

Knowledge of host protein properties is critical for developing purification methods for recombinant proteins from a specific host, or for choosing suitable hosts and targeted expression tissues for a specific recombinant protein. A method to obtain a three-dimensional (3D) map (surface hydrophobicity (SH), isoelectric point (pI) and molecular weight (MW)), of a host's aqueous soluble protein properties was developed. The method consists of hydrophobic partitioning in PEG 3350 (15.7%)-Na<sub>2</sub>SO<sub>4</sub> (8.9%)-NaCl (3%) system followed by quantitative, 2D-electrophoretic characterization of the proteins of each equilibrium phase and the original extract. The pI and MW of host proteins were obtained directly through 2D-electrophoresis. The partition coefficients of individual proteins, i.e. single spots on the 2D-gels, were obtained by matching the spots on the two gels of the proteins in the top phase and the bottom phase, respectively, and used to estimate their hydrophobicities according to a surface hydrophobicity scale based on the partition coefficients of several model proteins with known surface hydrophobicities in the same ATP system. The inclusion of the extract gel provided for a spot selection criterion based on satisfactory mass balance closure.

The method is illustrated by application to a mixture of model proteins and to complex mixtures i.e. corn germ proteins extracted at pH 7 and pH 4. Compared to corn germ extract at pH 7, the germ extract at pH 4 contains less protein and that protein is more hydrophobic, more basic and smaller. Therefore, extraction conditions will significantly affect purification processing.

The 3D-mapping method will be useful for 1) developing an optimal purification strategy for a protein expressed in a specific host; 2) predicting what kind recombinant proteins can be separated most easily if expressed in a specific host; and 3) choosing a suitable host or host fractions or the extraction condition for a specific recombinant protein to facilitate downstream purification.