Immobilisation and Characterisation of Glucose Dehydrogenase Immobilised on ReSynTM: A Proprietary Polyethyleneimine Support Matrix

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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 using inexpensive glucose as a substrate. Co-factors are expensive and not readily recoverable from biocatalytic systems. 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-factors utilising enzymes will result in a system which will recycle the co-factors allowing recovery and continuous reuse; thus reducing process costs.

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

METHODS
ReSynTM Particle Preparation
ReSynTM was manufactured using a bi-emulsion-based method. The first emulsion consisted of 50 μl monononol-4 (NB-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% glucose (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 glucose emulsion was added into the PEI emulsion. The reaction was allowed to take place for 60 min while mixing and after 60 min.

GDH Immobilisation
An aqueous solution preparation of GDH (final: 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 (Code 11430) 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 of NAD+ to NADH at 340 nm with constant conversion of glucose to gluconic-dehydroamine. One GDH Units (U) was defined as the amount of enzyme required to reduce 1 μmol of NAD+ per minute at 37°C. The assay mixture consisted of 1 μM NAD+, 100 μM D-glucose in 50 mM Tris-Cl pH 8.0. Maintenance in activity; pH profiling and temperature stability at 35 and 65°C of the free and immobilised GDH was determined.

RESULTS

Binding Capacity of GDH onto the ReSynTM
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.

Figure 1: Binding efficacy of Glucose Dehydrogenase on different ReSynTM matrices using destilled water at pH 8.0
Preparation A displayed the highest binding capacity of 122.2% (max), this was followed by Preparation C with 82.1%. Preparation D displayed the lowest binding capacity with 26.0% and 50.7% was obtained using Preparation B. These differences can be attributed to the degree of porosity within the polymer particles. Brion and colleagues (1997) immobilised GDH onto controlled-pore silica (CPS) with average pore sizes of 170 and 500 Å (100-1000 μm) and they obtained a low immobilisation yield of 0.73 and 1.18%.

Figure 2: pH profiling of free and immobilised GDH on various ReSynTM matrices:
The optimum pH for activity was 8.0. GDH immobilised on Preparation A and B; ReSynTM matrices上周 increase in activity is higher 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.

Figure 3: Graph depicting temperature stability of free and immobilised GDH on the various ReSynTM 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 ReSynTM preparations showed improved activity compared to the free form of the enzyme. Preparation B displayed the highest improved activity, followed by Preparations C and A respectively.

CONCLUSIONS
We successfully immobilised GDH onto ReSynTM 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.