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# Liquid Biphasic System: A Review on Modern Bioseparation Technologies

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**ABSTRACT**

Many researchers have been interested in a substitute bioseparation technology for different biomolecules, namely the liquid biphasic system (L.B.S.). The analysis starts with a detailed discussion of the basic concept of L.B.S. and follows by a debate on the further creation of the different components for phase-forming of L.B.S. Also, various advance L.B.S. technologies will be introduced. It was addressed that is beneficial to L.B.S.'s e-function for bimolecular extraction, separation, and purification. The L.B.S. key parameters for link were obtainable and assessed. Furthermore, a guideline for future growth was described as future opportunities and challenges of L.B.S. The measures outlined in this review gives an insight into potential studies of the techniques of liquid-liquid split.

**Kewords**: Aqueous biphasic system, Aqueous two-phase system, Biomolecules, Hiquid biphasic system, Purification, Separation, Recovery.

## 1. Introduction

In the downstream biotechnology sector, trendiest research concentrate on the development of many sustainable bio-based products, for example microalgae, fruit, the biomass of lignocellulos product secondary and waste crops purification and Separation techniques for biomolecular regeneration (for example, proteins, carotenoids and lipids) require specific operating conditions that ensure the obtaining of high-value products [1, 2]. Extraction techniques have been developed, for example, membrane separation, chromatographic methods, ultrafiltration, precipitation. Multiple stages, complex routes, Timely procedures, high energy supplies, and high Recovery and extraction [3, 4, 5].

Researchers are developing a new technique of purification and separation that can be achieved in a single- within a shorter time of the extraction process. However, extraction solvents are reduced the total manufacturing costs in a procedure that can be reprocessed and recycled [6]. About food and pharmaceutical uses, this includes alternatives that are not harmful and environmentally friendly [7].

A well-established technology attracted many researchers in biomolecules' bioseparation, namely liquid biphasic system (L.B.S.) [8].

In downstream processing, it's also named the technology for liquid-liquid extraction. Via liquid biphasic dividing techniques, issues related to traditional extraction method

have been overcome. The biphasic liquid extraction system consists of two fluids, separated by a layer of interfaces when the two-fluid combination reaches the critical state. Generally, Phase-forming component characteristics establish the physical-chemical interaction that can desensitize the target biomolecules extracted from the top to the base phase. On the selectivities of the. Component. Also, numerous. aided technologies. have been introduced into the S.B.L. to increase the efficacy of biomolecule separation such as electrolysis, ultrasound and bubbling [9, 12].

The L.B.S. application for extraction was applied, purification, and separation of carotenoids, triglycerides. and proteins, from microalgae [2, 13]. This analysis paper targets for mental knowledge summarization and previous L.B.S. studies to isolate and purify different biomolecules.

This evaluation starts with the L.B.S. concepts, following the different forms of biphasic structures. Latest works on advanced technologies including bubble- and ultrasound, The measurement and the evaluation of electricity-assisted L.B.S.

Further detail on quantification (i.e., separation efficiency, partition coefficient, recovery yield, and selectivity) and Sections 2 and 3, the L.B.S. composition was tabulated with supplied references. The readers will understand the advancement of L.B.S. technologies in each section of this study. Also, the main parameters to the extraction in L.B.S. are addressed, in-depth with the advantages and inconveniences of L.B.S. Furthermore, L.B.S.' future opportunities and obstacles there also been present. This appraisal article has a big effect on the purification and extraction of liquid liquids for different products in the biotechnological, which are an important tool for researchers in biomolecular extraction through L.B.S.

## 2. Liquid Biphasic System

Lengthy implementation of classification called liquid biphasic. (L.B.S.) or usually recognized a watery two-phase system (A.T.P.S.) is the latest trend of research into separation and purification technologies and for biomolecular separation. Martinus Willem Beijerinck blended gelatin with an aqueous starch solution in 1896 accidentally and developed the creation of an interconnected layer between the two water solutions [14, 15]. Per-Åke Albertsson was developed this concept of L.B.S. as a study of the separation technique, which discovered it by combining two di-treatment polymers - e.g. polyethene glycol and dextran [14], 16, 17]. The concept was then applied to a range of generations of researchers and engineers working in industrial biotechnology. The diagram of L.B.S. principles is shown in Fig. 1.

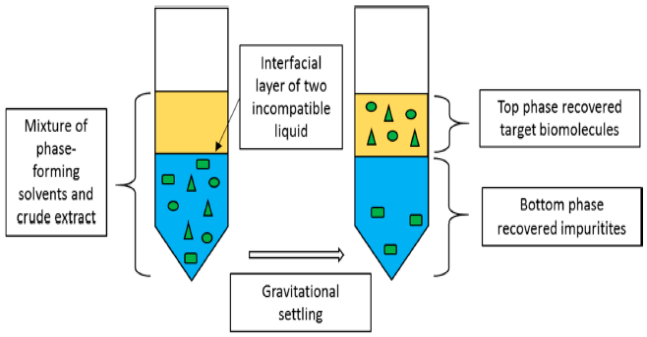


Fig. 1: Scheme figure of the mechanism in the liquid biphasic system (L.B.S.).

L.B.S. is renowned for extracting biotechnological substances like lipids, carotenoids and proteins. [1, 18, 19]. Comparison to conventional extraction by organic solvent, the speciality L.B.S. Techniques are the arrangement of phase-formation components that contain massive quantities of water but maintain a little interfacial layer that divides the two stages. It could either be expended to extract protein from cell remains. Likewise, L.B.S. can divide the mark biomolecules into Extraction Top Phase [20].

L.B.S. is widely used to isolate and purify BIMs sensitive to ionic conditions and have a low ionic system [16]. However, due to the lack of compatibility, polymer-based L.B.S. has ignored The costly components for phase forming and viscosity is very high y mechanism between high ionic strength biomolecules. To replace traditional polymerized L.B.S., L.B.S. was further produced using phase founding constituents include deep-eutectic solvents, alcohol, surfactants and ionic liquids.

Selectively partitioning the L.B.S., biomolecules can be extracted in a single-phase process compared with conventional techniques of extraction that involve many operational steps. L.B.S. has environmentally safe, affordable, and easy-to-scale, fast, and efficient biomollecular Recovery and purification techniques.

The importance of the physical and chemical interaction during the partitioning is essential to consider during the planning stage L.B.S. treatment [21].

Availability of different system-compatible parameters Properties is essential for optimum extraction, Recovery and cleaning. In selecting different parameters (e.g. the absence of biphasic systems, crystallization and salt precipitation,), an assessment of interactions is also important as this may

influence the findings. Finally, the test represents the Recovery and purity of any process parameter [21]. To replace the traditional LBS-based polymer-polymer base, the production in L.B.S. was used with different phased forming materials, such as alcohol, ionic liquids, eutectic dee solvents and surfactants. Ex is allowed by selective L.B.S. partitioning [22].

Comprehensive research on the link line size. and the gradient tie line. was previously assessed by Iqbal et al. [23].

Binodal. curves could be developed with three methods., including cloud point, turbid metric titration, and method for specified phase diagrams Node determination. [22, 24, 25]. Also, the coefficient L.B.S. partition was built to measure the balance among bottom and top in the L.B.S. process. Chemistry of these combinations in the L.B.S. forming process still lacks studies that are a void to be filled. Factors that a text can be manipulated using electrical, hydrophobic phasing, bio-specific and molecular scale and surface areas can also be manipulated to know its chemical and physical possessions.

## 2.1. Polymer Based L.B.S

The traditional polymer-based. L.B.S. generally consists of two Polymers like P.E.G. and P.E.G. - salt combos like citrate-based, sulfate and phosphate for components of the phase formation process P.E.G. is the most common polymer-based L.B.S. The aim of polymer-based L.B.S. is for non-ionic ionic characteristics to be consistent with low ionic biomolecules composition in an ionic setting. [16].

Furthermore, the polymer-based phase forming portion Could be retrieved and reutilized for the following mining, and This lowers costs of the phase formation part polymers [26].

The L.B.S., based on polymers, is widely used in protein extraction due to the hydrophobic and hydrophilic interaction of the L.B.S., which is based on polymer/salt. Even so, the concentration of salt solution must be controlled, since high salt concentrations can denature and harm the fragile protein. Most of the works have been done using traditional polymer-based L.B.S. as a stage-forming portion, to overcome the weakness of polymer-based L.B.S., include reprocessing and high viscosity [27, 28].

Random thermal separation polymers are propylene oxides (P.O.) of di-blocks and tri-blocks and copolymers of ethylene oxide (E.O.) and [29].

The low cloud temperature (47 C0) of thermo-separating polymers remains sufficient so that a mark protein can be retrieved from the polymer through a temperature-induced phase separation [30].

In general, the target protein is extracted from the polymer by an extraction procedure, include ultrafiltration, diafiltration and crystallization. However, the mechanisms of the polymer phase forming factor for biomolecular Recovery are still poorly understood. This indicates a void in the basic concepts of this L.B.S. extractive technique for prospective researchers. Several experiments with cyclodextrin glycosyltransferase (CGTase) from Bacillus cereus have been performed.

## 2.2. Organic Solvent-Based. L.B.S.

Liquid biphasic. is made up of various water mixing alcohols (like 2-Propanol mathyl alcohol, 1-propanol and ethanol). This L.B.S. was used to resolve the curb of polymer-based L.B.S. to boost biomolecular retrieval from phase formation compound [31].

Alcohol can quickly regenerate the biomolecules by evaporating the drink during the first step of using the phase formation elements. Recent studies have also shown a greener method For microalgal carotenoid extraction and restoration using alcohol of food quality, including ethanol and 2-propanol instead of traditional polymer L.B.S. [32].

The part that forms a step can also minimize process costs by using the rotary evaporators to recycle and reuse the alcohol during the following extrac method. Notwithstanding its gains, the difficulties of using alcohol, particularly, are toxic and risky for the environment because of the phase-forming component. Ooi et al. have published a Burkholderia pseudomallel lipase purification analysis using L.B.S. based on alcohol/salt [19]. In L.B.S. 16 per cent (w/w) of the best lipase recuperation was achieved The purification factor is 13.5 for both 2-propanol,4.5% (w/v) sodium chloride and 99% and 16% (w/w) potassium phosphate the attendance of alcohol in L.B.S. didn't inhibit either the distilled lipase's enzyme activity [33].

While concentration salt increases, they may produce an electrostatic potential which barred biomolecules from negative charging strongly. In the top step, the water-scattered alcohol results in a fast recovery rate. The study of alcohol / salt-based L.B.S. in the Recovery of human recombinant Interferon-to-2B (IFN-to-2b) intracellular from Escherichia coli has been conducted by Lin et al. [34].

Several alcoholic combinations (ethanol., 1-propanol., and 2-propanol.) and salt (ammonium sulphate., dipotassium hydrogen phosphate. and monosodium citrate.) were made. The best purification conditions for IFN-to2b purification with 16.2 have been identified as the L.B.S. consisting of 18 per cent (w/w) propanol and 22 per cent ammonium sulfite (w/w) in 1 per cent (w/w) sodium chloride. 74.6 per cent return. Because of its greatness pH level in the system and I.F.N. purification factor its high, ammonium sulfate salt was selected. With the increased pH environment within L.B.S., contaminant protein and protein from the I.F.N. were divided into the top-phase of water mixed alcohol. Mainly because of the harmful charging protein, which tends to break up and rejects the salt-rich lower stage [34].

Recent research was carried out with a recyclability test using the Padina australis and Sargassum binders 1-propanol. And the ammonium. Sulfate framework [35] 1.59 from Sargassum. Binder and Padina australis, with a purification factor of 2.49 % and the highest. Recovery of phlorotannin was 76.1 per cent and 91.67 per cent. After two cycles of the method, the reliable Recovery of phlorotannin was achieved. The use of alcohol-based L.B.S. for biomolecular extraction has shown a feasible and eco-friendly solution.

### 2.3. Ionic Liquid-Based. L.B.S.

An alternating organic compound and non-volatile green solvent were a recent research pattern through the use of ionic fluids (I.L.s) in downstream processes. Researchers have paid a great deal of attention to their excellent characteristics, for instance, thermal stability, low vapour pressure and melting point [36 ,37].

I.L.s have physical-chemical tuning characteristics of anion and cationic ions [38]. Ammonium cation, Choline cation, quaternary ammonium, or guanidium cation and phosphonium typically constitute the cationic component of the I.L. The anionic component is made up of eco-friendly sources including carboxylic acid, amino acid and organic buffers. Thus, it would be advantageous to replace I.L.s as the phase forming factor in the L.B.S. purification from the complex raw extract of unique target biomolecules [39].

Also, I.L.s for various applications have been used, such as electrical elements (e.g. sensors, fuel cells and batteries), fuel additives, CO2 detention and lubricants. The reactant costs for I.L.'s synthesis are costly.

Therefore, it is critical that I.L. is recyclable and reusable to make ILs-based L.B.S. more feasible and useful for the next extraction procedure in the bioprocessing industry. An analysis by Ostadjoo et al.bshows its possible landscapes in the area of lignocellulose biomass disbanding and biopolymer dispensation in the green and environmentally safe, 1-ethyl3-methylimidazole acetate (C2mim) (OAc) [40 – 42]. However, research related to their toxicity is still in progress and Environmentally outgoing for the escalation of these I.L.s, particularly I.L.s dependent on imidazole and pyridinium. We suggested that these I.L.s be further developed to mitigate their toxicity in various applications by substituting environmentally outgoing anionic components such as biological buffers, carboxyl acid, and amino acid.

Gutowski et al. have suggested that a biphasic system's development would result by fraternization imidazole dependent I.L.s and Cosmo tropic salt (i.e. K3PO4) [43].

This research was concerned with the phase-removal activity of IL-based L.B.S. Du et al. performed a single study on the extraction of protein with IL-based L.B.S. (2007). With a circulation of 10 and an improvement factor of 5, the investigators effectively extracted the urine protein [44].

Furthermore, NG et al, (2014) deliberate the decontamination of the Bacillus cereus fermentation broth from the CGTase in I.L./salt L.B.S. with the conformation of 35% (w/w) of (Emim) BF4 and 18% (w/w). The addition was 3% (w/w) of NaCl. The results showed that C8MIM- Br/salt L.B.S used the limit. Extraction e Facilities of C.P.C. were 99.0 per cent, 36.6 and 5.8, respectively. C.P.C. division coefficient is-based L.B.S. showed a feasible e-separation method for extracting diverse biomolecules from complex crude extracts. A current analysis examining the protein subdividing of ILs-based L.B.S. consisting of Iolilyte 221 PG and citrate salts has shown that this is feasibility but complex depending on different factors such as concentrations [45, 46].

The pH, temperature, chemical nature and ionic strength of the target biomolecules phase-forming element [47]. Therefore it favours a device pH of (a cumulative pH of 6.50) above the isoelectric protein limit. Proteins are negatively charged particles. The coefficient for tie-line length amid 38% and 76% was reference points for particular proteins to be partitioned in the top step (for example, bovine serum albumin and rubisco).

### 2.4. Deep-Eutectic-Solvent-Based L.B.S.

D.E.S.s are defined as an I.L. subclass due to their similarity with I.L.s' physical and chemical characteristics

[48]. Deep Eutectic Solvent behaviour is powered by hydrogen bonding, while ionic interactions dominate I.L.s [49]. Compared to I.L.s, which are poisonous and non-biodegradable, D.E.S.s are more environmentally friendly (e.g. imidazole and pyridinium-based I.L.s). The D.E.S.s synthesis is mixed between the accepters of hydrogen bond (e.g. phosphonium salts and quaternary ammonium) and the donors of hydrogen bond (such as carboxylic acid, amide and alcohols). D.E.S.s have a significant benefit in terms of relocation charges responsible for the melting point decrease compared to the raw material [50].

These D.E.S.s have overcome the flash gap created by the use of I.L.s, for instance, expenses and multifaceted synthesis routes. D.E.S.s are equally impressive in many fields, particularly in L.B.S. because it has similar features like I.L.s and shows certain distinctive features like ease of amalgamation, low price, and value for industrial use [51].

Choline chloride (ChCl) is a quaternary saline convention apply for the synthesis of D.E.S. D.E.S.s in ChCl have the same benefits as I.L.s, as well as their excellent biodegradability and low toxicity [52].

The extraction of bovine serum albumin (B.S.A.) by Zeng et al. was performed using four types of D.E.S.s: chloride (TMACl), choline chloride (ChCl), urea-tetrapropylammonium bromide (T.P.M.B.R.)-urea, and check-methyl urea [53]. Under optimal L.B.S. conditions, the extraction of 0.7 g mL a DES-based L.B.S. for protein extraction could reach up to 100.5 per cent for B.S.A.'s Chil-urea and 2,0 mL of dipotassium phosphatic, K2HPO4. Unfortunately, because of the hydrophilicity characteristic of D.E.S., ES-LBS target protein could not be derived from this analysis.

Pang et al. investigated similar work with di T.E.G.R.E.N.T. D.E.S.s consuming DES-based L.B.S. calm of choline chloride-polyethene glycol (ChCl-PEG or D.E.S.) and sodium carbonate for precise protein extract (i.e., B.S.A. and papain) [52]. ChCl-based D.E.S. has to be prepared to mix up to 100 cents to form a uniformly colourless with two mixtures 0.68 g mL to 1 ChCl and 0.1 g mL to 1 P.E.G. 2000 with a molar ratio of 20:1. The study found that the DES-NasCO3 L.B.S. had successfully obtained a high B.S.A. (95.16 per cent) and papain extraction under optimal conditions (90.95 per cent). The target protein has been extracted by extracting 1 mL D.E.S. top step, and ammonium (NH4)2SO4 and 0,45 mL ethanol Forms a new L.B.S. were introduced. It has been creating that the degree of back extraction is reduced by cumulative the concentration of the salt-rich bottom meditation. A modified DES-based L.B.S. was used to extract Cynomorium solarium Rupr ursolic acid with ultrasonic help [54].

This approach was contrasted with the traditional method of ultrasound extraction. The ursolic acid recovery rate was close. The presence of L.B.S., however, leads to more excellent ursolic acid purification. The retrieval rate of ursolic acid was 22.10 € for 0.42 mg/g, with purification factor 42.41 € for 0.84% compared to traditional extraction ultrasonic-\ where recovery yields were just 20.9 mg/g and a little decontamination factor of 20.17 € for 0.77% for the recovery factor.

## 2.5. Surfactant/Detergent-Based L.B.S.

The phase forming part of a typical polymer-based L.B.S. remains the surfactant-based L.B.S. transformation. The L.B.S. is designed on surfactants once mutually anionic and cationic surfactants are divided hooked on two insoluble, high concentration fluid phases with a high C.M.C well as a convinced molar ratio of the anionic and cationic surfactant formulation. This new method of surfactant-LBS has attracted significance, mostly because of the amalgamation process. There are various types (i.e. spherical micelles, tubal micelles, or vesicles) [55].

The Surfactants-based L.B.S. system is based on a cloud point extraction system (C.P.E.) that heats the non-ionic surfactant above the cloud's warmth and causes de-hydration of the detergent for phase separation [56].

The surfactant L.B.S. has rich one stage in surfactants and the second is a phase diluted in surfactant. The organic contaminant is separated into the degree with fatty surfactant and is then aggregated and condensed. The contaminant's existence of a limited volume of remedied water stays in the surfactants' dilute process. L.B.S. is widely used for the isolation, solubilization and partitioning of membrane-bound compounds, by hydrophobic and amphiphilic molecules. Triton X-100 and 20% (w/w) xylitol were used as surfactant-based L.B.S. composed of 24% (w/w) and lipase purification from pumpkin seeds [57].

The findings show that the L.B.S. dependent on surfactants could break the lipase hooked on the superior phase with fatty surfactant and leave the contamination in the lowest step is heavy in xylitol. The optimized solution had recovered the enzyme successfully 97 per cent and purification factor with 16.4. This study also showed that up

to 5 runs with a high percentage of 97% recovery could be recycled for the regeneration portion. Therefore, the phase-component retrieval after the fifth cycle was, significantly decreased, due to impurity accumulation in the phase-component.

Sankaran et al. used surfactant and xylitol to manufacture surfactant dependent L.B.S. in optimum conditions of 25% w/w of xylitol content, 15% (w/w) of TritonX-100, 80% w/w of crude Lipases, 4 mL of a high stage, 35 mL of a low setting, pH 7 and 15% of a standard set of surfactant based L.B.S. extraction. The maximum extraction of lipase and e leisure was 3.63 and 86.46 per cent at min flotation times [58]. This is an outstanding technique because of both L.B.S. surfactant extraction recycling components, so the biotech industry might use this revolutionary method to extract other biomolecules realistically and feasibly. Table 1 summarizes biomolecular extraction using different methods of forming phase in L.B.S., see Table 1.

## 3. Innovation Technologies Combined with L.B.S.

### 3.1. Bubble-Assisted LBS

Liquid biphasic initiation (L.B.F.) as a mixture of L.B.S. and solvent sublation (S.S.) is used to allow the adsorption during the separation of target biomolecules, by biphasic medium consisting of organic resolvent and saturated salt solution bubbling by air bubble (like oxygen and nitrogen). [8] (refer to Fig. 2-a). The L.B.F. technique theory is the surface-active biomolecular phenomenon getting an air bubble surface sorption mechanism. In an organic solvent phase, bubbles emerge and dissolve above the Water solution within the arrangement [11]. With bubble support in L.B.S., the bubble transport adsorption mechanism could intensively be improved, and this method is viable for biomolecular separation and extraction. Figure 2a displays the configuration of the L.B.S. assisted bubble.

### 3.2. Ultrasound-Assisted L.B.S.

The most critical mechanism for higher extraction and Recovery is cell disruption in biotechnology processes. Ultrasonic L.B.S. is a technique that researchers have widely recognized due to its cell disruption properties [62, 63].

The benefits of L.B.S. are low running costs, decreased energy usage and short periods [64]. The ultrasonic radiation is based on high cavitational forces created by ultrasound wave cavitation bubbles and mechanical shears, which have improved cell disruptions for biomolecules extraction [65].

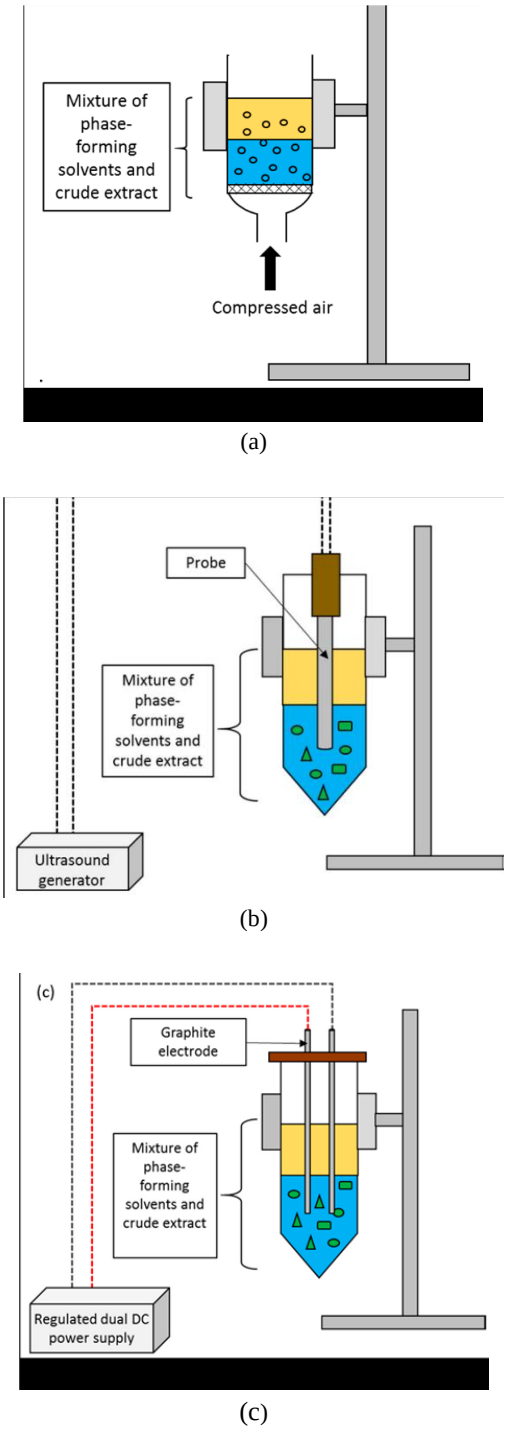


Fig. 2: Schem of (a) bubble-assisted L.B.S., (b) ultrasound-assisted L.B.S., and (c) electrical-assisted L.B.S. [86].

The ultrasound-assisted L.B.S. scheme setup is shown in Fig. 2-b. A built-in ultrasound and L.B.F. system were accustomed to associating relief protein retrieval in the extraction solution [66].

Table 1: Biomolecular extraction using different methods of phase formation in L.B.S. materials.

| Type of LBS | Composition of LBS | *Type of feedstock* | Biomolecule | Selectivity | Partition Coefficient, K | Purification Factor, PFT | Recovery Yield (%) | Ref. |
|---|---|---|---|---|---|---|---|---|
| Polymer/salt-based | EOPO 3900 and two phosphate salts | *Bacillus cereus cyclodextrin glycosyltransferase* | Cyclodextringlycosyltransferase (CGTase) | 3.19 | 17.54 | 5.30 | 87.0 | [31] |
| | 18% (w/w) PEG 8000 and 7.0% (w/w) potassium phosphate salts | *Bacillus cereus cyclodextrin glycosyltransferase* | Cyclodextringlycosyltransferase (CGTase) | - | - | 21.8 | 97.1 | [32] |
| | 15% (w/w) PEG 600 and 16% (w/w) dipotassium phosphate | *Amauroderma rugosum* | Lignin peroxidase | - | - | 1.33 ± 0.62 | 2.18 ± 8.50 | [33] |
| Alcohol/salt-based | 18% (w/w) 2-propanol and 22% (w/w) ammonium sulfate, (NH4 )2SO4 | *Escherichia coli* | Interferon (IFN)/ Glycoproteins | - | 0.82 | 16.24 | 74.64 | [34] |
| | 16% (w/w) 2-propanol and 16% (w/w) potassium phosphate | *Burkholderia pseudomallei* | Lipase | 287.5 | - | 13.5 | 99.3 | [19] |
| | 33.5% (w/w) of 2-propanol and 10% (w/w) ammonium sulfate | *Padina australis* | Phlorotannin | - | - | 2.49 | 76.1 | [35] |
| | 25% (w/w) of 2-propanol and 12.5% (w/w) ammonium sulfate | *Sargassum binderi* | Phlorotannin | - | - | 1.59 | 91.67 | [35] |
| Ionic-liquid based | 35% (w/w) of (Emim)BF4 and 18% (w/w) of sodium carbonate Na2CO3 | Fermentation broth | Bacillus cereus cyclodextrin glycosyltransferase (CGTase) | 9.66 | - | 51.0 | 96.00 | [45] |
| | C8MIM-Br and tri-potassium phosphate | *Spirulina platensis* | C-phycocyanin (CPC) | 5.8 | 36.6 | - | 99.00 | [46] |
| Deep-eutectic solvent based | 0.7 g mL−1 ChCl-urea and 2.0 mL dipotassium phosphate, K2HPO4 | Protein | Bovine serum albumin (BSA) | - | - | - | 99.6 99.7 and 100.0 BSA | [53] |
| | Choline chloride and PEG 2000, molar ratio of 20:1 | Protein | Bovine serum albumin and papain | - | - | - | Bovine serum albumin (95.16), papain (90.95) | [52] |
| | 36% (w/w) ChCl-glucose and 25% (w/w) dipotassium phosphate, K2HPO4 | Ursolic acid | Cynomorium songaricum Rupr. | - | - | 42.41 ± 0.84 | 22.10 ± 0.44 mg/g | [54] |
| Surfactant/detergent based | 24% (w/w) Triton X-100 and 20% (w/w) xylitol | *Cucurbita moschata* | Lipase | - | - | 16.4 | 97.0 | [57] |
| | 25% (w/w) of xylitol concentration, 15% (w/w) Triton X-100 | *Burkholderia cepacia* | Lipase | 262 | - | 2.56 | 86.46 | [58] |

The L.B.F. with ultrasonic help has better benefits than L.B.S. with ultrasonic assistance, powered by its refined coefficient concentration and the best departure. Much of it was because air bubbles were present which permitted the

adsorption from lower to top phase of surface-active proteins. As a result, the separation and recovery rates were improved. Extraction with ultrasound was also commonly used for Lignocellulose biomass cell disruption from plants [67].

Cistanche deserticola stems are ultrasonically aided in the extraction of phenyl ethanoide glycosides (e.g. acetonide and echinacoside). 27.56 and 30.23 mg/g were recovered successfully in L.B.S. [68]. It showed that ultrasonic L.B.S. is an eco-friendly, economical way to extract and enrich lignocellulose biomass biomolecules. Even so, while working with ultrasonic irradiation, it is essential to track process temperatures. The considerable shear strength created by sonic wave cavitational bubbles would make an enormous temperature process that would deform or distort the mark biomolecules, subsequent in a disapproving low extraction rate. The isolation and extraction of antioxidants like xylooligosaccharides (sugar) and phenolic compounds from wheat was also research that was supportive for the application of ultrasonic L.B.S.

### 3.3. Electricity-Assisted LBS

A promising mild cell decay technique for biomolecular Recovery, L.B.S. (Fig. 2-c), is an electricity aided approach. Electrical therapy, for example, the pulsed electric field shows a conceptualization in the direction of magnitude of ms or/or bis in the initiation order of short electric pulses subject to the load in the membrane that is so levitational to rearrange or interfere with the membrane, resulting in pores. This is also called electroporation. After all, an excellent condition is required as P.E.F. depends on the therapy strength and unit properties that pore-forming is revocable or irreversible [69, 70].

The mass transfer energy of the device also increased with P.E.F. treatment. In combining both P.E.F. and L.B.S., the extraction of a treated sample will be an advantage. This combination is called electropermeabilization, which increases the discharge of intracellular composites from the treated sample by using electrical or extractive solvents [71]. Besides offering greater extraction and bioscience, electrical dealing out also provides a lime solution in biotechnology industries. Lam et al. [73]. Description of the advanced biomolecule extraction technologies incorporated with L.B.S shows in Table 2.

## 4. Crucial Factors Affecting LBS

### 4.1. Polymer Molecular Weight

In L.B.S. established on polymer salt, the polymer phase portion is important because of the division of target biomolecules with different hydrophobicity degrees. The hydrophobicity increases with the increased molecular weight of a polymer even due to the long monomer hydrocarbon chain. This allows the free volume of the polymers' top stage to be decreased and the desired biomolecules to be separated into the lower stage. On the contrary, a small molecular polymer would minimize the mark biomolecules since it is partitioned in the top polymer-rich process with contaminant proteins [31, 32]. And so it is necessary to choose an optimal condition for the Polymer hydrophobicity to achieve optimal Recovery of target compounds.

The molecular weight effect of reaction for the Recovery of cyclodextringlycosylic transferase (CGTase) in Bacillus cereus was mentioned with polymers like P.E.G. and potassium phosphate salt. [32]. In this study, the L.B.F. method was used for CGTasa's extracting in a continual crude extract ratio of 1,0:30 to a volume of di-servant M.wt of P.E.G. (for example, PEG 4000, 6000, 8000, 10 000, and 20 000). The overall purification factor of CGTase retrieval of 7.26 and 97.1 per cent consisted of 18.0 per cent P.E.G. (w/w) 8000 and 7.0 per cent L.B.S. potassium phosphates. Well PEG 20,000 displayed a decontamination factor of 2.25 and 3.23, one-to-one, compared to the lowest M.wt, PEG 4000 and the highest M.wt. This suggested a more viscous process causing in a reduction in the free size of high-level polymer polymer-rich phase resulting from volume elimination and withdrawal of a low-molecular polymer (PEG 4000) into the polymer-rich top phase. In certain situations, based on product quality and optimizing the divisional condition, it is suggested to reduce molecular weight.

Furthermore, one of the disadvantages of using P.E.G. and salt in L.B.S. as phase components is that most are not recyclable the following step. The non-biodegradable portion of phase formation brands the total L.B.S. unfavorable for environmental reasons the pollution and costs rise [74]. Another related research substituted thermo-separate polymer (E.O.P.O.) as a phase constituent for decontamination and remediation of CGTase to increase the recyclability of the phase-forming components throughout L.B.S. [31].

Table 2: Biomolecular extraction using different forms of L.B.S. advanced advance technology.

| Assisted Technology | Composition of LBS | Type of Assisted Employed | Type of Feedstock | Biomolecule | Time | Extraction Efficiency, E (%) | Partition Coefficient, K | Recovery Yield (%) | Ref |
|---|---|---|---|---|---|---|---|---|---|
| Bubble-assisted LBS or Liquid biphasic flotation (LBF) | 50% (w/w) of 1-propanol and 250 g/L ammonium sulfate salt, (NH4 )2SO4 | Flotation system (compressed air 0.5 bar) | *Burkholderia cepacian* | Lipase | 30 min | 88.0 | - | 93.27 | [62] |
| | 100% ethanol, 20 mL of 200 g/L dipotassium phosphate K2HPO4 | Flotation system (compressed air 0.5 bar) | *Hylocereous polyrhizus* | Lipase | 15 min | E for peel and flesh were 88.361 ± 1.708%, 94.886 ± 0.060% | K value of peel and flesh were 24.168 ± 2.949, 21.195 ± 1.030 | Recovery for peel and flesh were 95.488 ± 0.213, 94.886 ± 0.060. | [8] |
| | 0.5 g/mL PEG 1000, 35 mL of 0.40 g/mL trisodium citrate Na3C6H5O7 | Flotation system (30 mL/min flow velocity) | Whey | a-lactalbumin | 42 min | 87.54 | - | - | [64] |
| Ultrasound-assisted LBS | 100% (w/w) acetonitrile and 200 g/L glucose solution. | Ultrasound irradiated for 5 min of 5 s ON/10 s OFF pulse mode and flotation system | *Chlorella vulgaris* FSP-E | Protein | 5 min | 86.38 | - | 93.33 of protein recovered | [6] |
| | 20% (w/w) ethanol and 23.5% ammonium sulfate | Ultrasound irradiated (300 W, 37 min) | *Cistanche deserticola Y. C. Ma stems* | Phenylethanoid glycosides | 37 min | Echinacoside and acteoside were 5.35 and 6.22 mg/g dry weight | - | Echinacoside and acteoside were 27.56 and 30.23 mg/g dry weight | [71] |
| | 24.3% (w/w) ethanol and 23.8% (w/w) ammonium sulfate | Ultrasound irradiated (30 Hz, 500 W, 10 min), | Wheat chaf | Xylooligosaccharides (sugar) and phenolic compound | 10 min | 72.79 ± 3.98 | 3.91 | Recovery of sugar and phenols were 16 mg/g and 2.67 mg/g | [72] |
| Electricity-assisted LBS | Without LBS | PEF treatment (5–7.5 kV/cm, 1–10 pulses and a pulse length of 0.05–0.2 ms) | Cell wall *C. reinhardtii* strain (cc-124) and cell wall *deficient mutant* strain (cc-400) | Protein | 10 min/pulse | - | - | Cell wall strain (cc-124) and cell deficient (cc-400) with average protein yield of 31 ± 6 protein and 11 ± 3 protein. | [77] |
| Bubble-assisted LBS or Liquid biphasic flotation (LBF) | 100% (w/w) ethanol, 200 g/L of dipotassium hydrogen phosphate (K2HPO4 ) | PEF treatment (3 V of voltage using graphitic electrodes) and 15 min flotation system | Peel and flesh of *Hylocereus polyrhizus* | Betacyanins | 15 min | E for peel and flesh were 98.383 ± 0.215 and 96.576 ± 0.083 | K for peel and flesh were 100.814 ± 7.324 and 24.883 ± 1.052 | Betacyanins concentration (98.383 ± 0.215 for peel and 96.576 ± 0.0083 for flesh | [76] |

After recycling, E.O.P.O. recovery checked the viability of recyclable characteristics by more than 80 per cent. The simplicity, speed and recyclability of the L.B.S. process is proof that the Recovery and purification of target biomolecules are the promising and attractive solution.

### 4.2. Sort and Strength of Alcohol

The total recovery production from the target biomolecules will be affected by tendency-nerine alcohols (e.g., mathyl alcohol, 2-propanol, 1-propanol and ethanol). The contact on organic solvent's active site helps hold the enzyme open and predicament the mark amalgams to the highest phase of alcohol. The more significant alcohol content is beneficial because it will increase the target biomolecules' buoyancy and constancy against the boundary.

In an experiment with caffeine from coffee seed extraction, Santos et al. reported on the possibility of manipulating the division of caffeine into either the top alcoholic phase or the salt-rich lower phase [75]. To isolate caffeine in the top step from alcohol, an increase in 2-propanol concentration resulted in increased caffeine -water connection. This would promote biomolecules to be separated in the highest process of alcohol. In the meantime, mathyl alcohol has been chosen to cave in the salt-rich lower process. The goal of selecting mathyl alcohol was to increase the propensity of caffeine split in the salt-rich bottom.

Current research into the Recovery from Chinese liquorice root (Glycyrrhiza uralensis Fisch) of glycyrrhizic acid (G.A.) and liquidity (L.Q.) has shown that 87% GA and 94% L.Q were effective in top-stage alcohol-rich conditions of 25% (w/w) ethanol and 30% (w/w) K2HPO4 in L.B.S. [76]. The dependency of 14-34% (w/w) of alcohol concentrations and removing dementia and partition coefficient were examined. The extraction and partition coefficient to bear the same for both G.A. and L.Q. Biomolecules are increased by the rise in alcohol concentration to 26 per cent in the system. However, as the alcohol concentration rose to 34 per cent, the extraction and partition coefficient divorce decreased. This resulted from many water-soluble spirits interacting with water molecules in the alcohol-rich top phase, which separated the biomolecules into the salt-rich bottom phase [77]. The word "volume exclusion" was also called e complete. The selection of alcohol, in general, depends primarily on the target biomolecules of the complex essential oil. The respective physical-chemical properties of each target biomolecule are critical to a particular optimum. L.B.S. separation and extraction specifications.

### 4.3. Salt Concentration

In L.B.S., salt type choice is crucial as a phase-forming factor because the solubility and the interaction of the target biomolecules can be significantly affected. The water's surface tension will increase when the salt is applied to a solution and thus increase the hydrophobic collaboration between protein and water [77]. Few studies demonstrated that high salt saturation causes a decrease in the target biomolecules' solubility due to increased salt-out ability [20, 36]. Lu et al. stated that Gibbs has been reliant mainly on the free energy of salt hydration for the capacity of salt solution and hydrophilic alcohol for the production of biphasic systems [79]. The modification of biomolecules partitioning's environmental processes and actions are used by the salt ingredients [78]. The L.B.S. salts were focused on their capacity to help the hydrophobic interaction of biomolecules [79]. The rise in salt levels helps to improve the Recovery in protein. According to Phong et al., salting-out was confirmed to be occurring in the presence of ions found to decline protein solubility in the salt-rich lower level [9].

### 4.4. pH System

Due to a shift in charge and solvent properties, the subdividing of mark biomolecules could be a method disassembled by L.B.S.' pH framework. Once the pH is larger than the pI and positive if the pH value is less than the pI, the target biomolecules' net load becomes negative. pH and pI are the same If the net bag is equal to zero [80]. A joyous dipole moment could increase the partition coefficient; hence, it favours dividing the target biomolecules into the polymer-rich top phase with a negative charge [81, 82]. In contrast to traditional PEG-based L.B.S., Polypyhydroxyalkanoate (P.H.A.) partitioning in the term separation-based L.B.S. of Cupriavidus necator H-16 was shown to adjust the pH method [83]. P.H.A. showed a washing and recovery factor of 3.67% and at pH 6, which was enhanced than traditional PEG-based L.B.S. with zero regeneration of P.H.A. in a high stage at the pH level of less than 7, 63.5% respectively. But the P.H.A. recovery rate abruptly decreased by 46.4% when the device's pH was changed to 8.0-8.8. Another B.S.A. extraction study found that the di targeted compound's net charge could change with the di residential pH values [84].

### 4.5. Temperature.

Temperature impact is reliant on the type and stability of the phase-forming constituents used by the L.B.S.A temperature adjustment as well as influences an L.B.S. interface's viscosity and density. For extreme recoveries and subdividing of the mark biomolecules, the optimum temperature was often used within the range of 20 to 40 ̈a C. The temperature impact of the C.P.C. extraction from Spirulina platensis microalgae has been studied. The greatest extraction elegance up to 99.0% has been obtained in the vicinity of the 308 K temperature range [46]. Down to 298 K, it was found that the rate of C.P.C. rebound decreased and that a low rebound was resulting [52]. However, studies have revealed that both the B.S.A. and the papain extractions fell with the temperature rise. The temperature rise induced this phenomenon to inhibit the surface water interaction with an amino acid with protein leading to lower protein extraction. [85]. Thus, the temperature outcome could be accounted as the biomolecular extraction efficiency depends on the temperature variations in the L.B.S.

## 5. Upcoming Outlook and Encounters of L.B.S. Application

Liquid biphases is an advantageous departure method to remove useful biomolecules. The L.B.S. is used to examine the chemical characteristics and comportment of target biomolecules. After all, designing L.B.S. as an alternative method of separating and purifying large-scale industries meets some significant challenges that must be tackled again. The splitting up of the biomolecules into the top step coefficient (K) based on critical parameters is one of the biggest concerns about the L.B.S. Each of the main parameters is time-consuming and crucial to establishing the optimal conditions for the optimum cleaning and restoration of biomolecules. Also, the biocompatibility, dangers and biodegradability of phase-forming components should be selected. Consequently, it favours a more ecological and highly biodegradable replacement phase-forming portion in the aquatic environment.

The extraction of biomolecules from natural sources is another problem to be tackled in L.B.S. Whatever the different studies that report the productivity of L.B.S. in biomolecular removal from natural sources and microbial bouillon fermentation, the biomolecular partition behaviour still needs to be understood, especially once a multifaceted crude feedstock is inserted into the L.B.S. Also, pollutants with destination biomolecules in the crude feedstock can have similar characteristics. During the contact with the extracting medium in the L.B.S., this will cause lower extraction. In the use of L.B.S. for biomolecular Recovery, a lack of awareness of partitioning behaviour remains an obstacle.

Also, there are still unknown technologies for improving biomolecular extraction and the handling of magnets and microwaves incorporated into L.B.S. Implementing these modern advanced technologies will increase awareness of L.B.S. It has also proven to be exciting prospects for biomolecular recoveries and purifications for the latest aided technology using bubble, ultrasound, and electrically assisted technologies. Nevertheless, these sponsored technologies need an in-depth analysis because of the lack of information among its LBS-aligned physicochemical mechanism.

To make the optimum use of L.B.S., the device requires an optimal optimization technique, in which the most significant result can be obtained for different applications. Univariate optimization is one of the most commonly used techniques the optimization or so-called O.F.A.T. is the selection of an individual parameter based on its highest performance following each other. Also, R.S.M. is another technique used for optimizing responses observed in the revisions of the most significant work. This optimization technique consists of a mathematical architecture that lets multiple parameters simultaneously vary from the one O.F.AT. parameter at a time.

## 6. Conclusion

The L.B.S. is a simple, selective method that is scalable and elegant and can be used to treat biomolecules downstream. Even so, the partitioning, sophistication is still desirable to use in business as it is expected. To ensure applicability in biotechnology industries, the problems related to the L.B.S. techniques, including economic viability and comprehension of partitioned performance. More creation with different LBS-integrated technologies is expected to be found in the future. Therefore, L.B.S. is being promoted for the recovery of other high-value bio-based products for industrial applications.

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