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Editorial:

Chromatographic Separation of Biomolecules Using Polymer/Surfactant Aqueous Two Phase System

Faiyaz Shakeel[1,2*], Nazrul Haq[1,2]

[1]*Center of Excellence in Biotechnology Research, King Saud University, Saudi Arabia.*

[2]*Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.*

The main drawbacks of 3-step tedious and cumbersome downstream processing of biomolecules includes complex procedural steps, production of excessive amount of by-products, huge amount of energy consumption and inefficient with respect to purity, yield and selectivity.[1] In order to overcome the limitations of downstream processing, aqueous two-phase systems (ATPS) have been applied as alternative, competent and environmentally benign approach (free of toxic organic solvents) for the separation and refinement of biomolecules from varying matrixes.[2] ATPS method has the inherent properties such as versatility, highly productive, economical, better purity profile, selectivity and rapid mass transfer rates.[3] It has achieved the position of leading technique in the last decade for separation and purification of enzymes[4], drugs[5], antioxidants[6], amino acids[7] and antibiotics.[8] These systems are usually formed by chemical entities that segregate into two different phases when mixed in specific ranges of composition and temperature e.g. by either mixing two polymers in aqueous media[9] or by a polymer with an inorganic salt[10] or a polymer with an organic salt.[11] Some other cheap polymers have also been explored in combination with polyethylene glycols (PEGs) e.g. polyethylenimine, maleic acid copolymer and poly(acrylic acid).[12-14] An interesting feature of poly(acrylic acid)-PEGs aqueous system was observed that if the system was amended with suitable amount of salt, a biphasic polymer–polymer system is formed at pH 5.0 in order to expedite the separation of highly polar polyelectrolytes in the phases.[15] With this ATPS, a comparatively quick separation can be achieved and also this ATPS has comparatively low viscosity with respect to other conventional ATPS.[16] These conventional ATPS (consisting of a polymer and an inorganic salt) has got an inherent disadvantage that polymer shows restricted solubility in systems containing salts as inorganic salts facilitates protein aggregation.[17] In order to overcome this limitation, potential of different class of chemicals as a component has been explored for the preparation of some alternative ATPS, for example salt in combination of short chain alcohol has been tested for the heat sensitive compounds from crude extract.[18] This alcohol-salt two-phase systems have an alcohol rich upper phase and a salt-rich lower phase, that possess the advantages of higher polarity compared with conventional organic–aqueous solvent system and lower viscosity compared with aqueous polymer two-phase systems, as well as the relatively low environmental toxicity. Clearly hydrophilic organic salt containing aqueous two-phase system has potential for use in countercurrent chromatography (CCC). This novel alcohol with salt ATPS possesses several advantageous features as compared to conventional polymer in combination with polymer or polymer in combination with salt systems like being economical, less viscosity and facile recovery of alcohol facilitated by evaporation.[19] With a view point of improving the economical as well as environmental friendly nature of the existing ATPS, Kumar et al suggested employing hyperbranched polymers (possessing special functional groups, having zero denaturation effect on biomolecules, excellent biocompatibility, high thermal/chemical stablity in addition to the lower viscosity in molten as well as in solutions)[20] as one of the component of ATPS because their presence in the fermentation broth doesn't have any deleterious effect on micro-organisms.[21] As a result of which these hyperbranched polymers can be easily recovered by thermally induced phase separation. Ethylene oxide (EO) and propylene oxide (PO) based thermo-separating copolymers in association with dextran for the formation of ATPS were explored for the first time in 1990s.[22] Gutowski et al in 2003 for the first time prepared ATPS in which ionic liquids (ILs) were as one of the components along with inorganic salts.[23] The driving force behind the use of ILs as a constituent of ATPS lies in their unique physicochemical properties such as low volatility, non-flammability, excellent thermochemical stability, good solubility in water, high solvency power for both organic and inorganic compounds and easy to prepare ILs of desired properties just by selection of salts.[24] Since then, many different class of solvents were tested for their ability to form ILATPSs with ILs such as IL + inorganic salt, IL + PEG and IL + carbohydrate.[23,25,26] ILATPSs have many unique properties common to both ILs and ATPSs such as less tendency to form emulsion, lower viscosity, use of volatile organic solvent eliminated, rapid phase separation, excellent separation capability and environmentally benign nature. ILs with inorganic

Dr. Faiyaz Shakeel, Center of Excellence in Biotechnology Research, King Saud University, Riyadh 11451, Saudi Arabia, Tel: +966-537507318, Email: faiyazs@fastmail.fm

salts were earlier used very frequently due to the tendency of inorganic salts to show salting-out effects. But elevated amount of inorganic salts is detrimental due to environment. Therefore, nowadays scientists focused on environmental friendly and biodegradable organic salts, such as the citrate, tartrate, acetate, carbohydrate and amino acid to be used for the preparation of IL-based ATPSs.[26-28]

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