ABSTRACT

The Flow Kinetics and Applications of
Immobilized β-Galactosidase Systems

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This thesis focuses on the immobilization of β-galactosidase on to Fractogel, glyceryl glass and nylon using a novel activating agent, 2-fluoro-1-methyl-pyridinium sulphonate (FMP). The work seeks to characterize these immobilized enzyme systems in terms of their kinetic parameters and properties as well as to evaluate their use for the determination of lactose in milk samples using flow injection analysis.

The results from flow kinetic studies for β-galactosidase immobilized on to the various support systems are analysed both by the Lilly, Hornby and Crook and the Kobayashi-Laidler theories. The Fractogel system shows good storage stability (retaining 55% of its initial activity after 547 days) when kept at 4°C.

Storage stabilities and kinetic studies are also investigated for the Fractogel system in water-miscible organic solvents such as dimethylsulphoxide, acetonitrile,

methanol, ethanol, propan-1-ol, glycerol and tetramethylurea. The alcoholic solvents, except ethanol, are found to enhance substrate hydrolysis whereas the other solvents suppress it.

In contrast to the Fractogel system, the β-galactosidase-glyceryl glass bioreactor retains only about 5% of its initial activity after 120 days. However, this system shows broad pH and temperature optima, characteristics which are very useful in analytical systems.

The characteristics of β-galactosidase, immobilized on to the inside of nylon tubing using glutaraldehyde, lysine and polyvinyl alcohol (PVA) as linking agents, are also investigated. The β-galactosidase-PVA-nylon system shows excellent storage stability. Kinetic studies on this system using water miscible organic solvents show that ethanol and propanol enhance whereas acetonitrile and glycerol suppress hydrolysis of the substrate.

This work also evaluates the development of flow injection analytical techniques (FIA) for routine quantitation of lactose. Both an enzymatic (detection limit = 16 $\mu g\ mL^{-1}$) and a non-enzymatic method (detection limit = 0.6 $mg\ mL^{-1}$) are developed and

evaluated. Commercial milk samples were analysed for their lactose content by these two methods. These FIA methods appear to have good commercial prospects.