ABSTRACT

Characteristics and Applications of Immobilized Enzymes in Ionic Liquids

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This thesis reports on investigations into the favorable alteration of the catalytic properties of immobilized enzymes in ionic liquid (IL) media, when compared with aqueous solvent systems. The first part of this report describes the successful immobilization of two enzymes, β-galactosidase and β-1,4-galactosyltransferase onto Sepharose CL-4B®, as well as details of some of the kinetic properties of these (free and immobilized enzymes) in IL media compared with phosphate buffer (0.1M, pH 7.4) or de-ionized water. Immobilized β-galactosidase demonstrated a 30-fold increase in activity in the ionic liquid 1-butyl-3-methylimidazolium dicyanamide when compared with free β-galactosidase enzyme in the phosphate buffer. Immobilized β-1,4-galactosyltransferase gave a 45% increase in activity in 1-butyl-3-methylimidazolium hexafluorophosphate when compared with de-ionized water. The storage stability of the two above stated enzymes, as well as immobilized glucose oxidase and xanthine oxidase were monitored in IL media, and comparable shelf-lives (retention of ≥ 90% relative activity after six months) were obtained with the best storage conditions quoted in the literature.

Secondly, the applicability of the immobilized β-1,4-galactosyltransferase enzyme in an ionic liquid system was demonstrated by virtue of the efficient synthesis of three disaccharides which effectively comprise the main constituents of the pharmaceutically important compound, Globo-H. High yields were obtained (>95%) in these one-pot syntheses, and recyclability of solvent and catalyst was also realized.

Also described is the development and optimization of a glucose biosensor, constructed using a p(HEMA) hydrogel matrix, with the IL, 1-butyl-3-methylimidazolium tetrafluoroborate acting as the solvent medium. The synthesis and characterization of a novel peptidic squarate mediator, and inclusion of this mediator together with the conducting polymer, p(pyrrole) in the design of the sensor, made for a highly sensitive glucose biosensor. Performance characteristics include a wide linear glucose calibration range of 1.0×10^{-5} to 1.6×10^{-2} M, an optimal working potential of

260mV, and a sensitivity of 1.3×10^4 μA/M, which are comparable to some of the better glucose biosensors described in the literature.

Finally, the successful solvent-free synthesis of an ionic liquid, 1-ethylacetate-3-methylimidazolium hexafluorophosphate, utilizing a typical household microwave was carried out. This IL was characterized and its ability to efficiently dissolve cholesterol was harnessed in its use as the solvent medium for a cholesterol biosensor.

Key words: ionic liquid, glucose biosensor, β-galactosidase, glucose oxidase, kinetic parameters, β-1,4-galactosyltransferase, squarate mediator, p(HEMA), pyrrole, xanthine oxidase, cholesterol oxidase