

## BEHAVIOR OF SOLUBLE AND PRECIPITATED TRYPSIN IMMOBILIZED TO EUDRAGIT-100

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Trypsin of two different concentrations was coupled to Eudragit S-100 by using carbodiimide hydrochloride as the coupling agent. When 5 and 500 mg trypsin was used, the protein coupled per mole of Eudragit was 0.022 and 2.0 mg respectively. Eudragit can be precipitated in presence of ethanol and  $\text{CaCl}_2$  on the activities of free and immobilized trypsin ( $5\text{mg g}^{-1}$  Eudragit) were determined with N  $\alpha$ -Benzyl-DL-Arginine p-Nitroanilide hydrochloride (BAPNA, low molecular weight substrate) and Azocasein (high molecular weight substrate).  $\text{CaCl}_2$  did not affect the activities of free and immobilized trypsin with BAPNA and Azocasein, while ethanol, and ethanol &  $\text{CaCl}_2$  reduced the activities of both free and immobilized enzyme. The  $K_m$  value of free trypsin to BAPNA was higher ( $22.2\mu\text{g}$ ) than for soluble immobilized preparations ( $94.9$  and  $116\mu\text{g}$  with 5 and 500mg trypsin  $\text{g}^{-1}$  Eudragit respectively). The  $K_m$  in presence of ethanol and  $\text{CaCl}_2$  was  $544.7\mu\text{g}$  while the precipitated forms of 5 and 500mg trypsin  $\text{g}^{-1}$  Eudragit respectively showed 295.6 and  $493.6\mu\text{g}$ . When Azocasein was used as the substrate, the free and 5 and 500 mg trypsin  $\text{g}^{-1}$  Eudragit immobilized preparations had the  $K_m$  value of 0.91, 1.17 and 1.33 respectively. The same preparations in presence of  $\text{CaCl}_2$  and ethanol showed 1.9, 1.7 and 2.4mg of  $K_m$ . to inhibit  $15\mu\text{g}$  of trypsin  $15\mu\text{g}$  of soy bean trypsin inhibitor (STI) was required when either BAPNA or Azocasein was used as the substrate and in presence or absence of ethanol and  $\text{CaCl}_2$ . The activities of soluble and precipitated forms of 5 and 500mg trypsin  $\text{g}^{-1}$  Eudragit never reached zero in presence of different concentrations of STI. The activities of soluble and precipitated immobilized ( $5\text{mg trypsin g}^{-1}$  Eudragit) with Azocasein reached zero in presence of 25 and  $15\mu\text{g}$  of STI respectively. The activity of soluble form of 500mg trypsin  $\text{g}^{-1}$  Eudragit reached zero in presence of 1.8mg of STI, while the activity of the precipitated form was completely inhibited by 0.75mg of STI.