ABSTRACT

Activating agents for hydroxyl supports and their use in enzyme immobilization

Charmaine Lord

There continues to be a constant search for reagents to be used as viable alternatives for activating hydroxyl supports. This is due to the number of limitations associated with many of the currently used activating agents. The present study thus focuses on the evaluation of several new reagents as viable alternatives for the activation of hydroxyl supports for the immobilization of enzymes. These were the aza-arenophilic ligand, p-nitrophenylacrylate, methacryloyl chloride and bis(4-nitrophenyl)carbonate.

The aza-arenophilic activated supports, first reported on by Ngo and Khatter in 1990, were used exclusively for the purification of immunoglobulins. This thesis reports for the first time on the use of the aza-arenophilic ligand as a novel alternate method for the activation of hydroxyl supports for the covalent immobilization of urease, β-galactosidase, glucose oxidase and trypsin. The optimized conditions for this system were found to be as follows: a relatively short activation time of 1 hour; a 1:1 mole ratio of the activating agents DMAP/DCTFP; mild coupling conditions – 0.01M $NaHCO_3$ at pH 9.0; good loading capacities – 4.8mg and 2.8mg urease per mL for Sepharose CL-4B and Fractogel supports respectively. The system showed excellent storage stability (>95%) after 11 months and no

appreciable evidence of leakage of the protein into the storage buffer of the gels.

The activating agent p-nitrophenylacrylate, synthesized by reacting p-nitrophenol and acryloyl chloride, has a melting point between 61.8-62.2°C and is very stable and easy to handle. The optimized compromised conditions for activation of Sepharose CL-4B followed by enzyme coupling were found to be as follows: 10.36 mmoles p-NPA per mL of Sepharose CL-4B; activation time of 15 hours; mild enzyme coupling conditions - 0.01M $NaHCO_3$ at pH 9.0; a loading capacity of 6.5 mg urease per mL gel and excellent storage stability over a period of 524 days. Flow kinetic studies were carried out on the immobilized β–galactosidase system in a packed bed reactor and the Lilly, Hornby and Crook model was used to analyze this kinetic data. The maximum reaction capacity of the immobilized β–galactosidase system was 322 μmol min^{-1} with an apparent Michaelis constant, $K'_{m(app)}$ of 0.40 mmol L^{-1}. This enzyme system has a broad pH optimum of 7.0 to 8.0 with an optimum temperature of 35°C.

Bis(p-nitrophenyl)carbonate was also used as an activating agent. Results show this activating agent is very stable when stored at either room temperature or −10°C with maximum activation of the hydroxyl support being achieved in approximately 30 minutes for Sepharose CL-4B and 2 hours for Fractogel. Coupling of β–

galactosidase and protein A to the activated support proceed under mild conditions (pH 7.0) at room temperature with 72% and 87% of protein bound on Sepharose CL-4B and Fractogel supports respectively. Additionally, these systems showed negligible leakage of the protein into the storage buffer of the gels over a thirty-day period.

Methacryloyl chloride was also investigated with respect to its suitability as an activating agent. With this reagent, optimum activation of the hydroxyl support was achieved in 9-10 hours when 10 mmoles of the activating agent was used per mL of the hydroxyl support. Optimum enzyme coupling was observed at pH 9.0 in a 0.05M $NaHCO_3$ buffer. All three enzymes immobilized (glucose oxidase, urease and β-galactosidase) showed good storage stabilities (79%, 92% and 96% for respectively) at 4°C over sixty days.

Key words: p-nitrophenylacrylate; methacryloyl chloride; bis(4-nitrophenyl) carbonate; aza-arenophilic ligand.