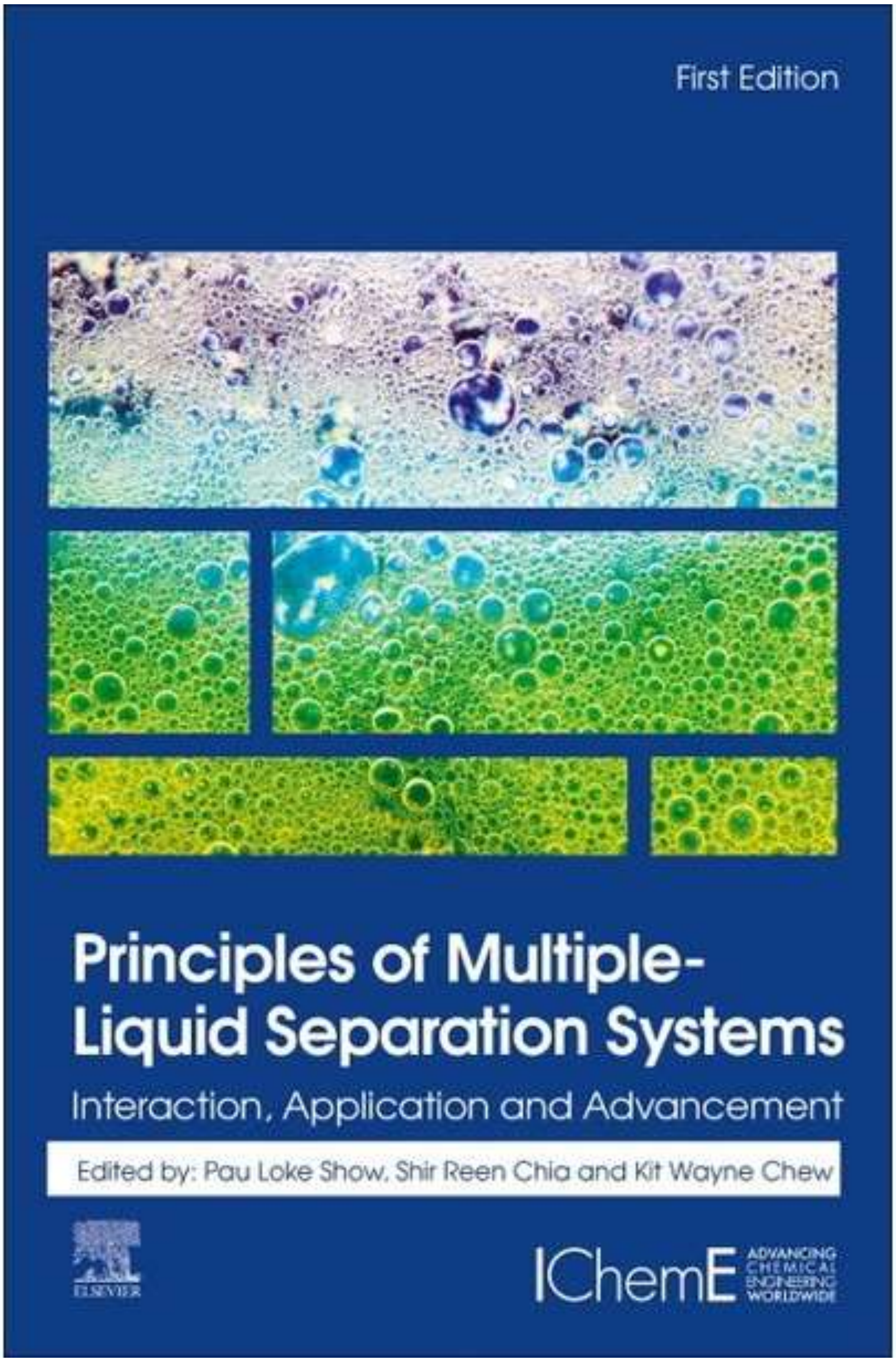

First Edition

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

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Polymer–salt interaction

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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

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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 noninflammable 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 accidently 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).

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