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IMPROVING TRYPSIN COUPLING TO EUDRAGIT S-100 WHILE AVOIDING NON-SPECIFIC PROTEIN BINDING

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The optimum time required for the coupling of trypsin to Eudragit S-100 by carbodiimide (EDC) method was 1.5 (with N α-Benzymal-DL-Arginine p-Nitroanilide hydrochloride and BAPNA) and 3h (with azocasein). With an increase in trypsin concentration, protein coupled to Eudragit decreased and 29-39% of the total activity was expressed. Trypsin treated under coupling conditions while avoiding Eudragit and mixed with EDC and ethanol amine was 37.1% and treated only with EDC was 56.4%). 3M Glycine-NaOH buffer (pH 10.6), 1N HCl, 0.0 - 1.25M $CaCl_2$ and 0.2M $(NH_4)_2SO_4$ did not remove the non-specifically bound trypsin completely. Non-specifically bound trypsin was completely removed by 0.1M Tris^{-3}gl^{-1} Triton X-100 buffer (pH 7.6, Buffer W3) in the second cycle. The expected activity expressed by trypsin was 21.9, while at the end of the coupling procedure when the preparation was washed with Buffer W3, 34.7% of the activity was expressed. Removal of the excess EDC after activation prior to coupling has given an activity of 29.3%. When the activated polymer was precipitated, washed with 0.01M acetate buffer (pH 4.5) and used for coupling 39.8% of the activity was expressed, while washing two times with Buffer W3 decreased the activity to 13%. The normal coupling procedure while avoiding EDC and 54.7% of the activity was expressed while washing with Buffer W3 at the end of coupling completely removed the enzyme activity. When trypsin (50mg) was mixed with benzamidine (3.2mg) prior to coupling to Eudragit for 3rd and 24th and after final coupling step, washed two times with Buffer W3, activity expressed was 76.6 and 45% respectively. The activated polymer was precipitated, washed with acetate buffer (pH 4.8) and normal coupling procedure was adopted after treating the trypsin with benzamidine, the activity expressed was 14.7 and 24.3 respectively. To improve the activity expressed, the normal coupling procedure can be followed while protecting the active site of trypsin with benzamidine and finally washing with 0.15M Tris-3gl^{-1} Triton X-100 buffer (pH 7.60) two times.