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INVITED REVIEW PAPER

Microfluidic extraction using two phase laminar flow for chemical and biological applications

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Abstract−We review the state of the art in microfluidic separation technique based two-phase laminar flow with an application focus on chemical and biological sample. As we describe herein, two-phase laminar flow in the microfluidic extraction has several biological and engineering advantages over other methods including high reproducibility, biocompatibility, and selectivity. We review advances in applications of two-phase laminar flow and examine key parameters such as flow rate, phase composition, and surface charge property, how these can affect extract performance with the technology including microfluidic separation system. A special technology focus is given to emerging novel integrative microfluidic extraction, which aims to merge aqueous phase laminar flow and electric field technologies into simple packages. We conclude with a brief discussion of some of the emerging challenges in the field and some of the approaches that are likely to enhance their application.

Key words: Microfluidic Device, Two-phase System, Separation, Extraction, Protein, Cell

INTRODUCTION

Two-phase systems (TPSs) are appealing separation methods as clean alternatives for traditional organic-water solvent extraction systems. TPSs as a useful extraction technique is formed by mixing two aqueous solutions of structurally different polymers or by mixing a polymer and salt aqueous solution together when concentrations of the components are higher than a critical value. This phenomenon was first observed by Beijerinck, in 1896, and an 'incompatibility' was first noted in solutions of agar, a water-soluble polymer, with soluble starch or gelatin. Albertsson demonstrated partitioning of bacteria and cell fragments in 1956 [1]. Since then TPS has been shown to be effective at separating erythrocytes based on age [2] and species in 1976 by Walter et al. [3]. TPS has also been used to analyze the surface charge properties of blood cells and to fractionate leukocyte subtypes [4]. From the beginning stages of TPS with these kinds of applications, TPS has been widely used for the concentration, purification and separation of metal ions, proteins, cells, and other biological products [5].

For the concentration and separation of metal ions, Akama et al. have developed TPS for the extractive preconcentration and separation of Cr(VI) and the selective extraction of trace Cd^{2+} from large amounts of Co^{2+}, Cu^{2+}, Fe^{3+} and Zn^{2+} using tetrabutylammonium bromide (TBAB)/$(NH_4)_2SO_4$ system [6,7]. And poly(ethylene glycol) (PEG)/$(NH_4)_2SO_4$ system has been established for the extraction of Ni-dimethylglyoximato complex by Yoshikuni et al. [8].

TPS has also been applied for the separation and purification of proteins from the cultivation media constitutes a major bottleneck for the widespread commercialization of recombinant proteins [9]. The prefractionation of a complex mixture of proteins increases the resolution in analytical separations of proteins from cells, tissues or organisms [10]. Johansson et al. have investigated TPS for separation and partitioning of green fluorescent protein from escherichia coli using PEG/sodium-poly(acrylate) system [11]. To achieve high recovery yields and to avoid limitations of single-stage TPS, Rosa et al. used a counter-current multi-stage TPS. It could be possible to achieve a more effective process in terms of selectivity, target product enrichment, raw materials consumption and throughput. They performed a multi-stage equilibrium TPS of human antibodies and respective purification from all CHO cells supernatant contaminants [12,13].

For the separation of cells, in 2001, a new type of TPS has been developed in which a temperature-sensitive polymer, poly-*N*-isopropylacrylamide was used as a ligand carrier for the specific separation of animal cells [14]. Bradley et al. used membrane grafting of methoxypoly(ethylene glycol) to produce immunocamouflaged red blood cells [15].

Although standard molecular biology protocols for plasmid DNA (pDNA) purification have been available, economical and efficient production and purification processes are still demanded to produce pure DNA. An alternative approach based on TPS for initial purification of plasmid DNA (pDNA) from main contaminations has been described in several publications [16-19] and is a promising alternative for chromatographic methods. Some of the studies applied hydrophobic interaction chromatography or Ultrafiltration/diafiltration processes to recover the partitioned pDNA from the salt-rich phase of the TPS. Both processes are, however, either costly or/and

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require extended processing times. To circumvent these disadvantages, a membrane-based process enabling phase acting at the same time as a desalting and conditioning/formulation step. Frerix et al. integrated TPS-membrane capture process developed earlier [16,20] by a polish procedure followed by rapid recovery of the supercoiled pDNA using membrane adsorption [21]. Gomes et al. used ATPS based on PEG 600, sodium citrate and ammonium sulfate were used to partially purify pDNA from Escherichia coli alkaline lysates to overcome the drawback that the wastewater streams generated in the polymer/salt TPS have high concentrations of phosphate or ammonium ions [22]. Mashayekhi et al. investigated partitioning behavior of mammalian genomic DNA fragments in TPS with micellar system which was generated using the nonionic surfactant Triton X-114 and phosphate buffered saline (PBS) [23]. The integrated process for purification of pDNA using TPS combined with other procedures such as chromatography has been also investigated [19, 24,25].

Separation of blood components with TPS at the macroscale requires large sample volumes and can take long time to complete. Microfluidic devices can potentially enhance TPS because the two-phase interface can easily be made in a microfluidic channel with laminar flow. It is well known that a stable laminar flow can be obtained in a microchannel because of its low Reynolds number. The surface area-to-volume ratio of the streams is very large, decreasing the distance a cell must travel before coming into contact with a phase interface and potentially making separation faster in microchannel [26]. Microfluidics also allows for continuous cell separation, something that is difficult with traditional TPS.

SooHoo et al. and Tsukamoto et al. have used PEG/dextran TPS to isolate leukocyte and erythrocyte cells from whole blood cells in a microfluidic chip [27,28]. TPS in microfluidic device also facilitates the monitoring of live and dead cells were fractionated by continuous-flow extraction in a microchannel [29].

TPS, as described above applications, exhibits advantages as follows; [30]

• TPS provides mild conditions that eliminate the use of hazardous solvent.

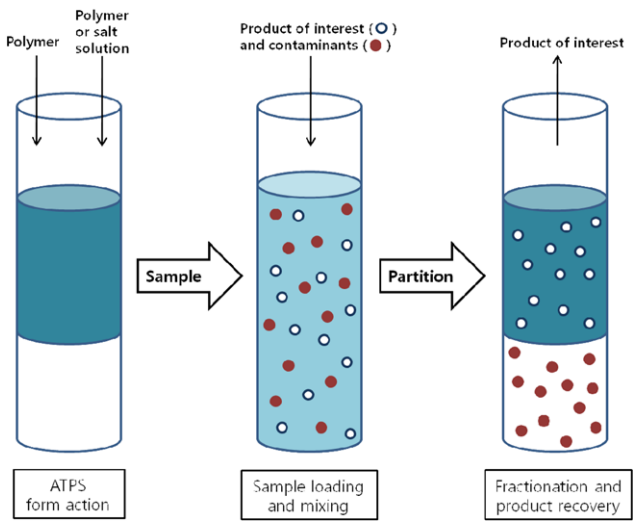

Fig. 1. Simplified representation of the fractionation of bioparticles in aqueous two-phase processes [5].

• The development of specified processes is facilitated by adjusting the variables such as temperature, concentration, degree of polymerization, presence of certain ions etc.).

• TPS shows ease of use, low costs and shorter times for phase splitting and recycling of the system components.

The first subsection below provides a theoretical background of TPS for understanding the mechanism of extraction. Section 2 describes some of these techniques which have integrated microfluidic channels and developed for enhancing extraction efficiency with flow rate, phase composition, and so on. The final section provides a review of the future trend and challenges which have recently been demonstrated for two-phase extraction based bimolecular separation.

THEORETICAL BACKGROUNDS

1. Phase Separation

The basic facts in aqueous two-phase extraction are that (1) phase separation occurs with the addition of either polymer-polymer or polymer-salt pairs into an aqueous solution, and (2) the solute material is predominantly partitioned into one of the separated phases. Because it is essential to understand and predict the system behavior for extensive application of this technology, many research groups have aimed to predict the phase separation and partition behavior more accurately based on thermodynamic theories or experimental measurements of characteristic constants describing interactions between system elements.

Two kinds of approaches could be generally considered for the prediction of phase diagrams: one based on polymer solution theory such as the lattice theory of Flory [31] and Huggins [32], and the other derived from the osmotic virial equation.

The basic problem with thermodynamic properties of polymers in solution is to derive an expression for the change of Gibbs free energy associated with the formation of a polymer solution from

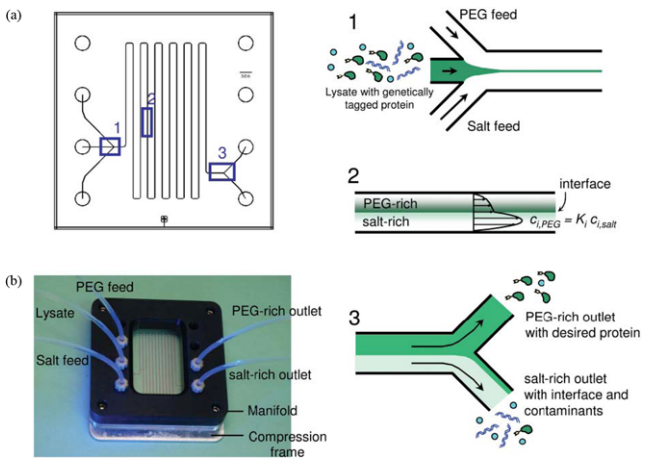

Fig. 2. (a) Schematic of laminar flow extraction. In region 1, a cell lysate stream is hydrodynamically focused between inlet streams containing PEG and salt (potassium phosphate, pH 8). In region 2, farther downstream, laminar two-phase flow with a stable interface is observed. At the end of the channel (region 3), the flow is split into two outlet streams. (b) Photograph of the microfluidic chip mounted in a Delrin manifold, with inlet and outlet tubing connections [13].

pure components, i.e., the free energy of mixing, ΔG_m, using the following usual relationship:

$$\Delta G_m = \Delta H_m - T\Delta S_m \quad (1)$$

where ΔH_m, ΔS_m and T is the enthalpy of mixing, the entropy of mixing, and the absolute temperature, respectively. The expression for ΔG_m based on the lattice theory of Flory and Huggins is expressed mainly with the fraction of lattice sites occupied by polymer segments, relative molecular volumes, and the Flory interaction parameters. The critical conditions for miscibility and the incipient appearance of two phases lead to the conclusion that phase separation occurs if the interaction between segments of the two polymer types is energetically unfavorable. A detailed mathematical description is well introduced by Brooks et al. [33]. The lattice model is easy way to simulate the phase separation behavior with the advantage of simple approach. However, it is not accurately describing the details of the interactions present in the polymer-polymer-water system.

Another approach for the prediction of phase diagram is using the osmotic virial equation in which the chemical potentials of components are described by a power series in the polymer concentrations with interaction coefficients. Edmond and Ogston [34] firstly suggested to apply this equation truncated at the second-virial-coefficient term for dilute ternary aqueous solutions of polymers (or proteins). The researchers in Berkeley [35,36] and Cabezas et al. [37] extended their research to study the effects of salts in their calculations. However, their approaches were not the same in the aspect of the way treating the effects of salt and molecular weight [38].

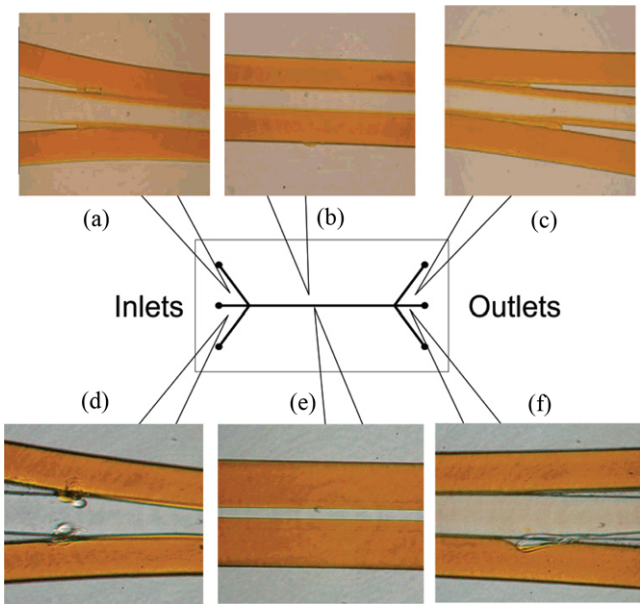

Fig. 3. Photographs of the three-phase flow in the microchannel. (a) Junction near the inlets of the microchannel without surface modification. (b) Center of the confluent microchannel without surface modification. (c) Junction near the outlets of the microchannel without surface modification. (d) Junction near the inlets of the microchannel modified with octadecylsilane groups. (e) Center of the confluent microchannel modified with octadecylsilane groups. (f) Junction near the outlets of the microchannel modified with octadecylsilane groups [53].

The addition of salt into polymer-water solution may also induce phase separation. Exclusion mechanism which correlates protein partitioning in aqueous two-phase systems derived from polymer-salt-water was provided by several researchers [39,40]. Kabiri-Badr et al. applied fluctuation theory to describe salt polymer aqueous two phase system [36]. Kang and Sandler [41] predicted binodals using UNIQUAC equation. However, it required many adjustable parameters.

The phase diagram is a useful tool or guideline to build an extraction system for a particular two-phase system. The diagram gives us information including the initial amount of each component necessary to form two phase system in equilibrium, the concentration of each component in both phases, and the ratio of phase volumes. Depending on location of the initial coordinates of the mixture, it is determined if the system will form two immiscible aqueous phases.

A binodal curve and tie line are necessary to learn for reading the phase diagram. Binodal curve divides the phase diagram into two regions: one-phase and two-phase region. The tie line is a line connecting two nodes on the binodal curve, and the two nodes indicate the equilibrium composition of phase components in each phase. Therefore, the initial coordinates for two-phase system should be placed above the binodal curve and on the tie line. The tie-line length (TLL) has the same units as the component concentrations and is often related with physical properties such as density difference between the resulting phases, viscosity, and interfacial tension [42].

2. Prediction of Protein Partition Coefficients

For the theoretical consideration of protein partitioning, the Flory-Huggins model was again used with the assumption of one more polymer component being inserted and partitioned. The partition coefficient, K, in terms of the polymer concentration differences between the phases, the molecular weights of the two polymers and the added macromolecules and χ_{ij} parameters for the macromolecule interacting with the solvent and each polymer [38]:

$$\ln K = P_p[(\phi_1^t - \phi_1^b)(1 - x_{1p}) + (\phi_2^t - \phi_2^b)(1/P_2 - x_{2p}) + (\phi_3^t - \phi_3^b)(1/P_3 - x_{3p})], \quad (2)$$

where

P_i=(molecular volume of component i)/(molecular volume of solvent)

i=1, 2, 3, or p refers to solvent, polymer 1, polymer 2, or protein, respectively

ϕ_i=fraction of solution volume occupied by component i; t or b refer to top or bottom phase

χ_{ij}=interaction parameters describing the i-j interaction.

Several features can be known from this equation. Firstly, K depends exponentially on the relevant properties of partitioned material and the phase system. The partition behavior becomes more one-sided with increasing protein molecular weight and with increasing the difference in concentration between the phases of either polymer. Partition also occurs preferably towards the less weighted polymer side.

A modified lattice theory was suggested to predict protein partitioning without taking into account the salt effects [43]. Although it was difficult to get all the parameters from experiment, it was confirmed by this study that the exclusion effects are the dominant forces between nonionic polymers and proteins in aqueous solutions.

Besides the lattice model, the virial expansion method was also used for the prediction of protein partition. The constant pressure

theory and the extended constant volume equation were suggested and the former is known as more realistic. The basic equation, for isoelectric proteins in the absence of slats, can be expressed as [38]

$$\ln K = A_{2p}(m_2^b - m_2^t) + A_{3p}(m_3^b - m_3^t) \quad (3)$$

where mi is the molality of component i in the indicated phase and A_{2p} and A_{4p} are the virial coefficients to be evaluated.

The salt effect, the interactions with the other components, is still not considered in this equation. Forciniti and Hall calculated the virial coefficients assuming no net attractions between any of the components. In their studies, the shape of protein and polymer was assumed to be rigid sphere and the sphere, rods, or Gaussian coils, respectively [38]. It has been shown that certain common salts such as phosphate, sulfate, citrate may affect the partition of a highly charged biomolecule [36,44]. When the salt effect is taken into account, it should be considered that the charged proteins are distributed by an electrical potential difference which is caused by unequal distribution of a salt between the phases. The influence of this electrical potential can be described by an additional term in chemical potential difference equation. The Berkeley group also modified the Eq. (3) by including salt interaction terms and concentrations.

Other groups suggested a simple model that correlates the physicochemical properties of proteins with their partition coefficient in ATPSs based on the so-called modified group contribution approach: [45]

$$K = K_{hphob} \cdot K_{el} \cdot K_{size} \cdot K_{sol} \cdot K_{aff}$$

Where K_{hphob}, K_{el}, K_{size}, K_{sol}, and K_{aff} denote the contribution of hydrophobicity, electrostatic forces, size solubility, and affinity to the overall partition coefficient, respectively.

APPLICATIONS OF TWO-PHASE MICROFLUIDIC SYSTEM

Research on two-phase laminar flow systems indicated that phase properties including viscosity and interfacial tension, and condition of flow rate play key roles in determining the formation of stable phase separation in the microfluidic device [27,28,46-48]. In addition, for the development of the more efficient two-phase extraction based microfluidic channel, new approaches have been recently demonstrated by applying an additional electric field and introducing the affinitive metal ions to target sample [49]. In this section, a number of applications based on two-phase extraction system are introduced.

1. Formation of Parallel Laminar Flow

In two-phase laminar flow system, it is crucial to form a stable liquid-liquid interface in the microfluidic channels [50-52]. To handle the continuous parallel laminar flow on the microfluidic channel, the use of surfactants or surface treatment should be considered depending on the different phase composition [53-56].

In case of an organic-aqueous system [57,58], the liquid-liquid profile is well formed due to the large interfacial tension between the two immiscible fluid phases. However, we usually observed that the aqueous flow invaded the upper channel for the organic phase at the end-junction of the microchannel without any surface modification of microchannel [53-56,59]. To resolve this problem, many researchers have applied to introduce organic-aqueous solution after chemical modification of the surface at the outlet channel. Maruyama et al. [55] demonstrated the surface modification of the microchannel by octadecylsilane groups induced spontaneous phase separation of the two-phase flow in the chip. For the details of surface modification experiments, one-half of the glass wall was modified to be hydrophobic by the flow of the hydrocarbon such as octadecyltrichlorosilane (OTS) and a self-assembled monolayer (SAM) of OTS was formed on it. According to the reported results, we found that the only surface treatment was sufficient to confine the organic flow on the hydrophobic regions to form a stable organic-aqueous interface in the microfluidic channel.

Compared with aqueous-organic systems of large polarity difference, aqueous-aqueous two-phase system (ATPS) has a benefit to form stable parallel laminar flow without surfactants or special surface treatments [5,29,60-62]. This is because ATPS has low interfacial tension (10^{-4}-10^{-1} mNm^{-1}) and consists of two aqueous phases showing similar affinity with substrate [63]. In condition of ATPS, even at low flow rate no spherical flow was observed due to relatively low interfacial tension between two phases [27,47]. And this characteristic enables the operation at the condition of low flow rates. Moreover, the stable flow between top phase and bottom phases

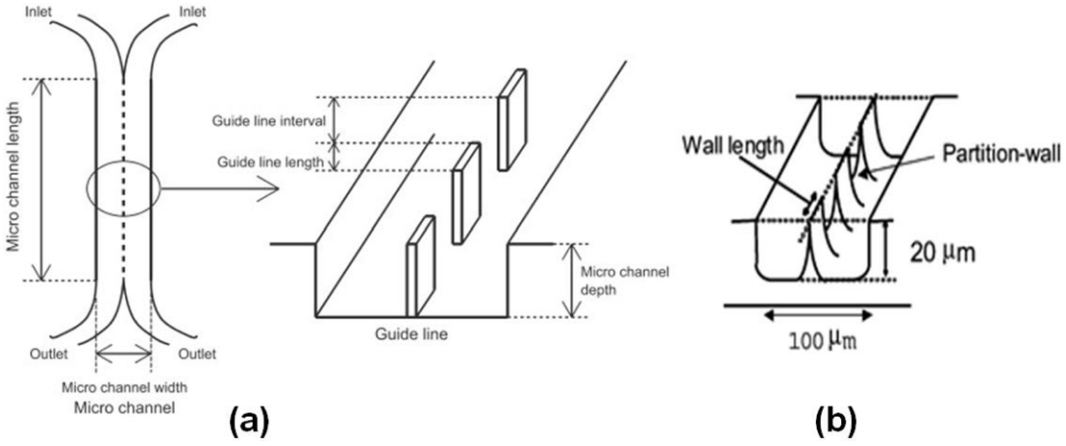

Fig. 4. (a) Schematic view of a micro-channel reactor with a guideline structure [62]. (b) Representative illustration of a microchannel with partition walls [65].

and the clear liquid-liquid interface formed well over the entire microchannel.

As an another approach to make stable organic-aqueous flow in the chip, Tagawa et al. [64] presented a special guide structure of the microchannel which helped to keep the two-phase flow system stable for extraction of target sample from study of the molecular transport. In this system, partition walls with guideline structure were designed at the middle of a micro-channel with adequate intervals in order to stabilize and to maintain a laminar two-phase flow. As shown in Fig. 4, this technique is to stabilize and maintain a two-phase laminar flow and a good phase separation which we will describe in greater detail in section 3.1 [65,66].

2. Characteristics of Two-phase Extraction in Microfluidic Device

2-1. Effect of Flow Rate on Microfluidic Extraction

Mass transfer in a microchannel, where the turbulence flow cannot occur, is governed by molecular diffusion as the Reynolds number in such a system is less than 1 [51,53,60,61,67,68]. The flow rate of the solution, therefore, is a profoundly important parameter to determine the efficiency of extraction and the stable formation of the two-phase laminar flow as well. For example, Huh et al. [69] presented the development of microfluidic ATPS that utilizes protein purification technology which was successfully carried out for the high purity and recovery rate of bacteriorhodopsin (BR). As can be seen in Fig. 5, the recovery rate of BR decreased slightly with increasing the flow rate of the buffer phase. It is supposed that the microfluidic channel, which is able to form a thickness of the sample phase with controlling the flow rate of the buffer phase, would enlarge the contact area and contact time. In an another example, Tsukamoto et al. [28] proposed an aqueous two-phase laminar flow in a microfluidic chip and used the system to isolate leukocyte and erythrocyte cells from whole blood cells based on ATPS comprised of polyethylene glycol (PEG) and dextran (Dex). In this system, at the condition of low flow rate, two-phase interface formation was not observed and emulsions formed in the microchannel, and an emulsion was formed where the PEG solution interacted with the Dex layer. This result indicated that the diffusion coefficient of the solution was larger than the flow rate.

In addition, the interfacial contact area in the microfluidic system adds significantly to the extraction of target sample from the sample flow. However, increasing the interfacial contact area in an effort to enhance the removal of the target sample in the microfluidic device posed some difficulties. Thus, the larger the difference in the concentration, the greater the transport of target sample from the sample flow. For example, Huh et al. [60] reported a novel five-flow microfluidic desalting system using the metal ions of Mn^{2+}, Zn^{2+} and Fe^{3+}, which have urea affinity-capturing properties. As can be seen in Fig. 6, the urea in the sample phase was removed using diffusion and the difference in the concentrations from the sample phase to the both buffer phases in addition to the affinity-capturing of urea by the metal ions of Mn^{2+}, Zn^{2+}, and Fe^{3+}. In this experiment, as another interesting approach, the affinity flow of metal ions into the both sides of sample flow has been newly applied, resulting in facilitating the removal of urea by the complex interactions between the metal ions and the urea [70].

To achieve the high efficiency of extraction in microfluidic device, the optimum condition of flow rate and concentration of sample should be determined depending on various kinds of phase composition.

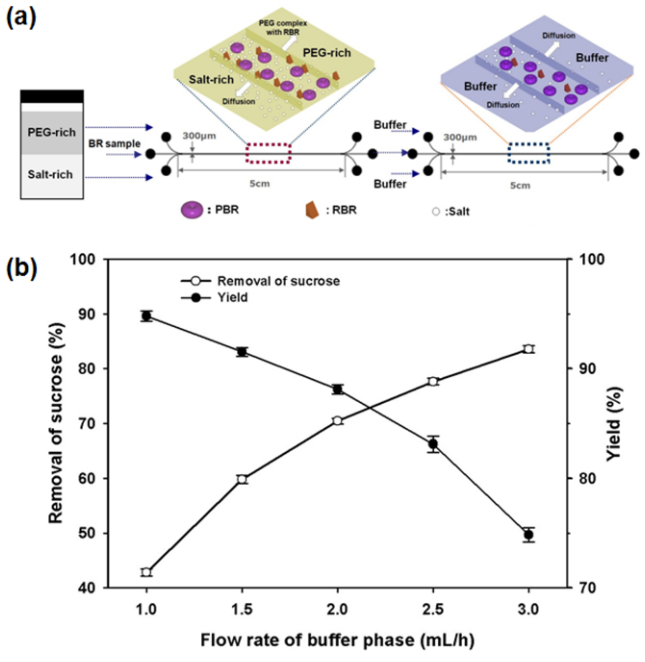

Fig. 5. (a) Schematic diagram of (a) ATPS and ionic liquid two phase system (ILTPS), and dialysis for the removal of contaminated proteins and excessive sucrose in the microfluidic device. (b) Effect of flow rate of both buffer phases on the recovery rate and purity of bacteriorhodopsin (BR) in the micro-dialysis system [69].

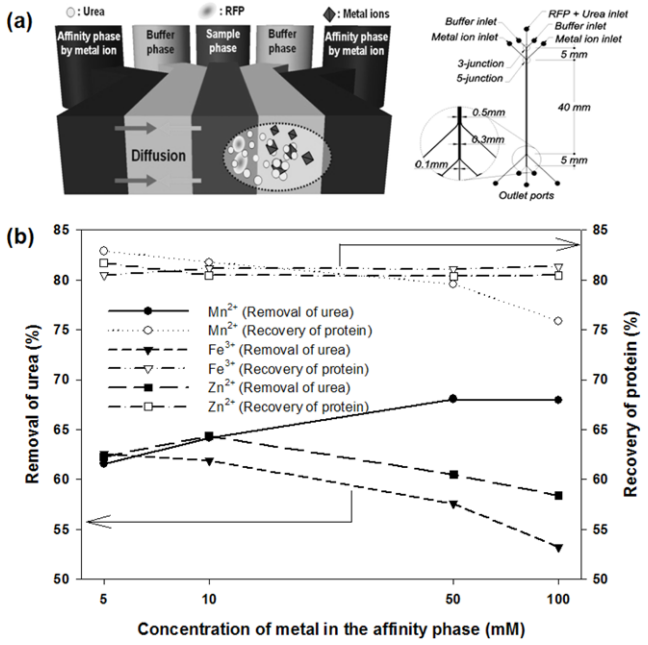

Fig. 6. (a) Schematic diagram of the desalting process using the affinity-capturing characteristics of the metal ions in the microfluidic device. (b) Effect of a concentration of 5 mM-100 mM Mn^{2+}, Zn^{2+} and Fe^{3+} metal ions on the removal efficiency of urea and on the recovery of protein [60].

2-2. Aqueous Two-phase Extraction for Bio-application in Microfluidic System

As described above, ATPS has good biocompatibility, easy adjustment, and special selectivity. Thus, ATPS has received much attention in recovery and purification of a variety of biological products including protein, genetic materials, and cells [28,29,47,49,69]. Microfluidic devices can potentially enhance ATPS because the surface area-to-volume ratio of the streams is very large, decreasing the distance a cell must travel before coming into contact with a phase interface and potentially making separations faster.

For the separation of biological sample, most experiment results have been mainly focused on the affinity between the surface of biological sample and solution of ATPS owing to differences in size, charge, and hydrophobicity [13,27,49,71-73]. Nevertheless some researchers suggested the mechanisms of phase forming and partitioning of biomolecules, but the exact mechanisms are still not well understood.

In cell extraction system, particular surface properties and net charge are key parameters for selectively separating cells using ATPS in microfluidic devices. Yamada et al. [74] presented a microfluidic device for separating plant cell aggregates with the different affinity of cell aggregates to each phase (the PEG-rich phase or Dec-rich phase) using salts. As another example, Nam et al. [29] described the separation of live and dead cells of Chinese hamster ovary cells by a change of overall surface properties of animal cells such as hydrophobicity and surface net charge. In this experiment, all live cells were partitioned to PEG-phase flow at the junction of outlet microchannels, while most of the dead cells were kept on the interface flow at pH 6.6. SooHoo and Walker [27] have recently presented the leukocyte concentration at the PEG-rich phase from whole blood using a microfluidic ATPS. In this study, three different patterns of three polymer streams each were evaluated for their effectiveness in concentrating leukocytes. Among them, the most effective configuration was the Dex-PEG-Dex stream pattern because the interfacial surface area-to-volume ratio was four times greater than in the one interface system.

Similar to the cell separation approaches, protein partitioning in such an ATPS takes place due to different affinities of a protein species to the polymers. The affinities and by that the migration across the phase boundary depend on the physico-chemical properties of the protein such as isoelectric point, surface hydrophobicity, molecular mass as well as on medium properties like polymer molecular mass, pH, and salt concentrations [49,69,74]. By controlling these factors, the selective partitioning and recovery of a target protein can be achieved. For example, Huh et al. [61] have recently demonstrated the partitioning of the protein sample in ATPS with the various pHs and flow rate of the buffer phase. Corresponding ATPSs have also been used for the partitioning of nucleic acids and organelles.

3. Novel Approaches of Microfluidic Extraction Using ATPS

3-1. Shape Control of Microfluidic Channel for Two-phase Extraction

Several research groups presented the shape effect of microchannel on mass transfer and separation efficiency [65,74-80]. In a microfluidic system, the change of geometry affects the flow pattern and shape of the interface between two different phases. These changes have been reported to increase mass transfer to the interface and enhance extraction efficiency. Yamada et al. [74] demonstrated a pinched microchannel where a PEG/Dextran aqueous two phase system was applied. In this system diffusion to the interface was a rate-limiting step for extraction of cells. By fabricating a pinched point in the middle of the microchannel, they succeeded in separating cells effectively. In pinched point diffusion the cell length was decreased, so relatively large cells were more easily and quickly attached to the interface of two different phases. As a result, this pinched region enhanced extraction efficiency of cells. They also proceeded to separate particles depending on their sizes in a pinched microchannel with similar mechanism.

Another interesting recent example was presented by Ueno et al. [81] that involved the channel shape effect on mass transfer by changing geometry of the microchannel wall. They demonstrated the unique extraction scheme that triangular wall shaped microchannels, asymmetric and symmetric, were fabricated by polystyrol imprinting method. Only an asymmetric triangle-shaped wall made the interface not planar but sinusoidal. As the deeper the shape was, the more enhanced result was obtained. They assumed that the geometric change of interface affected mass transfer of extractants. This sinusoidal interface increased contact time between two phases, which is the key factor of extraction efficiency. This approach offers the advantage of improving the extraction efficiency near the sinusoidal interface owing to the interfacial change [81].

Maruyama et al. [65] also recently reported intermittent partition walls within a microchannel. In this and similar systems, the interface

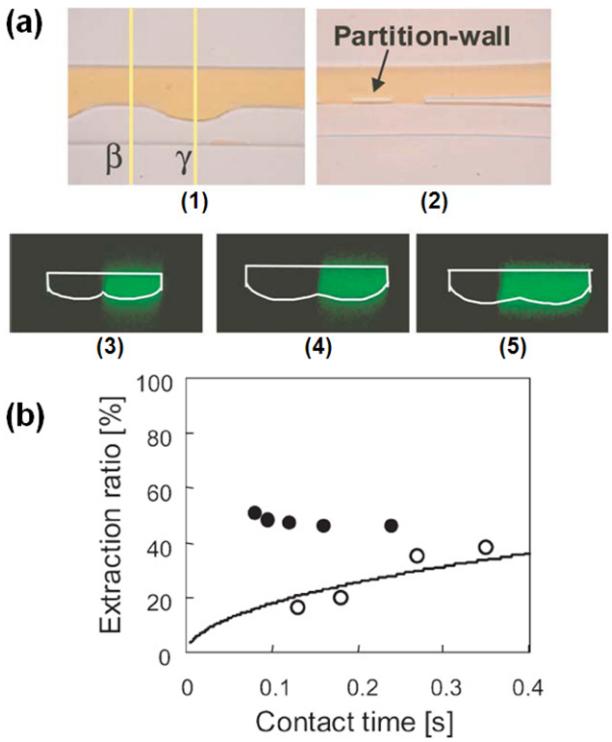

Fig. 7. (a) Photographs of the two-phase flow in the microchannel. (1) Center of a confluent microchannel with intermittent partition walls. (2) End-junction near the outlets of the microchannel with intermittent partition walls. (3) Cross-section of the two-phase flow in the microchannel without partition walls. (4, 5) Cross-sections of the two-phase flow in the microchannel with partition walls. (b) Effect of contact time on extraction ratio [65].

between two phases is stable and flat with laminar flow. As shown in Fig. 7, the intermittent partition walls caused not planar but sinusoidal interface and promoted extraction efficiency of Yttrium to organic phase. This allows it to form a small amount of turbulence in the sinusoidal interface of the microfluidic channel, inducing the mixing of the aqueous phase. It is now well recognized that the interface between two phases is a rate-limiting step in microchannel extraction. The mixing near interface area induced concentration profile changes, hence enhancing mass transfer of the solutants.

3-2. Effect of Electric Field on Extraction of Protein

ATPS is favorable in many potential applications for the stable analysis of wide range of chemicals since its aqueous nature enables hydrophilic and biocompatible condition. Many experiments have demonstrated protein partitioning in ATPS supported by an additional electric field [82-91]. For example, Clark et al. [92] proposed the two-phase electrophoretic separation based on two different properties of biomolecules: their physico-chemical affinities to the fluid phases of an ATPS and their electrophoretic mobilities under applied electric field [93,94]. Levine and Bier [95,96] have examined the transport of proteins across the phase boundary through the measurement of partitioning coefficients and electrophoretic mobilities of proteins inside a U-tube.

As another example, Munchow et al. [91] reported a study on protein transport phenomena discovered in partitioning experiments with a new setup for continuous-flow two-phase electrophoresis consisting of a microchannel in which a phase boundary is formed in the flow direction. As shown in Fig. 8, an electric field perpendicular to the phase boundary can be applied, enabling transport not only from the phase with the lower partitioning coefficient to the preferred phase but also vice versa. Huh et al. [61] have recently reported the protein separation by coupling aqueous two-flow system and free flow zone electrophoresis (FFZE). This interesting approach shows the highly purified target protein can be obtained by applying FFZE after introducing sample into aqueous laminar microfluidic flow (Fig. 9). As a result, this method enables a simple and

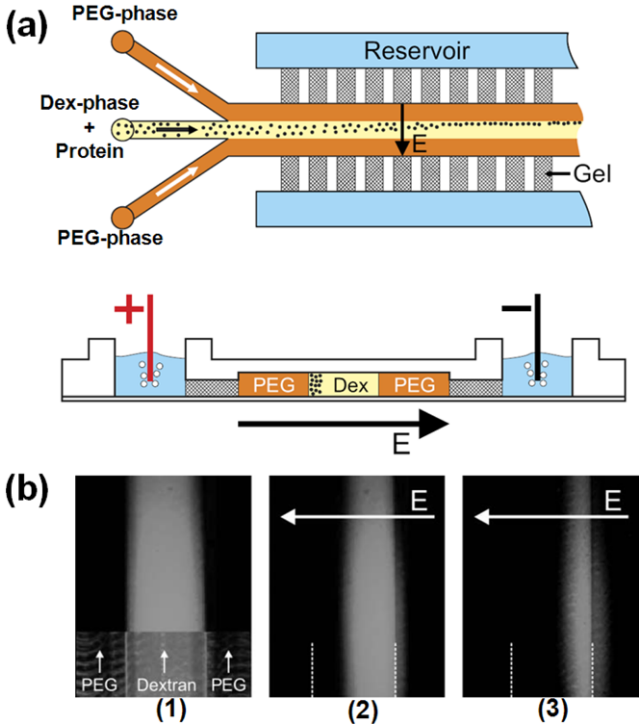

Fig. 8. (a) The microfluidic chip comprises a separation channel with three inlets and one outlet. (b) (1) Uniform distribution of BSA within the Dex-phase without electric field. (2)-(3) Proteins concentrate at the right phase boundary at 3.5 and 4.5 V dc. [91].

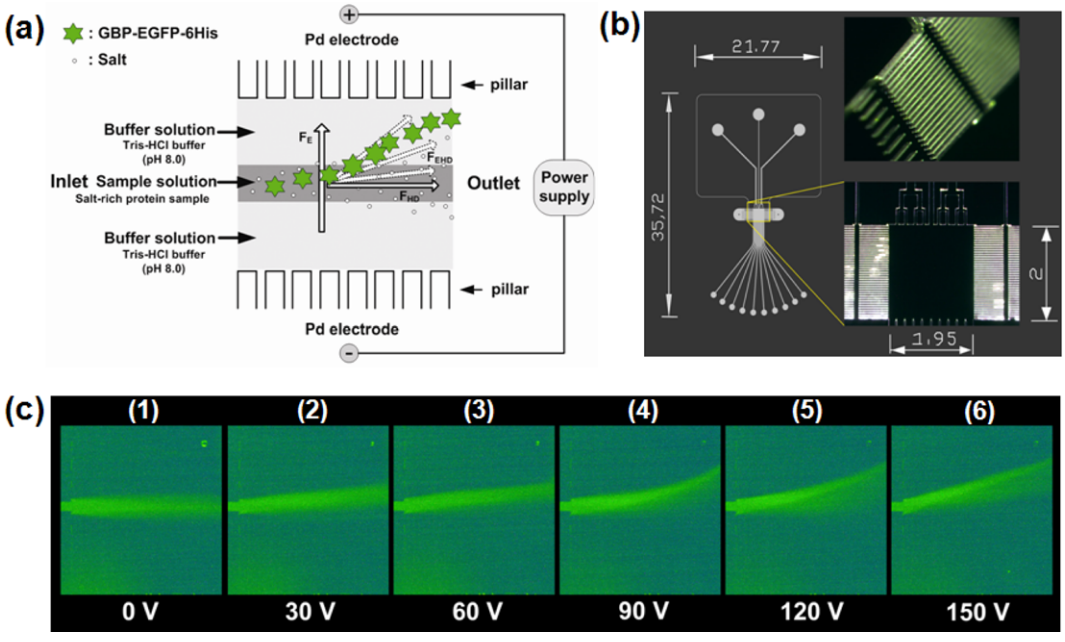

Fig. 9. (a) Conceptual diagram of the electro-desalting process using the electric field of 0-150 V in the microfluidic device. (b) Schematic diagram and lay-out of desalting process in the electro-microfluidic device. (c) Image of EGFP behavior in the micro channel with the increase of electric field in the range from 0 V to 150 V [61].

rapid electro-microfluidic desalting system as an effective means of cleaning up protein sample, depending on charged characteristics of protein.

This is a new separation approach of biomolecules, for which the combination of electrophoresis and aqueous laminar flow may enhance the extraction efficiency or offer new possibilities.

SUMMARY AND CONCLUSIONS

We have reviewed microfluidic separation technology based two-phase laminar flow with an application focus on biological products. As described above, a microfluidic reaction channel is so small that a low volume of reactants is required for separation, and the channel is not affected by gravitational force. Furthermore, a two-phase interface can easily be made in a microfluidic channel with laminar flow. Compared with other general separation methods, ATPS have advantages, especially biomolecules, because of low osmotic pressure, low interfacial force and high biocompatibility. This is because microfluidic extraction-based two-phase laminar flow is suitable for simple and rapid separation and concentration of biological products. For more efficient- and high-yield separation, we reviewed microfluidic extraction system-based two-phase laminar flow in microfluidic channel by considering those experimental parameters such as flow rate of each flow, phase composition of aqueous two-phase, surface charge property, and concentrations of components. In this system, the determination of optimum flow rate of the solution is crucial to forming a stable liquid-liquid interface in the microfluidic channels and to improve the efficiency as well. In addition, depending on the different phase composition and various kinds of bio-samples, the charge property to the pH of solution and viscosity of introducing solution should also be considered.

As we have attempted to focus on here, recent advances in experimental design have led to the increasing application of ATPS to the microfluidic separation of biomolecules. The change of geometry affected the flow pattern and shape of the interface between two different phases, resulting in increasing mass transfer to the interface and enhanced extraction efficiency. An important advancement related to the integrative microfluidic extraction has been also investigated by combining an electric field and two-phase laminar flow. These types by coupling with external force, electric or magnetic and affinity agents will certainly play an even more important role in next generation methods of microfluidic extraction-based two-phase laminar flow systems.

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