Characterization of Aqueous Two-Phase Systems and Their Potential New Applications

2

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Abbreviations

ADSA	Axisymmetric drop shape analysis
ATPS	Aqueous two-phase system
DARA	Distribution analysis of radiolabeled analytes
HPLC	High-performance liquid chromatography
HTS	High throughput screening
K_P	Partition coefficient
LHS	Liquid-handling stations
PEG	Polyethylene glycol
TLL	Tie-line length
V_R	Volume ratio

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2.1 Introduction

Successful implementation of aqueous two-phase system (ATPS) strategies requires a complete understanding of the different physical and chemical phenomena happening within a particular system. This said, the design of ATPS involves the correct selection of parameters such as phase-forming chemicals (i.e., polymers, salts, ionic liquids, alcohols, etc.), pH, tie-line length (TLL), and phase volume ratio (V_R) for the particular product or products being recovered (Rito-Palomares 2004). The mixed interactions of these parameters upon the physicochemical characteristics of the products being fractionated will cause the molecules of interest to partition to either of the phases either by concentration or by separation from the rest of the contaminants in the mixtures (González-Valdez et al. 2013).

In most cases, the appropriate selection of system design parameters requires a thorough experimentation of all these variables since, to date, models fail to predict the behavior of solutes (especially in mixtures) in ATPS (Mistry et al. 1996). This is particularly important because, being a primary recovery operation, ATPS are usually involved in the separation of a particular set of products from streams carrying mixtures of contaminants that must be eliminated (Rito-Palomares 2004). More importantly, because of its characteristics, a well-designed ATPS operation is usually regarded as a viable option for substituting other more complicated or time-consuming procedures including chromatography (Mayolo-Deloisa et al. 2011). With this in mind, the engineering design of a biphasic separation should be optimal, and in doing so, ATPS should be completely characterized and understood from an intrinsic (i.e., the physicochemical interactions affecting it) and operational point of view. This chapter aims to present in a logical manner the different procedures to characterize the physicochemical properties of an ATPS and the solutes that are partitioned within them and to present novel and potential new uses where ATPS strategies could be successfully implemented.

2.2 Aqueous Two-Phase System Characterization

The first step in implementing an ATPS operation involves the selection of the phase-forming components to be used in the system. These chemicals can be broadly categorized into six main groups: polymers, salts, alcohols, ionic liquids, micelle-forming agents, and low molecular weight solvents (Benavides et al. 2011). The selection and use of at least one of the chemicals in a group with at least one of another group (or the same group in polymer-polymer and ionic liquid systems) above a critical concentration generate a biphasic system with two immiscible phases that may be used for extraction and fractionation of different products (González-Valdez et al. 2013). Each one of these types of ATPS formed (i.e., polymer-polymer, polymer-salt, alcohol-polymer, alcohol-salt, and ionic liquid-based and micellar systems) is mainly used for the recovery and fractionation of specific kinds of molecules depending on the properties they possess as it has been extensively described in literature (Diamond and Hsu 1992; Rosa et al. 2010).

The most common ATPS are generated with the use of two incompatible polymers or one polymer and a salt. For many of these cases, there is published equilibrium data (i.e., binodal curves) that might be used to then choose the concentration of the phase-forming chemicals in each of the phases (Zaslavsky 1995). However, this data is only available for commonly used systems and should be generated for particular operations which is becoming a common practice since the use of novel phase-forming chemicals such as "smart" polymers, new ionic liquids, or affinity ligands for product fractionation is gaining momentum (Leong et al. 2016; Montalvo-Hernández et al. 2012). Furthermore, published equilibrium data should only be used as a reference since in most cases, experimental errors, variations in the molecular weights of the species, poor temperature control, and uncontrolled or unreported addition of additives are rarely reported, and all of these aspects might cause variations in the position of the binodal curve (Forciniti 2000).

Therefore, the generation of binodal curves and phase diagrams is one of the first steps to be made toward the characterization of ATPS. On their part, phase diagrams are unique to each combination of phase-forming chemicals and additives under specific pH and temperature conditions. These diagrams are delineated by their corresponding binodal curve, under which no phase formation can be achieved and above which all the different operation conditions are found. Coordinates for all of these possible systems will then give the information needed for the total composition of the system and will lie on a specific tie-line length (TLL) that denotes the composition of the phase-forming chemicals in both the top and bottom phases (Kaul 2000). Even more, the specific "working coordinates" for that particular system will also indicate according to its position along the tie-line and the volume ratio (V_R) of the top and bottom phases of the system which has also a particular influence in product partition (Diamond and Hsu 1989).

Generation of binodal curves can be achieved by different experimental approaches. For instance, in turbidimetric titration, a series of mixed stock solutions of phase-forming chemicals in known concentrations is prepared and then diluted with an appropriate solvent. If, when agitated, these mixtures become visually turbid, a clear indication of being "above" the binodal curve is given. Then, solvent addition is made in a dropwise manner until the mixture becomes clear indicating that the mixture has reached the binodal curve. The final concentrations of the phase-forming chemicals at that point will then indicate one of the coordinates of the binodal curve that can then be completed with the other points obtained from the series of mixed stock solutions (de Oliveira et al. 2008). In a similar manner, in the cloud point method, a concentrated stock solution of one of the ATPS components is added dropwise to a known amount of a concentrated solution of the other component. At a certain point, the mixture will become turbid indicating the chemical compositions that lay on the binodal curve which again can be extrapolated with a series of similar experiments varying the known amounts of the second component (Kaul 2000). Within this context, a novel approach involving the use of microdevices for the characterization of binodal curves is starting to show promising results since the appearance of two distinct phases can be controlled by tuning the volumetric flow of the phase components. By knowing the concentrations, densities, and

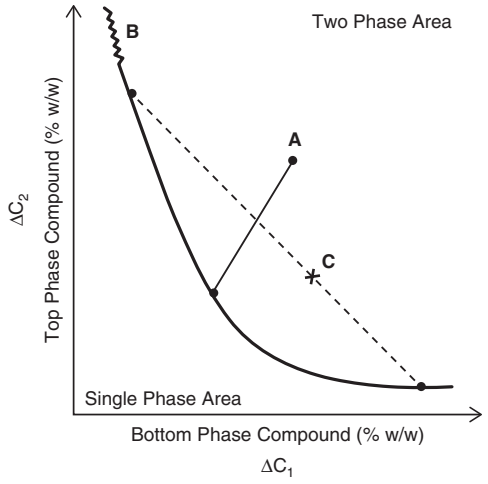

Fig. 2.1 Schematic representation of the different methodologies available for binodal curve characterization. (*A*) Shows the procedure of turbidimetric titration where a series of known two-phase points are titrated until one single phase is observed indicating the point where the curve lies. (*B*) Presents the cloud point method procedure (indicated by the *zigzag line*) where stocks of either system components are mixed and taking the solution above and below the cloud point locating the place where the cure lies. (*C*) Illustrates the determination of nodes, where the composition of each of the phase-forming components is calculated for each of the phases and graphed to determine the binodal curve. This also helps with determining the tie-line length composition of that particular system

flows of the said stock phase-forming solution, the visual formation of two distinct phases in the microdevice can serve as an indicator to calculate the concentrations laying in the binodal curve (Vazquez-Villegas et al. 2016). Finally, in another more traditional method, calculation of the binodal curve can also be achieved by determining the nodes in a series of systems by analysis of the amounts of phase-forming chemicals in each of the generated phases providing the different points in the binodal. In fact, this is also helpful in calculating the different TLL values in the diagram where TLL = $(\Delta X^2 + \Delta Y^2)^{1/2}$. In this case, ΔX and ΔY represent the difference between the compositions (in % w/w) between the top and bottom phases of each of the phase-forming chemicals. Other TLL values can then be calculated by extrapolating the slopes of these lines since in most cases tie-lines are parallel to each other (Kaul 2000). Figure 2.1 presents a schematic representation of the use of the different methodologies available to generate binodal curves.

Understanding the physicochemical parameters that will affect product partition and being able to measure them provide the means of developing better ATPS strategies and have a better quality control in industrial processes. As noted in Fig. 2.2, several of these parameters may have an important influence in solute partition behavior. For instance, interfacial tension provides a significantly more sensitive measurement of the effective concentration of a polymer solution than does the determination of TLL or the phase diagram which is important in repetitive batch

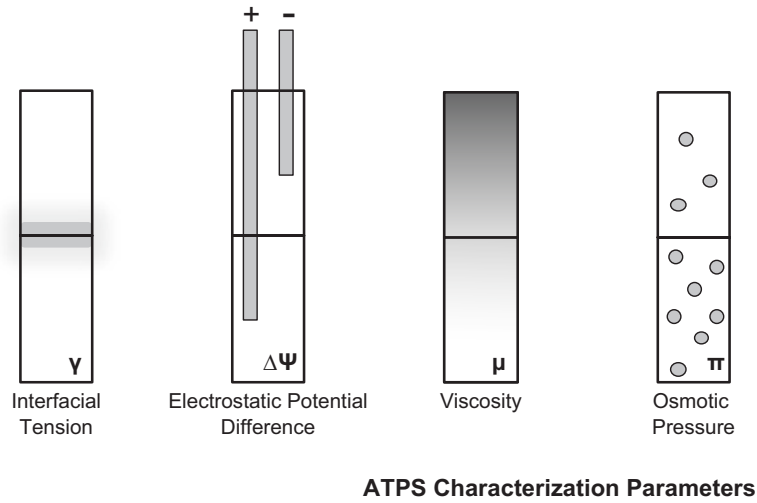

Fig. 2.2 Important parameters for characterization of the intrinsic properties of aqueous two-phase systems. Interfacial tension (γ) can be estimated by the rotating drop method or the sessile drop method. Electrostatic potential difference ($\Delta\Psi$) is acquired with the use of reversible electrodes connected to salt bridges. Viscosity (μ), on its part, is simply estimated with the use of viscometers and/or pycnometers. Finally, osmotic pressure (π) can be obtained with the use of a vapor pressure or membrane osmometer

operations where polymer batches are changed frequently (Brooks and Jones 2000). To calculate interfacial tension in the typical low tension values observed in ATPS, two methods are recommended. First is the rotating drop method that considers that when a fluid drop (top phase) is placed in a liquid of higher density (bottom phase) contained in a rotating horizontal tube, its elongation along the axis will continue until the deformation forces due to the centrifugal field are balanced by the interfacial tension. Using the proper equipment and a series of equations, the interfacial tension can then be calculated (Princen et al. 1967; Walter 1994). In the other recommended method (i.e., the sessile drop method), the contact angle between the phase interface and the solid support can also be calculated besides interfacial tension. This is important because this contact angle might be closely related to the partition behavior of small suspended solids (Brooks and Jones 2000). In this method, a sufficiently large droplet of a phase solution is deposited over a surface and becomes deformed due to the gravitation force action. The surface area of the sphere is proportional to its squared radius, and the gravitational deformation depends on its volume which is proportional to the radius raised to the third power. If the effects of surface gravitational forces are comparable, then the interfacial tension (or in this particular case surface tension) of the phase can be calculated from the droplet shape (Staicopolus 1962). Modern computer program analyses of the droplet shapes have proven successful in the calculation of this parameter. In fact, recent developments in this area have allowed the use of entropic edge detector

schemes to enable edge detection of drops of ternary liquid systems with ultralow interfacial tensions. For instance, axisymmetric drop shape analysis (ADSA), a well-established methodology for interfacial tension measurements in conventional fluid-liquid systems, has given comparable results within an uncertainty of 0.001 mJ/m^2 with the sessile drop technique (Atefi et al. 2014).

The presence of some salts makes ATPS behave as if there was an electrostatic potential difference ($\Delta\Psi$) between them. This potential is driven by the unequal partition of some ion species because of the partial exclusion from the polymer-rich phase and is supported by the partitioning behavior of charged macromolecules in ATPS (Walter 1994). In this sense, potentials in biphasic systems are only a few millivolts at the most, but since most interaction energies in these systems are small, these electrostatic potentials can be able to dominate all partitioning behavior. Hence, measurements of this parameter can be crucial in the understanding of partition mechanisms. However, this is not a crucial task since potential measurements may lay on the detection limits of the equipment being used and do not provide thermodynamic potential difference between the phases due probably to the presence of liquid junction potentials at the tips of the salt bridges used for this purpose (Brooks and Jones 2000). In electrostatic potential measurements, reversible electrodes are connected by salt bridges to the two phases of the systems, one electrode is then slowly moved through the interface into the bottom phase, and the change in voltage is noted in time. The differences between the readings from the time when both bridges are immersed in one phase while one of the capillaries is slowly moved into the other phase provide an estimate for $\Delta\Psi$ (Walter 1994). It is important to mention, however, that this procedure needs to be repeated at least ten times for reliable estimations. Furthermore, if the change of potential across the interface is of interest, the measurement of electrophoretic mobilities of drops of one phase to another could provide this information. Nonetheless, the interpretation of these mobilities remains uncertain and requires further investigations, but it is believed that the potential reverses sign at the phase boundary implying the presence of a potential well on each side of the interface that could accumulate material with a net charge (Brooks and Jones 2000). This provides further information on the usual observation of product accumulation between both phases besides the effects of reduction of free volume and free energy that also contribute to this phenomenon.

All of the biomolecule transport processes in an ATPS and other aspects such as phase separation rate, mixture, centrifugation, and pumping energy (especially in large-scale operations) heavily depend on viscosity. Phase viscosity on its part depends on the phase-forming chemicals being used and the location of the system in the phase diagram. In this manner, concentration and polymer molecular weight are proportional to the viscosities obtained. In the particular case of polymer-polymer systems, viscosity measurements become complicated when a big difference in the molecular weight of the polymers being used exists. In these cases, the viscosity of the low molecular weight polymer phase remains practically constant, while the viscosity of the other phase (i.e., the high polymer molecular weight phase) strongly increases with TLL increments (Walter 1994). Despite this, viscosity measurements in ATPS are straightforward, inexpensive, and sensitive

procedures since they can be done with a simple capillary viscometer, a controlled temperature bath, an electronic timer, and a pycnometer (Brooks and Jones 2000).

Finally, when partitioning cells, organelles, or liposomes, the characterization of the osmotic pressure in the system is also recommended. In ATPS, buffer salts are the phase-forming chemicals that most contribute to this parameter, while the contribution of polymers is almost null (Walter 1994). Therefore, the low molecular weight components in the system should be isotonic with the products being partitioned. When necessary, osmotic pressure can be measured with the use of a vapor pressure or membrane osmometer. Membrane osmometers in the context of ATPS are used by placing a pre-equilibrated phase sample separated from pure water by a semipermeable membrane. The phase is not able to cross this membrane but water flow across it to dilute the phase. The pressure required to stop this water flow corresponds to the osmotic pressure. In the procedure involving vapor pressure osmometers, the concentration of osmotically active particles that reduce the vapor pressure of a particular ATPS phase is calculated, but this procedure is not regularly used.

As it can be seen, the successful implementation of an ATPS operation is heavily dependent on developing or adapting a phase diagram all the way to the measurement of the different properties of the system. None of these tasks are trivial, but their understanding and study can represent a great advantage in processing yields and reproducibility. These aspects are of great importance particularly in large-scale operations where numerous runs have to be made with a robust and reliable operation which to some point has been one of the main drawbacks in finding a larger amount of adapted ATPS processes in the biotechnological industry.

2.3 Solute Partition Characterization

As it has been stated in the previous section, partition of the different biotechnological products has a dependence on both the system characteristics and the physicochemical properties of the molecules being recovered in a rather complex thermodynamic scenario. However, to study this behavior, a simple description of the partition coefficient of the molecule (K_P), defined as the relation of the concentration of a particular product between the top and bottom phases of the system, is regularly used (Rito-Palomares and Lyddiatt 2002). The molecules involved in an ATPS operation interact with the phase-forming chemicals with ionic and hydrophobic interactions, hydrogen bonding, van der Waals forces, and noncovalent interactions and can also interact with themselves when high solute concentrations are being used (Benavides et al. 2011). The net effect of these interactions will then cause a selective partition of the molecule to either of the phases depending on the most favorable energy state that can be reached in each of the cases.

The three most influential solute physicochemical properties that influence partition in ATPS are size, electrochemical charge, and hydrophobic character (Olivera-Nappa et al. 2004). In the ATPS context, phase-forming chemicals are usually larger than the solutes, subjecting these molecules to steric effects and changing the available volume for them to partition toward a particular phase in a phenomenon

generally known as free volume effect. For the cases where some of the system materials are ionizable, electrochemical partition effects play an important role in the operation. Therefore, pH control in the system becomes crucial to maximize these types of interactions between ionizable solutes like proteins and the phase-forming components by generating charges that may provoke a selective fractionation. Finally, the hydrophobic interactions that play a role in solute partitioning in ATPS can be categorized in two well-known effects: phase hydrophobicity and the salting-out effect. Both effects generate hydrophobic interactions that promote solutes with lower amphipathicity and hydrophobicity to partition toward the most hydrophobic phase (Andrews et al. 2005; Schmidt et al. 1996). Furthermore, it should also be mentioned that addition of affinity ligands to ATPS is another exploitable strategy to promote ad libitum solute partitioning. In this case, ligands whose partition behavior in ATPS is already known and that present a certain biological activity or affinity toward a specific molecule are included in the system to increase the selectivity of the biphasic system (Ruiz-Ruiz et al. 2012).

With these interactions in mind, once the systems under study have reached equilibrium, the measurement of the concentrations of the solutes in the top and bottom phases of the system and in many cases that of the accumulated mass in the interface is required. As mentioned, this measurement allows the quantification of the partition coefficients of the molecules and allows a final characterization of the systems. To do so, the selection of appropriate analytical techniques should be considered. Commonly, this can be done spectrophotometrically either with the use of a calibration curve prepared at the maximum absorbance of the solutes under study or with specific colorimetric assays for that particular solute. In the case of systems where the product of interest can be visually observed with the aid of a microscope, like cells, or nanoparticles, concentration can be estimated in this way. More advanced analytical techniques such as high-performance liquid chromatography (HPLC), infrared, or mass spectroscopy, to mention some examples, can also be used depending on the level of accuracy needed in each case. However, it should always be taken into consideration that the phase constituents may interact with the selected analytical technique and appropriate blanks or precautions should always be used in order to obtain a reliable measurement (González-Valdez et al. 2011). Also, it should be remembered that the partition behavior of molecules in ATPS is difficult to model and that empirical approaches are usually used (Benavides et al. 2011). This can sometimes become a tedious task mainly because of all of the parameters that need to be evaluated in each of the partition procedures and the increasing degrees of freedom that each one of the possibilities represents within the system. In this context, high throughput characterization of solute partitioning is becoming an important tool that aids in the acceleration of finding the best conditions to acquire a particular behavior of a specific set of molecules.

The use of high throughput screening (HTS) platforms has gained momentum in the biotechnological industry during the last decade (Amrhein et al. 2014). This has allowed the development of highly automated and optimized processes with advantages that mainly include the reduction of operation materials, sample, and experimental efforts with more accurate analytics. These platforms are already being used in upstream operations such as the screening of strain mutants, substrates, and

microbial cultivation processes but are less used in downstream operations where most advances have been made in chromatographic operations. Nonetheless, this does not mean that ATPS have not been subject to optimization efforts using these kinds of technologies. In doing so, liquid handling stations (LHS) have provided the means to prepare, characterize, and optimize ATPS (Bensch et al. 2007). With this technology, binodal curves and tie-lines can be characterized without previous knowledge of the system behaviors. Binodal curves are obtained with dyes that are dissolved in the system components and by later correlating the top phase volume to the concentrating factor of the dye. Tie-lines are calculated by applying the lever arm rule on the phase volume, and the effect of density differences may be calculated with the use of lab-on-a-chip technologies (Amrhein et al. 2014). But furthermore, solute partition coefficients and yields can be quickly obtained with coupled sensors and measurement equipment compatible to robotic systems like turbidity measurements for the determination of cell debris and ELISA activity assays for the characterization of specific proteins (Bensch et al. 2007). The current capabilities of HTS in the context of ATPS allow the characterization of between 600 and 1000 phase systems per day. Problems and error sources such as liquid handling inaccuracies, top and bottom phase sampling, and meniscus forming because of the phase compositions that influence measurements are being addressed with the use of other integrated chip technologies within the HTS operations. It is important to mention that HTS procedures are in most manners compatible with traditional techniques used in ATPS solute characterization and can be also adapted to include more novel approaches such as distribution analysis of radiolabeled analytes (DARA) with rapid reversed phase chromatography analysis that aid with decision-making with regard to controlled conversion of monophasic systems into bi- or triphasic ones because of the addition of biomass and the addition of phase-forming chemicals to fine-tune a separation, variations between batches, and ATPS operation with phase-recycling (Lebreton et al. 2002). With this, the implementation of HTS methods in ATPS will certainly allow the coping with industrial relevant issues such as time to market demands, material consumption, cost efficiency, and process robustness according to quality-by-design requirements which are often some of the limitations that ATPS face before its industrial implementation. In this context, Fig. 2.3 presents the different considerations to have for the characterization of solute partitioning in ATPS.

2.4 Novel Operational Strategies and Potential New Applications for Aqueous Two-Phase Systems

Being a highly biocompatible operation, traditional ATPS strategies involve mainly the primary recovery and purification of proteins, nucleic acids, virus, viruslike and other bionanoparticles, cells, organelles, and low molecular weight compounds, and in this matter, extensive reviews and scientific publications can be found on this subject (Benavides and Rito-Palomares 2008). But furthermore, ATPS are starting to be implemented in novel strategies and in different manners that are extending their use while opening new application possibilities.

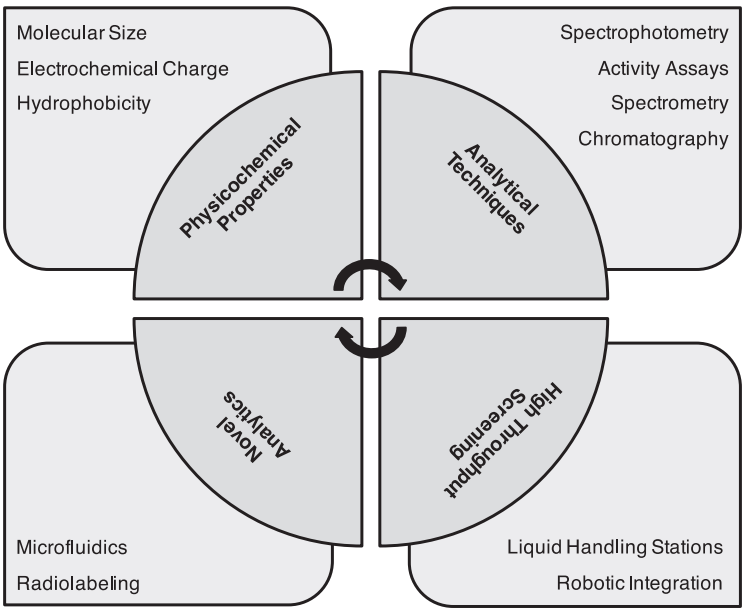

Fig. 2.3 Considerations for solute partition characterization. The diagram presents the different aspects to be taken into account to establish the partition behavior of the different solutes loaded in an aqueous two-phase system. These include the physicochemical properties of both the solutes and the phase-forming chemicals, the appropriate common and novel analytical techniques for concentration measurements, and the possibility of using high throughput screening techniques for this purpose

One of the new strategies being implemented with ATPS involves unit operation integration. In this manner, ATPS offer the possibility of serving as an extraction and primary recovery operation with the implementation of cell disruption within the system in a single operation. With this strategy, after culture and centrifugation, cells can be transferred to the system where cell disruption can be achieved by mechanical or chemical methods liberating the products and offering a partition environment where products will partition toward a specific phase while cell debris and other molecules preferably partition toward the other (Rito-Palomares and Lyddiatt 2002). Even more, going back a few stages, ATPS offer as well the possibility of serving as a reactor or fermenter and a recovery operation. In these approaches, ATPS can theoretically be designed to be formed by a "culture medium" phase and a polymeric one where, after inoculation, cells grow in one of the phases and liberate the products toward the other phase. This is achievable especially by those organisms that grow under saline conditions and where products may represent a growth inhibitor since once in the system products can be continuously released to the opposite phase. Enzymatic reactions can

also be accommodated in specific ATPS with an appropriate knowledge of the partition behaviors of the substrates, products, and enzymes. Furthermore, certain chemical reactions can also be achieved in biphasic systems in a similar fashion (Andersson and Hahn-Hägerdal 1990).

The use of "intelligent" or "smart" polymers capable of responding to external physical stimuli by changing some of their mechanical properties is a current matter of interest in ATPS development. The use of the said polymers is allowing the appearance of novel systems that besides presenting interesting partition and recovery yields for many products allow the simplification of the operations needed to later remove the phase-forming components from the processing streams. For example, thermosensitive polymers are being used in the generation of ATPS for the recovery of proteins. After phase separation, the proteins can be removed from the polymeric solution by allowing the precipitation of the polymer with the addition of heat. Besides interchanging a rather expensive operation like ultrafiltration for the removal of the polymers for a much simpler one where only heat is applied, this procedure allows the recovery of the polymer that was used that can later be recycled back into the ATPS operation (Lladosa et al. 2012).

Another novel approach in ATPS usage refers to the refolding of proteins. Usually, recombinant molecules expressed in certain microorganisms are obtained in insoluble forms or inclusion bodies. Recent studies have shown that ATPS possess an interesting potential in becoming a protein refolding operation, specifically because of their high-water content that favors the reduction of chaotropic agents used in the solubilization of these molecules and because polymers like polyethylene glycol (PEG) usually used in ATPS operations can function as chaperone molecules that promote the correct refolding of the proteins in solution. In the same line, refolding operations can be optimized with the addition of additives that depending on the protein can enormously enhance its correct refolding like is the case for the addition of metal ions to ATPS to refold denatured metalloenzymes (Sánchez-Trasviña et al. 2015). In fact, the advantages of ATPS usage in protein refolding have shown multiple advantages over more traditional refolding procedures involving the use of packed columns (Kuboi et al. 2000).

The use of microfluidic devices in ATPS operations is also a major trend in the area. The advantages presented by these devices like their operation in laminar flow, portability, and low solution usage are starting to become an interesting opportunity for product recovery especially in those systems where the product of interest is in very low concentrations (Munchow et al. 2007; Vázquez-Villegas et al. 2013, 2016). As mentioned, microfluidic devices can also be exploited for ATPS characterization purposes making this approach versatile in the sense in which it can be used as both a processing and an analytical technique. In fact, the use of microdevices in ATPS operations is allowing the characterization of ATPS operation in continuous mode in contrast to the traditional batch operation. In fact, ATPS operating in continuous mode are now operational up to a pilot scale with comparable results to those observed in batch mode operations (Vázquez-Villegas et al. 2015).

As an analytical tool, ATPS provide the means of obtaining and measuring some of the physicochemical properties of the molecules, particles, or cells being partitioned. The correlations between their partition coefficients and the known properties of the systems with the superficial physicochemical properties of the solutes allow the calculation of their superficial properties and hydrophobicity in a simple manner that can later be of utility in sorting and other separation procedures (Andrews et al. 2005; Trindade et al. 2005). In the same line, ATPS are being used as in three-dimensional proteomic analysis in combination with 2D electrophoresis where crude protein extracts can be characterized according to their molecular weight, isoelectric point, and partition coefficient that serves as an indicator of hydrophobicity (Aguilar et al. 2009).

As seen, the uses of ATPS go much further than their traditional application in the primary recovery of biotechnological products. Novel ATPS are constantly being developed for more and more particular applications with the use of novel components or techniques both for operational and analytical purposes. ATPS is a versatile and robust technique that can be used in a macro- or a microscale with different advantages for specific operations and available also for its use in batch and continuous processes. This technique that has been constantly used during more than half a century continues to be of importance in the research and industrial biotechnological fields. Figure 2.4 presents a graphical summary of the novel operational strategies and applications for ATPS presented in this section.

2.5 Concluding Remarks

The successful implementation of ATPS strategies requires a careful consideration of many designs and physicochemical aspects to meet the required purification and recovery standards needed in the operation. The characterization of ATPS starts with the appropriate selection of the phase-forming chemicals, the generation of the binodal curve, and all of the thermodynamic parameters like TLL and V_R for those specific components. The measurement of the intrinsic properties of each of the generated systems is as well a desirable procedure to better understand the observed partition behaviors of the solutes or to predict such behavior in an educated manner. Afterwards, the study of the partition of molecules in them should be performed extensively.

ATPS characterization is important because this liquid-liquid extraction strategy continues to be an important operation because of the great advantages it has in comparison to other more complicated, expensive, and time-consuming operations. Furthermore, ATPS is not only a purification strategy found in some biotechnological processes but is also a strategy that allows process integration and an interesting tool that can be employed in analytical measurements.

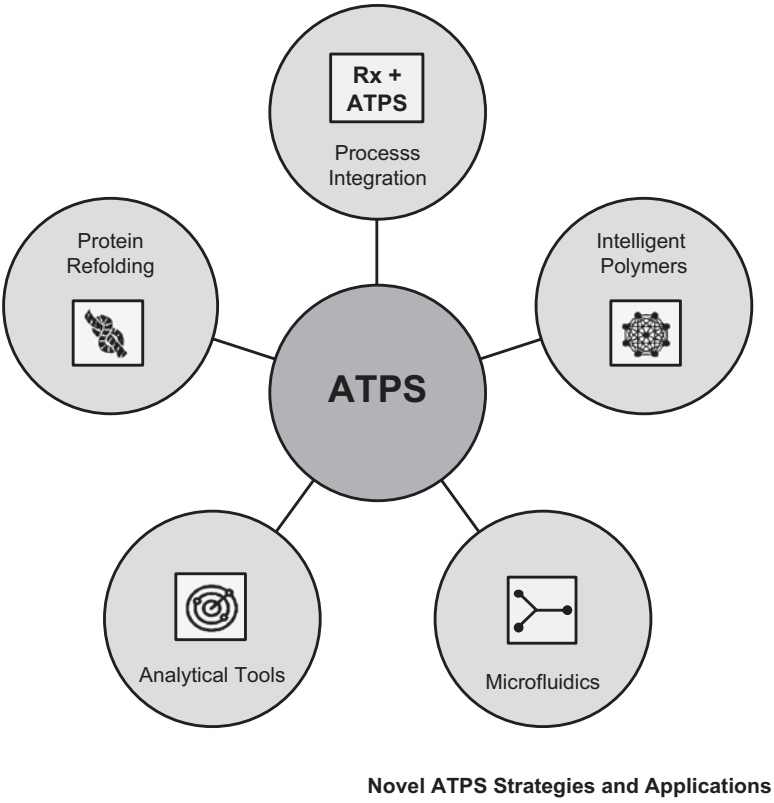

Fig. 2.4 Schematic summary of the novel and forthcoming ATPS-based strategies and applications

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