Effect of Pore Size on the Performance of Immobilised Enzymes

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Supplementary Information

Guidelines for Establishing Confidence Ratings of Parameters Plotted –

1) Support Data: *Pore Diameter, Total Pore Volume, Surface Area*

Requirements to Achieve High Confidence

- Support is clearly and comprehensively identified in the paper with detail of all/any chemical treatment
- Data has been measured with details of experimental method and interpretation or, support is a fully identified commercial material whose characteristics have been properly measured in other studies.

Reasons for Loss in Confidence

- Support is not clearly identified – partial/missing code, details of any treatment not clearly communicated

2) Guidelines for Performance Data: *Retention of Activity, Protein Loading, Specific Activity of Enzyme in Immobilisate, Specific Activity of Immobilisate.*

Requirements to Achieve High Confidence

- Value is clearly reported in paper with clear definition of parameter and/or
- Value can be confidently calculated from information clearly provided in paper

- It is clear that sufficient protein has been offered for immobilisation so that the saturated protein loading value has been reached and employed in subsequent studies

Reasons for Loss in Confidence

- Unclear definition of performance data measured e.g. if protein loading estimated is based on measurements of catalytic activity, not protein mass.
- Protein loading reported as molarity with no further details given to allow parameter to be converted mg/g.
- Values implausibly high or low, suggesting orders of magnitude error. Values corrected by 10^3 may be used with low confidence.

Sections 1) and 2) will then be rated as having *high, medium* or *low* and given a colour – *green, medium* or *low* respectively. An overall confidence rating is then judged and the data is plotted as a shape – *high confidence* is represented as a square, *medium confidence* a triangle and *low confidence* a circle. The final plotted point is therefore given as shape (establishing an overall confidence) split in half with the top colour representing the

confidence given to the support data and the bottom half representing the confidence associated with the performance data.

Below is an example of how confidence rating is established and represented in the pore characteristics vs. performance data graphs.

Support Data: Pore Diameter

- Medium confidence rating (▲)

Eupergit CM is of medium pore size, as stated by supplier. However there are not enough reports of pore size characterisation to establish a general consensus of high confidence. Pore size ranges are reported between 10-100 nm as stated by the commercial supplier, therefore 50 nm has been taken as an 'average' pore size but with only medium confidence.

Performance Data: Protein Loading

- High confidence rating (■)

Confidence Rating Awarded = Medium (▬)

Methods of Pore Size Analysis

Table 1S: *Most commonly cited advantages and disadvantages of N_2 adsorption/desorption and Hg porosimetry.*

Method of Characterising Pores	Advantages	Disadvantages
Hg Porosimetry	• Faster than N_2 adsorption/desorption – single analysis = 30-45mins • Wider range of detection than N_2 adsorption/desorption (can obtain data from porous materials with pore diameters between 3 nm and 200 μm) • Gives more accurate data for the largest pores	• High pressures may compress sample • Overestimates the volume of the smallest pores • Underestimates pore diameter of materials with large pores
N_2 Adsorption/Desorption	• Detects smaller pores than Hg porosimetry (can obtain data from porous materials with pore diameters between 0.3 nm and 300 nm)	• Fine pore structures are difficult to degas • Overestimates large pore diameters • Potentially destructive due to

		low temperature of liquid N_2 (77 °K)

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