ABSTRACT

This thesis is centered around the immobilization of three enzyme systems, namely trypsin, glucose oxidase/peroxidase and β-glucuronidase and their use in two types of enzyme reactors viz: packed bed and open tubular heterogeneous systems (OTHERS).

CHAPTER ONE considers some of the reasons for the considerable interest which has risen over the last few years in the field of solid supported enzymes. CHAPTER ONE also discusses some general aspects of supported enzymes and an attempt is made to explain their behaviour in terms of theories put forward by various investigators. Also included in CHAPTER ONE are the general characteristics of flow injection analysis (f.i.a.) and its role in various areas of analytical chemistry. Finally a brief review of the enzymes investigated is presented.

CHAPTER TWO describes the details of the experimental and kinetic analysis of trypsin immobilized on a newly marketed support, an FMP-activated Fractogel. A packed bed reactor was constructed from the immobilized trypsin gel and a detailed kinetic study was done with

respect to the effects of pH, temperature, inhibitors and the water miscible organic solvents dimethyl-sulphoxide, acetonitrile, methanol and tetramethylurea.

CHAPTER THREE describes the work done in developing an analytical technique for routine quantification of D-glucose in serum. A bienzyme reactor was prepared by immobilizing glucose oxidase and peroxidase on an FMP-activated Fractogel support. Kinetic work was done to establish the pH, temperature and flow rate optima for the f.i.a. reactor. The possibility of using this f.i.a. system for routine analysis of D-glucose in serum was also investigated. The results obtained were statistically compared with the results obtained using the free enzyme method done at the Port-of-Spain General Hospital in Trinidad.

CHAPTER FOUR describes the work done on β-glucuronidase immobilized on the inside of a nylon tube. pH, temperature and flow rate - substrate concentration studies were carried out on this system. The results were analysed in terms of the Kobayashi-Laidler theory outlined in Chapter One. The results show that over the various substrate concentrations and flow rates employed, the reactors were largely diffusion free. The values of $K_{m(app)}$ varied only slightly with flow rate. The extrapolated $K'_{m(app)}$ value obtained at very high flow

rate compares favourably with the K_m value for native β-glucuronidase.