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# Examining structure–activity correlations of some high activity enzyme preparations for low water media

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ABSTRACT

A first study of the comparison of structures of enzymes (by FT-IR and CD) in different high activity (in low water media) preparations is reported. Using chymotrypsin and subtilisin as models, we have studied various factors that distinguish enzyme precipitated and rinsed with propanol (EPRP), crosslinked enzyme aggregates (CLEA), protein coated microcrystals (PCMC) and crosslinked protein coated microcrystals (CLPCMC). The suspensions in organic media were assayed for catalytic activity, and structures were probed by FT-IR and CD measurements. CD studies of enzyme suspensions were possible by using a rotating cell accessory. There was a generally good correlation between higher catalytic activity and retention of native structures. Activity and retention of native structure was always higher if aqueous enzyme solution was added to propanol rather than vice versa in the precipitation step of these preparations. The work identifies factors which may lead to better biocatalyst designs for low water media.


## 1. Introduction

Use of enzymes in low water media has emerged as an attractive option in carrying out biotransformations. One longstanding concern has been about improving their performance in such media (Arnold, 1990; Mattiasson and Adlercreutz, 1991; Hudson et al., 2005; Carrea and Riva, 2008). Many methods have been presented that yield catalysts with enhanced activity in organic and related media. Several published methods to produce high activity catalysts have in common the dehydration of the enzymes from aqueous solution by mixing with a water-miscible organic solvent. This occurs in preparation of: (a) cross-linked enzyme aggregates (CLEA), when the organic solvent is used as precipitant (Schoevaart et al., 2004); (b) enzymes precipitated and rinsed with propanol (EPRP) (Roy and Gupta, 2004); (c) protein-coated microcrystals (PCMC) (Kreiner et al., 2001); and (d) cross-linked protein coated microcrystals (CLPCMC) (Shah et al., 2008). At first sight these methods appear quite different, and they are indeed distinct. However, they have in common the exposure of the enzyme molecules to aqueous-organic mixtures of various compositions during the precipitation. One obvious difference between procedures is whether or not the precipitated enzyme is cross-linked. A second distinction lies in the amount of co-solute (excipient) also present in the enzyme solution, which is sufficient to produce core microcrystals in the case of PCMCs. By varying the concentration of co-solute it is possible to span the whole range from EPRPs to PCMCs, with a step-change in behavior at the point where co-solute crystallization becomes significant (Solanki and Gupta, 2008). A third variable is the order of addition of the aqueous enzyme solution and the organic solvent. For PCMCs and CLPCMCs, the usual procedure is to add the aqueous solution to the organic solvent. The sequence of medium compositions experienced by the enzyme molecules will be quite different if instead organic solvent is added to the aqueous solution. The earliest papers using organic solvent precipitation in making EPRP and CLEAs described adding the organic solvent to the aqueous enzyme solution (Lopez-Serrano et al., 2002; Roy and Gupta, 2004), which would be the conventional approach in precipitating proteins. More recently it is reported that the addition can also be made the other way round (Schoevaart et al., 2004), although without comment on the significance of this. Note that other agents may also be used to precipitate enzymes for CLEAs, but organic solvents are a common choice.

*Abbreviations:* EPRP, enzyme precipitated and rinsed with *n*-propanol; PCMC, protein coated microcrystals; CLEA, cross linked enzyme aggregates; CLPCMC, cross linked protein coated microcrystals; REPRP, reverse enzyme precipitated and rinsed with *n*-propanol; RPCMC, reverse protein coated microcrystals; RCLEA, reverse cross linked enzyme aggregates; RCLPCMC, reverse cross linked protein coated microcrystals; CD, circular dichroism; FT-IR, Fourier transform infrared spectroscopy.

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