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Characterization of aqueous two phase systems by combining lab-on-a-chip technology with robotic liquid handling stations

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ABSTRACT

Over the last decade, the use of design of experiment approaches in combination with fully automated high throughput (HTP) compatible screenings supported by robotic liquid handling stations (LHS), adequate fast analytics and data processing has been developed in the biopharmaceutical industry into a strategy of high throughput process development (HTPD) resulting in lower experimental effort, sample reduction and an overall higher degree of process optimization. Apart from HTP technologies, lab-on-a-chip technology has experienced an enormous growth in the last years and allows further reduction of sample consumption. A combination of LHS and lab-on-a-chip technology is highly desirable and realized in the present work to characterize aqueous two phase systems with respect to tie lines. In particular, a new high throughput compatible approach for the characterization of aqueous two phase systems regarding tie lines by exploiting differences in phase densities is presented. Densities were measured by a standalone micro fluidic liquid density sensor, which was integrated into a liquid handling station by means of a developed generic *Tip2World* interface. This combination of liquid handling stations and lab-on-a-chip technology enables fast, fully automated, and highly accurate density measurements. The presented approach was used to determine the phase diagram of ATPSs composed of potassium phosphate (pH 7) and polyethylene glycol (PEG) with a molecular weight of 300, 400, 600 and 1000 Da respectively in the presence and in the absence of 3% (w/w) sodium chloride. Considering the whole ATPS characterization process, two complete ATPSs could be characterized within 24 h, including four runs per ATPS for binodal curve determination (less than 45 min/run), and tie line determination (less than 45 min/run for ATPS preparation and 8 h for density determination), which can be performed fully automated over night without requiring man power. The presented methodology provides a cost, time and material effective approach for characterization of ATPS phase diagram on base on highly accurate and comprehensive data. By this means the derived data opens the door for a more detailed description of ATPS towards generating mechanistic based models, since molecular approaches such as MD simulations or molecular descriptions along the line of QSAR heavily rely on accurate and comprehensive data.

1. Introduction

The development of biopharmaceuticals is a cost and time consuming procedure which is driven by time to market demands and administrative requirements in regard to process robustness and product quality. In the theme of high throughput process development (HTPD) several methods have been developed which can cope with the industrial relevant issues regarding time to market demands, material consumption, cost efficiency and process robustness according to quality-by-design (QbD) requirements [1–5].

Seeking for alternative drug purification technologies aqueous two phase systems (ATPSs) have arouse enormous interest due to cost efficiency, tolerance of solid particles, solid particle removal, scalability and gentle conditions required for biopharmaceuticals [6,1].

Aqueous two-phase systems are composed of two polymers (e.g. polyethylene glycol (PEG) and dextran) or of polymer and salt (e.g. PEG and phosphate). These systems expose a miscibility gap which is defined by the respective binodal curve. A system point located in the two-phase region leads to phase separation along the tie line, whose ends show the compositions of the top and bottom phases that exist in equilibrium with each other.

A number of thermodynamic predictive approaches were reported for the calculation of physiochemical properties, density and activity [7,8]. However, due to complexity of ATPSs the

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application of these models on industrial relevant ATPSs processes might be challenging. There is still a lack of understanding of ATPS phase formation and protein partitioning mechanisms. Thus process development and optimization is still realized heuristically requiring cost and time intensive experimental screening. In order to react on these shortcomings a number of studies focused on the screening for adequate ATPSs for a number of highly selective purification tasks for valuable biopharmaceutical products in the last years [1,9–11].

To shed some light in the mechanistic understanding of phase formation but also to improve ATPS screening for industrial challenges, the initial characterization of ATPSs is highly important regarding the determination of binodal curve and tie lines which comprise the most important system properties such as phase composition and mass ratio of top and bottom phase. These properties could provide an insight into the mechanisms responsible for the protein partition [6] and could be used for molecular dynamics simulations or quantitative structure activity relationship (QSAR) models, which have been shown to be potential approaches for mechanistic modeling protein partitioning [12,13]. The binodal curves are used to design the screening space as well as for tie line determination. The tie lines enable the calculation of phase composition of top and bottom phase. This information can be highly important for the comparability to precipitation by phase forming component or conditioning processes for further chromatography.

The commonly used binodal curves are evaluated by cloud point method also known as turbidity titration which exploits the transition from a clear to a turbid solution when entering the two phase region. This methodology was established as a high throughput technique by Bensch et al. [14].

A number of different techniques for tie line determination have been reported previously, such as gravimetric analysis [15] or the use of conductivity (salt) and refractive index (PEG) analysis [6,16,17]. However these techniques are not able to cope with high-throughput requirements.

Bensch et al. [14] have reported on extensive high throughput screening techniques in downstream processing with respect to the preparation, characterization and optimization of ATPS. For evaluating the tie lines a dye is dissolved in the ATPS, which exclusively partitioned into the top phase and can be quantified by absorbency measurement within a few minutes. The top phase volume correlates to the concentrating factor of the dye. The tie lines can be calculated approximately by applying the lever arm rule on the phase volumes under the consideration that density differences of phase solutions are negligible. This approach has been shown to be sufficient for fast ATPS process development [1,2,14].

However, there are some requirements to the selected ATPS with respect to dye stability and partitioning. Moreover, a serious drawback is the assumption of negligible densities alluding to an increasing simplification with increasing tie line length. Liquid handling inaccuracies at top and bottom phase sampling as well as meniscus forming depending on phase compositions can be error sources for absorbency measurement.

To get a higher accuracy in characterizing ATPSs additional measurement methods seem necessary. Apart from HTS techniques, another promising technology is the 'micro total analysis system', also called lab-on-a-chip system, which has experienced an enormous growth over the last years [18,19] and offers unique advantages in sample handling, reagent mixing, separation and detection [20]. This technology is applied in a number of different fields from standard operations like cell cultivation, reactors and mixers or cell counting and flow cytometry to applications in clinical diagnostics, cancer research and drug discovery and screening [21]. Lab on chip systems seem promising to fulfill the requirements of to be compatible to HTS demands as they require a low sample volume.

Unfortunately, a number of lab-on-a-chip devices are not compatible to liquid handling stations (LHSs) because they do not meet design requirements to be supplied with samples using a robotic manipulator arm (RoMa) or using a liquid handling arm (LHA). A combination of lab-on-a-chip technologies with liquid handling stations can improve the process development and process optimization enormously and is highly desirable. Waldbaur et al. [22] have reported previously on a generic microfluidic interface design which enables the use of microfluidic chips on LHSs.

In this work we have combined both technologies in order to characterize ATPSs with respect to tie lines. We present a new high-throughput compatible approach for tie line determination by means of phase densities. Phase densities of top and bottom phase are determined in a HTS method by combining LHSs and micro fluidic density sensor and are used for evaluating tie lines. This offers the unique advantage of considering differences in phase densities and gaining density data within the screening, which are important information with respect to feasibility and processivity issues downstream and further development of thermodynamic models for ATPS. A standalone lab-on-a-chip micro fluidic liquid density sensor was converted into a integrated fully automated liquid handling station by using a *Tip2World* interface we present and explain in detail. This *Tip2World* interface is capable to transform standalone lab-on-a-chip systems along other analytical devices into fully automated instruments integrated into liquid handling stations. ATPSs composed of low molecular polyethylenglycols and potassium phosphate at pH 7 with and without NaCl were characterized with respect to binodal curves, phase densities and tie lines by applying the new approach described in detail in this work.

2. Materials and methods

2.1. Preparation of stock solutions

Polyethylenglycol of analytical grade with different molecular weights was purchased from Sigma Aldrich (St. Louis, MO, USA). Stock solutions of polymers were prepared by mixing masses of polymer and water, purified by an Ariums^{TM}pro UV system (Sartorius Stedim Biotech, Göttingen, Germany), in order to reach the desired polymer concentration, which was 70% (w/w) (PEG300, PEG400), 60% (w/w) (PEG600) and 40% (w/w) (PEG1000).

Dipotassium phosphate and potassium phosphate of analysis grade were purchased from BDH Prolabo (Radnor, PA, USA). The added mass of potassium was included into the calculation of the total mass ratio of 40% (w/w) potassium phosphate solutions. Masses of the basic and acid component of potassium phosphate were combined in order to reach a pH of 7.0 at room temperature.

Masses of sodium chloride of analysis grade (Merck KGaA, Darmstadt, Germany) and ultra pure water were combined in order to reach a sodium chloride stock solution with 25% (w/w) NaCl.

2.2. Liquid handling stations

All experiments were performed in a high throughput format using two fully automated liquid handling stations (LHS), namely Freedom Evo^{TM}200 and Freedom Evo^{TM}100 stations (Tecan, Crailsheim, Germany).

Freedom Evo^{TM}200 was used for ATPS preparation and binodal determination. This LHS exposed an 8-channel liquid handling arm with teflon coated fixed tips connected to 1 ml dilutors. In addition, this LHS was equipped with a centric gripper, and a orbital shaker (*Te-shake*^{TM}, Tecan, Crailsheim, Germany).

Tie line determination and density screening respectively were performed by means of a Freedom Evo^{TM}100. This LHS was equipped with 8-channel liquid handling arm with stainless