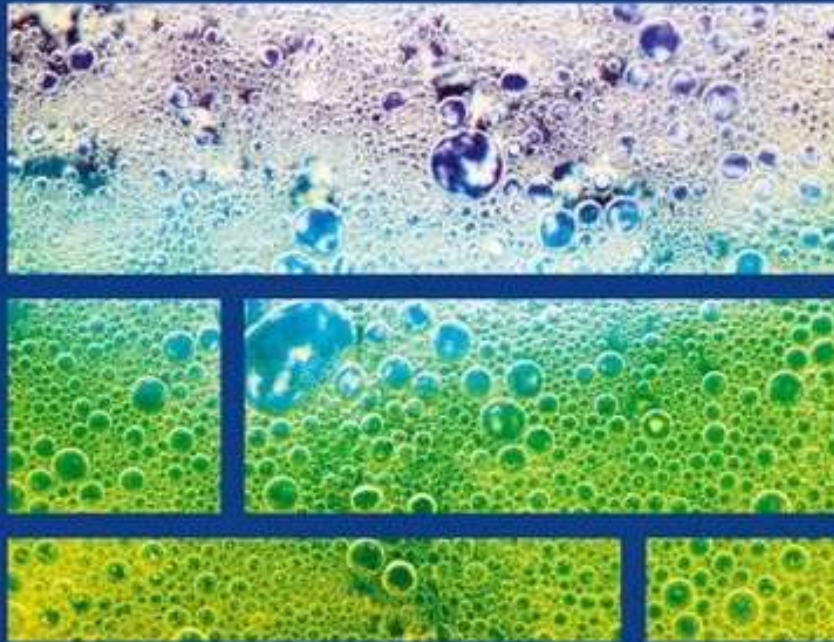


First Edition



# Principles of Multiple-Liquid Separation Systems

Interaction, Application and Advancement

Edited by: Pau Loke Show, Shir Reen Chia and Kit Wayne Chew



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# Contents

<b>Contributors</b>	<b>xi</b>
<b>1. Polymer–polymer interaction</b>	<b>1</b>
<i>Kah Rong Chew, Revathy Sankaran, Kit Wayne Chew and Pau Loke Show</i>	
1.1 Introduction	2
1.2 Phase diagram	3
1.3 Parameters influencing phase diagram	4
1.4 Application of aqueous two-phase system	9
1.5 Genetic materials	14
1.6 Future perspective	15
1.7 Conclusion	15
References	16
<b>2. Polymer–salt interaction</b>	<b>21</b>
<i>Jun Wei Roy Chong, Zatul Iffah Mohd Arshad, Kit Wayne Chew and Pau Loke Show</i>	
2.1 Introduction	21
2.2 Mechanism and working principles	22
2.3 Key process parameters	26
2.4 Applications	30
2.5 Limitation and future challenges	37
2.6 Conclusion	38
References	38
<b>3. Alcohol–salt interaction</b>	<b>45</b>
<i>Hui Yi Leong, Chih-Kai Chang, Krisya Nicole Garcia Aung, Dong-Qiang Lin and Pau Loke Show</i>	
3.1 Introduction	46
3.2 Background and basic principle of alcohol/salt-based liquid biphasic system	46
3.3 Influence of key parameters	48
3.4 Applications of alcohol/salt-based LBS	51
3.5 Limitations and advancements to the alcohol/salt-based liquid biphasic system	56
3.6 Conclusions	58
References	59

<b>4. Sugar-based deep eutectic solvent-aqueous two-phase system</b>	<b>64</b>
<i>Sophie Jing Nee Chai, Xiao-Qian Fu, Dong-Qiang Lin and Pau Loke Show</i>	
4.1 Introduction	64
4.2 Sugar-based deep eutectic solvent	65
4.3 Sugar-based deep eutectic solvent-aqueous two-phase system	67
4.4 Effect of parameters	69
4.5 Application of sugar-based deep eutectic solvent-aqueous two-phase system	72
4.6 Advancement of sugar-based deep eutectic solvent-aqueous two-phase system over the last 5 years	74
4.7 Recycling of sugar-based deep eutectic solvent	75
4.8 Conclusions	76
References	77
<b>5. Ionic liquid–salt interaction</b>	<b>81</b>
<i>Wang Sze Kuan, Malcom S.Y. Tang, Wen Yi Chia and Kit Wayne Chew</i>	
5.1 Introduction	81
5.2 Fundamentals of ionic liquid–salt: thermodynamic and properties	83
5.3 Determination of solution concentration in both phases	85
5.4 Factors that influence the two-phase separation in ionic liquid/salt ATPS	85
5.5 Applications of Ionic liquid/salt ATPS	87
5.6 Conclusion	93
References	93
<b>6. T-butanol–salt three-phase interaction</b>	<b>95</b>
<i>Yan Jer Ng, Yoong Kit Leong, Wen Yi Chia, Kit Wayne Chew and Pau Loke Show</i>	
6.1 Introduction	95
6.2 Process description	96
6.3 Principle of three-phase partitioning	97
6.4 Application of three-phase systems	98
6.5 Future perspectives and challenges	104
6.6 Conclusion	106
References	106
<b>7. Green solvents for multiphase systems</b>	<b>111</b>
<i>Jia Rhen Loo and Wai Yan Cheah</i>	
7.1 Introduction	111
7.2 Green extraction solvents, principles, and reasons for its use	112
7.3 Increase of usage and future trend	125
7.4 Economical factor	128
7.5 Conclusion	128
References	129

---

<b>8. Recyclability and reusability of the solvents</b>	<b>133</b>
<i>Heam Boon Quah, Xuwei Liu, Shir Reen Chia, Saifuddin Nomanbhay and Pau Loke Show</i>	
8.1 Introduction	133
8.2 Solvents for bioseparation	135
8.3 Benefits of recycling solvents	139
8.4 Requirements on solvent recycling	140
8.5 Solvent recycling	142
8.6 Methods for solvent recovery and recycling	143
8.7 Feasibility of solvent recovery process	154
References	155
<b>9. Conventional designs for multiphase liquid separation</b>	<b>171</b>
<i>Apurav Krishna Koyande, Teoh Rui Hong, Kit Wayne Chew and Pau Loke Show</i>	
9.1 Introduction	171
9.2 Principles of three-phase partitioning	172
9.3 Variables that affect TPP	175
9.4 Types of assisted TPP	178
9.5 Applications of TPP	180
9.6 Future prospects	182
9.7 Conclusion	183
References	183
<b>10. Advancement in system designs for multiphase liquid separation</b>	<b>187</b>
<i>Nguyen Minh Duc, Shir Reen Chia, Saifuddin Nomanbhay and Vishno Vardhan Devadas</i>	
10.1 Introduction	187
10.2 Liquid biphasic system	188
10.3 Liquid biphasic flotation	191
10.4 Ultrasound-assisted liquid biphasic system	194
10.5 Magnetic-assisted liquid biphasic system	196
10.6 Electricity-assisted liquid biphasic system	199
10.7 Microwave-assisted liquid biphasic system	201
10.8 Future prospects	202
References	203
<b>11. Economical sustainability of multiphase systems</b>	<b>211</b>
<i>Kien Xiang Bong, Wai Siong Chai and Pau Loke Show</i>	
11.1 Economic sustainability	211
11.2 Advantages of liquid–liquid separation over conventional method	212
11.3 Three-phase interactions	214
11.4 Costing in liquid separation system	214
11.5 Value of end product from biochemical engineering separation	222
11.6 Cost–benefit analysis of ATPS and conventional separation method	224

11.7	ATPS process cost/benefits evaluation—polymer–salt interaction	225
11.8	Conventional protein A affinity chromatography cost/benefits analysis	230
11.9	Conclusion	235
	References	236
<b>12.</b>	<b>Environmental sustainability of multiphase systems</b>	<b>241</b>
	<i>Hock Chee Lu, Sze Shin Low, Shuet Fen Lai and Kuan Shiong Khoo</i>	
12.1	Introduction	241
12.2	Environmental impact caused by conventional extraction method	242
12.3	Nonconventional extraction method	245
12.4	Comparison between alternative extraction methods	250
12.5	Environmental sustainability-related industrial applications	252
12.6	Conclusion	256
	References	256
<b>13.</b>	<b>Potential upscaling of multiphase systems</b>	<b>259</b>
	<i>Jasmine Tiong Sie Ming, Chin Kui Cheng, Shuet Fen Lai, Kit Wayne Chew and Kuan Shiong Khoo</i>	
13.1	Introduction	259
13.2	Chromatography	260
13.3	Membrane	265
13.4	Aqueous two-phase system	270
13.5	Precipitation	280
13.6	Conclusion	283
	References	284
<b>14.</b>	<b>Integrated systems for multiphase development</b>	<b>289</b>
	<i>Kho Wan You, Shir Reen Chia and Saifuddin Nomanbhay</i>	
14.1	Introduction	289
14.2	Ultrasonic-assisted extraction	290
14.3	Microwave-assisted extraction	295
14.4	Enzyme-assisted extraction	303
14.5	Conclusion	309
	References	309
<b>15.</b>	<b>Precursors for promoting liquid–liquid phase separation</b>	<b>317</b>
	<i>Mei Yuen Siau, Shuet Fen Lai, Kuan Shiong Khoo and Pau Loke Show</i>	
15.1	Introduction	317
15.2	Fundamentals of aqueous two-phase systems and its application	318
15.3	Parameters affecting ATPS	321
15.4	Future prospects and challenges of ATPS	325
15.5	Conclusion	327
	References	327

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<b>16. Considerations in designing a multiphase separation system</b>	<b>331</b>
<i>Sridaran Raguraman, Wen Yi Chia, Kit Wayne Chew and Pau Loke Show</i>	
16.1 Introduction	331
16.2 Basis of separation	332
16.3 Considerations for designing multiphase bioseparation system	335
16.4 Conclusion	342
References	342
<b>17. Life-cycle environmental and economical assessment of multiphase systems</b>	<b>345</b>
<i>Zhi Ting Ang, Shuet Fen Lai, Kuan Shiong Khoo and Pau Loke Show</i>	
17.1 Introduction	345
17.2 Introduction of liquid biphasic system/technologies	346
17.3 Application of multiphase systems	348
17.4 Life-cycle assessment of multiphase systems	355
17.5 Case studies	357
17.6 Challenges	370
17.7 Conclusion	372
References	372
<b>Index</b>	<b>375</b>

# Polymer–salt interaction

# 2

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## 2.1 Introduction

There are various conventional liquid–liquid extraction techniques such as Soxhlet extraction, microwave-assisted extraction, supercritical fluid extraction, and solvent extraction for the separation and purification of biomolecules and bioactive compounds. Nevertheless, the above-mentioned methods lead to several drawback limitations, such as the following: (1) solvent extraction uses a large quantity of solvents, long duration time, causes toxicity, and possibility of thermal degradation of bioactive ingredients (Kumar et al., 2017); (2) supercritical fluid extraction has a very complicated system and expensive (Arun et al., 2020); (3) microwave-assisted extraction has complex mass transfer affecting the chance of scaling up (Chan et al., 2015), and (4) Soxhlet extraction methods are time-consuming, causes thermal degradation, less extraction selectivity, costly solvents, and nonenvironment friendly (Arun et al., 2020). Recently, researchers have proposed another liquid–liquid separation method which is the aqueous two-phase system (ATPS). As mentioned by Mastiani et al. (2019), ATPS can be formed by dissolving two incompatible polymers or a polymer and salt such as polyethylene glycol (PEG)/dextran (DEX) and PEG/salt, respectively (Mastiani et al., 2019). Usually, phase separation occurs above the critical concentrations which result in two aqueous phases each enriched with one of the components, producing a polymer-rich, salt-poor top phase, and *vice versa* (Song et al., 2013; Hatti-Kaul, 2000).

ATPS has sparked various attention due to its high potential for separation, extraction, enrichment, and purification of several biomolecules such as membranes, proteins, enzymes, viruses, and nucleic acids (Iqbal et al., 2016). The advancement of ATPS systems can provide low operational cost, low toxicity, large-scale up, good mass transfer, biocompatible, low energy requirement, and able to adhere to the economic and environmental protection (Shaker Shiran et al., 2020; Tang et al., 2014; Yau et al., 2015). The polymer–salt and two polymer ATPS are much more beneficial compared to conventional liquid–liquid fractionation due to some reasons: (1) water is used as the solvent instead of organic solvent which provides a mild environment to prevent denaturation of biomolecules; (2) ATPS is efficient and easy to operate; and (3) scaling



up of equipment based on ATPS is relatively simple and easy (Berlo et al., 1998). Furthermore, polymer–salt ATPS is more favorable than that of polymer–polymer ATPS because salt is less costly as compared to dextran and lower viscosity between phases leading to less time consumption for phase separation (Berlo et al., 1998). There are some drawbacks of polymer–salt ATPS in which incompatible when dealing with high ionic strength environment. In contrast, polymer–polymer ATPSs poses low ionic strength, thus are more suitable for recovery, separation, and purification of solutes sensitive to ionic conditions (Albertsson, 1961).

In most ATPS, PEG is the most commonly used polymer due to cost-efficient, greener, and nonflammable reagent (de Oliveira et al., 2008). The principle behind PEG polymer is as such (1) solubilization in water enables the hydrogen bonding process; (2) the decrease in solubility of polyethylene-oxide due to the addition of monovalent cations; and (3) the cloud point decreases due to the high concentration of salt ions which reduces the amount of free water required for the solubilization of polyethylene (Mazzola et al., 2008). Some of the commonly used polymer–salt ATPSs are PEG-citrate, PEG-phosphate, and PEG-sulfate. Phosphate and sulfate are considered to a lesser degree as compared to citrate, as citrate is more environmental friendly due to nontoxic and biodegradability when discharged into sewage or wastewater treatment plants (de Oliveira et al., 2008).

## 2.2 Mechanism and working principles

The concept of ATPS started back in 1896 in The Netherlands, when a person named Martinus Willem Beijerinck accidentally found and observed that the aqueous starch solution is immiscible in gelatine aqueous solution. Instead, the application of ATPS is discovered by Per-Åke Albertsson when attempting to purify chloroplast, and managed to publish his first studies on the application of ATPS for the purification of biological molecules (Grilo et al., 2014; Iqbal et al., 2016). Firstly, ATPS can be performed by mixing two incompatible aqueous solutions such as one polymer with a type of high concentration salt (Albertsson and Tjerneld, 1994). Unlike conventional liquid–liquid separation using organic solvents, as the term suggests “Aqueous” comprises high concentration of water content (80%–95%) in both phases, forming low interfacial tension systems, a nontoxic and gentle environment for the separation of biomolecules, such as proteins and enzymes, thus less chances of damage to biomolecules and enable polymers to stabilize their structure and biological activities (Albertsson and Tjerneld, 1994; Asenjo and Andrews, 2011; Hatti-Kaul, 2000). The basic graphical process pathway representing the basic working mechanism of a polymer–salt system for the partitioning and purification of desired protein molecules is shown in Fig. 2.1. In contrast to polymer–polymer ATPS, the interaction of physical properties between the two phases of polymer–salt systems has greater differences, thus partitioning of biomolecules is often unequal, favors one of the phases, and extraction process can be carried out at a faster rate. Moreover, molecules with increasing molecular weight such as proteins, nucleic acids, and peptides will tend to be more uneven and sensitive to phase compositions (Johansson, 1994).

## References

Albertsson, P.Å., 1961. Fractionation of particles and macromolecules in aqueous two-phase systems. *Biochem. Pharmacol.* 5, 351–358. [https://doi.org/10.1016/0006-2952\(61\)90028-4](https://doi.org/10.1016/0006-2952(61)90028-4).

Polymer–salt interaction

39

Albertsson, Per-Åke, 1970. Partition of cell particles and macromolecules in polymer two-phase systems. In: Anfinsen, C. B., Edsall, John T., Richards, Frederic M. (Eds.). *Advances in Protein Chemistry*. Advances in Protein Chemistry, 24. Academic Press, pp. 309–341. [https://doi.org/10.1016/s0065-3233\(08\)60244-2](https://doi.org/10.1016/s0065-3233(08)60244-2).

Albertsson, P.-Å., Tjerneld, F., 1994. [1]Phase diagrams. *Methods in Enzymology*. Academic Press, San Diego, pp. 3–13 Vol. 228. [https://doi.org/10.1016/0076-6879\(94\)28003-7](https://doi.org/10.1016/0076-6879(94)28003-7).

Alves, J.G.L.F., Chumpitaz, L.D.A., da Silva, L.H.M., Franco, T.T., Meirelles, A.J.A., 2000. Partitioning of whey proteins, bovine serum albumin and porcine insulin in aqueous two-phase systems. *J. Chromatography B: Biomed. Sci. Applications* 743, 235–239. [https://doi.org/10.1016/S0378-4347\(00\)00111-0](https://doi.org/10.1016/S0378-4347(00)00111-0).

Ananthapadmanabhan, K.P., Goddard, E.D., 1987. Aqueous biphasic formation in polyethylene oxide-inorganic salt systems. *Langmuir* 3, 25–31. <https://doi.org/10.1021/la00073a005>.

Andrews, B.A., Schmidt, A.S., Asenjo, J.A., 2005. Correlation for the partition behavior of proteins in aqueous two-phase systems: effect of surface hydrophobicity and charge. *Biotechnol. Bioeng.* 90, 380–390. <https://doi.org/10.1002/bit.20495>.

Andrews, B.A., Asenjo, J.A., 2010. Theoretical and experimental evaluation of hydrophobicity of proteins to predict their partitioning behavior in aqueous two phase systems: a review. *Sep. Sci. Technol.* 45, 2165–2170. <https://doi.org/10.1080/01496395.2010.507436>.

Arun, K.B., Madhavan, A., Sindhu, R., Binod, P., Pandey, A., R, R., Sirohi, R., 2020. Remodeling agro-industrial and food wastes into value-added bioactives and biopolymers. *Ind. Crops Prod.* 154. [10.1016/j.indcrop.2020.112621](https://doi.org/10.1016/j.indcrop.2020.112621).

Asenjo, J.A., Andrews, B.A., 2011. Aqueous two-phase systems for protein separation: a perspective. *J. Chromatogr. A* 1218, 8826–8835. <https://doi.org/10.1016/j.chroma.2011.06.051>.

Babu, B.R., Rastogi, N.K., Raghavarao, K.S.M.S., 2008. Liquid–liquid extraction of bromelain and polyphenol oxidase using aqueous two-phase system. *Chem. Eng. Process.* 47, 83–89. <https://doi.org/10.1016/j.ccep.2007.08.006>.

Banik, R.M., Santhiagu, A., Kanari, B., Sabarinath, C., Upadhyay, S.N., 2003. Technological aspects of extractive fermentation using aqueous two-phase systems. *World J. Microbiol. Biotechnol.* 19, 337–348. <https://doi.org/10.1023/A:1023940809095>.

Benavides, J., Mena, J.A., Cisneros-Ruiz, M., Ramírez, O.T., Palomares, L.A., Rito-Palomares, M., 2006. Rotavirus-like particles primary recovery from insect cells in aqueous two-phase systems. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 842, 48–57. <https://doi.org/10.1016/j.jchromb.2006.05.006>.

Benavides, J., Rito-Palomares, M., 2008. Practical experiences from the development of aqueous two-phase processes for the recovery of high value biological products. *J. Chem. Technol. Biotechnol.* 83, 133–142. <https://doi.org/10.1002/jctb.1844>.

Berlo, M., Luyben, K.Ch.A.M., Wielen, L.A.M., 1998. Poly(ethylene glycol)–salt aqueous two-phase systems with easily. *J. Chromatogr. B* 711, 61–68.

Beyene, T., 2015. Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. *J. Veterinary Sci. Technol.* 7, 1–7.

Carlson, A., 1988. Factors Influencing the use of aqueous two-phase partition for protein purification. *Sep. Sci. Technol.* 23, 785–817. <https://doi.org/10.1080/01496398808063140>.

Cascone, O., Andrews, B.A., Asenjo, J.A., 1991. Partitioning and purification of thaumatin in aqueous two-phase systems. *Enzyme Microb. Technol.* 13, 629–635. [https://doi.org/10.1016/0141-0229\(91\)90076-M](https://doi.org/10.1016/0141-0229(91)90076-M).

Chan, C.-H., Yusoff, R., Ngoh, G.-C., 2015. Assessment of scale-up parameters of microwave-assisted extraction via the extraction of flavonoids from cocoa leaves. *Chem. Eng. Technol.* 38, 489–496. <https://doi.org/10.1002/ceat.201400459>.