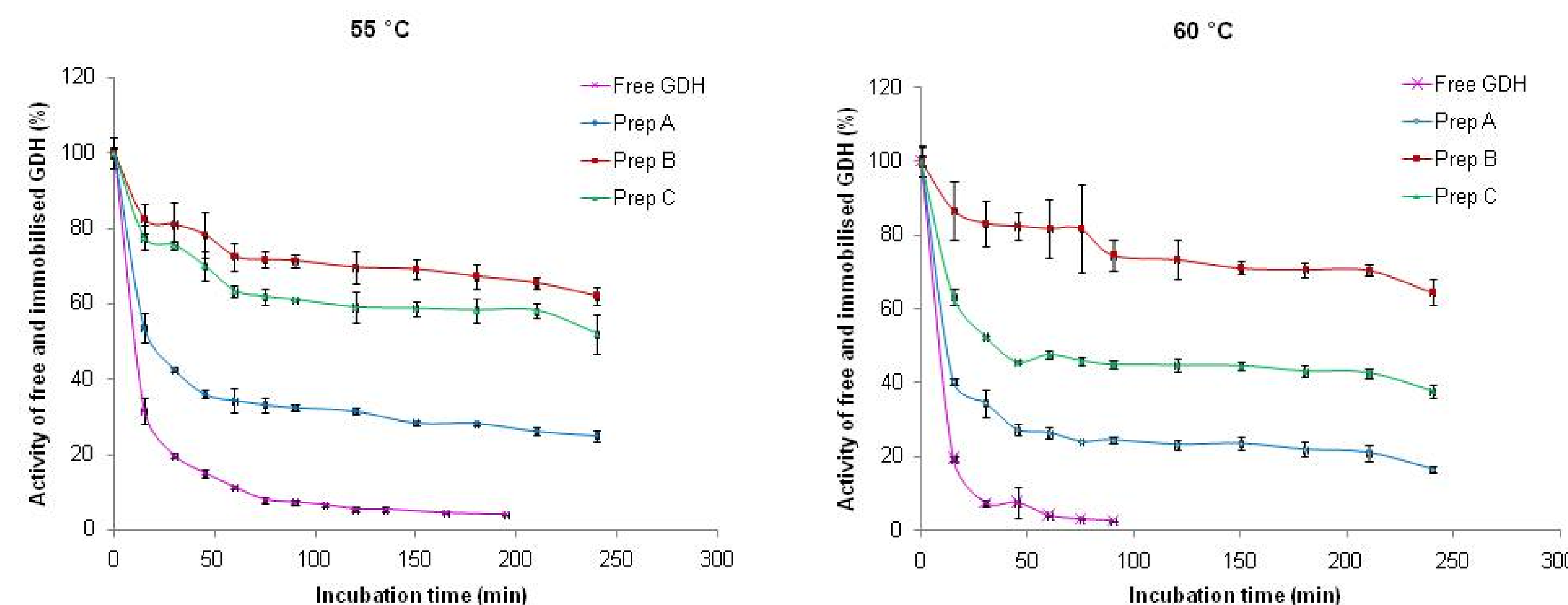

Figure.3: Graphs displaying temperature stability of free and immobilised GDH on the various ReSyn™ matrices. Preparations during incubation at 55 and 60 °C.

Immobilised GDH retained more activity during incubation at 55 and 60 °C. At both temperatures, 50 and 60 C, GDH immobilised on ReSyn™ preparations showed improved activity compared to the free form of the enzyme. Preparation B displayed the highest improved activity, follwed by Preparation C and A respectively.

CONCLUSION(S)

We successfully immobilised GDH onto ReSyn™ particles. The immobilised GDH maintained improved activity, broadened the pH profile and conferred thermal stability. These results could expand the possible applications of this enzyme for biocatalysis.

REFERENCES

[1] Werner H., *Trends in Biotechnology*, Vol. 17, 487 (1999).
[2] Zhao H. and van der Donk W. A., *Current Opinion in Biotechnology*, Vo.l. 14, 583 (2003).
[3] Baron M., Fontana J.D., Guimaraes M.F. , and Woodward J., Applied Biochemistry and Biotechnology, Vol. 63-65, 257 (1997).
[4] Jordaan J., Simpson C., Brady D., Gardiner N.S., and Gerber I.B., *Wo2009/057049 A2*, 7 May (2009).
[5] Santos A.M.P., Oliveira M.G., and Maugeri F., *Bioresource Technology*, Vol. 98, 3142 (2007).

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SPES BONA