Enhancement of Vanillin Partitioning and Recovery in Nanoparticle-based Aqueous Two-phase System Containing PEG and Dextran Polymers

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doi: https://doi.org/10.15255/CABEQ.2023.2201

Original scientific paper
Received: March 18, 2023
Accepted: June 1, 2023

Vanillin, widely utilized in the food, medicinal, and pharmaceutical industries, requires an improved extraction process that is cost-effective and environmentally friendly to meet the growing industrial demand. To tackle this challenge, we conducted an investigation on a nanoparticle-based aqueous two-phase system (ATPS), incorporating polyethylene glycol (PEG) and dextran (DEX). The primary objective was to develop an ATPS that is non-alkaline, operates under mild environmental conditions, and is both non-toxic and cost-effective. The study focused on identifying a suitable nanoparticle that could improve the partitioning of vanillin in ATPS and facilitate economically favorable separation processes. Various nanoparticles were evaluated as additives to enhance vanillin partitioning. The study explores the influence of parameters, such as polymer weight percentages and DEX molecular weight on vanillin partitioning and recovery percentage. Additionally, the impact of incorporating different nanoparticles was assessed in the optimized system composed of 6.5 wt% PEG6000 and 7.8 wt% DEX15000. Results indicate that the addition of only 0.001 g of silver nanoparticles to the optimal system improved the partition coefficient by 42 % and the vanillin recovery percentage by approximately 8 % compared to the nanoparticle-free ATPS.

Keywords

vanillin, polyethylene glycol, dextran, aqueous two-phase system, nanoparticle, partitioning

Introduction

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is an aromatic compound including functional groups such as aldehyde, ether, and phenol[1]. It has various uses in the food and beverage industries as a flavoring agent and preservative, besides its applications as starting material in the formulation of pharmaceuticals[2,3]. Vanillin has diverse medicinal properties such as neuroprotective, antimicrobial, inflammation, and stress reduction activity[4,5]. Moreover, this valuable biomolecule plays an important role as an antioxidant and anticancer agent by preventing oxidative damage in tissues[5].

There are two commercial types of vanillin in the global market, natural and synthetic. The natural source of vanillin is the vanilla bean. Natural vanillin constitutes only 1 % of the products in the market due to the complex separation of vanillin from other components contained in vanilla beans, and the costly harvesting and processing of beans[6]. On the other hand, synthetic vanillin is produced by biotechnological and chemical approaches. Due to the increasing awareness of consumers about chemical health risks, using microorganisms for bioconversion of substances such as ferulic acid, lignin, eugenol, etc., to produce vanillin have attracted much attention[3,7].

The global consumption of vanillin in 2016 was more than 18,000 tons, and is expected to reach 20,000 tons by 2025. Therefore, due to the increasing demand for vanillin consumption in the food and pharmaceutical industries, the improvement of the vanillin extraction process through cost-effective and environmentally friendly methods is a significant issue[5].

In biotechnological processes, a large amount of the final cost of the product belongs to the down-

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stream stage including extraction, and purification parts[8,9]. The liquid-liquid extraction has been widely considered due to its high impact, simplicity, and superior performance. Aqueous two-phase systems (ATPSs) have been recognized as a common liquid-liquid extraction media, and because of their aqueous environment and low surface tension between two phases, these media were introduced as the most appropriate systems for biomolecules[8]. Industrial processes with ATPSs could be an alternative to chromatography in order to reduce operating costs and facilitate the separation process[10]. The behavior of biomolecules in ATPSs is influenced by various factors, including the characteristics of the biomolecule itself, type of phase component, and its molecular weight. These factors interact with each other, making it challenging to predict and interpret how biomolecules partition in ATPSs due to the numerous parameters involved. The complexity and diversity of biomolecule behavior in ATPSs hinder the establishment of a general framework[11]. Nevertheless, it is important to recognize that ATPSs possess unique properties that make them highly valuable for study. ATPSs are composed of more than 80 % water, making them highly suitable environments for biomolecules. Another significant advantage of ATPSs is their potential to reduce the reliance on harsh organic solvents, which can have detrimental environmental impacts. By using ATPSs, there can be a reduction in energy consumption and greenhouse gas emissions associated with the production and disposal of these solvents[12,13]. In addition, the use of more sustainable polymer components, such as dextran, in ATPSs can further enhance the sustainability of the extraction and separation process. Dextran, being a naturally occurring polysaccharide, is biocompatible and biodegradable, making it a more environmentally friendly alternative to synthetic polymers[14].

ATPSs are composed of mutually immiscible components that are both soluble in water[15]. Typical ATPSs are classified based on their components such as polymer/polymer; polymer/salt; alcohol/salt, and ionic liquid/salt systems[12].

Polymer/polymer ATPSs are the most common systems that have been used for biomolecule partitioning, and phase separation in these mixtures is affected by the molecular weight and interaction of polymer components[16,17]. Polyethylene glycol (PEG) and dextran (DEX) form one of the most common and classic ATPSs[18]. PEG is a non-branched polymer composed of ethylene oxide units, and DEX is branched, consisting of glucose units connected with α-1,6 glycosidic linkages. In addition, their use as nontoxic substances in the pharmaceutical and food industries has been confirmed based on literature[19–22].

ATPSs including PEG and DEX have been increasingly used to separate a wide range of biomolecules, so it is time to improve the partitioning of biomolecules in these ATPSs with a novel approach. The use of nanoparticles in biological applications has increased widely, and besides its advantages, such as biocompatibility and creating an inert atmosphere without changing biological activities, it can help improve biomolecule partitioning in ATPSs[23,24]. The basis of this approach is the spontaneous partitioning of nanoparticles in ATPSs, in which the nanoparticle transfers biomolecules to one of the phases as a carrier[25].

Metal and metal oxide nanoparticles have various applications in targeted drug delivery, gene, and cancer therapy due to their antimicrobial, antioxidant, and antibacterial properties[26]. The main uses of metal oxide nanoparticles are the treatment of anemia and cancer with iron oxide nanoparticles[27], the use of titanium dioxide nanoparticles as an antibacterial agent[28], and the use of zinc oxide nanoparticles as an antibacterial agent and skin infection reducer[29]. Carbon nanotubes are another category of nanoparticles that have attracted attention due to their chemical stability and high specific surface area[30]. Moreover, the widespread use of silver nanoparticles in food packaging, sunscreen, antibacterial and antiviral drugs has been mentioned in the literature[31,32].

Several researchers have extensively investigated the partitioning of vanillin in ATPS using various components. The following references provide valuable insights into this research area:

Claudio *et al.* investigated the partition coefficient of vanillin in an improved ionic liquid (IL)-based aqueous two-phase systems. The main parameters in the partitioning process: concentration of vanillin in the system, ionic liquid cation and anion structure, and the temperature of equilibrium were evaluated, and they obtained the highest partition coefficient (98.08) in IL-rich phase for optimized conditions and systems[33]. Cardoso *et al.* used acetonitrile (ACN) and carbohydrate to study vanillin partitioning in ATPSs. They attained a partition coefficient of vanillin higher than 3, and recoveries ranged between 75 % and 91 % in the ACN-rich phase[34]. Cardoso *et al.* assessed the partitioning of vanillin in novel ATPSs composed of ACN and polyols. They observed partition coefficient and recoveries higher than 7 and 89 % in the ACN-rich phase, respectively[35]. Cardoso *et al.* formed ATPSs composed of poly (vinyl alcohol) and ACN, and found the highest partition coefficient and recovery of vanillin 2.24, 79 % at 5 °C in ACN-rich phase, respectively[36]. Shahriari *et al.* used cholinium chloride and salt (K_3PO_4) to study vanillin partitioning in ATPSs by conditions and systems as followed:

temperature, cholinium chloride, and salt weight fractions and vanillin concentration, and they attained partition coefficient and recoveries higher than 3 and 72 %, respectively[37].

Recently, the novel nanoparticle-based ATPS to study the partitioning of vanillin was published by our team. We enhanced the partition coefficient of this valuable biomolecule by almost 127 % by adding small amounts of modified carbon nanotubes (CNT) in ATPS composed of PEG and Na_2SO_4[25]. However, it is important to address a significant concern associated with the use of inorganic salts in ATPS, namely, their tendency to induce alkalinity. This alkalinity can potentially damage the structure of pH-sensitive biomolecules, which is a crucial consideration in various applications. To overcome this concern, our study aimed to evaluate the feasibility of incorporating DEX as an alternative component alongside PEG in ATPS. By replacing mineral salts with DEX, we aimed to create a greener and more sustainable approach for the partitioning of vanillin. Our goal was to minimize the potential risks associated with alkalinity. We believe that this alternative approach offers a more favorable environment for the separation of biomolecules, while minimizing the potential detrimental effects on their structure.

In the current study, we intentionally selected two distinct ATPSs consisting of polymer (PEG6000) and DEX with different molecular weights (DEX15000 and DEX35000). It is important to emphasize that the partitioning behavior of biomolecules in ATPSs with different components is highly intricate and can exhibit substantial variations. By investigating different ATPSs, we aim to gain a more comprehensive understanding of the behavior of vanillin within these systems. This approach allows us to explore the complex interactions between vanillin and different system components, thereby contributing to the advancement of knowledge in this field. Moreover, it is important to note that our previous study did not specifically examine the influence of n-Ag and functionalized n-Fe_2O_3 on the partition coefficient of vanillin in ATPSs. However, we specifically investigated the impact of these two types of nanoparticles, as well as other nanoparticles, as agents for enhancing the partitioning behavior and recovery of vanillin.

In this work, the experiments were performed in three stages. In the first stage, the phase diagram for two individual ATPSs (PEG6000+DEX-15000+H_2O and PEG6000+DEX35000+H_2O) were obtained by cloud point titration method. In the second stage, the efficiency of variables: polymer molecular weight and its weight percentage on vanillin partition coefficient and its recovery percentage were assessed in these ATPSs, and in the third stage, the effect of nanoparticles (silver / metal oxides / single and multiwall carbon nanotubes (SWCNT, MWCNT) / functionalized MWCNT and iron oxide) on the partitioning and recovery percentage of vanillin were investigated in ATPS with the highest partition coefficient as an optimal system.

Materials and methods

Materials

Vanillin (4-hydroxy-3-methoxy benzaldehyde, ≥99 wt% pure) and DEX with different molecular weights (M_w = 15000, 35000 g mol^{-1}, ≥99 wt% pure) were purchased from Sigma-Aldrich. PEG (M_w = 6000 g mol^{-1}, ≥99 wt% pure) was supplied by Merck. Silver nanoparticles (n-Ag, ≥99 wt% pure), zinc oxide nanoparticles (n-ZnO, ≥99 wt% pure), and iron oxide nanoparticles (n-Fe_2O_3 with a purity of ≥95 wt%) were obtained from US Research Nanomaterials, Inc., and titanium dioxide nanoparticles (n-TiO_2, ≥99 wt% pure) were supplied from Degussa Evonik. Single-walled and multiwalled carbon nanotubes (SWCNT, MWCNT with a purity of ≥95 wt%) were provided by US Research Nanomaterials, Inc., and Neutrino company, respectively. To achieve the functionalization of n-Fe_2O_3, precise quantities of $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 6H_2O$ were refluxed with an acetic acid solution and chitosan for 6 hours at a temperature of 80 °C. Following the addition of ammonia and a stirring period of 30 minutes, the resulting sediments underwent multiple rinses with deionized water to eliminate any impurities. Subsequently, the sediments were dried in a vacuum oven at 50 °C for a period of 24 hours to ensure complete removal of moisture. In line with the methodology employed in our previous research, MWCNTs underwent treatment using a solution comprising sulfuric acid and nitric acid in a ratio of 3:1. The resulting mixture was then subjected to ultrasonic agitation in a bath for 4 hours at room temperature, facilitating the complete dispersion of MWCNTs throughout the solution. Subsequently, the obtained suspension was refluxed for 8 hours at 80 °C, followed by centrifugation to separate the sediments. The sediments were thoroughly rinsed with deionized water on multiple occasions. Finally, the sediments, now referred to as functionalized MWCNTs, were dried in a vacuum oven at 40 °C for 24 hours to eliminate any remaining moisture[25].

Methods

PEG/DEX phase diagrams

To determine the phase diagram and achieve separation between the single-phase and two-phase

regions, a cloud point titration method was employed at a controlled temperature of 25 °C (±1 °C) and atmospheric pressure. The method used in this study follows the established approach described in our previous works as well as in the studies of other researchers in this field[33,38]. It is a commonly employed method for conducting similar investigations and has proven to be effective in studying the desired phenomenon. The process involved adding drops of a 50 % (by weight) PEG solution to a 10 % (by weight) DEX solution until the mixture became cloudy. Subsequently, water drops were added with continuous stirring to form a single-phase region, characterized by transparency. Next, the composition of the mixture was determined. To calculate the composition of the entire system, points on the binodal curve were obtained by considering the composition of the initial mixture and the amount of solution added. This iterative procedure was repeated until the required data points for the binary curves were obtained. The mass fraction of the components in the ternary system was estimated with precision, accounting for the weight of all added components[37].

Partitioning of vanillin

ATPSs were prepared by mixing certain weight percentages of components including PEG, DEX (with different molecular weights), vanillin, and distilled water. The working points within the two-phase zone were chosen using the phase diagram. In the polymer-polymer system, by changing the weight percentage of polymers and the molecular weight of DEX at a constant temperature, the optimal system was selected to investigate the effect of nanoparticles. In addition, the effect of different nanoparticles on the partition coefficient of biomolecule was studied.

PEG, DEX, and distilled water were precisely weighed using a digital balance with an accuracy of ± 10^{-4} g. The components were then stirred for 30 minutes. Subsequently, 0.01 gram of vanillin was added to the mixture, and stirring continued until complete dissolution of the vanillin was achieved. To investigate the partitioning of vanillin in the presence of nanoparticles within the ATPSs, initially, 0.001 g of the nanoparticles was introduced into 2 mL of distilled water. The mixture was sonicated for 1 hour to ensure thorough dispersion of the nanoparticles throughout the distilled water. Next, the nanoparticle suspension was added to the ATPS containing vanillin while the mixture was agitated for 30 minutes. Following this, the system was placed in an incubator (Memmert, Germany), and left for a day to reach equilibrium and ensure complete partitioning between the two phases. The incubator maintained a controlled temperature of 25 °C with an accuracy of ± 0.01 °C. Finally, the top and bottom phases were separated using a glass Pasteur pipette, and transferred to separate containers for further analysis[37].

Vanillin concentration in the top and bottom phases was measured by spectrophotometry (UV/Vis Model: SP-2100UV, Nanjing T-bota Scietech Instruments & Equipment Co., Ltd. (TBT), China) regarding its maximum absorption at the wavelength of 280 nm. The concentration of this biomlolecule in two phases was obtained using the calibration curve. The partition coefficient of vanillin was determined according to Eq. 1. It was obtained as the ratio of vanillin concentration in the top phase (PEG-rich phase) to its concentration in the bottom phase (DEX-rich phase)[38].

$$K_{\mathrm{Van}} = \frac{[\mathrm{Van}]_{\mathrm{T}}}{[\mathrm{Van}]_{\mathrm{B}}} \tag{1}$$

where K_{Van} is the partition coefficient of vanillin, and $[\mathrm{Van}]_{\mathrm{T}}$, $[\mathrm{Van}]_{\mathrm{B}}$ are the vanillin concentrations in the top (PEG-rich) and bottom (DEX-rich) phases, respectively. The recovery percentage of vanillin in the top phase (R_{T}) was obtained based on the following equation:[37]

$$R_{\mathrm{T}} = \frac{[\mathrm{Van}]_{\mathrm{T}}}{[\mathrm{Van}]_{\mathrm{T}} + [\mathrm{Van}]_{\mathrm{B}}} \tag{2}$$

For the thermodynamic analysis of biomolecule partitioning, the molar Gibbs free energy of vanillin partitioning was obtained by Eq. 3[39].

$$\Delta_{\mathrm{part}} G_{\mathrm{m}}^{\circ} = -RT \ln K_{\mathrm{Van}} \tag{3}$$

where K_{Van} T, and R represent the partition coefficient of vanillin, temperature (K), and universal gas constant (8.314 J mol^{-1} K^{-1}), respectively.

Results and discussion

The separation of biomolecules by ATPSs is assessed as a function of their partition coefficient. In this study, the partitioning of vanillin as a natural additive widely used in the food and pharmaceutical industries and its recovery percentage were measured in aqueous two-phase polymer/polymer (PEG/DEX) systems. The effect of parameters such as the weight percentage of both polymers and the molecular weight of DEX on the partition coefficient of vanillin was evaluated. In addition to determining the recovery percentage and the molar Gibbs free energy of vanillin partitioning for these ATPSs, the effect of adding different nanoparticles on the quantity of the aforementioned dependent variables was assessed for the optimal system.

PEG/DEX phase diagrams

In order to study the separation process and determine the partition coefficient in ATPS, the phase diagram was used. The ability to form ATPS including PEG and DEX at constant temperature (25 °C) and atmospheric pressure was evaluated. The polymers showed the ability to form biphasic systems and the phase diagram using PEG (6000 g mol^{-1}) and DEX (15000, 35000 g mol^{-1}) is presented in Fig. 1. According to this figure, the binodal curve shifts to the left (i.e., closer to the origin – pure water) by increasing molecular weight of the polymer, and as a result of reducing the solubility of the polymer in water, the components have a greater ability to form ATPS. Therefore, the volume available for binding with water molecules decreases and the two-phase region expands[36]. The result is in agreement with other observations regarding polymer-based ATPSs[36,40]. In addition, as the chain length increases and the available volume decreases in the polymer-rich phase, less space is available for biomolecules[41].

Effect of weight percentage of components on vanillin partitioning

The effect of weight percentage of PEG and DEX on the partition coefficient of vanillin at 25 °C and atmospheric pressure was evaluated. The experimental data of the vanillin partition coefficient and its standard deviation in different molecular weights of DEX without adding nanoparticles are reported in Figs. 2 and 3. For both systems, the partition coefficient greater than one indicates the tendency of vanillin to migrate to the top phase (PEG-rich phase) and the results could be evaluated based on Hansen solubility parameters (HSP). The total solubility parameter (δ_t) and the difference of solubility parameters ($\Delta\delta$) were calculated based on the following equations[42]:

$$\delta_t = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2} \quad (4)$$

$$\Delta\delta = \sqrt{\left(\delta_{d1} - \delta_{d2}\right)^2 + \left(\delta_{p1} - \delta_{p2}\right)^2 + \left(\delta_{h1} - \delta_{h2}\right)^2} \quad (5)$$

where δ_d, δ_p, and δ_h represent the dispersion, polar, and hydrogen bonding solubility parameters, respectively. The values of HSPs for all components and the difference in HSPs of all materials with each other are reported in Table 1[42,43].

$\Delta\delta$ determines the compatibility of two components, so its decrease indicates the stronger interaction between them[42]. According to the results, the values of $\Delta\delta$ for vanillin related to PEG is less than DEX and water. This indicates that PEG has a stronger interaction with vanillin compared to other components. So, it can be concluded that the tendency of vanillin for migration is in the PEG-rich phase (top phase).

According to Figs. 2 and 3, by increasing the concentration of the PEG, the maximum partition coefficient of vanillin was observed in both systems. The initial increase can be due to the interaction of more units of the polymer with the biomolecule as a result of increasing the concentration and viscosity of the polymer in the top phase; thus, the partition coefficient increases. After reaching the maximum, an excessive increase in the weight percentage of PEG makes less space available for biomolecules, which decreases the partition coefficient of biomolecule. Also, by increasing the concentration of DEX at a fixed weight percentage of PEG, the similar trend was observed, i.e., a maximum at a certain mass fraction of DEX. The initial increase in the weight percentage of DEX makes less space available for biomolecules in the bottom phase, thus

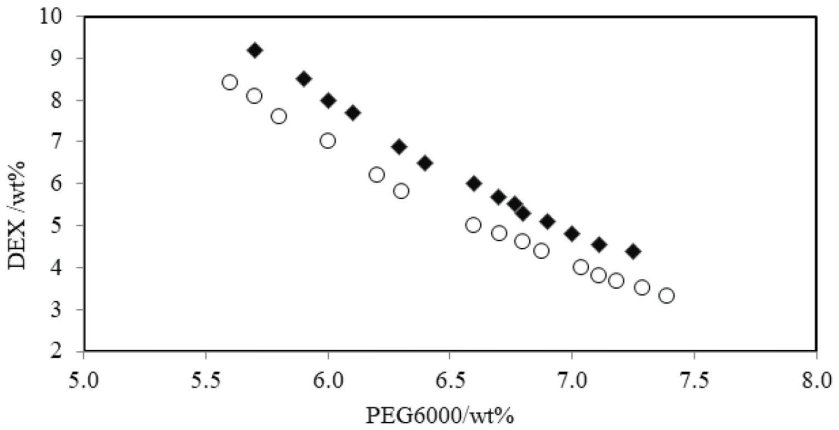

Fig. 1 – *Phase diagrams for ternary systems including PEG6000 +DEX+H_2O at 25 °C and atmospheric pressure: (♦) DEX15000; (○) DEX35000*

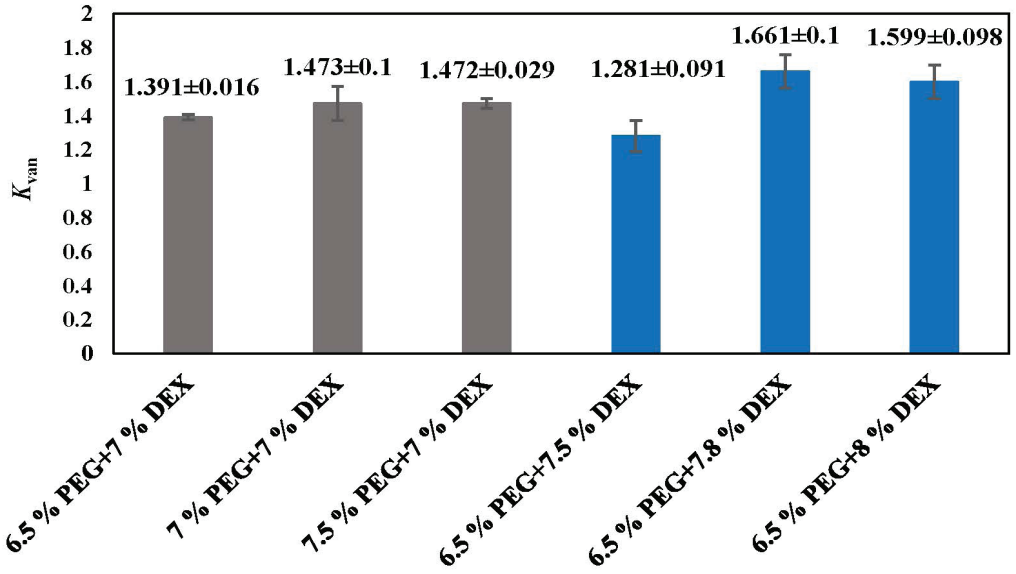

Fig. 2 – *Effect of weight percentage of components on the vanillin partitioning in PEG6000+DEX15000 ATPS at 25 °C and atmospheric pressure*

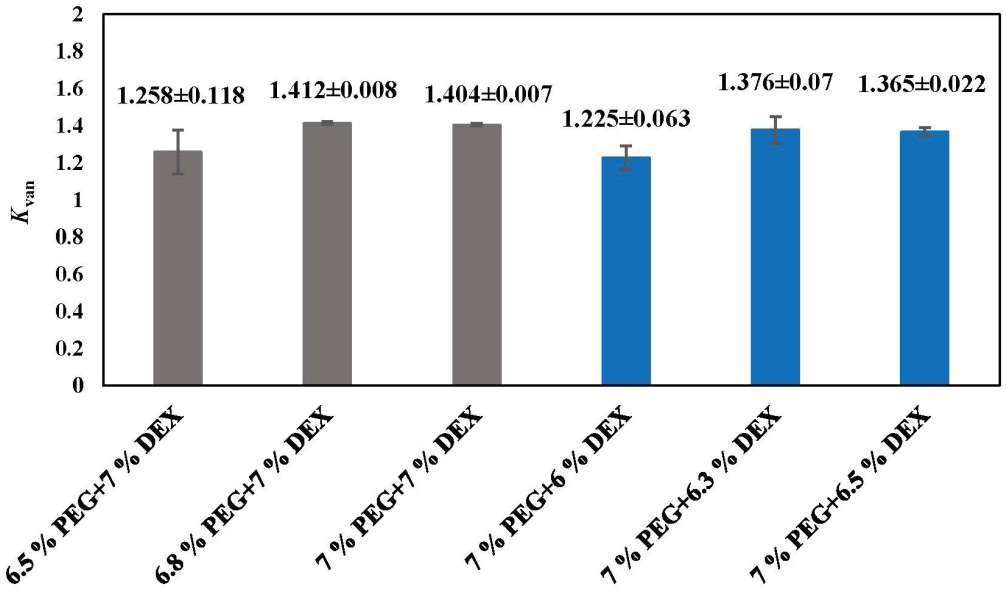

Fig. 3 – *Effect of weight percentage of components on the vanillin partitioning in PEG6000+DEX35000 ATPS at 25 °C and atmospheric pressure*

Table 1 – *Hansen solubility parameters and their difference for components of ATPS*

Component	δ_d^a	δ_p^b	δ_h^c	δ_t^d	$\Delta\delta_{PEG}$	$\Delta\delta_{DEX}$	$\Delta\delta_{Water}$
	MPa$^{0.5}$						
Vanillin	19.4	9.8	11.2	24.45	9.48	11.56	31.95
PEG	21.02	0.96	8.19	22.58	0	19.24	37.68
DEX	23.21	15.66	20.41	34.65	19.24	0	23.21
Water	15.50	16.00	42.30	47.80	37.68	23.21	0

[a]dispersion solubility parameter; [b]polar solubility parameter;
[c]hydrogen bonding solubility parameter; [d]total solubility parameter

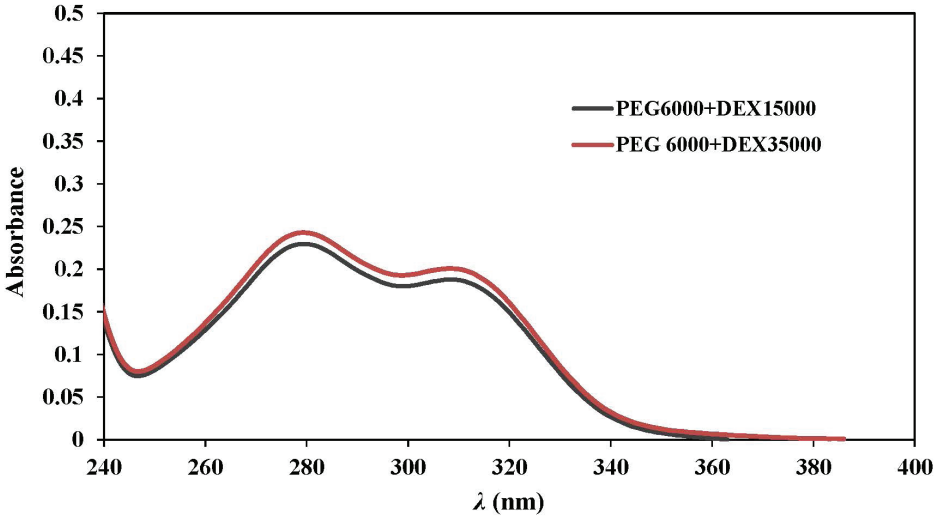

Fig. 4 – *Absorbance of vanillin in the PEG-rich phase (top phase) for PEG6000 and DEX15000, 35000 ATPSs at 25 °C and atmospheric pressure as a function of wavelength, λ*

pushing the vanillin biomolecules to the top phase and increasing the vanillin partition coefficient. After reaching the maximum, the decrease in the partitioning of vanillin with an excessive increase in the weight percentage of DEX can be attributed to salting-in effect caused by DEX. According to the results, the highest and the lowest partition coefficient of vanillin were observed for PEG6000 (6.5 wt%) + DEX15000 (7.8 wt%) and PEG6000 (7 wt%) + DEX35000 (6 wt%) ATPSs, respectively.

The conformation of vanillin in the PEG-rich phase of the ATPS was analyzed using UV-vis spectra. Initially, the maximum absorbance of vanillin in distilled water was determined at a wavelength of 280 nm. Subsequently, the maximum absorbance of vanillin in the PEG-rich phase was assessed in ATPSs containing PEG6000+DEX15000 and PEG6000+DEX35000. The results obtained from the UV-vis spectra are presented in Fig. 4. Upon comparing the spectra, it was observed that the maximum absorbance of vanillin (at 280 nm) remained consistent across samples. This indicated that there is no significant alteration in the electronic or structural properties of vanillin, suggesting that the biomolecule remains stable and maintains its original conformation throughout the separation process. The absence of a shift in the absorption maximum indicates that there are no significant chemical reactions or interactions occurring between the biomolecule and the surrounding components, such as the PEG molecules in the PEG-rich phase. Therefore, the biomolecule undergoes no substantial changes or disruptions in its structure during the separation process. The results are in agreement with our team's previous observations for PEG+Na_2SO_4 ATPS in the presence and absence of functionalized MWCNT[25].

Effect of molecular weight of DEX on the vanillin partitioning

Based on Figs. 2 and 3, the effect of DEX molecular weight on the partition coefficient of vanillin is evident, and by its increase, the partition coefficient of vanillin is decreased. Thus, the ATPS containing PEG6000+DEX15000 has a higher partition coefficient compared to ATPS containing PEG6000+DEX35000. Ebrahimi *et al.* also confirmed the decrease in this dependent variable by increasing molecular weight. The reason for this reduction is the effect of decreasing the volume of available water in the DEX-rich phase and increasing the hydrophobicity of this phase. It should be remarked that by increasing the length of the polymer chain for a certain polymer mass fraction, the number of available hydroxyl groups decreases, and as a result, the hydrophobicity of the polymer increases[8].

For further understanding of the vanillin partitioning, the recovery percentage of a biomolecule in the top phase and the molar Gibbs free energy of vanillin partitioning in both ATPSs are given in Table 2. The effect of increasing the weight percentage of polymers and the molecular weight of DEX on the recovery percentage of vanillin had a similar trend by its partition coefficient. The maximum recovery of vanillin was 62.42 % in ATPS containing PEG6000 (6.5 wt%) and DEX (7.8 wt%).

As presented in Table 2, the negative values of $\Delta_{part}G^{\circ}_{m}$ shows the spontaneous transfer of vanillin to the top phase, and the more negative the molar Gibbs free energy of partitioning, the greater the salting-out of vanillin by DEX[39].

Effect of nanoparticles on the vanillin partitioning

The PEG6000 (6.5 wt%) + DEX15000 (7.8 wt%) was determined as the optimal system in or-

Table 2 – *Recovery of vanillin in the top phase and the values of $\Delta_{part}G^{\circ}_{m}$ in PEG+DEX+H_2O ATPSs at 25 °C and atmospheric pressure*

ATPS	PEG (wt%)	DEX (wt%)	H_2O (wt%)	R_T (%)	$\Delta_{part}G^{\circ}_{m}$ (kJ mol^{-1})
PEG6000+DEX15000	6.5	7	86.5	58.17	–0.82
	7	7	86	59.56	–0.96
	7.5	7	85.5	59.54	–0.96
	6.5	7.5	86	56.15	–0.61
	6.5	7.8	85.7	62.42	–1.26
	6.5	8	85.5	61.52	–1.16
PEG6000+DEX35000	6.5	7	86.5	55.71	–0.57
	6.8	7	86.2	58.54	–0.85
	7	7	86	58.4	–0.84
	7	6	87	55.06	–0.5
	7	6.3	86.7	57.91	–0.79
	7	6.5	86.5	57.71	–0.77

der to investigate the effect of different nanoparticles, and the results related to the effect of adding n-Ag and metal oxide nanoparticles (n-TiO_2, n-Fe_2O_3, functionalized n-Fe_2O_3, n-ZnO), SWCNT, MWCNT and functionalized MWCNT on the partition coefficient of vanillin are reported in Fig. 5.

According to Fig. 5, the partition coefficient of vanillin in the optimal ATPS system was determined to be 1.661 in the absence of nanoparticles. However, upon the addition of certain nanoparticles, such as n-Ag, functionalized n-Fe_2O_3, n-ZnO, and functionalized MWCNT, the partition coefficient of vanillin increased. On the contrary, the addition of other nanoparticles had no significant impact on the partition coefficient of vanillin. Specifically, among the investigated nanoparticles, n-Ag exhibited the most significant effect in enhancing the partitioning of vanillin. The partition coefficient of vanillin in the presence of n-Ag was reported as 2.357. These results indicate that n-Ag demonstrated the highest capability in promoting the partitioning of vanillin. A similar effect was reported by Afzal *et al.*, who studied the influence of various nanoparticles on the partition coefficient of Cephalexin in ATPS containing poly (ethylene glycol) and organic salts[23].

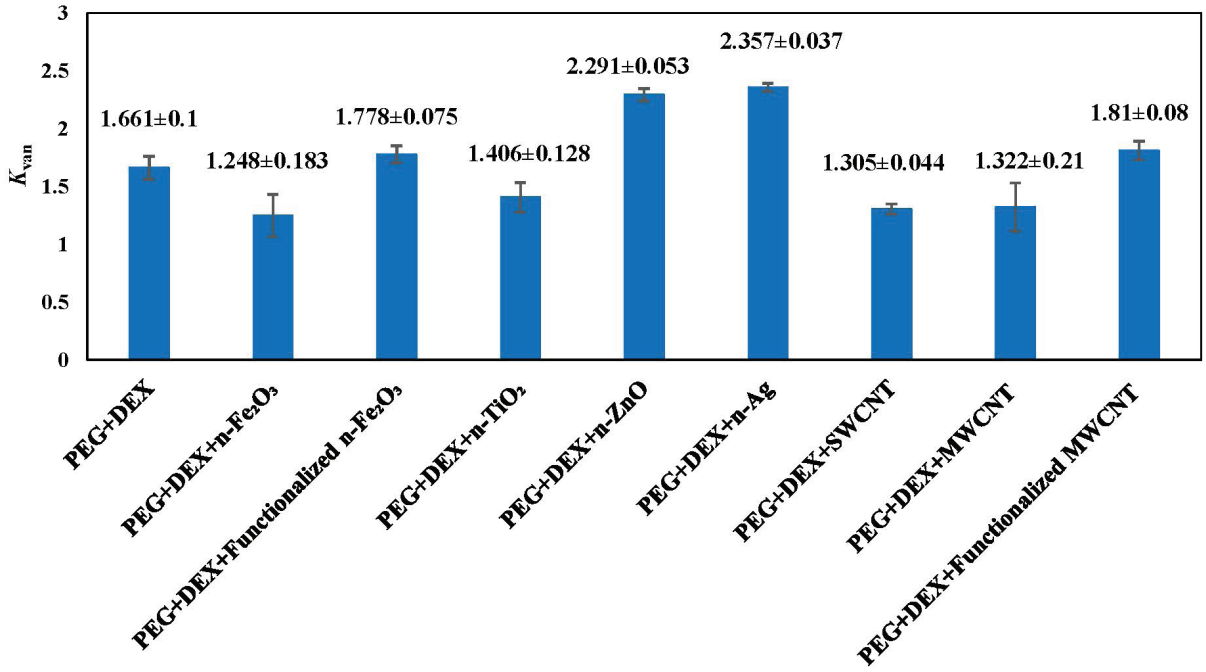

Fig. 5 – *Effect of nanoparticles addition on vanillin partitioning in PEG6000 (6.5 wt%) + DEX15000 (7.8 wt%) ATPS at 25 °C and atmospheric pressure*

Table 3 – *Recovery of vanillin in the top phase and the values of $\Delta_{part}G^{\circ}_{m}$ in PEG6000 (6.5 wt%)+DEX15000 (7.8 wt%) + nanopaticles ATPS at 25 °C and atmospheric pressure*

Nanoparticles	R_T (%)	$\Delta_{part}G^{\circ}_{m}$ (kJ mol^{-1})
Without nanoparticles	62.42	–1.26
n-Fe_2O_3	55.51	–0.55
SWCNT	56.62	–0.66
MWCNT	56.93	–0.69
n-TiO_2	58.44	–0.84
Functionalized Fe_2O_3	64	–1.42
Functionalized MWCNT	64.41	–1.47
n-ZnO	69.62	–2.05
n-Ag	70.21	–2.12

The change in partition coefficient when nanoparticles are added to the system can be attributed to the unique properties and behavior of nanoparticles in ATPSs. When nanoparticles are introduced into ATPSs, they undergo spontaneous partitioning, meaning that they distribute themselves between the two phases. This partitioning behavior of nanoparticles allows them to act as carriers, facilitating the transfer of biomolecules to one of the phases. By acting as carriers, nanoparticles can influence the distribution of biomolecules and alter their partitioning behavior in the ATPSs. This, in turn, can affect the partition coefficient of the biomolecule, as observed in our study. The specific mechanisms by which nanoparticles interact with biomolecules and affect their partitioning are complex and can vary depending on the nanoparticle properties and the nature of the biomolecule.

The recovery percentages of vanillin in the top phase and the values of $\Delta_{part}G^{\circ}_{m}$ in PEG6000 (6.5 wt%) + DEX15000 (7.8 wt%) + nanoparticles ATPS at 25 °C are reported in Table 3. Results show that the maximum recovery percentage of vanillin (70.21 %) belongs to the optimal system including silver nanoparticles with an ≈8 % increase compared to this ATPS in absence of nanoparticles.

As mentioned in Table 3, the negative values of $\Delta_{part}G^{\circ}_{m}$ indicate the spontaneous transfer of vanillin to the top phase in the absence and presence of nanoparticles[39]. It should be remarked that the nanoparticles including n-Ag, n-ZnO, functionalized MWCNT and functionalized n-Fe_2O_3 led to more negative values of molar Gibbs free energy of partitioning compared to the optimal system in the absence of nanoparticles.

Comparison of partition coefficient and recovery of vanillin with literature values

The results found in the literature for the partition coefficient and recovery of vanillin using different ATPSs at 25 °C and atmospheric pressure are presented in Table 4. As reported in Table 4, the highest partition coefficient and recovery of vanillin belongs to ionic liquid ([C_7H_7mim]Cl)+K_3PO_4+H_2O and Sorbitol+Acetonitrile+H_2O ATPSs, respectively[35,37]. In this study, the novel nanoparticle based ATPS containing PEG+DEX+H_2O was used to form a nontoxic and economical system that has the ability to be used in the food and pharmaceutical industries. In addition, the partition coefficient and recovery percentage of vanillin were improved by 42 % and ≈8 % by adding small amounts of n-Ag (0.001 g) compared to ATPS without nanoparticles.

Conclusions

In this study, we present the phase diagram for PEG6000 + DEX15000 or DEX35000 ATPSs. We investigated the partition coefficient of vanillin, its recovery percentage, and the molar Gibbs free energy of vanillin partitioning in these systems with varying weight percentages of PEG6000 and DEX15000 or DEX35000 in the absence of nanoparticles. The partition coefficient values in all systems studied were greater than one, indicating that vanillin tends to partition to the top phase (PEG-rich phase). We then evaluated the effect of nanoparticles on the partition coefficient of vanillin in the optimal system (6.5 wt% PEG6000 + 7.8wt%

Table 4 – *Comparison of the reported maximum partition coefficient and recovery of vanillin with literature values in ATPSs at 25 °C and atmospheric pressure*

ATPS	K_{Van}	Recovery (%)	Reference
[C_7H_7mim]Cl+K_3PO_4+H_2O	98.69	--	[33]
Mannose+Acetonitrile+H_2O	≈10	91	[34]
Sorbitol+Acetonitrile+H_2O	≈65	≈95	[35]
Poly(vinyl alcohol)+Acetonitrile+H_2O	≈1.5	≈77	[36]
[Ch]Cl+K_3PO_4+H_2O	11.40	94.02	[37]
PEG4000+Na_2SO_4+Functionalized MWCNT	43.07	--	[25]

DEX15000). Our results show that the optimal system with n-Ag has the highest partition coefficient and recovery percentage, with values of 2.36 and 70.21 %, respectively. The lowest partition coefficient and recovery percentage values were observed for the system with n-Fe_2O_3, by values of 1.25 and 55.51 %, respectively. It is worth noting that the addition of only 0.001 g of n-Ag to the optimal system leads to a 42 % increase in partition coefficient and ≈8 % increase in recovery percentage compared to the ATPS without nanoparticles.

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