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Preparation and evaluation of inclusion complexes of Papain with HP-β-CD

A. Swapna

Gokaraju Rangaraju College of Pharmacy, India

Abstract

Inclusion complexes of β-cyclodextrin are useful to improve solubility and stability of drugs and therapeutic proteins. Protein molecule actives were incorporated in to HP- β-CD for improving the dissolution, bioavailability. Studies permit to understand their properties and possible therapeutic applications. Papain (digestive enzyme) was chosen and the factors, namely substrate concentration, pH of the medium, and temperature influencing enzyme activity were investigated. A suitable analytical method was established for papain at λ_{max} 660 nm. It was verified that there is no interference of hydroxy propyl β-cyclodextrin on the analytical wavelength of enzyme. Enzymatic activity of papain was assayed at increasing concentrations of hydroxy propyl β-cyclodextrin. The enzyme activity was decreased as the concentration of HP-β-CD increased, indicating inaccessibility of papain to the substrate and also increasing complexation. There was no shift in optimum pH 7.5 on adding HP-β-CD. Optimum temperature for papain was 30^{o}C and temperature stability was achieved till 55 °C, in presence of HP-β-CD. Complexes of different ratios were prepared from 1:10 to 1:200 by physical mixing, solvent evaporation and neutralization and assayed. By physical mixtures, the enzymatic activity was found to be enhanced compared to papain alone and optimum for 1:40. The free activity was reduced gradually in alkaline pH, but inclusion complex preserved its activity even in alkaline pH, and extreme temperatures. Inclusion complexes were estimated by IR and shown the presence of enzyme and HP-β-CD in the complexes bands.