ABSTRACT

Use of Modified Poly(HEMA) Hydrogels in Biosensor Construction and Controlled Release of Insulin

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This thesis reports on investigations into three important bioanalytical applications of pH-responsive hydrogels – their use as matrixes for the immobilization of enzymes, for biosensor construction and for the controlled release of biomolecules.

The first part of the thesis describes the use of pH-responsive hydrogels as matrixes for the immobilization of two enzymes, glucose oxidase (GOx) and glutamate oxidase (GlutOx). Spherical hydrogel beads were prepared by an inverse suspension polymerization process and enzymes were immobilized via both physical entrapment as well as by covalent linkage. Bioreactors were subsequently prepared and incorporated into flow injection (FI) systems for the quantitation of glucose and monosodium glutamate (MSG) respectively. For the FI manifold incorporating immobilized GOx, calibration curves were found to be linear over the glucose concentration range of 0.1 – 15.5 mM with a detection limit of 0.08 mM. Up to 20 samples can be manually analyzed per hour, with hydrogel-GOx bioreactor exhibiting good within-day (0.19 %) and day-to-day (1.30 %) precisions. The optimized FI manifold for MSG quantitation yielded a linear range of up to 8 mM with a detection limit of 0.2 mM and a throughput of 30 samples per hour. Analysis of soup samples gave within-day and day-to-day precisions of 3.55 % and 4.80 % respectively. Bioreactors containing physically entrapped enzymes retained > 60 % of their initial activities after a storage period

of up to 1 year at 4°C, thus indicating that they are quite stable and demonstrate the potential for use in microanalytical systems.

Chapter THREE investigates the potential of hydrogels for biosensor construction. Poly(HEMA) hydrogels were intimately combined with another polymer, polypyrrole, to synthesize an electroactive hydrogel. Such a composite material served as an immobilization matrix for three oxido-reductase enzymes – glucose oxidase, cholesterol oxidase and galactose oxidase. Thin films of bioactive polymer were subsequently cast upon platinum electrodes which functioned as amperometric biosensors for their relevant substrates. Calibration curves generated in a serum matrix were found to be linear over the concentration ranges of 0.05 – 20 mM, 0.20 – 15 mM and 0.03 – 10 mM, with detection limits of 25 μM, 120 μM and 25 μM for glucose, cholesterol and galactose in serum respectively. All biosensors displayed good precisions towards their respective analytes in serum with mean CVs < 5 %. The three biosensors exhibited very good storage stabilities (> 70 %) in the absence of any buffer at 4°C. The composite polymer film was also found to be very effective in screening the common endogenous interferents (ascorbic acid and cysteine) present in serum which are known to cause fouling of sensors.

Chapter FOUR describes the potential of these p(HEMA) hydrogels to function as controlled drug release devices. The p(HEMA) hydrogel was made pH-sensitive by addition of a co-monomer, dimethylaminoethyl methacrylate, and its swelling properties were investigated. Such gels exhibited up to 90 % degree of swelling. Glucose oxidase (GOx) was also entrapped within these pH-sensitive

hydrogels, thus making them glucose-responsive. The swelling properties of such gels in the presence of varying glucose concentrations were also studied, with some gels exhibiting ~30 % change in swelling with the introduction of glucose (50 mg/dL) into the external swelling medium. Two biomolecules, insulin and protamine, were loaded into pH-sensitive and glucose-responsive hydrogels and their release kinetics were characterized at different pH's and glucose concentrations respectively. Results show that metabolite release from these systems increased with decreasing solution pH and cross-linker composition as well as with increasing GOx loading into the hydrogel. Finally, in an attempt to model a more controlled in-vivo insulin release system, a multilaminated hydrogel device was synthesized. Such a device demonstrated a more controlled release of insulin now sustained over a longer time period and closer to a zero-order release profile rather than a first-order profile with the sudden burst of drug.

Keywords : poly(HEMA), hydrogel, pyrrole, polymer, biosensors, enzymes, glucose, cholesterol, galactose, insulin, protamine, controlled drug release.