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Zwitterionic Buffering Agent as an Alternative to Improve Carboxylic Acid Extraction Performance with 1-Heptanol Solvent

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Abstract. Separation of carboxylic acids by liquid-liquid extraction method using 1-heptanol solvent and the addition of EPPS biological buffer was studied in this research. Liquid-liquid equilibrium (LLE) data on carboxylic acid systems (butyric acid/propionic acid), water, 1- heptanol, biological buffer EPPS at 303.15 K and atmospheric pressure were obtained. Experimental data were correlated well using the NRTL model with the RMSD below 1%. Based on experimental data, the effect of adding EPPS biological buffer can increase extraction performance as indicated by the value of separation factor (S) of butyric acid increasing two times higher, while separation factor values of propionic acid can be increased 4 times higher than the system without EPPS biological buffer. These results can be used as a reference for the design of the extraction column in the carboxylic acid extraction process so that the extraction process can run more efficiently and optimally.

Introduction

Carboxylic acid is an organic compound often used in various industries [1], such as propionic and butyric acid [2]. Propionic/butyric acid have main roles in the food and beverage industry as additives and preservative products. In addition, this carboxylic acid has a special role in the pharmaceutical industry as the main compound for producing medicines, including aspirin and phenacetin, which is very prospective in industrial development [3]. This carboxylic acid (propionic/butyric acid) is usually obtained by chemical reaction or fermentation. In the fermentation process, the carboxylic acid (propionic/butyric acid) produced usually has a low yield. It contains impurities such as high water content (aqueous solution) [4]. Thus, it is necessary to recover, concentrate, and purify carboxylic acid (propionic/butyric acid) from its aqueous solutions [5].

Several methods are used to separate solutes from aqueous solutions, including adsorption, membrane separation, distillation, and extraction [6]; however, each has limitations that reduce their efficiency. A significant challenge is achieving high recovery rates from dilute solutions. For instance, adsorption often shows low selectivity and capacity in complex mixtures, leading to incomplete recovery of target acids [7,8]. Traditional liquid-liquid extraction requires large amounts of organic solvents, raising operational costs and environmental concerns. While membrane techniques are promising, they face fouling and scaling issues that decrease effectiveness and lifespan [9]. Moreover, distillation tends to be ineffective for carboxylic acids due to their higher boiling points and azeotropes complicating the process [7,9]. Research indicates that liquid-liquid extraction (LLE) is more effective for recovering carboxylic acids from aqueous solutions than traditional techniques such as distillation, adsorption, and membrane separation. For instance, Karp et al. [10] reported that using trioctylamine dissolved in a hydrophobic alcohol significantly enhanced the extraction of propionic acid and acetic acid from fermentation broth. This method can improve recovery rates and minimize the loss of valuable acids during extraction [11].

The addition of salt, known as the "salting-out" effect, has been shown to enhance the extraction performance of carboxylic acids in liquid-liquid extraction. Vakili-Nezhaad et al., [12] demonstrated that introducing sodium chloride and potassium chloride into the aqueous phase significantly increased the separation factor for acetic and propionic acids when using isopropyl methyl ketone and isobutyl methyl ketone as the organic solvent. This improvement occurs because salt reduces the solubility of carboxylic acids in the aqueous phase, promoting their transfer to the organic phase. Similarly, Wannachod et al.[13] reported that incorporating salt altered the solubility and tie-line data for formic acid in liquid-liquid extraction systems, improving extraction performance. However, it is important to note that salt is often used in high concentrations, which can affect the optimal pH of the extraction medium, potentially denature biomolecules, and damage equipment. On the other hand, biological buffers, particularly EPPS (4-(2-hydroxyethyl)-1-piperazine propane sulfonic acid), is a zwitterionic sulfonic acid buffering agent that has the potential as a green agent for removing carboxylic acids from its aqueous solutions. It contains a piperazine ring that buffers and maintains pH within 7.3 - 8.7 [14]. Tiwikrama and Altway found that buffers could maintain a consistent pH, which is crucial for the stability of carboxylic acids during extraction [15] .The buffering capacity helps prevent unwanted side reactions at extreme pH levels, thus preserving the integrity of the extracted acids. Biological buffers can provide a more controlled extraction environment, reducing the risk of degradation of sensitive compounds [16].

This research will be carried out on the liquid-liquid extraction of carboxylic acids (propionic acid/butyric acid) from an aqueous solution using 1-heptanol. 1-heptanol is a protic compound that has the potential to be an effective solvent in separating carboxylic acids from water because it is polar and has low vapor. Apart from that, the addition of EPPS biological buffer can improve extraction performance, as indicated by the separation factor and distribution coefficient values. The equilibrium data, organic and raffinate composition obtained will be correlated using Aspen Plus V12 to obtain NRTL model parameters to present a new system for the recovery and purification process of carboxylic acids (propionic/butyric acid) with 1-heptanol solvent and the addition of EPSS biological buffer. Liquid-liquid equilibrium data and model parameters in this system can be used as a reference in designing columns for separating carboxylic acids from aqueous solutions in the chemical industry.

Materials and Methods

Materials. Butyric acid ($\geq$99 %mass), propionic acid ($\geq$99 %mass), 4-(2-hydroxyethyl)-1-piperazine propane sulfonic acid (EPPS) ($\geq$99.5 %mass), 1-heptanol ($\geq$98 %mass), were purchased from Merck Sigma-Aldrich Reagent Pte, Singapore.

Methods. In this research, liquid-liquid equilibrium measurements were conducted for a system consisting of carboxylic acids (propionic and butyric acid), water, 1-heptanol, and the biological buffer EPPS, using a jacketed equilibrium cell maintained at 303.15 K. Different concentrations of carboxylic acids (ranging from 5% to 30% mass) were dissolved in water. Following that, 1-heptanol and the biological buffer EPPS were added according to specific calculations. The solution was stirred for four hours until it became homogeneous, after which a settling phase was allowed to occur for an additional four hours, resulting in the formation of two distinct phases: an acid-rich phase and a water-rich phase.

To maintain the temperature within the equilibrium cell, thermostatic water from a water bath was circulated. Samples from both the acid-rich and water-rich phases were collected and analyzed. Their compositions were analyzed using Gas Chromatography (GC, SCION 436-GC) equipped with a flame ionization detector (FID) and a SCION-5 capillary column to detect propionic acid, butyric acid, and 1-heptanol. Hydrogen was used as the carrier gas at a flow rate of 10 mL/min. The oven temperature was set at 50°C for 10 minutes, then raised to 200°C at a ramp rate of 5°C/min. Both the injector and detector temperatures were maintained at 250°C. Each sample was analyzed three times, and the average value was used to calculate mass fractions. The water composition was studied using Karl Fischer, while the composition of EPPS was analyzed gravimetrically. The resulting equilibrium data were then correlated using the NRTL model and further simulated with Aspen Plus software.

Result and Discussion

Liquid-liquid Equilibrium of Experimental Data. In this research, the liquid-liquid equilibrium measurement of carboxylic acid (propionic/butyric acid) + water + 1-heptanol + EPPS biological buffer with the independent variable, the composition of butyric acid and propionic acid each 5-30% by mass, and with temperature 303.15 K and under atmospheric pressure (101.3 kPa) as dependent variables. Obtained liquid-liquid equilibrium experimental data are presented in **Table 1**. The symbols W_1, W_2, W_3, and W_4 represent the mass fraction of carboxylic acid, water, EPPS biological buffer, and solvent. Meanwhile, superscript I represents the organic phase, and superscript II represents the aqueous phase.

Table 1. Liquid-Liquid Equilibrium of Experimental Data for Carboxylic Acid (1) + Water (2) + EPPS (3) + Solvent (4) System at 303.15 K and101.3 kPa

SYSTEM	***ORGANIC PHASE***				***AQUEOUS PHASE***			
	W_1^I	W_2^I	W_3^I	W_4^I	W_1^{II}	W_2^{II}	W_3^{II}	W_4^{II}
Butyric Acid + Water + EPPS + 1-Heptanol	0.0036	0.0285	0.0000	0.9679	0.0006	0.7648	0.2340	0.0006
	0.0190	0.0219	0.0000	0.9591	0.0044	0.7287	0.2661	0.0008
	0.0727	0.0408	0.0000	0.8865	0.0136	0.7183	0.2672	0.0008
	0.1593	0.0702	0.0000	0.7705	0.0292	0.7406	0.2296	0.0006
Propionic Acid + Water + EPPS + 1-Heptanol	0.0676	0.0272	0.0000	0.9053	0.0180	0.7594	0.2211	0.0015
	0.0801	0.0382	0.0000	0.8816	0.0230	0.7230	0.2520	0.0020
	0.0891	0.0410	0.0000	0.8699	0.0270	0.7104	0.2600	0.0026
	0.0920	0.0437	0.0000	0.8642	0.0370	0.6303	0.3300	0.0027

The addition of EPPS biological buffer in this research aimed to improve the extraction performance of carboxylic acid (butyric acid/propionic acid) + water + 1-heptanol. The mechanism by which a biological buffer promotes phase separation is similar to that of salt, which is called the buffering-out effect. The EPPS biological buffer utilized in this work is zwitterionic amino acid that can exhibit both positive and negative charges inside the same molecule, resulting in a net charge of zero for the complex ion. Furthermore, zwitterions, similar to electrolytes, possess substantial dipole moments and engage in electrostatic interactions with solvents and other charged molecules in the solution [17]. Biological buffer in water exhibits a substantial dipole owing to its zwitterionic characteristics, and it possesses both hydrogen bond donor and acceptor sites. Consequently, it is posited that the biological buffer significantly interacts with water molecules through mechanisms such as hydrogen bonding and electrostatic interactions, compelling the solute molecules to be separated from the aqueous solution into another liquid phase.

Based on the results in this study, the excellent extraction performance is demonstrated by the high composition of carboxylic acid (propionic/butyric acid) in the organic phase. As seen in **Table 1**, the solvent composition (1-heptanol) in the aqueous phase (W_4^{II}) and the water composition in the organic phase (W_2^I) show minimal values, <0.003 and <0.007, respectively. This value indicates that during the extraction process, minimal solvent loss occurs, which can save energy requirements in the product purification process and solvent recycling. Thus, the addition of biological buffer EPPS as an auxiliary material for the separation of butyric acid/propionic acid from aqueous solutions is very prospective because the electrostatic interaction and van der Waals interaction between EPPS and water is more significant than with solvent due to the zwitterion nature of the biological buffer which has a significant dipole moment [18,19].

Distribution Coefficient and Separation Factor. The separation factor (S) represents the ability of 1-heptanol, with the addition of EPPS biological buffer as the solvent, to extract carboxylic acid (propionic/butyric acid) from its aqueous solution. A high separation factor value indicates satisfactory selectivity of a solvent and a buffering-out effect.

$$S=\frac{D_1}{D_2}=\frac{w_1^I/w_1^{II}}{w_2^I/w_2^{II}} \tag{1}$$

The distribution coefficient represents the capability of a solvent to carry the solute from its aqueous solution, where superscript I refers to the solvent phase, II refers to the aqueous phase, w_1 stands for the mass fraction of water, and w_2 stands for that of carboxylic acid. Higher values of (D_2) and the separation factor (S) would be desirable due to a lower required amount of solvent for separation and indicate satisfactory selectivity of a solvent and buffering-out effect [20]. The D and S calculation results from liquid-liquid equilibrium experimental data at 303.15 K and 101.3 kPa are presented in **Table 2**.

Table 2. Distribution Coefficient (Di) and Separation Factor (S) for Quaternary System at Temperature 303.15 K and 101.3 kPa

SYSTEM	W_1^{II}	D_1	D_2	S
Butyric Acid + Water + EPPS + 1-Heptanol	0.0006	6.57	0.037	176.6
	0.0044	4.34	0.030	144.4
	0.0136	5.33	0.057	93.8
	0.0292	5.46	0.095	57.6
Propionic Acid + Water + EPPS + 1-Heptanol	0.0180	3.75	0.036	104.8
	0.0230	3.48	0.053	65.9
	0.0270	3.30	0.058	57.2
	0.0370	2.49	0.069	35.8

Based on the previous results, the highest separation factor values in the system (Butyric Acid/Propionic Acid) + Water + 1-heptanol were 98.65 and 25.96 at room temperature, respectively [21]. Thus, the separation factor values of butyric acid and propionic acid increased up to 1.6 times, and up to 4 times higher with the addition of EPPS biological buffer than the system without the addition of biological buffer. The increase in the separation factor value and distribution coefficient indicates the buffering-out effect of the biological buffer, i.e., the interaction of buffer ions with water molecules. This interaction reduces the solubility of propionic acid in the aqueous phase. This results in a buffering effect that transfers propionic acid to the organic solvent phase [12]. The separation factor values of butyric acid and propionic acid in the system with the addition of EPPS biological buffer respectively reached 57.6-176.6 and 35.8-104.8, as presented in **Table 2**. Meanwhile, the separation factor for butyric acid and propionic acid for systems without using biological buffer EPPS studied by (21) only obtained values of 40.7-96.8 and 7.6-25.8, respectively. The separation factor value with the EPPS biological buffer addition system is higher than without the addition of EPPS biological buffers. This shows that EPPS can increase the separation factor value in the system with the addition of biological buffers. In addition, EPPS biological buffers can improve and optimize the performance of the extraction column and prospective due to the buffering-out effect, creating an extraction process of butyric acid and propionic acid from an aqueous solution more efficient and selective [22]. The benefits of buffering-out compared to salting-out include a reduced risk of equipment corrosion and lower energy requirements for the recovery and recycling of extraction solvent. Subsequent to the extraction of butyric acid/propionic acid, the recovery of biological buffer can be utilized in the separation process. In general, the extraction of carboxylic acid without a biological buffer resulted in a decreased separation factor and distribution coefficient, necessitating increased energy and costs for subsequent product purification and extraction solvent recovery [21,23]. Consequently, the application of biological buffer presents a potential and competitive approach for the separation of carboxylic acid.

Correlation Results of the Non-Randomness Two-Liquid (NRTL) Model with Aspen Plus. The experimental data obtained were correlated using the *Non-Randomness Two-Liquid* (NRTL) equation, which can used to predict fractions and operating conditions, calculate equilibrium liquid-liquid multicomponent composition, and be used in mixtures that form azeotropes such as carboxylic acids and water. The model can calculate the equilibrium composition of a multicomponent liquid-liquid using only the constituent binary parameters. This model consists of the non-randomness parameter (α_{12}) which can be used for mixtures with significant variations. The *non-randomness* parameter (α_{12}) with the NRTL model was set at 0.2. The following is the NRTL model equation for multicomponent mixtures:

$$ln\gamma_1 = \frac{\sum_J^N \tau_{ji} G_{ji} x_j}{\sum_K^N G_{ki}\, x_k} + \sum_J^N \frac{x_j G_{ij}}{\sum_K^N G_{kj} x_k}\left[\tau_{ij} - \frac{\sum_k^N x_k \tau_{kj} G_{kj}}{\sum_k^N G_{kj} x_k}\right] \tag{2}$$

$$\tau_{ij} = \frac{b_{ij}}{T}, \qquad \tau_{ij} = \frac{b_{ji}}{T} \tag{3}$$

$$G_{ij} = \exp(-\alpha_{ij}\tau_{ij}) \ ; \ G_{ji} = \exp(-\alpha_{ij}\tau_{ij}) \tag{4}$$

$$\tau_{ii} = \tau_{jj} = 0 \ ; \ G_{ii} = G_{jj} = 1 \ ; \ a_{ij} = a_{ji} \tag{5}$$

b_{ij} is the interaction parameter between molecules i and j, and αij is a non-randomness parameter representing the local composition. The non-randomness parameter (αij) is set to 0.2 for pair (i -j).

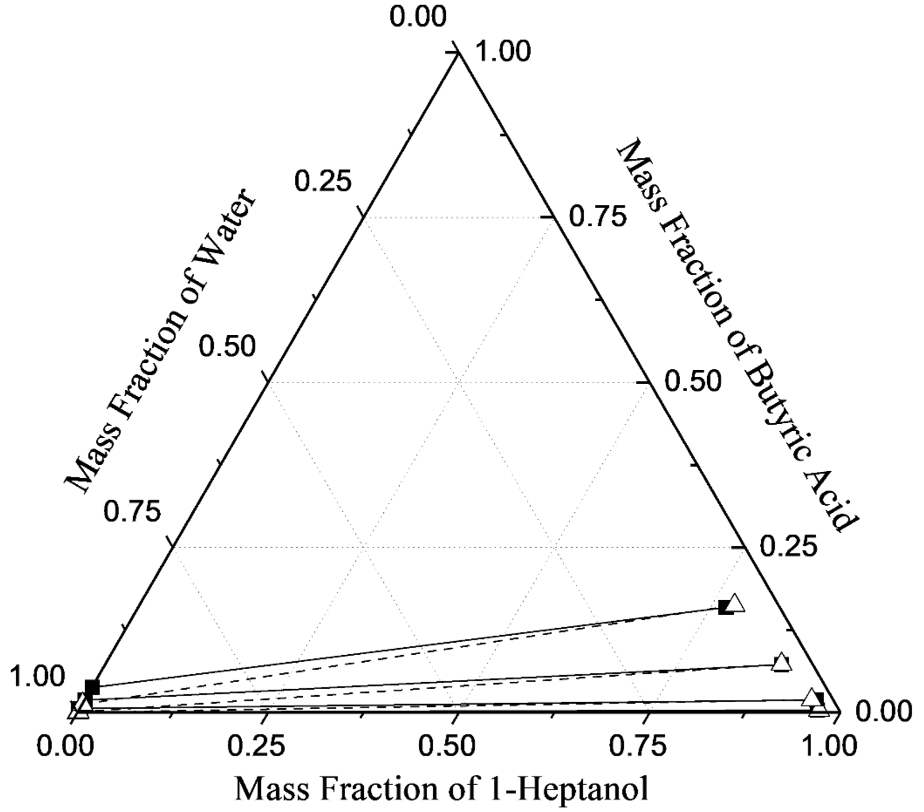

Fig. 1. Phase Diagram of Butyric Acid + Water + EPPS + 1-Heptanol System;
-■- experiment data, --△-- NRTL correlation data

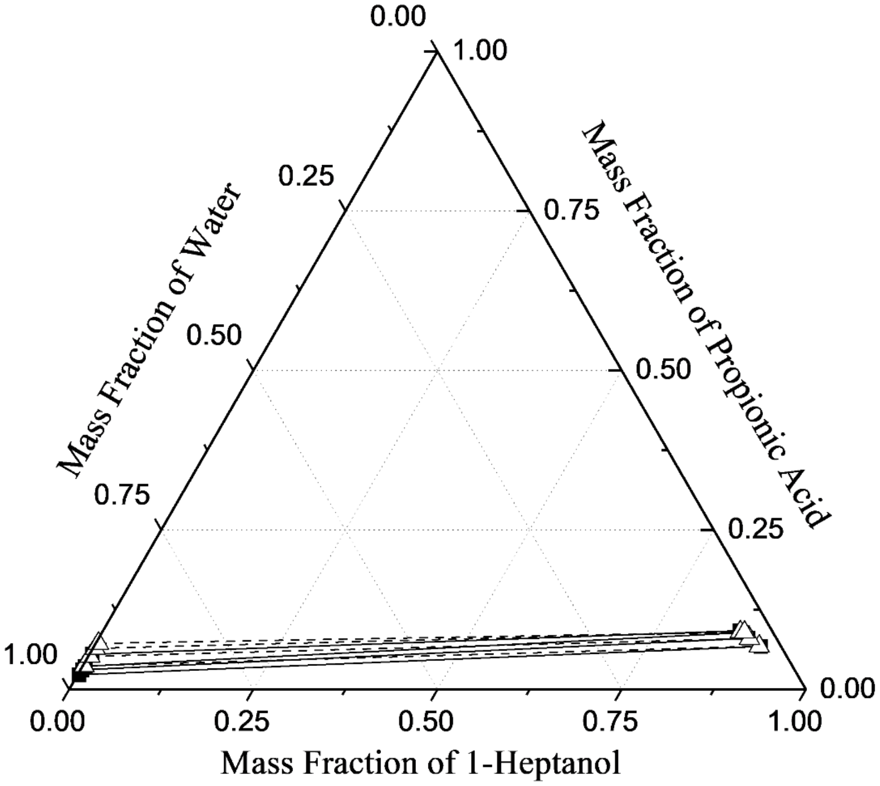

Fig. 2. Phase Diagram of Propionic Acid + Water + EPPS + 1-heptanol;
-■- experiment data, --△-- NRTL correlation data

The correlation of the experimental data is based on the Root Mean Square Deviation (RMSD) value reference, which is the absolute deviation of the average composition between experimental data and calculation results using a model equation.

Table 3. Interaction parameters of the NRTL model for quaternary system Acid (1) + Water (2) + *Biological Buffer* (3) + Solvent (4) at 303.15 K (30°C) and P=101.3 kPa

SYSTEM	i-j	$b_{ij}(K)$	$b_{ji}(K)$	*aij*	%RMSD
Butyric Acid + Water + EPPS + 1-Heptanol	1-4	-928.355	-1933.64	0.2	0.8340
	2-4	3698.38	376.687	0.2	
	3-4	-876.438	215.287	0.2	
	1-2	-178.972	-1308.09	0.2	
	1-3	1741.92	-213.193	0.2	
	2-3	-1.21304	-1979.02	0.2	
Propionic Acid + Water + EPPS + 1-Heptanol	1-4	-1374.78	-8881.58	0.2	0.7332
	2-4	2277.16	604.698	0.2	
	3-4	1127.02	9116.73	0.2	
	1-2	-1044.24	-8818.80	0.2	
	1-3	-6099.75	-7371.43	0.2	
	2-3	-3128.74	-2145.93	0,2	

Based on **Table 3**, the RMSD value from the NRTL model obtained is 0.8340% for Butyric acid + Water + EPPS + 1-Heptanol system and 0.7332% for Propionic acid + Water + EPPS + 1-Heptanol system. This value shows that the NRTL Model can correlate liquid-liquid equilibrium experimental data well by obtaining the RMSD value of less than 1%. The smaller deviation value indicates that the model is appropriate for the experiment. Based on the ternary diagram shown in **Figure 1** for the Butyric acid + Water + EPPS + 1-Heptanol system and **Figure 2** for Propionic acid + Water + EPPS + 1-Heptanol system also indicates that the NRTL model is correlated with well-proven tie-line results correlation and adjacent experimental data.

Conclusions

In this work, the LLE data for carboxylic acid + water + 1-heptanol + EPPS biological buffer systems were measured in 303.2 and 101.3 KPa. The capability of 1-heptanol with the addition of EPPS biological buffer can extract butyric acid greater than that of propionic acid. This result indicates that the addition of EPPS biological buffer can improve the extraction performance of butyric acid and propionic acid with 1-heptanol as the solvent, with the separation factor of butyric acid reaching 176.6, which shows an increase of 2 times higher compared to systems without EPPS biological buffers. Meanwhile, separation factor values of propionic acid can reach 104.8, which increases 4 times higher compared to a system without EPPS biological buffer. Experimental LLE tie-line data from the studied system were well correlated using the NRTL model with RMSD below 1%. These results can be used as a reference for extraction column design in the carboxylic acid extraction process so that the extraction process can run more efficiently and optimally.

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