Summary of the Work
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- The feasibility of aqueous two phase extraction for the isolation and purification of ADH and invertase from baker’s yeast was demonstrated.

- During purification of ADH precipitation with PEG followed by ATPE (comprising PEG 20000/potassium phosphate), has resulted in specific activity of 449.6 U/mg with 6.6 fold enrichment and about 93% enzyme activity recovery at the standardized conditions.

- ATPE employing PEG 3350/magnesium sulphate system has resulted in specific activity of 33 U/mg with ~8.81 fold purification of invertase with 77% enzyme activity recovery at the standardized process conditions.

- Degree of enzyme (invertase) purification increased with an increase in nanoparticle concentration due to partitioning of contaminant proteins to the opposite phase.

- Highest enrichment of invertase was observed in case of PEG-3350/Magnesium sulphate system with gold nanoparticles with 32.72 U/mg specific activity, enzyme activity recovery of 92% and 9 fold enrichment (compared to crude extract) among all the systems studied.

- Integration of MF (employing nanofibrous membranes) followed by aqueous two phase extraction has resulted in increased purification of selected enzymes (ADH and invertase) without losing the yield (enzyme activity recovery).

- Integration of microfiltration (employing nanofibrous membranes) with ATPE (Mode 3) was found to be the best, resulting in 647.32 U/mg specific activity of ADH with 8.12 fold enrichment and 95% enzyme activity recovery.
Highest degree of purification of 14.09 fold with 54.52 U/mg specific activity and 92.41% enzyme activity recovery of invertase was obtained during integration of MF with ATPE among all the integration methods employed.

The study revealed that electroextraction (field assisted transfer of a given biomolecule) is possible also in polymer/salt systems despite their high electrical conductivity.

Electroextraction of ADH employing PEG/potassium phosphate system resulted in a gradual increase in the partitioning of ADH towards the bottom phase in normal polarity and towards the top phase in reverse polarity, with respect to time of electroextraction (0-60 min) at any given pH.