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Protease-mediated syntheses of polypeptides in compressed 1,1,1,2-tetrafluoroethane media

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Protease from Subtilisin Carlsberg and liquid 1,1,1,2-tetrafluoroethane (R134a) mediated the syntheses of high molar mass poly(L-phenylalanine ethyl ester), poly(L-leucine ethyl ester) and copolymers of poly(L-phenylalanine ethyl ester-co-L-leucine ethyl ester) with yields of ca. 50%. This hydrophobic and relatively polar compressed fluid medium allows for the protease activity and solubilities of the amino acid esters and products. The homopolypeptides displayed higher crystallinity than the copolypeptides with random incorporation of both L-aminoacid ethyl esters. The affinity of the protease enzyme was significantly higher for L-phenylalanine ethyl ester than for the leucinate substrate. Secondary structure assessment by FTIR and Circular Dichroism indicates a non-common folding of peptides. Calculations using molecular dynamics, Flory-Huggins parameter and Gibbs energy of mixing to obtain insight on the solubility behavior of these systems predict that solutions of R134a and copolymer will be thermodynamically miscible at all the temperatures and volume fractions of copolymer studied. Additionally, R134a-polymer systems will present phase separation at specific temperatures and volume fractions of the polymer. The prediction also shows that the obtained copolypeptide is more soluble in R134a than the more crystalline homopolypeptides separately.

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