A PLURONIC COUPLED METAL CHELATING LIGAND FOR MEMBRANE AFFINITY CHROMATOGRAPHY

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A technique for bio-specific affinity chromatography using synthetic non-porous membranes and a novel metal chelating Pluronic® surfactant is described. Synthetic organic polymers such as polysulfone and poly(vinyldiene fluoride) were used as solid, hydrophobic adsorption matrices for the affinity ligand Pluronic-N,N-dicarboxymethyl-3,6-diazaoctanedioate (Pluronic-DMDDO). The hydrophobic polypropylene oxide moiety of Pluronic® F108 allowed non-covalent adsorption of the ligand to the affinity membrane. The protein repellent-properties of the hydrophilic polyethylene oxide brush layer of Pluronic, was used to preserve the bio-specific activity of the ligand and to increase ligand accessibility. An ethylene diamine tetraacetic acid dianhydride was coupled to the terminal hydroxyl end groups of Pluronic to create a metal chelating ligand. The sorption capacities for Cu(II), Zn(II) and Ni(II) were determined under non-competitive conditions. Proton induced X-ray analysis was used to generate quantitative data on the metal chelate capacity and regeneration properties of this metal affinity polymeric system. Recombinant pantothenate kinase and commercial carbonic anhydrase were used as test proteins. Histidine tagged pantothenate kinase bound to Pluronic-DMDDO modified membranes only in the presence of Ni^{2+} while the metallo-enzyme carbonic anhydrase also bound to membranes functionalised with Pluronic-DMDDO-Ni^{2+}. Eluted histidine tagged proteins retained much of their biochemical activity and the membranes were capable of being regenerated and re-used.