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Thermoseparating Aqueous Two-Phase System: Recent Trends and Applications

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Aqueous two-phase system (ATPS) has the benefits of being environmental-friendly, providing a mild environment for bioseparation, ability to handle high capacity and can be easily scaled up. Among all ATPS techniques, thermoseparating ATPS outshine the others due to its distinctive thermoseparating properties and easy-recyclability and has increasingly capture attractions from industries and researches for its application on isolation and recovery of biomaterials. This review starts with a brief introduction of ATPS, including its history, range of applications, unique characteristics, partitioning-influencing factors, and lastly, the different types of ATPS together with their descriptions and general applications. This followed by a summary of recent applications is listed, focusing on thermoseparation-based ATPS. Thermoseparating ATPS is concluded to have huge potential as a cost-effective and environmental-friendly industrial-scale bioseparation and recovery technique.

1. Introduction

Conventionally formed by mixing polymer/polymer or polymer/salt above critical concentration (Prinz et al., 2012), aqueous two-phase system (ATPS) plays a role in purification and recovery in a wide range of applications as well as analytical and preparation tool on biochemistry (Rosa et al., 2010). Nontoxic and relatively environmental-friendly phase-forming components are employed in ATPS compared to volatile and toxic solvents utilized in conventional solvent extraction methods (Ng et al., 2012). ATPS also provides satisfactory product's quality and yield in purification of both low and high value products with low cost and energy consumption, in opposition to expensive chromatography techniques which more economic favourable to high-end products (Silvério et al., 2013).

The partitioning behaviours of biomolecules in ATPS are affected by their physicochemical properties such as net charge, size and relative hydrophobicity (Ferreira et al., 2008); and can be manipulated by altering system variables including types and concentration of polymers and/or salts, volume ratio (V_r), pH and temperature (Ng et al., 2012). Strategies such as peptide tags addition (Jiang et al., 2015), pH altering (Moreira et al., 2013), and cosolutes additions (such as non-ionic surfactants or salt) (Dembczynski et al., 2010) can be employed to enhance or direct the partitioning of biocomponents to the desired phase.

Due to limitations of conventional ATPSs including difficulty of phase-forming components recycling, temperature-induced ATPS formed by thermoseparating polymers (TSPs) has caught the attentions of researchers and industries. The common types of TSPs include random, diblock, and triblock copolymers of ethylene oxide and propylene oxide (EOPO) and separate into water and polymer phases after heated above cloud point (CP) or lower critical solution temperature (LCST). The target product can then be recovered exclusively in the water phase and the polymers can be recycled and reutilized easily (Chen et al., 2014). This review presents the recent advances in the development of thermoseparating polymer-

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based ATPS. The paper starts with a brief description of ATPS, followed with an in-depth summary of applications and researches on thermoseparation-based ATPS in the recent years.

2. Applications of thermoseparating ATPS

2.1 Lysozyme

The separation and purification of lysozyme from crude egg white has been investigated by Dembczyński and his coworkers utilizing $EO_{50}PO_{50}$/potassium phosphate ATPS (Dembczyński et al., 2010). The partitioning of lysozyme were all done at pH value of 9 and 850 mol/m^3 NaCl. It is found that increment in $EO_{50}PO_{50}$ concentration as well as addition of NaCl will promotes the yield of lysozyme in top phase. This is because the lysozyme (pI = 10.7) becomes positively-charged at pH 9 and is attracted to the hydrophobic counterion (Cl^-) partitioned to the top phase. Another reason for high partition of lysozyme is due to the hydrophobic interactions between its high hydrophobic surface and the system components. Considering yield, purity, high concentration factor, simplicity of phase harvesting and cheap chemical cost for industrial-scale process, the optimal conditions of 10 wt% potassium phosphate, 40 wt% $EO_{50}PO_{50}$ and 0.85 M NaCl at pH 9.0 is chosen for primary extraction. After back-extraction, the specific activity, yield and purification factor of lysozyme overall is 32,300 U/mg, 85 % and 16.9. (Dembczyński et al., 2010).

Continue from previous work (Dembczyński et al., 2010), the study of four consecutive lysozyme recovery from egg white were done by recycling both top and bottom phase components. It was shown that the parameters in lysozyme separation (such as volume ratio (V_r) and protein concentration in top phase) are quite stable, regardless of high accumulation of contaminant protein in phosphate-rich bottom phase. Comparing with affinity techniques that provided similar purification level and both chromatographic and multi-step filtration methods which require significantly higher cost though gave better quality, thermoseparation-based ATPS has indeed proven as one of the most cost-saving and simplest lysozyme purification methods. Last but not least, improvements such as fast and precise NaCl concentration measurement method and complete elimination of lysozyme in bottom phase have been suggested to increase the yield of lysozyme (Dembczynski et al., 2010).

Scaled-up to pilot scale separation process, thermoseparation-based ATPS coupled with cross-flow membrane separation technique was investigated as the use of centrifugation for industrial-scale process is uneconomical. In order to enhance lysozyme yield at reasonable cost, the isolation of protein from phosphate-rich phase utilizing ultrafiltration technique was studied and proposed to be done after every second recycling. This integrated process provided specific activity and yield of 34,000 U/mg and 47.5 % with polymer recovery up to 83.9 % in single extraction step (Dembczyński and Białas, 2013).

In order to simplify the tediousness and reduce the number of experiment, a response surface methodology (RSM), named Box-Behnken design has been employed to determine optimal conditions of lysozyme recovery by thermoseparation-based ATPS. It is concluded that concentration of NaCl added is the most influential factor among all, further proving electrostatic interaction plays an important role in partitioning of lysozyme. An optimal conditions of 17.4 wt% $EO_{50}PO_{50}$/ 22.67 wt% potassium phosphate with 0.85 mol/L of NaCl concentration and pH 9.0 was achieved with good agreement between the predicted results and experimental data (Dembczyński et al., 2013). In the study, it can be observed that lysozyme has a better partitioning efficiency at slight acidic condition (pH 6) in most cases during primary partitioning step which agree with the literature (Lu et al., 2013). However, the system generally exhibited a higher yield at slight basic condition (pH 9) during second thermoseparation step. Therefore, it is suggested that the lysozyme partitioning can be further enhanced by modifying the system pH to higher than 10.7 or adding triethylammonium phosphate salt during thermoseparation step. As the optimal EOPO concentration of previous study is 40 wt/wt %, the study can be further improved by cover a wider pH range and EOPO concentration to obtain a global optimum, instead of local optimum.

2.2 Cyclodextrin glycosyltransferase (GCTase)

Isolation and recovery of GCTase from Bacilius cereus have been studied by Ng and his colleagues utilizing thermoseparation-based ATPS (Ng et al., 2012). Agreeing with literature, it was found that thermoseparating polymer with EO:PO ratio of 50:50 is more suitable for partitioning of GCTase as the polymer phase provides greatest solubility for GCTase, thus avoiding the precipitation in interphase. Among thermoseparation systems of different TLLs, EOPO 3900 with TLL of 41.2 wt/wt % has been chosen for recovery of GCTase as the partitioning behaviour was promoted by the increase in hydrophobicity difference between two phases. Although EOPO 970 has the 50:50 EO:PO ratio as well, it needed higher concentrations of EOPO to form two phases that might cause precipitation at the interphase and volume exclusion effect, which causes negative impacts on K and P_F. V_r is also an important parameter in GCTase extraction as low V_r reduces the top phase's free volume and limited the solubility of GCTase. On the other hand, it is also indicated that addition of excessive crude load might alter the ATPS

composition and V_r which resulted in precipitation and yield reduction. With the optimal conditions of 42.1 wt% TLL, 1.25 V_r, 20 wt% crude load and pH 7, yield and purification factor of 87 % and 13.1 have been achieved. In the recycling studies, the GCTase's yield and purity fall following the recycling cycles due to increasing amount of contaminants in bottom phase after each cycle, thus, recommending that the EOPO copolymer can only be recycled and reused twice (Ng et al., 2012). However, it appears that the recycling can be performed continuously as long as new salt phase is used after every second recycling since constant polymer recovery was achieved throughout the studies.

2.3 Laccase

As an alternative oxidoreductase to conventional chemical oxidation with wide range of applications, development of new extraction and recovery method for laccase are of considerable interest (Silvério et al., 2013). There are several studies on its separation and purification utilizing ATPS, including PEG 3000/phosphate system (Prinz et al, 2012), PEG 1000/phosphate system (Mayolo-Deloisa et al., 2009) and PEG 4000/phosphate system (Ratanapongleka, 2012). Lladosa and his colleagues is among the first who study the extraction of laccase utilizing thermoseparation-based ATPS systems (Lladosa et al., 2012). The shortest TLL of four systems which composed of Ucon with citrate and formate salts were investigated as least phase-forming components are required and have lowest viscosities. All systems present K value around 0.5 which means that laccase has 2 times higher concentration in bottom phase compared to that of top phase, suggesting these systems have potential for laccase recovery and can be further optimized (Lladosa et al., 2012).

Silverio et al. (2013) have then proposed the recovery of laccase using ATPS-based extractive fermentation, thus performed a preliminary study to obtain a suitable ATPS. Comparing among 21 different ATPS systems, PEG/Li_2SO_4 (K = 2.081), UCON/phosphate salt (K<0.604), Dextran/Ucon (K = 0.487) and PES/dextran (K = 1.911) have shown the potential for purification of commercial laccase from *Trametes versicolor*. Continue on enzyme stability study, it is worth noting that acidic pH influences the stability of laccase more significantly than basic pH. PEG maintains or improves the enzyme stability better compared to Ucon in both polymer/salt system and polymer/polymer system. Due to high loss of enzymatic activity, both Ucon/sulfate and Ucon/phosphate systems were not suitable for laccase extraction although offer the advantage of polymer recycling. Sulfate salts is more effective in provides enzyme stability than phosphate salts as the latter are more chaotropic according to Hofmeister series, but this is not the case in Ucon/sulfate system. All in all, PEG/sulfate is the most potential laccase recovery system in terms of both partitioning and enzyme stability (Silvério et al., 2013). Other than PEG with lower molecular weight, addition of sodium chloride has proven to have significant influence on laccase partitioning according to literature above. Optimization of these parameters can be done for this study together with effect of polymer and salt concentration (tie-line length).

Continue on the research, UCON/phosphate salt system was first time studied as an extraction technique of laccase from Peniophora cinerea fermented using corn steep liquors (Moreira et al., 2013). Laccase activity was found to be stimulated in the presence of acidic salt solution (i.e. KH_2PO_4 with pH 4.6) as it normally has optimal activity in acidic pH range. The laccase partition preferentially to the bottom phase for both phosphate salts (K_2HPO_4 and KH_2PO_4) and phosphate buffer, with activity yield more than 100 % in some cases. This might due to partitioning of contaminants in the complex fermentation medium to the top phase. For increasing amounts of crude extract, laccase activity and purification factor decreased due to saturation of contaminant in top phase, but there is no significant influence on the protein yield. Compared with extraction using PEG/phosphate ATPS in literature stated above, UCON/phosphate salt systems showed higher laccase activity yield, but lower purification factor. Referring to the previous literature, the purity of the product enzyme can be further enhanced by addition of sodium chloride. Credited for their high protein yield and enzyme activity together with recycling of phase-forming components, thermoseparation-based ATPS proved to be a potential isolation and recovery method of laccase from complex fermentation medium compared to conventional methods, such as lyophilization, ammonium sulphate precipitation, ultrafiltration and ion exchange chromatography (Moreira et al., 2013).

2.4 Ciprofloxacin (CIP)

Applications of traditional detection techniques for ciproflaxin (CIP) such as high-performance capillary electrophoresis and spectrofluorimetry have been limited due to their high detection limit and complex pretreatment (Chen et al., 2014). Thermoseparation-based ATPS coupled with HPLC/UV detection system has been proposed by Chen and his colleagues for isolation and concentration of CIP (Chen et al., 2014). K_2HPO_4 was chosen among various salt to form ATPS with EOPO L31 as alkaline salts have higher extraction efficiency compared to acid and neutral salts. As an acidic component, CIP converted to salts and excluded to EOPO-rich phase as the pH increase, which is also the reason pH 11 chosen as the optimal extraction pH. The thermoseparating copolymer was recommended to be recycled and reutilized

not more than twice in the recycling study as the phase-forming salt losing its salting-out ability and reduction in EOPO recovery after every cycle. It was suggested that residual CIP and contaminant should be removed from the phosphare-rich phase before reutilized in subsequent system. The isolation and enrichment of CIP from real samples, such as milk, egg and shrimp was performed using optimized ATPS and presented a lower value that maximum residual limits (Chen et al., 2014).

2.5 L-asparaginase

Serve as an effective treating agents in various types of human cancer including leukemia, downstream processing of L-asparaginase using Triton X-100/K_2HPO_4 aqueous two-phase micellar system has been studied (Qin and Zhao, 2003). However, the studies showed accumulation of L-asparaginase in salt-rich bottom phase which could be difficult to recover in subsequent step and discrete operation steps require storage period which may causes modification and denaturation of target products. In order to overcome these obstacles, Zhu and his coworkers have proposed in-situ extraction of L-asparaginase from ATCC Escherichia coli 11303 using thermoseparating triblock copolymers (PEO-PPO-PEO) (Zhu et al., 2007). Performance of triblock copolymers of various molecular mass and different EO:PO ratio have been compared to PEG 1000, 3000 and 6000. For PEG/phosphate salt system, L-asparaginase partitioned to the PEG-rich top phase and followed a common trend of increasing K_a and PF with rise of both salt concentration and PEG molar mass. This is due to enhanced hydrophobic interactions between the bioproducts and the polymer-phase (Zhu et al., 2007).

On the other hand, for the case of PEO-PPO-PEO, partitioning of L-asparaginase to the top phase increase as the salt concentration increase until 21.5 wt% with a sudden fall afterward, especially those with higher molar mass. With increasing triblock copolymer molar mass, PF generally showed an increasing trend, while K_a did not show any specific trend, but those with higher molar mass also gave higher partitioning. In comparison, thermoseparating copolymer systems gave K_a 5 fold higher and PF 2 fold purer compared to that of PEG systems as well as showing the advantage of polymer recycling capability. Subsequently, in-situ ATPE integrated with high pressure homogenizer was performed to minimize adsorption of L-asparaginase on cell debris and eliminate centrifugation and storage process. A higher yield and improved specific activity is obtained through this integrated process. Optimum pH of 5.0 was obtained around the p*I* of L-asparaginase (4.9) as the electrostatic attraction of bioproducts on cell debris is minimized and contaminant partitioned preferably into top phase at higher pH (Zhu et al., 2007).

2.6 Extractive fermentation of lipase

Extractive bioconversion is a technique which integrates fermentation together with downstream processing (clarification, concentration and partial purification) using a continuous process has gained increasing interests (Ooi et al., 2011). The main principle of extractive fermentation using ATPS is to promote the partitioning of the target product in one phase, while cell and substrates accumulated in the other phase (Ooi et al., 2011). The integration of ATPS with extractive fermentation shows huge potential as a substitute for conventional cultivation processes for simultaneous fermentation and purification of bioproducts and proteins (Moreno-Cid et al., 2012).

Isolation and purification of lipase from Burkholderia cepacia has been done using thermoseparating polymers/phosphate salts ATPS. The bottom phase volume reduced as the TLL increased with consistent partitioning behavior, favoring partitioning of lipase to the polymer-rich top phase. The increase in volume ratio led to an increase in partition coefficient (K), selectivity (S) and purification factor (P_F) of lipase. There is a change in composition of ATPS, decrease in V_r and accumulation of precipitate at the interface as the crude feedstock exceeded 20 wt%. The optimal partitioning conditions of lipase were EOPO 3900, TLL of 48.5 wt%, V_r of 2.23, pH 7 and crude feedstock of 20 wt%. High yield and purification factor of 99 % and 14 has been achieved in four successive recovery of lipase (Show et al, 2012a).

Continue on previous work, extractive bioconversion of lipase from Burkholderia cepacia using thermoseparating ATPS was investigated. The viscosity of fermentation medium increased with molecular weight and concentration of TSPs, which might reduce the oxygen mass transfer in the medium, leading to retarded cell growth and reduction in lipase production. The lipase partitioned favourably into polymer-depleted top phase at pH 8.5 due to the dominant electrostatic forces. The production of inhibitors was minimized as the orbital speed during fermentation increased. The optimal conditions for extractive fermentation of lipase was EOPO 3900 of concentration 10 wt%, pH 8.5, 200 rpm orbital speed and temperature of 30 °C. The extractive bioconversion has been done in repetitive batch for lab scale (shake flask) and scaled-up to 2.0 L bioreactor (Show et al, 2012b). Table 1 summarizes the recent applications of thermoseparation-based ATPS on separation of bio-components with their respective yields and recycling properties.

Table 1: Summary of recent application of thermoseparation-based ATPS on bioseparation

Bioproduct	System	Recovery Yield	Polymer Recycling	Reference
CGTase	EOPO 3900/phosphate	87 % yield / 13.1 PF	2 times	(Ng et al., 2012)
Ciprofloxacin	EOPO L31/K_2HPO_4	97.7 % extraction efficiency	2 times	(Chen et al., 2014)
Laccase	UCON/KH_2PO_4	134 % yield / 1.31 PF	N.A.	(Moreira et al., 2013)
Laccase	UCON/K_2HPO_4	K = 0.272	N.A.	(Silvério et al., 2013)
Lipase	EOPO 3900/phosphate	99 % yield / 14 PF	3 times	(Show et al., 2012a)
Lipase	EOPO 3900	99 % yield / 14 PF	N.A.	(Show et al., 2012b)
Lysozyme	$EO_{50}PO_{50}/K_2HPO_4$	85 % yield / 16.9 PF	3 times	(Dembczyński et al., 2010), (Dembczynski et al., 2010)

3. Conclusions

With the knowledge and insights obtained, it is hoped that this paper will be able to shed some light on the recent trends and applications of thermoseparation-based ATPS. The potential applications of thermoseparating ATPS include enzyme, DNA, antibody, antibiotic and others. All in all, thermoseparating polymer-based ATPS has a huge potential for industrial-scale cost effective and environmental-friendly biomaterials separation and recovery.

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References

Chen B., Han J., Wang Y., Sheng C., Liu Y., Zhang G., Yan Y., 2014, Separation, enrichment and determination of ciprofloxacin using thermoseparating polymer aqueous two-phase system combined with high performance liquid chromatography in milk, egg, and shrimp samples. Food Chem, 148, 105-11.

Dembczyński R., Białas, W., 2013, Pilot-scale separation of lysozyme from hen egg white by integrating aqueous two-phase partitioning and membrane separation processes. Process Biochemistry, 48, 1992-1998.

Dembczynski R., Bialas W., Jankowski T., 2010, Recycling of phase components during lysozyme extraction from hen egg white in the EO50PO50/K2HPO4 aqueous two-phase system. Biochemical Engineering Journal, 51, 24-31.

Dembczynski R., Bialas W., Jankowski T., 2013, Partitioning of lysozyme in aqueous two-phase systems containing ethylene oxide-propylene oxide copolymer and potassium phosphates. Food and Bioproducts Processing, 91, 292-302.

Dembczyński R., Białas W., Regulski K., Jankowski T., 2010. Lysozyme extraction from hen egg white in an aqueous two-phase system composed of ethylene oxide–propylene oxide thermoseparating copolymer and potassium phosphate. Process Biochemistry, 45, 369-374.

Ferreira I.F., Azevedo A.M., Rosa P.A., Aires-Barros M.R., 2008. Purification of human immunoglobulin G by thermoseparating aqueous two-phase systems. J Chromatogr A*,* 1195**,** 94-100.

Jiang Z.G., Zhang H.D., Wang W.T., 2015. Purification of papain by metal affinity partitioning in aqueous two-phase polyethylene glycol/sodium sulfate systems. J Sep Sci*,* 38**,** 1426-32.

Lladosa E., Silvério S.C., Rodríguez O., Teixeira J. A., Macedo E.A., 2012. (Liquid+liquid) equilibria of polymer-salt aqueous two-phase systems for laccase partitioning: UCON 50-HB-5100 with potassium citrate and (sodium or potassium) formate at 23 °C. The Journal of Chemical Thermodynamics, 55, 166-171.

Lu Y., Lu W., Wang W., Guo Q., Yang Y., 2013. The optimization of aqueous two-phase extraction of lysozyme from crude hen egg white using response surface methodology. Journal of Chemical Technology & Biotechnology, 88, 415-421.

Mayolo-Deloisa K., Trejo-Hernández M. D. R., Rito-Palomares M., 2009. Recovery of laccase from the residual compost of Agaricus bisporus in aqueous two-phase systems. Process Biochemistry, 44, 435-439.

Moreira S., Silverio S.C., Macedo E.A., Milagres A.M., Teixeira J.A., Mussatto S.I., 2013. Recovery of Peniophora cinerea laccase using aqueous two-phase systems composed by ethylene oxide/propylene oxide copolymer and potassium phosphate salts. J Chromatogr A, 1321, 14-20.

Moreno-Cid J.A., Canales M., De La Fuente J., 2012. Production of recombinant Aedes albopictus akirin in Pichia pastoris using an aqueous two-phase semicontinuous fermentation process. Biochemical Engineering Journal, 68, 114-119.

Ng H.S., Tan C.P., Mokhtar M.N., Ibrahim S., Ariff A., Ooi C.W., Ling T.C., 2012. Recovery of Bacillus cereus cyclodextrin glycosyltransferase and recycling of phase components in an aqueous two-phase system using thermo-separating polymer. Separation and Purification Technology, 89, 9-15.

Ooi C.W., Hii S.L., Kamal S.M.M., Ariff A, Ling T.C., 2011. Extractive fermentation using aqueous two-phase systems for integrated production and purification of extracellular lipase derived from Burkholderia pseudomallei. Process Biochemistry, 46, 68-73.

Prinz A., Zeiner T., Vossing T., Schuttmann I., Zorn H., Górak A., 2012. Experimental investigation of laccase purification using aqueous two-phase extraction. Chemical Engineering Transactions, 27, 349-354.

Qin M.J., Zhao F.S., 2003. L-Asparaginase Release from Escherichia coli Cells with Aqueous Two-Phase Micellar Systems. Applied Biochemistry and Biotechnology, 110, 11-21.

Ratanapongleka K., 2012. Partitioning Behavior of Laccase from Lentinus polychrous Lev in Aqueous Two Phase System. Songklanakarin J. Sci. Technol., 34, 69-76.

Rosa P.A.J., Ferreira I.F., Azevedo A.M., Aires-Barros M.R., 2010. Aqueous two-phase systems: A viable platform in the manufacturing of biopharmaceuticals. Journal of Chromatography A, 1217**,** 2296-2305.

Show P.L., Tan C.P., Anuar M.S., Ariff A., Yusof Y.A., Chen S.K., Ling T.C., 2012a. Primary recovery of lipase derived from Burkholderia cenocepacia strain ST8 and recycling of phase components in an aqueous two-phase system. Biochemical Engineering Journal, 60, 74-80.

Show P.L., Tan C.P., Anuar M.S., Ariff A., Yusof Y.A., Chen S.K., Ling T.C. 2012b. Extractive fermentation for improved production and recovery of lipase derived from Burkholderia cepacia using a thermoseparating polymer in aqueous two-phase systems. Bioresour Technol, 116, 226-233.

Silvério S.C., Rodríguez O., Tavares A.P.M., Teixeira J.A., Macedo E.A., 2013. Laccase recovery with aqueous two-phase systems: Enzyme partitioning and stability. Journal of Molecular Catalysis B: Enzymatic, 87, 37-43.

Zhu J.H., Yan X.L., Chen H.J., Wang Z.H., 2007. In situ extraction of intracellular L-asparaginase using thermoseparating aqueous two-phase systems. J Chromatogr A, 1147, 127-134.