

## Liquid–Liquid Equilibria and Vanillin Partitioning in PEG–Sodium Sulfate Aqueous Two-Phase Systems

Mateus Guedes Araújo<sup>1</sup> , Maria Eduarda Andrade Ruas<sup>1</sup> ,  
William James Nogueira Lima<sup>1</sup> , Janaína Teles de Faria<sup>1\*</sup> 

### Abstract

Vanillin is one of the most widely used flavoring compounds. Although the synthetic form dominates the global market, biotechnological routes have been explored, and economical and sustainable downstream processes are still required. Aqueous two-phase systems offer an attractive alternative due to their advantages, including biocompatibility, as they are composed primarily of water. This study evaluated the effects of polyethylene glycol molecular mass (1500 and 6000 g/mol), temperature (25, 35, and 45 °C), tie-line length, and phase mass ratio on liquid–liquid equilibria and vanillin partitioning in polyethylene glycol –sodium sulfate aqueous two-phase system. Binodal curves were satisfactorily correlated using the Merchuk model, and an increase in temperature caused a slight expansion of the biphasic region. PEG molecular mass had a negligible effect on phase-forming ability but influenced vanillin distribution, with lower molecular mass favoring partitioning. Vanillin preferentially partitioned into the top phase, with the best performance observed in 25% (w/w) polyethylene glycol 1500 and 8.5% (w/w) sodium sulfate at 35 °C (partition coefficient = 33.0 and recovery = 98.7%). Variations in mass ratio along the same tie-line only slightly affected partitioning. These results provide insights into optimizing aqueous two-phase system as a sustainable approach for vanillin recovery.

**Keywords:** aqueous biphasic systems; flavor compound; phenolic molecule; liquid–liquid extraction; partitioning; polymer–salt system.

**Practical Application:** Aqueous two-phase system allows evaluation of vanillin partitioning under different system conditions.

## 1 INTRODUCTION

Vanillin (3-methoxy-4-hydroxybenzaldehyde), a phenolic aldehyde, is a notable flavor compound commonly used in food, beverage, pharmaceutical, and cosmetic industries (Banerjee & Chattopadhyay, 2019; Venkataraman et al., 2024). It is recognized for its characteristic vanilla odor and flavor (Peña-Barrientos et al., 2023). According to Venkataraman et al. (2024), annually, around 16,000 metric tons of vanillin are manufactured, corresponding to approximately USD 650 million. The global demand for this compound is high and growing, with the market dominated by synthetic vanillin (Martău et al., 2021), since the natural supply is much lower than market demand (Venkataraman et al., 2024). Furthermore, synthetic vanillin has greater flavoring strength and a much lower price than the natural form, extracted from vanilla beans (Martău et al., 2021). Currently, about 95% of vanillin is produced chemically from lignin and guaiacol (Venkataraman et al., 2024).

In recent years, research has demonstrated human health benefits (Martău et al., 2021), as well as antioxidant and antimicrobial properties (Venkataraman et al., 2024) associated with vanillin. However, food safety control agencies worldwide have restricted the application of chemically derived vanillin in the pharmaceutical and food industries due to its low-quality final

products (Banerjee & Chattopadhyay, 2019; Martău et al., 2021). Therefore, the biotechnological production of “nature-identical” vanillin through bioengineering and fermentation technologies, the bioconversion of agro-industrial wastes and by-products, and the use of enzymatic or immobilized systems have been explored for vanillin production (Banerjee & Chattopadhyay, 2019; Martău et al., 2021; Venkataraman et al., 2024).

Considering the formation or extraction of by-products or unwanted products in vanillin production, downstream processes for the separation, recovery, and purification of this compound from culture media or extracts hold significant importance. According to Cláudio et al. (2010), the recovery and purification of vanillin by economical and sustainable processes remain a major concern. In this context, studies on sustainable techniques characterized by low energy and organic solvent consumption, low cost, and the preservation of functional characteristics are essential. A potential technique that meets these requirements and may contribute to the separation and recovery of biomolecules is the application of aqueous two-phase systems (ATPSs). ATPSs have been recognized for their advantages, such as simplicity, low cost, scalability, safety, short processing times (Segaran & Chua, 2024), versatility, biocompatibility, and flexibility (Jorge & Pereira, 2024).

Received: Oct. 09, 2025.

Accepted: Oct. 21, 2025.

<sup>1</sup>Universidade Federal de Minas Gerais, Agricultural Sciences Institute, Montes Claros, Minas Gerais, Brazil.

\*Corresponding author: [janainafaria@ufmg.br](mailto:janainafaria@ufmg.br)

Conflict of interest: nothing to declare.

Funding: This research received financial support from the Programa de Apoio à Pós-Graduação (PROAP) of the Coordination for the Improvement of Higher Education Personnel (CAPES).

ATPS are formed by mixing a variety of two or more incompatible phase-forming components in water, such as polymer–polymer, polymer–salt, polymer–ionic liquid (IL), polymer–surfactant, polymer–sugar, polymer–deep eutectic solvent, IL–salt, and salt–surfactant, among others (Sadeghi & Coutinho, 2024). Composed primarily of water, ATPSs offer a biocompatible environment for biomolecules (Chen et al., 2023; Jorge & Pereira, 2024). These aqueous biphasic systems have been studied for the extraction, concentration, and separation of various biological molecules of industrial interest (Chen et al., 2023; Segaran & Chua, 2024). However, before applying ATPS for these purposes, preliminary studies evaluating the effects of different parameters on the partitioning of pure compounds are necessary to optimize the process (Sadeghi & Coutinho, 2024). The selection of efficient and economical parameters that enhance the separation and purification of molecules in ATPS is among the primary goals of researchers (Nouri et al., 2019). Therefore, studies on the effect of factors such as the type and concentration of the phase-forming components, polymer molecular mass (MM), type of salt, ionic strength of the system, phase mass ratio, temperature, and pH are required to determine the optimal conditions for their application.

Several studies have investigated vanillin partitioning in ATPS formed by various components, including IL-based systems (Arzideh et al., 2025; Cheng et al., 2024; Cláudio et al., 2010), deep eutectic solvent-based systems (Souza et al., 2024), acetonitrile-based systems (Cardoso et al., 2015), polymer–polymer systems (Nouri et al., 2023), alcohol–salt (Veloso et al., 2020), and polymer–salt (Padilha et al., 2020) systems. However, few studies have assessed the effects of individual parameters, such as PEG MM, temperature, composition (tie-line length, *TLL*), or phase mass ratio, on vanillin partitioning in PEG–sulfate ATPS. The present study therefore evaluates these parameters separately in a PEG–sodium sulfate system to provide insights for optimizing vanillin partitioning.

### 1.1 Relevance of the work

This study evaluated vanillin partitioning in aqueous two-phase systems (ATPSs) composed of PEG and sodium sulfate under different conditions. Vanillin is one of the most important flavoring compounds, with wide applications in the food, pharmaceutical, and cosmetic industries. Although biotechnological routes for vanillin production have been explored, economical and sustainable downstream processes are still necessary. In this context, this work contributes to the application of ATPS for the partitioning and recovery of vanillin. The findings provide valuable insights for designing greener and more efficient processes for the recovery of high-value biomolecules.

## 2 MATERIAL AND METHODS

### 2.1 Chemicals and apparatus

Polyethylene glycol (PEG) of average MMs of 1500 g/mol (PEG 1500, CAS number: 25322-68-3) and 6000 g/mol (PEG

6000, CAS number: 25322-68-3) was obtained from Dinâmica Química (Brazil). Sodium sulfate (CAS number: 7757-82-6; purity > 99%) was provided by Synth (Brazil). Vanillin (CAS number: 121-33-5; purity > 97%) was purchased from Êxodo Científica (Brazil). All chemicals were of analytical grade and used without further purification. Deionized water (Milli-Q, Millipore, Australia) was used to prepare the solutions and ATPSs.

An analytical balance (AW220, Marte, Brazil) with an accuracy of  $\pm 10^{-4}$  g was used to prepare the solutions and mixtures. A vortex (Warmnest, Brazil) was employed to ensure homogeneous mixing of the systems. A temperature-controlled bath (Q214M2, Quimis, Brazil) and a biochemical oxygen demand (BOD) incubator (SSBOD, Solidsteel, Brazil) were used for temperature equilibration. A UV–VIS spectrophotometer (k-37, Kasvi, Brazil) was used to determine vanillin concentration. All concentrations are reported in % (w/w).

### 2.2 Phase diagrams and tie-lines correlations

Binodal curves of PEG (1500 and 6000 g/mol)–sodium sulfate systems were obtained by the cloud point titration at 25, 35, and 45 °C under atmospheric pressure (Sadeghi & Coutinho, 2024). Stock solutions of  $\text{Na}_2\text{SO}_4$  (30% w/w) and PEG (50% w/w) were prepared in deionized water. The salt solution was added dropwise to a known amount of PEG solution (50% w/w) under constant stirring until the mixture became turbid, indicating the formation of two phases. The mixture was then diluted with a defined amount of water until it became clear, bringing the system below the cloud point. Systems were equilibrated in a temperature-controlled bath ( $\pm 1$  °C). The composition of each point on the binodal curve was calculated from the initial mass and concentration of the PEG solution, the amount and concentration of the added salt solution, and the dilution water. This procedure was repeated to obtain sufficient points for binodal curve construction. Experimental binodal data were correlated using the Merchuk model (Equation 1) (Merchuk et al., 1998), a nonlinear mathematical model:

$$[w_1] = A \cdot \exp(B[w_2]^{0.5} - C[w_2]^3) \quad (1)$$

where  $[w_1]$  and  $[w_2]$  correspond to the total concentration (in % w/w) of the polymer and salt, respectively, and  $A$ ,  $B$ , and  $C$  are the parameters obtained by regression of the experimental data.

The tie-lines (TLs) were determined by the gravimetric method described by Merchuk et al. (1998), based on the mass balance and experimental binodal data fitted. For each PEG and each temperature, three points were selected within the two-phase region, with the following global compositions: 7.0% (w/w) of salt and 22.0% (w/w) of PEG (point 1), 8.5% (w/w) of salt and 25.0% (w/w) of PEG (point 2), and 10.0% (w/w) of salt and 28.0% (w/w) of PEG (point 3). Appropriate amounts of water, salt, and polymer stock solutions were weighed into graduated tubes, and the total mass of each system was adjusted to 8 g. The mixtures were vortexed for 2 min and then allowed to stand in a BOD incubator for 24 h to reach phase equilibrium at 25, 35, and 45 °C. After this period, the volume of each phase was recorded, and both phases were carefully separated using

long-needle syringes and weighed individually. The lever-arm rule, which relates the mass composition of the top and bottom phases to the global system composition, was applied to determine the concentration of each component in the phases (Michel et al., 2015). Therefore, the TLs were obtained using the following four equations (Equations 2–5) and four unknown variables ( $[w_1]_t$ ,  $[w_1]_b$ ,  $[w_2]_t$ , and  $[w_2]_b$ ) (Merchuk et al., 1998):

$$[w_1]_t = A \cdot \exp(B[w_2]_t^{0.5} - C[w_2]_t^3) \quad (2)$$

$$[w_1]_b = A \cdot \exp(B[w_2]_b^{0.5} - C[w_2]_b^3) \quad (3)$$

$$\frac{m_b}{m_t} = \frac{[w_1]_m - [w_1]_t}{[w_1]_b - [w_1]_m} \quad (4)$$

$$\frac{m_b}{m_t} = \frac{[w_2]_m - [w_2]_t}{[w_2]_b - [w_2]_m} \quad (5)$$

where the subscripts  $t$ ,  $b$  and  $m$  correspond to the top phase, bottom phase, and mixture composition, respectively, and  $m_b$  and  $m_t$  represent the masses of the top and bottom phases.

The length ( $TLL$ ) and slope ( $TLS$ ) of the TLs are useful parameters for describing the liquid–liquid equilibrium behavior. These parameters were calculated using the following equations (Equations 6 and 7) (Jorge & Pereira, 2024):

$$TLL = \sqrt{([w_1]_t - [w_1]_b)^2 + ([w_2]_t - [w_2]_b)^2} \quad (6)$$

$$TLS = \frac{[w_1]_t - [w_1]_b}{[w_2]_t - [w_2]_b} \quad (7)$$

## 2.3 PARTITION OF VANILLIN

Partition experiments were conducted in 15 mL graduated tubes. Each system (total mass of 8 g) was prepared using the same global compositions previously employed for TL determination (Section 2.2) and supplemented with a 100  $\mu$ L aliquot of vanillin solution (0.3 mg/mL). After vortexing for 2 min, the mixtures were left to stand at 25, 35, and 45  $^{\circ}$ C for 24 h in a BOD incubator ( $\pm 1$   $^{\circ}$ C) to reach phase equilibrium. Subsequently, the top and bottom phases were carefully withdrawn using a long-needle syringe and transferred to clean tubes. The vanillin partition coefficient ( $k$ ) and vanillin recovery in the top phase ( $R_v$ ) were determined according to Equations 8 and 9, respectively:

$$k = \frac{[van]_t}{[van]_b} \quad (8)$$

$$R_v = \frac{100}{1 + \frac{1}{k \cdot R_v}} \quad (9)$$

where  $[van]_t$  and  $[van]_b$  are the concentrations of vanillin in the top and bottom phases, respectively, and  $R_v$  represents the volume ratio of the top and bottom phases.

To evaluate the effect of the phase ratio (i.e., the mass ratio of the top to bottom phase), blank systems with a total mass of 30 g were prepared as described in Section 2.2. After 24 h to ensure phase equilibrium, the top and bottom phases were collected, leaving a small volume to prevent phase mixing. Each phase was

then weighed and combined to obtain ATPS with a total mass of 8 g and mass ratios of 1:3, 1:1, and 3:1 (w/w, top:bottom). The partition experiments were carried out as described above.

## 2.4 Determination of vanillin

The concentration of vanillin in each phase was quantified using a UV–VIS spectrophotometer at 280 nm, according to Cláudio et al. (2010), employing a previously established calibration curve (2–10 mg/L). A possible interference of PEG and sodium sulfate with the analytical method was investigated and found to be negligible.

## 2.5 Statistical analysis

All experiments were performed in triplicate, and the results were reported as mean  $\pm$  standard deviation. The Merchuk model (Equation 1) was fitted to the experimental binodal data using nonlinear regression and evaluated using the adjusted coefficient of determination ( $R^2$ ), root mean square error ( $RMSE$ ), and analysis of variance at a 5% significance level. To assess the effects of the polymer MM,  $TLL$ , temperature, and phase mass ratio on vanillin partitioning, partition coefficient values were compared using Tukey's test ( $p \leq .05$ ). Statistical analyses were performed using SAS software (University Edition, USA), and the system of equations (Equations 2–5) was solved with the Excel Solver tool (Microsoft, USA).

## 3 RESULTS AND DISCUSSION

### 3.1 Phase diagrams

The binodal curve is the boundary between the monophasic and biphasic regions. Each phase diagram consists of a binodal curve and is an important characteristic of an ATPS, distinctive for each system under specific conditions of composition, temperature, pH, etc. Analysis of binodal curves is important for understanding phase separation and optimizing biomolecule partitioning. The binodal curves obtained for PEG 1500/6000 + sodium sulfate + water systems at different temperatures (25, 35, and 45  $^{\circ}$ C) are shown in Figure 1. As shown in Figure 1, the phase diagram was divided by the binodal curves, with the two-phase region above and the monophasic region below. The larger the biphasic region, the greater the efficiency of phase separation.

The effect of temperature on phase separation has been studied in several systems. According to Pirdashti et al. (2021), this parameter has a complex and variable effect on ATPS. Figure 1 shows that the binodal curves at 25 and 35  $^{\circ}$ C are nearly superimposed for both systems, indicating that temperature in this range has little effect on the size of the biphasic region. This suggests that, for these systems, phase separation is not influenced by temperature within the evaluated range. At 45  $^{\circ}$ C, however, the biphasic region shows a slight increase, indicating that higher temperature favored phase separation. As temperature increases, interactions between salt ions and water molecules (mainly charge–dipole electrostatic interactions) become more pronounced than those between PEG and

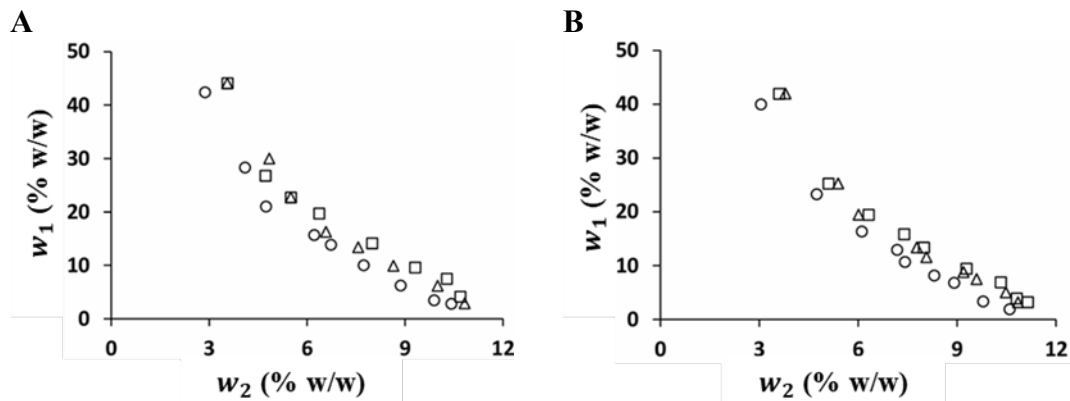
water (mostly dipole–dipole) (Lüsse & Arnold, 1996), thereby enhancing phase separation.

Many studies have shown that increasing temperature leads to an expansion of the biphasic region in polymer–salt systems (Azimaie et al., 2010; Mokarizadeh & Nemati-Kande, 2022; Pirdashti et al., 2021; V. S. Sampaio et al., 2020). For such systems, higher temperature facilitates phase separation, leading to a larger biphasic region. However, González-Amado et al. (2016) observed that temperature has little or no effect on the biphasic region of PEG 4000/8000 + ammonium sulfate/sodium sulfate + water systems, similar to the present study. Nascimento et al. (2018), who studied ATPS composed of PEG 400 and sodium sulfate, also reported that the biphasic region area did not vary significantly with increasing temperature.

The effect of polymer MM on the two-phase formation ability of the systems was further evaluated, and the results are shown in Figure 2. Graphical analysis showed no significant differences in the biphasic region with increasing PEG MM at 25, 35, and 45 °C, indicating that systems formed by both PEGs have the same phase-forming ability. Wysoczanska and Macedo (2016b) observed only a slightly larger biphasic area with increasing PEG molecular weight for aqueous systems composed of PEG 4000/6000/8000 and potassium citrate/potassium sodium tartrate at 25 °C.

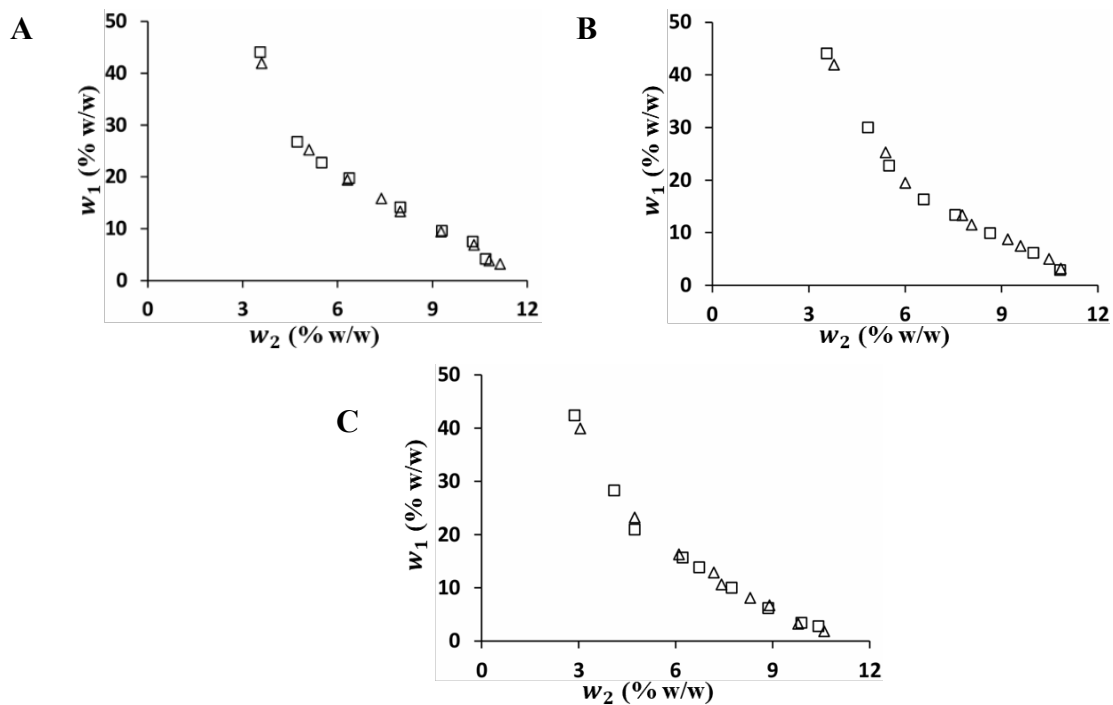
### 3.2 Correlation of binodal curve

The binodal data were fitted using the Merchuk equation (Equation 1) via the least-squares regression method, and the



Temperature (°C): 25 (□), 35 (Δ), and 45 (○). Pressure: 0.1 MPa. w: concentration (% w/w).

**Figure 1.** Experimental binodal curve of systems composed of (A) polyethylene glycol 1500 ( $w_1$ ) +  $\text{Na}_2\text{SO}_4$  ( $w_2$ ) + water, and (B) polyethylene glycol 6000 ( $w_1$ ) +  $\text{Na}_2\text{SO}_4$  ( $w_2$ ) + water.



Polyethylene glycol molecular mass: 1500 (□) and 6000 (Δ) g/mol. Pressure: 0.1 MPa. w: concentration (% w/w).

**Figure 2.** Experimental binodal curve of systems composed of polyethylene glycol ( $w_1$ ) +  $\text{Na}_2\text{SO}_4$  ( $w_2$ ) + water at (A) 25 °C, (B) 35 °C, and (C) 45 °C.

fitting parameters for PEG 1500 and PEG 6000 at three different temperatures, along with  $R^2$  and  $RMSE$  values, are presented in Table 1. For all systems, the model fit was significant ( $p < .05$ , data not shown). For both systems, the coefficients of determination were higher than 0.975, and  $RMSE$  values were  $\leq 1.3585$ . Equation 1 can be satisfactorily used to correlate the present experimental data, ensuring that the model is well fitted to the binodal data points. The Merchuk equation has been used to

correlate binodal data of diverse polymer-salt ATPS (González-Amado et al., 2016; Herbst & Pott, 2019; Mokarizadeh & Nemati-Kande, 2022).

### 3.3 Tie-line, tie-line slope, and tie-line length

Table 2 presents the  $TL$  data: global compositions, top and bottom phase compositions,  $TLLs$ , and tie-line slopes ( $TLSs$ ) for

**Table 1.** Values of regression coefficients of the Merchuk equation, coefficients of determination, and root-mean-square errors for systems containing polyethylene glycol 1500 or 6000 +  $Na_2SO_4$  + water at different temperatures.

Temperature (°C)	A	B	C	$R^2$	$RMSE$
<b>PEG 1500 + <math>Na_2SO_4</math> + water</b>					
25	282.03110	-1.017910	0.00043	0.9909	0.8017
35	266.55510	-0.98681	0.00053	0.9920	0.8017
45	475.09090	-1.34899	0.00017	0.9774	1.3088
<b>PEG 6000 + <math>Na_2SO_4</math> + water</b>					
25	301.04180	-1.03554	0.00049	0.9900	0.8990
35	285.01100	-0.99506	0.00046	0.9922	0.7907
45	586.88880	-1.41030	0.00011	0.9751	1.3585

$R^2$ : coefficients of determination;  $RMSE$ : root-mean-square errors; A, B, and C: parameters obtained by fitting experimental data to the Merchuk equation.

**Table 2.** Experimental (liquid-liquid) equilibrium concentrations, tie-lines lengths (% w/w) and slopes for systems composed of polyethylene glycol 1500 or 6000 ( $w_1$ ), sodium sulfate ( $w_2$ ) and water at different temperatures.

T (°C)	TL	Global composition		Top phase		Bottom phase		TLL	TLS
		$[w_1]$	$[w_2]$	$[w_1]$	$[w_2]$	$[w_1]$	$[w_2]$		
<b>PEG 1500 + <math>Na_2SO_4</math> + water</b>									
25	1	22.00	7.00	31.77	4.44	4.50	11.58	28.20	-3.80
	2	25.00	8.50	40.10	3.60	0.68	16.39	41.50	-3.10
	3	28.00	10.00	47.30	3.00	0.09	20.05	50.20	-2.80
35	1	22.00	7.00	32.04	4.41	3.99	11.64	29.00	-3.90
	2	25.00	8.50	35.43	4.04	0.09	19.16	38.40	-2.30
	3	28.00	10.00	41.57	3.46	0.00	23.49	46.10	-2.10
45	1	22.00	7.00	33.18	3.87	3.07	12.31	31.30	-3.60
	2	25.00	8.50	37.12	3.55	0.50	18.50	39.60	-2.40
	3	28.00	10.00	44.95	3.04	0.18	21.42	48.40	-2.40
<b>PEG 6000 + <math>Na_2SO_4</math> + water</b>									
25	1	22.00	7.00	32.71	4.42	4.82	11.14	28.70	-4.20
	2	25.00	8.50	41.84	3.55	0.77	15.62	42.80	-3.40
	3	28.00	10.00	47.76	3.11	0.07	19.74	50.50	-2.90
35	1	22.00	7.00	32.09	4.62	6.16	10.74	26.60	-4.20
	2	25.00	8.50	42.00	3.62	1.07	15.37	42.60	-3.50
	3	28.00	10.00	47.87	3.16	0.11	19.60	50.50	-2.90
45	1	22.00	7.00	34.13	4.05	4.36	11.30	30.60	-4.10
	2	25.00	8.50	41.88	3.49	1.51	15.47	42.10	-3.40
	3	28.00	10.00	47.98	3.14	0.51	19.43	50.20	-2.90

Pressure = 0.1 MPa.  $w$ : concentration (% w/w). T: temperatures; TL: tie-lines; TLL: tie-lines lengths; TLS: tie-line slopes; PEG: polyethylene glycol.

PEG 1500/6000 + sodium sulfate + water systems, measured at 25, 35, and 45 °C. For all investigated systems, the top phase contained > 30% w/w PEG and < 5% w/w sodium sulfate, while the bottom phase contained > 10% w/w salt and < 7% w/w polymer. In some systems, PEG was nearly excluded from the bottom phases. Table 2 shows that the top phase is PEG-rich, whereas the bottom phase is salt-rich, a behavior characteristic of polymer–salt ATPS.

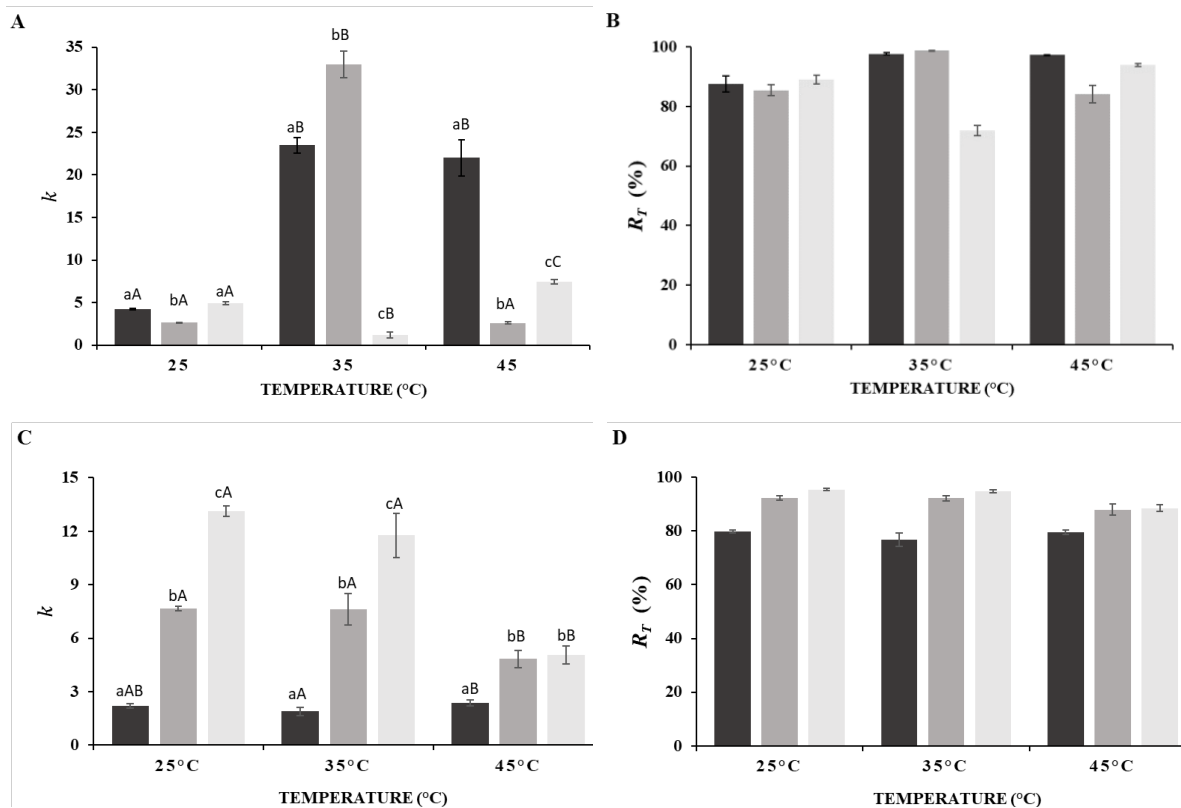
It can be seen from Table 2 that the higher the salt concentration in the bottom phase and the lower the polymer concentration, the higher the *TLL*. This result agrees with previous studies on other polymer–salt systems (Carvalho et al., 2008; Parmoon et al., 2019). In addition, for the same global composition, *TLL* increased with PEG MM, consistent with observations for other PEG–zinc sulfate ATPS (Pirdashti et al., 2021).

Regarding the *TLs*, no clear trend was observed between temperature and *TLs* for all systems. However, an increase in global composition resulted in a reduction in the *TLs* magnitude (lower absolute value) for both systems at all temperatures (Table 2). Similar behavior was reported by Wyszczanska and Macedo (2016a, 2016b), who observed a decrease in the absolute value of *TLs* and an increase in *TLL* with increasing global composition in systems composed of PEG with different MMs and various salts at 25 °C.

### 3.4 Vanillin partitioning by aqueous two-phase system

To evaluate the potential of the studied biphasic systems for use in extractive processes, the partition coefficient (*k*) and the recovery in the top phase (*R<sub>t</sub>*) were determined for ATPS composed of PEG 1500/6000 and sodium sulfate (Figure 3). The effects of global phase composition, PEG MM, temperature, and phase mass ratio on vanillin partitioning were assessed. As shown in Figure 3, the partition coefficients were higher than 1, indicating that the target biomolecule preferentially partitioned to the PEG-rich (top) phase. This result agrees with the 1-octanol/water partition coefficient of vanillin at 25, 35, and 45 °C, with log *K<sub>ow</sub>* values of 1.19, 1.11, and 0.99, respectively (Noubigh et al., 2010), reflecting its affinity for hydrophobic environments such as the PEG-rich phase. This preferential interaction between vanillin and PEG, compared with the other phase-forming component, is consistent with previous studies on polymer-based ATPS. For instance, Nouri et al. (2019) reported *k* = 19.26 in PEG 4000–sodium sulfate ATPS and *k* = 1.38 in PEG 4000–dextran 15000 ATPS, both at 25 °C. More recently, Nouri et al. (2023) found *k* values ranging from 1.28 to 1.66 in systems composed of PEG 6000 and dextran 15,000, and 1.23–1.41 in PEG 6000–dextran 3500 ATPS, both at 25 °C.

According to Figure 3, the partition coefficient of vanillin ranged from 1.17 to 33.0 and from 1.90 to 13.13 for ATPS



\*Means sharing the same lowercase letter for different global compositions at the same temperature, or the same uppercase letter for the same global composition at different temperatures, do not differ significantly according to Tukey's test ( $p > .05$ ).

*k*: Partition coefficients; *R<sub>t</sub>*: recovery of vanillin in the top phase; dark gray bar: 22% w/w PEG + 7.0% w/w salt; Medium gray bar: 25% w/w PEG + 8.5% w/w salt; Light gray bar: 28% w/w PEG + 10.0% w/w salt. Error bars are standard deviations. Pressure = 0.1 MPa.

**Figure 3.** Partition coefficients and recovery of vanillin in the top phase of aqueous two-phase systems composed of polyethylene glycol 1500 and sodium sulfate (A and B, respectively) and polyethylene glycol 6000 and sodium sulfate (C and D, respectively), evaluated at three global composition points and different temperatures.

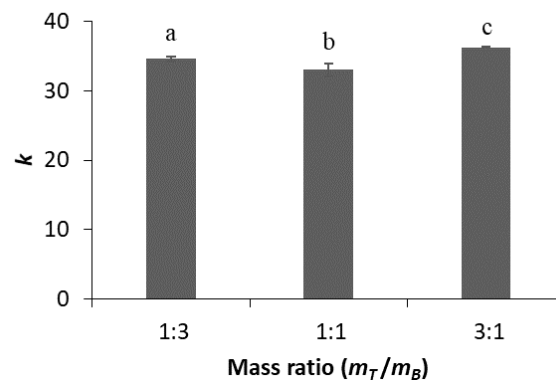
composed of PEG 1500 and PEG 6000, respectively, with recovery in the top phase higher than 71.9 and 76.8%. In ATPS composed of PEG 6000, the preferential migration of vanillin to the top phase was positively influenced by the increase in global composition and hence in the *TLL* of the systems. For these systems, the partition coefficient increased by almost 600% as the global composition (and corresponding *TLL*) increased, at all temperatures (Figure 3). This suggests that the interaction of vanillin with the top phase was intensified, since differences in the compositions and properties of the equilibrium phases are known to increase with *TLL*.

However, a different trend was observed in systems composed of PEG 1500. For PEG 1500–ATPS, vanillin showed the highest *k* value in the second and first *TLL* at 35 and 45 °C, respectively, indicating that partitioning was favored at medium and low global concentrations (Figure 3). The increase in vanillin partitioning with rising PEG concentration can be attributed to stronger interactions with a greater number of PEG molecules, resulting from the higher polymer concentration in the system. Nevertheless, excessive PEG content may reduce the space available to accommodate vanillin in the top phase, favoring its migration to the opposite phase and thus lowering the partition coefficient (Nouri et al., 2023). This phenomenon, known as the volume exclusion effect, may also be related to the polymer MM. An increase in the polymer chain length reduces the free volume of the polymer-rich phase, leading to partitioning of biomolecules into the bottom phase and consequently decreasing the partition coefficient (D. A. Sampaio et al., 2016).

Since the partitioning behavior of biomolecules can be affected by polymer MM, the effect of PEG MM on vanillin partitioning was evaluated. According to Figure 3, in general, the partition coefficient of vanillin decreased with increasing PEG MM. In these systems, the migration of vanillin to the salt-rich (bottom) phase (decrease in *k* value) may be attributed to the more pronounced volume exclusion effect of the polymer. The recovery of vanillin (*RT*) also decreased in these ATPS with increasing polymer MM, due to the higher concentration of the biomolecule in the bottom phase.

The effect of temperature on the partition coefficient of vanillin was also evaluated. From Figure 3, it can be seen that temperature had varying effects on the distribution of the target molecule in the systems studied. In some cases, an increase in temperature favored the preferential migration of vanillin to the top phase, as observed for TL 1 and TL 3 in PEG 1500–ATPS (*k* values increased from 4.2 to 22.0 and from 4.9 to 7.4, respectively). In contrast, higher partition coefficients were obtained at lower temperatures for PEG 6000–ATPS at TLs 2 and 3 (*k* = 7.7 and 13.1, respectively, at 25 °C). Therefore, depending on the global composition, an increase in temperature either favored or hindered vanillin partitioning to the top phase under the conditions evaluated.

In other studies on vanillin partitioning, Cardoso et al. (2015) reported a decrease in the partition coefficient with increasing temperature in poly(vinyl alcohol) + acetonitrile ATPS. Similarly, Cláudio et al. (2010) observed the presence of a maximum in vanillin partitioning as a function of temperature



*k*: partition coefficient of vanillin;  $m_T$ : mass of the top phase;  $m_B$ : mass of the bottom phase. Means sharing the same letter do not differ significantly according to Tukey's test ( $p > .05$ ).

**Figure 4.** Effect of different mass ratios between the top and bottom phases of aqueous two-phase systems composed of polyethylene glycol 1500 and sodium sulfate, with a tie-line length of 34.8% (w/w), on the partition coefficient of vanillin at 35 °C and 0.1 MPa.

in IL–ATPS, in addition to reporting a decrease in *k* values with increasing temperature.

For any ATPS, a *TL* on a phase diagram is defined as a line connecting the global, top, and bottom phase compositions after equilibrium is reached (Yin et al., 2022). Mixture points along the same *TL* result in systems with identical top and bottom phase compositions, but with differences in global composition, mass ratio, and relative volume (Silva & Loh, 2006; Yin et al., 2022).

The effect of the phase mass ratio on vanillin partitioning was studied at 35 °C, testing mass ratios of 1:3, 1:1, and 3:1 (top phase/bottom phase) of phases obtained from the ATPS composed of 25.0% w/w PEG 1500 and 8.5% w/w sodium sulfate (Table 2, *TLL* = 38.4% w/w). According to Figure 4, the partition coefficients showed little variation with mass ratio, indicating that vanillin partitioning was minimally affected by changes in global composition along the same *TL*. The 3:1 mass ratio resulted in the highest partition coefficient ( $k = 36.2 \pm 0.07$ ), possibly because a greater number of polymer molecules can accommodate more of the target molecule (Assis et al., 2020). In contrast, the 1:3 system showed a slightly lower *k* value ( $34.6 \pm 0.38$ ). This finding is notable, as preconcentration of the target molecule in the top phase when its mass is smaller than the bottom phase may reduce process costs, primarily by lowering polymer consumption, which is typically the most expensive phase-forming component in ATPS. Moreover, preconcentration in a smaller phase can reduce cost in downstream processing steps.

## 4 CONCLUSIONS

ATPS composed of PEG (1500 and 6000 g/mol) and sodium sulfate were studied in this work. Binodal curves at 25, 35, and 45 °C were obtained and satisfactorily correlated with the Merchuk model. An increase in temperature from 35 to 45 °C caused a slight expansion of the biphasic region. The effect of PEG MM on the phase-forming capability of the systems was negligible.

Phase equilibrium compositions were obtained for three systems for each PEG–ATPS and temperature. Higher global compositions resulted in greater *TLL* and lower (absolute) *TLS*. Vanillin showed a higher affinity for the hydrophobic PEG-rich phase. Different effects of *TLL* and temperature were observed in systems composed of PEG 1500 or 6000. In general, the partition coefficient decreased with increasing PEG MM. The best vanillin partitioning was observed in the ATPS composed of 25% w/w PEG 1500 and 8.5% w/w sodium sulfate at 35 °C, with *k* and *RT* values of 33.0 and 98.7%, respectively. Biomolecule partitioning was only slightly affected by changes in phase mass ratio (different global compositions) along the same TL, enabling preconcentration in a smaller phase.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) and the Laboratório de Química Instrumental (LQI-UFMG), at the Universidade Federal de Minas Gerais

## REFERENCES

- Arzideh, S. M., Movagharnejad, K., & Pirdashti, M. (2025). Thermodynamic insights into vanillin partition in 1-decyl-3-methylimidazolium chloride and potassium phosphate salts aqueous two-phase systems at (298.15 to 313.15) K. *Physical Chemistry Research*, 13(2), 217–232. <https://doi.org/10.22036/pcr.2025.472769.2564>
- Assis, R. C., Mageste, A. B., Lemos, L. R., Orlando, R. M., & Rodrigues, G. D. (2020). Application of aqueous two-phase systems for the extraction of pharmaceutical compounds from water samples. *Journal of Molecular Liquids*, 301, Article 112411. <https://doi.org/10.1016/j.molliq.2019.112411>
- Azimaie, R., Pazuki, G. R., Taghikhani, V., Vossoughi, M., & Ghotbi, C. (2010). Liquid–liquid phase equilibrium of MgSO<sub>4</sub> and PEG1500 aqueous two-phase system. *Physics and Chemistry of Liquids*, 48(6), 764–772. <https://doi.org/10.1080/00319100903046112>
- Banerjee, G., & Chattopadhyay, P. (2019). Vanillin biotechnology: the perspectives and future. *Journal of the Science of Food and Agriculture*, 99(2), 499–506. <https://doi.org/10.1002/jsfa.9303>
- Cardoso, G. B., Souza, I. N., Pereira, M. M., Costa, L. P., Freire, M. G., Soares, C. M. F., & Lima, Á. S. (2015). Poly(vinyl alcohol) as a novel constituent to form aqueous two-phase systems with acetonitrile: Phase diagrams and partitioning experiments. *Chemical Engineering Research and Design*, 94, 317–323. <https://doi.org/10.1016/j.cherd.2014.08.009>
- Carvalho, C. P., Coimbra, J. S. R., Costa, I. A. F., Minim, L. A., Maffia, M. C., & Silva, L. H. M. (2008). Influence of the temperature and type of salt on the phase equilibrium of peg 1500 + potassium phosphate and PEG 1500 + sodium citrate aqueous two-phase systems. *Química Nova*, 31(2), 209–213. <https://doi.org/10.1590/S0100-40422008000200004>
- Chen, Y., Liang, X., & Kontogeorgis, G. M. (2023). Optimal design of aqueous two-phase systems for biomolecule partitioning. *Industrial & Engineering Chemistry Research*, 62(28), 11165–11177. <https://doi.org/10.1021/acs.iecr.3c00710>
- Cheng, Y., Liu, C., & Guo, X. (2024). Formation of aqueous two-phase systems based on p-sulfonatocalix[4]arene and imidazolium bromide and their selectivity toward vanillin and phenylalanine. *Journal of Molecular Liquids*, 413, Article 125911. <https://doi.org/10.1016/j.molliq.2024.125911>
- Cláudio, A. F. M., Freire, M. G., Freire, C. S. R., Silvestre, A. J. D., & Coutinho, J. A. P. (2010). Extraction of vanillin using ionic-liquid-based aqueous two-phase systems. *Separation and Purification Technology*, 75(1), 39–47. <https://doi.org/10.1016/j.seppur.2010.07.007>
- González-Amado, M., Rodil, E., Arce, A., Soto, A., & Rodríguez, O. (2016). The effect of temperature on polyethylene glycol (4000 or 8000)–(sodium or ammonium) sulfate Aqueous Two Phase Systems. *Fluid Phase Equilibria*, 428, 95–101. <https://doi.org/10.1016/j.fluid.2016.06.019>
- Herbst, J., & Pott, R. W. M. (2019). The effect of temperature on different aqueous two-phase diagrams of polyethylene glycol (PEG 6000, PEG 8000, and PEG 10000) + potassium sodium tartrate + water. *Journal of Chemical & Engineering Data*, 64(7), 3036–3043. <https://doi.org/10.1021/acs.jced.9b00133>
- Jorge, A. M. S., & Pereira, J. F. B. (2024). Aqueous two-phase systems – versatile and advanced (bio)process engineering tools. *Chemical Communications*, 60(84), 12144–12168. <https://doi.org/10.1039/D4CC02663B>
- Lüsse, S., & Arnold, K. (1996). The interaction of poly(ethylene glycol) with water studied by 1H and 2H NMR relaxation time measurements. *Macromolecules*, 29(12), 4251–4257. <https://doi.org/10.1021/ma9508616>
- Martão, G. A., Călinoiu, L.-F., & Vodnar, D. C. (2021). Bio-vanillin: Towards a sustainable industrial production. *Trends in Food Science & Technology*, 109, 579–592. <https://doi.org/10.1016/j.tifs.2021.01.059>
- Merchuk, J. C., Andrews, B. A., & Asenjo, J. A. (1998). Aqueous two-phase systems for protein separation: Studies on phase inversion. *Journal of Chromatography B: Biomedical Sciences and Applications*, 711(1–2), 285–293. [https://doi.org/10.1016/S0378-4347\(97\)00594-X](https://doi.org/10.1016/S0378-4347(97)00594-X)
- Michel, B., Neves, M. T., Sousa, R. C. S., Chagas, M. M., Martins, B. A., & Coimbra, J. S. R. (2015). Partitioning of whey proteins using aqueous two-phase systems with ionic liquids. *Química Nova*, 38, 1148–1152. <https://doi.org/10.5935/0100-4042.20150123>
- Mokarizadeh, M., & Nemati-Kande, E. (2022). Temperature effect on the phase equilibrium of polyethylene glycol 2000 + trilitium citrate + water aqueous two-phase systems at T = 288.15, 298.15, 308.15, and 318.15 K. *Journal of Chemical & Engineering Data*, 67(5), 1237–1249. <https://doi.org/10.1021/acs.jced.2c00091>
- Nascimento, R. G., Fontan, R. C. I., Ferreira Bonomo, R. C. F., Veloso, C. M., Castro, S. S., & Santos, L. S. (2018). Liquid–liquid equilibrium of two-phase aqueous systems composed of PEG 400, Na<sub>2</sub>SO<sub>4</sub>, and water at different temperatures and pH values: Correlation and thermodynamic modeling. *Journal of Chemical & Engineering Data*, 63(5), 1352–1362. <https://doi.org/10.1021/acs.jced.7b00947>
- Noubigh, A., Mgaidi, A., & Abderrabba, M. (2010). Temperature effect on the distribution of some phenolic compounds: An experimental measurement of 1-octanol/water partition coefficients. *Journal of Chemical & Engineering Data*, 55(1), 488–491. <https://doi.org/10.1021/jc900271h>
- Nouri, M., Shahriari, S., Nabi, P., & Pazuki, G. (2023). Enhancement of vanillin partitioning and recovery in nanoparticle-based aqueous two-phase system containing PEG and dextran polymers. *Chemical and Biochemical Engineering Quarterly*, 37(2), 55–65. <https://doi.org/10.15255/CABEQ.2023.2201>

- Nouri, M., Shahriari, S., & Pazuki, G. (2019). Increase of vanillin partitioning using aqueous two phase system with promising nanoparticles. *Scientific Reports*, 9(1), Article 19665. <https://doi.org/10.1038/s41598-019-56120-8>
- Padilha, C. E. A., Nogueira, C. C., Almeida, H. N., Medeiros, W. R. D. B., Oliveira Filho, M. A., Araújo, J. S., & Santos, E. S. (2020). Separation and concentration of bioactive phenolic compounds by solvent sublation using three-liquid-phase system. *Food and Bioprocess Technology*, 120, 151–157. <https://doi.org/10.1016/j.fbp.2020.01.008>
- Parmoon, G., Mohammadi Nafchi, A., & Pirdashti, M. (2019). Effects of the polymer molecular weight and type of cation on phase diagrams of polyethylene glycol + sulfate salts aqueous two-phase systems. *Hemijiska Industrija (Chemical Industry)*, 73(6), 375–385. <https://doi.org/10.2298/HEMIND191003035P>
- Peña-Barrientos, A., Perea-Flores, M. J., Martínez-Gutiérrez, H., Patrón-Soberano, O. A., González-Jiménez, F. E., Vega-Cuellar, M. Á., & Dávila-Ortiz, G. (2023). Physicochemical, microbiological, and structural relationship of vanilla beans (*Vanilla planifolia*, Andrews) during traditional curing process and use of its waste. *Journal of Applied Research on Medicinal and Aromatic Plants*, 32, Article 100445. <https://doi.org/10.1016/j.jarmap.2022.100445>
- Pirdashti, M., Heidari, Z., Fashami, N. A., Arzideh, S. M., & Khoiroh, I. (2021). Phase equilibria of aqueous two-phase systems of PEG with sulfate salt: effects of pH, temperature, type of cation, and polymer molecular weight. *Journal of Chemical & Engineering Data*, 66(3), 1425–1434. <https://doi.org/10.1021/acs.jced.0c01029>
- Sadeghi, R., & Coutinho, J. A. P. (2024). Sugaring-out assisted organic-aqueous biphasic systems: Characteristics, mechanisms and applications. *Separation and Purification Technology*, 350, Article 127919. <https://doi.org/10.1016/j.seppur.2024.127919>
- Sampaio, D. A., Mafra, L. I., Yamamoto, C. I., Andrade, E. F., Souza, M. O., Mafra, M. R., & Castilhos, F. (2016). Aqueous two-phase (polyethylene glycol + sodium sulfate) system for caffeine extraction: Equilibrium diagrams and partitioning study. *The Journal of Chemical Thermodynamics*, 98, 86–94. <https://doi.org/10.1016/j.jct.2016.03.004>
- Sampaio, V. S., Alves, A. N., Souza Júnior, E. C., Veríssimo, L. A. A., Castro, S. S., da Fontan, R. C. I., Veloso, C. M., Ferrão, S. P. B., & Bonomo, R. C. F. (2020). Thermodynamic modeling of aqueous two-phase systems composed of macromolecules and sulfate salts at pH 2.0. *Journal of Chemical & Engineering Data*, 65(1), 9–18. <https://doi.org/10.1021/acs.jced.9b00557>
- Segaran, A., & Chua, L. S. (2024). Review of recent applications and modifications of aqueous two-phase system for the separation of biomolecules. *International Journal of Biological Macromolecules*, 276(Part 1), Article 133856. <https://doi.org/10.1016/j.ijbiomac.2024.133856>
- Silva, L. H. M., & Loh, W. (2006). Sistemas aquosos bifásicos: fundamentos e aplicações para partição/purificação de proteínas. *Química Nova*, 29(6), 1345–1351. <https://doi.org/10.1590/S0100-40422006000600033>
- Souza, I. N., Rodrigues, L. C. V., Soares, C. M. F., Buarque, F. S., Souza, R. L., & Lima, Á. S. (2024). Deep eutectic solvent-based aqueous two-phase systems and their application in partitioning of phenol compounds. *Molecules*, 29(18), Article 4383. <https://doi.org/10.3390/molecules29184383>
- Veloso, A. V., Silva, B. C., Bomfim, S. A., Souza, R. L., Soares, C. M. F., & Lima, Á. S. (2020). Selective and continuous recovery of ascorbic acid and vanillin from commercial diet pudding waste using an aqueous two-phase system. *Food and Bioprocess Technology*, 119, 268–276. <https://doi.org/10.1016/j.fbp.2019.11.011>
- Venkataraman, S., Athilakshmi, J. K., Rajendran, D. S., Bharathi, P., & Kumar, V. V. (2024). A comprehensive review of eclectic approaches to the biological synthesis of vanillin and their application towards the food sector. *Food Science and Biotechnology*, 33(5), 1019–1036. <https://doi.org/10.1007/s10068-023-01484-x>
- Wysoczanska, K., & Macedo, E. A. (2016a). Effect of molecular weight of polyethylene glycol on the partitioning of DNP-amino acids: PEG (4000, 6000) with sodium citrate at 298.15 K. *Fluid Phase Equilibria*, 428, 84–91. <https://doi.org/10.1016/j.fluid.2016.07.009>
- Wysoczanska, K., & Macedo, E. A. (2016b). Influence of the molecular weight of PEG on the polymer/salt phase diagrams of aqueous two-phase systems. *Journal of Chemical & Engineering Data*, 61(12), 4229–4235. <https://doi.org/10.1021/acs.jced.6b00591>
- Yin, K., Chen, L., Liu, F., Fan, T., Wu, Y., Li, S., & Yan, Z. (2022). Liquid-liquid equilibrium data for novel aqueous two-phase systems composed of betaine-xylitol deep eutectic solvent + alcohol (1-propanol/2-propanol/tert-butanol) + water. *Journal of Chemical & Engineering Data*, 67(9), 2475–2485. <https://doi.org/10.1021/acs.jced.2c00167>