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A Review on Aqueous Two-Phase Systems with a Special Mention on Application of Ionic Liquids

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Abstract: ***Aqueous two phase system (ATPS) is an excellent biocompatible extraction methodology having wide applications in the purification of various biomolecules. In this method water comprises almost 80-85 % in both phases and hence the biomolecules are highly stable during extraction. ATPS system offers the advantage of process integration and there by increases the yield of biomolecules. Optimization of different parameters like type and concentration of phase forming compounds, addition of external agents like salts, temperature, physicochemical nature of biomolecules are influential in determining the partitioning of biomolecules towards a phase. In this review the methodology of ATPS and its application, challenges and future prospects are discussed in detail with a special mention on application of ionic liquids in ATPS.***
Keywords: Aqueous two phase system, Ionic Liquids, Biomolecules, Extraction

I. INTRODUCTION

Aqueous two-phase systems (ATPS) are formed when two polymers or polymer/ salt system are mixed above a critical concentration[1]. Both phases are predominated by water and this aqueous environment provides stability, solubility and integrity of biomolecules that are subjected to extraction[2]. This method is reliable to clarify, purify and concentrate the products[3]. ATPS generally minimizes the number of steps involved in the purification steps there by increasing the yield and results in decreased cost of labor. This method also poses disadvantages like difficulty in polymer regeneration and excess of sewage containing inorganic salts leading to environmental pollution[2];[3]. ATPS has better resolution in purification and able to separate closely related enzymes effectively[4].

A. Aqueous Two Phase systems

ATPS are mostly polymer-polymer and polymer-salt systems. The conventional polymers used in phase formation include Polyethylene glycol and dextran. The salts are mostly inorganic salts of phosphate. However easily biodegradable organic salts like citrate salts have advantages [2]. Detergent based ATPS systems are also used in the purification of membrane protein owing to their amphiphilic nature[3]. Since the cost of polymers used is expensive, cheaper alternative to dextran namely poly acrylic acid is shown to be efficient extracting 95.2 % of myoglobin[2]. Other possible cheaper alterantive incudes cashew nut gum as reported in the parttion of BSA by Sarubbo et al .,2004 [5]. Aqueous two phase micellar systems created using Triton X-114 a non ionic surfactant enables for concentration of mammalian genomic DNA which has potential application in early detection of cancer biomarkers[6].

B. Tie line

Phase diagram is a representation to depict the extent of separation of two phases under various composition of top and bottom phases. Binodal curve separates the region of homogenous and heterogeneous phases [7]. Tie line length (TLL) is defined as the distance between the top and bottom phase compositions in equilibrium. Tie lines are necessarily parallel, enabling to construct a new tie line using the slope of other. Tie line lengths are calculated using the formula

$TLL = \sqrt{(\Delta P_1^2) + (\Delta P_2^2)}$, Here in ΔP refers to the difference in polymer concentrations of both phases[2].

C. Partition Coefficient

Partition coefficient defined as the ratio of the concentration of biomolecule in upper phase to lower phase depends on the charge, hydrophilicity, hydrophobicity, electrostatic interactions and charge distribution. The volume of two phases is significant in defining the final compositions of phase forming components in top and bottom phase. The phase volume ratio is given as the ratio of volume of top phase to bottom phase[3].\

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D. Factors Affecting Partition Coefficient

The partition of biomolecule is affected by mass transfer resistance, interphase tension, extent of mixing , time for settling, insufficient separation, molecular weight of polymers and post translational modifications of proteins like glycosylation [3]. Addition of neutral salts lie NaCl and its concentration alters the microenvironment and influences the partition of biomolecules[2]. Increase in PEG molecular weights of larger the chain length increases the excluded volume and minimizes the free volume available for protein partition in PEG rich phase[8],[9]. While changing the pH of the systems the proportion of ions in both the phases are varied and results in voltage difference between two phases[10]. Affinity partitioning of biomolecules selectively towards a single phase is possible by conjugating polymers with affinity ligand for biomolecule of interest[11].

E. Applications

The following table 1 shows the potential application of ATPS in recovery and purification of various biomolecules

S.No	Biomolecule	ATPS system	% yield	Purification factor	References
1	Myoglobin	PEG 4000-PAA	95.2	-	[2]
2	α-galactosidase	PEG 4000- PO_4 salt	87.71	3.6	[12]
3	Bovine serum Albumin	PEG-1500- Cashew nut gum	-	-	[5]
4	Lipase	PEG 2000/ $(NH_4)_2SO_4$, with 5% Na_2CO_3	99	13.5	[13]
5	Elastase	PEG /KH_2PO_4-K_2HPO_4	75.4	-	[10]
6	Amylase	PEG 1000 / Pot.Phosphate	45.5	5.4	[8]
7	β-1,3-1,4-glucanase	PEG 2000-Magnesium Sulphate	65.3%	-	[14]
8	Papain	PEG–ammonium sulfate	88	-	[4]
9	Polysacharride	K_2HPO_4/ethanol	12.47	2	[15]
10	C-Phycocyanin	PEG-POt.Phosphate	79	4.32	[16]

Table 1: Representative example of different classes of biomolecules extracted using conventional ATPS

ATPS is used in primary stages of purification of recombinant therapeutic proteins expressed in prokaryotic (*E.coli*) and eukaryotic (hybridoma, CHO and transgenic plant cells) expression systems and its proved to be cost effective than conventional membrane filtration[17]. A study comparing the yield of α -galactosidase from *Aspergillus oryzae* using ATPS and conventional ion exchange chromatography showed upto 87% increase in yield of the enzyme by using ATPS[12].

F. Novel Polymers

Novel polymers referred a smart polymers respond to differences in temperature, pH, electric fields and magnetic fields and hence enable better separation of polymers after biomolecule removal[17]. Thermoseparating polymer Ucon 50-HB-5100 (a random copolymer of 50% ethylene oxide (EO) and 50% propylene oxide (PO) is reported in the purification of endo-polygalacturonase. This system has an advantage of easy polymer separation by changing the temperature[18]. As polymer removal is a major barrier in industrial scale up of ATPS, recently Ionic liquids (IL) like Ammoeng110™ is reported to be successfully used in the partition of alcohol dehydrogenase towards IL rich upper phase containing sat in lower phase[19].

G. Ionic liquids in ATPS based extraction

In the conventional ATPS systems involving polymer-polymer or polymer- salt the applicability is limited by the polarities of phase forming compounds. As an alternative, Ionic liquids (ILs) possessing positive, negative and alkyl chain could be manipulated and conditions of extraction can be optimized to obtain better purification. Ionic liquids are molten salts with large organic cation and an

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inorganic/organic anion. Being ionic in nature most ILs are chemically stable with greater solvation ability, insignificant volatility and non-flammability[20]. A study using imidazolium-based ILs as adjuvants to conventional PEG-salt system for purification of lipase from *Bacillus* sp. reported purification factor of 245 [21]. 100% recovery of Bovine serum albumin is reported using phosphonium and ammonium-based ILs [22]. 1-Butyl-3-methylimidazolium tetrafluoroborate based ILs enabled the preferential partitioning of wheat esterase from wheat extracts towards ILs rich phase with a recovery of 88.93% [23].The practical advantage of tailoring the ionic liquids enabled even for the separation of enantiomers of aminoacids from racemic mixture[24]. In a study to understand the driving forces influencing extraction by ionic liquids, electrostatic interaction between surface charged residues of aminoacids and positive charge of cation of ionic liquid is determined to be the most prominent [25]. Optimization of extraction of gallic acid using ATPS involving different salts and ILs revealed that the composition of phase forming compounds influence the pH in salt and IL rich phase and hence the partition coefficient [26]. Studies on influence of temperature exposed the importance of temperature maintenance to ensure maximum extraction efficiency of vanillin [27]. A detailed research on how increase in cation side chain of ILs impact the partitioning towards the IL rich phase is explained using alkaloids as model compounds [28]. A comparative study on the application of extraction of biomolecules using phosphonium-based ILs and imidazolium-based counterparts with similar anions, Phosphonium based ILs are superior in enabling partitioning behavior of biomolecules [29]. The examples of biomolecules extracted using ATPS involving ionic liquids is listed in table 2.

S.No	Biomolecule	ATPS system with ionic liquid	% yield/Partition coefficient (K)	Reference
1	Penicillin G	1-butyl-3-methylimidazolium chloride and NaH_2PO_4	91.5%	[30]
2	Lipase	$[C_8mim]Cl$ and KH_2PO_4/K_2HPO_4	-	[31]
3	Vanillic and syringic acids	PEG and Na_2SO_4 and $[C_4mim]Cl$)	99%	[32]
4	Rubisco	Iolilyte 221 PG and sodium potassium phosphate buffer	K is 3 to 4 times higher than PEG salt system	[33]
5	Roxithromycin in real water samples	1-butyl-3-methylimidazolium tetrafluoraborate, $[Bmim]BF_4$) and Na_2CO_3,	90.7%	[34]
6	Colorants from broth of *Penicillium purpurogenum* DPUA 1275	$[N_{2,2,2,2}]$Br based IL and potassium citrate buffer	K== 24.4 ± 2.3	[35]
7	Flavonoids	Choline amino acids ionic liquid	-	[36]
8	*Panax Ginseng* C. A Saponins	n-alkyl-tropinium and n-alkyl-quinolinium bromide ionic liquids (ILs) + salt	99.5% and K=651	[37]
9	L-phenylalanine	PEG/salt+ IL as adjuvant	K= 4.458	[38]

Table 2: Application of Ionic liquids in extraction of biomolecules

II. CONCLUSION

The review highlighted the key aspects of Aqueous two phase systems in the extraction of biomolecules of different characteristics. The advantages of this method of extraction are discussed with major applications. A detailed mention of role of ionic liquids in the formation of ATPS , the designing ability by altering the chain lengths and their influence in partitioning of biomolecules are briefed. The cost economics comparison for large scale utilization of this method with conventional methods of purification of bio pharamaceuticals has to be studied in future to make large scale application of Aqueous two phase purification feasible.

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REFERENCES

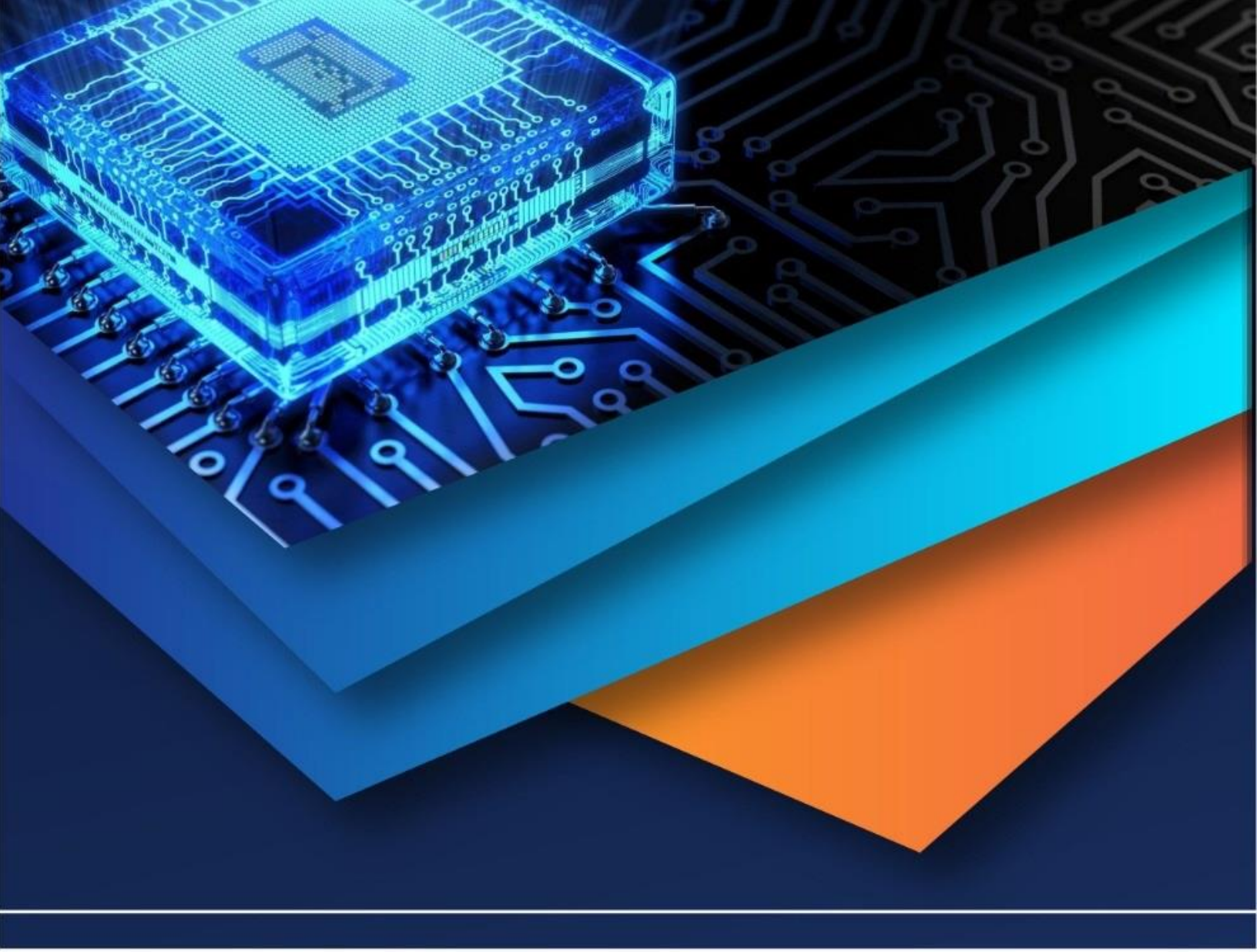
[1] P. Albertsson, “Partition of cell particles and macromolecules in polymer two-phase systems,” Adv. Protein Chem., 1970.
[2] S. Saravanan, J. R. Rao, B. U. Nair, and T. Ramasami, “Aqueous two-phase poly(ethylene glycol)-poly(acrylic acid) system for protein partitioning: Influence of molecular weight, pH and temperature,” Process Biochem., 2008.
[3] K. Selber et al., “Large-scale separation and production of engineered proteins, designed for facilitated recovery in detergent-based aqueous two-phase extraction systems,” Process Biochem., 2004.
[4] S. Nitsawang, R. Hatti-Kaul, and P. Kanasawud, “Purification of papain from Carica papaya latex: Aqueous two-phase extraction versus two-step salt precipitation,” Enzyme Microb. Technol., 2006.
[5] L. A. Sarubbo et al., “New aqueous two-phase system based on cashew-nut tree gum and poly(ethylene glycol),” J. Chromatogr. B Biomed. Sci. Appl., 2000.
[6] F. Mashayekhi, A. S. Meyer, S. A. Shiigi, V. Nguyen, and D. T. Kamei, “Concentration of mammalian genomic DNA using two-phase aqueous micellar systems,” Biotechnol. Bioeng., 2009.
[7] J. A. Asenjo and B. A. Andrews, “Aqueous two-phase systems for protein separation: A perspective,” Journal of Chromatography A. 2011.
[8] R. P. Bezerra, F. K. S. L. Borba, K. A. Moreira, J. L. Lima-Filho, A. L. F. Porto, and A. C. Chaves, “Extraction of amylase from fermentation broth in poly (ethylene glycol) salt aqueous two-phase system,” Brazilian Arch. Biol. Technol., 2006.
[9] G. Patil and K. S. M. S. Raghavarao, “Aqueous two phase extraction for purification of C-phycocyanin,” Biochem. Eng. J., 2007.
[10] Y. Xu, G. He, and J. Li, “ Effective extraction of elastase from Bacillus sp. fermentation broth using aqueous two-phase system ,” J. Zhejiang Univ. Sci., 2005.
[11] Y. Xu, M. A. Souza, M. Z. R. Pontes, M. Vitolo, and A. Pessoa, “Liquid-liquid extraction of enzymes by affinity aqueous two-phase systems,” Brazilian Archives of Biology and Technology. 2003.
[12] K. Naganagouda and V. H. Mulimani, “Aqueous two-phase extraction (ATPE): An attractive and economically viable technology for downstream processing of Aspergillus oryzae α-galactosidase,” Process Biochem., 2008.
[13] M. Anvari, “Extraction of lipase from Rhizopus Microsporus fermentation culture by aqueous two-phase partitioning,” Biotechnol. Biotechnol. Equip., 2015.
[14] G. He, X. Zhang, X. Tang, Q. Chen, and H. Ruan, “Partitioning and purification of extracellular β-1,3-1,4-glucanase in aqueous two-phase systems,” J. Zhejiang Univ. Sci., 2005.
[15] H. Ma et al., “Aqueous two-phase extraction of polysaccharide from Potentilla anserine L.,” Adv. J. Food Sci. Technol., 2015.
[16] S. Chethana, C. A. Nayak, M. C. Madhusudhan, and K. S. M. S. Raghavarao, “Single step aqueous two-phase extraction for downstream processing of C-phycocyanin from Spirulina platensis,” J. Food Sci. Technol., 2015.
[17] A. M. Azevedo, P. A. J. Rosa, I. F. Ferreira, and M. R. Aires-Barros, “Chromatography-free recovery of biopharmaceuticals through aqueous two-phase processing,” Trends in Biotechnology. 2009.
[18] M. Pereira, Y. T. Wu, A. Venâncio, and J. Teixeira, “Aqueous two-phase extraction using thermoseparating polymer: A new system for the separation of endo-polygalacturonase,” Biochem. Eng. J., 2003.
[19] S. Dreyer and U. Kragl, “Ionic liquids for aqueous two-phase extraction and stabilization of enzymes,” Biotechnol. Bioeng., 2008.
[20] K. R. Seddon, “Ionic liquids: A taste of the future,” Nat. Mater., 2003.
[21] R. L. Souza, S. P. M. Ventura, C. M. F. Soares, J. A. P. Coutinho, and Á. S. Lima, “Lipase purification using ionic liquids as adjuvants in aqueous two-phase systems,” Green Chem., 2015.
[22] M. M. Pereira, S. N. Pedro, M. V. Quental, Á. S. Lima, J. A. P. Coutinho, and M. G. Freire, “Enhanced extraction of bovine serum albumin with aqueous biphasic systems of phosphonium- and ammonium-based ionic liquids,” J. Biotechnol., 2015.
[23] B. Jiang, Z. Feng, C. Liu, Y. Xu, D. Li, and G. Ji, “Extraction and purification of wheat-esterase using aqueous two-phase systems of ionic liquid and salt,” J. Food Sci. Technol., 2015.
[24] D. Wu, Y. Zhou, P. Cai, S. Shen, and Y. Pan, “Specific cooperative effect for the enantiomeric separation of amino acids using aqueous two-phase systems with task-specific ionic liquids,” J. Chromatogr. A, 2015.
[25] S. Dreyer, P. Salim, and U. Kragl, “Driving forces of protein partitioning in an ionic liquid-based aqueous two-phase system,” Biochem. Eng. J., 2009.
[26] A. F. M. Cláudio, A. M. Ferreira, C. S. R. Freire, A. J. D. Silvestre, M. G. Freire, and J. A. P. Coutinho, “Optimization of the gallic acid extraction using ionic-liquid-based aqueous two-phase systems,” in Separation and Purification Technology, 2012.
[27] A. F. M. Cláudio, M. G. Freire, C. S. R. Freire, A. J. D. Silvestre, and J. A. P. Coutinho, “Extraction of vanillin using ionic-liquid-based aqueous two-phase systems,” Sep. Purif. Technol., 2010.
[28] H. Passos, M. P. Trindade, T. S. M. Vaz, L. P. Da Costa, M. G. Freire, and J. A. P. Coutinho, “The impact of self-aggregation on the extraction of biomolecules in ionic-liquid-based aqueous two-phase systems,” Sep. Purif. Technol., 2013.
[29] C. L. S. Louros et al., “Extraction of biomolecules using phosphonium-based ionic liquids + K3PO4 aqueous biphasic systems,” Int. J. Mol. Sci., 2010.
[30] Q. Liu et al., “Partitioning behavior of penicillin G in aqueous two phase system formed by ionic liquids and phosphate,” Sep. Sci. Technol., 2006.
[31] S. P. M. Ventura, R. L. F. De Barros, J. M. De Pinho Barbosa, C. M. F. Soares, Á. S. Lima, and J. A. P. Coutinho, “Production and purification of an extracellular lipolytic enzyme using ionic liquid-based aqueous two-phase systems,” Green Chem., 2012.
[32] M. R. Almeida, H. Passos, M. M. Pereira, Á. S. Lima, J. A. P. Coutinho, and M. G. Freire, “Ionic liquids as additives to enhance the extraction of antioxidants in aqueous two-phase systems,” Sep. Purif. Technol., 2014.
[33] R. K. Desai, M. Streefland, R. H. Wijffels, and M. H. M. Eppink, “Extraction and stability of selected proteins in ionic liquid based aqueous two phase systems,” Green Chem., 2014.
[34] C. X. Li, J. Han, Y. Wang, Y. S. Yan, X. H. Xu, and J. M. Pan, “Extraction and mechanism investigation of trace roxithromycin in real water samples by use of ionic liquid-salt aqueous two-phase system,” Anal. Chim. Acta, 2009.
[35] S. P. M. Ventura, V. C. Santos-Ebinuma, J. F. B. Pereira, M. F. S. Teixeira, A. Pessoa, and J. A. P. Coutinho, “Isolation of natural red colorants from fermented broth using ionic liquid-based aqueous two-phase systems,” J. Ind. Microbiol. Biotechnol., 2013.
[36] E. Gómez, P. F. Requejo, E. Tojo, and E. A. Macedo, “Recovery of flavonoids using novel biodegradable choline amino acids ionic liquids based ATPS,” Fluid Phase Equilib., 2019.
[37] A. He, B. Dong, X. Feng, and S. Yao, “Extraction of bioactive ginseng saponins using aqueous two-phase systems of ionic liquids and salts,” Sep. Purif.

©IJRASET: All Rights are Reserved

Technol., 2018.

[38] H. Yang, L. Chen, C. Zhou, X. Yu, A. E. G. A. Yagoub, and H. Ma, "Improving the extraction of L-phenylalanine by the use of ionic liquids as adjuvants in aqueous biphasic systems," Food Chem., 2018.

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