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A critical assessment of the Flory-Huggins (FH) theory to predict aqueous two-phase behaviour

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ABSTRACT

This article provides an analysis on published models used to calculate phase separation in aqueous two-phase systems (ATPS) based on Flory-Huggins (FH) theory, in terms of problem formulation and mathematical solving algorithm. An integrated algorithm is presented, showing different mathematical approaches of using the FH theory. The algorithm involves the estimation of interchange energy, and the calculation of phase compositions. Based on experimental data, the thermodynamic model can provide a useful framework to perform a sensitivity analysis on parameters, in order to understand the influence of salt type, polymer molecular mass, and ionic strength on phase separation. However, this model, restricted to entropic and enthalpic terms, cannot quantitatively describe the data. This occurs mainly because of the strong influence of random experimental errors on the estimation of interchange energy and FH not being an exact description of phase separation in salt based ATPS. After providing a literature overview and mathematical analysis, we bring to the field the application of the FH theory for selecting ATPS and its limitations.

1. Introduction

Aqueous two-phase systems (ATPS) are well-known as downstream processing unit operation to separate valuable compounds from fermentation broth and complex systems [1]. Food industry can rely on this method to separate proteins with the advantages of mild operating conditions, high recovery yield and ease of scaling up [2]. Moreover, this technique can also be applied at the biorenewable energy field, providing solubilisation of the lignocellulosic substrate during the pretreatment of the biomass [3]. Thermodynamic analysis of ATPS can assist not only to a theoretical understanding of phase separation [4], but also in the prediction of optimal parameters for a specific application [5]. A reliable thermodynamic model can also be applied to re-predict phase separation when systems are disturbed due to phase recycle and removal of solutes in a specific application. Consequently, a process design involving ATPS can highly benefit when a thermodynamic model is integrated throughout the design stage.

Among a wide variety of thermodynamic models [6], Flory-Huggins (FH) [7] presents several advantages, such as a clear distinction between entropic and enthalpic effects, and a relatively simple and analytical set of equations. The FH equations [7] were developed for polymer solutions (high molecular weight macromolecules dissolved in low-molecular weight solvents). This theory has been applied broadly to polymer blends and block copolymers [8]. In 1998, Johansson and co-workers [9] introduced a framework based on the FH theory for the prediction of phase separation of ATPS composed of polymer and salt. Among the lattice models, UNIQUAC and UNIFAC have also been used to describe phase behaviour in polymer-salt based ATPS [10].

Because of the charge of the salt ions, the models have been extended to consider the electrostatic interactions between these ions. This long-range contribution is expressed by the Debye-Hückel (DH) and Pitzer-Debye-Hückel (PDH) terms [11,12]. Apart from the extra term to incorporate the ionic interactions, some models also present ion specific parameters. The electrolyte perturbed chain statistical associating fluid theory (ePC-SAFT) is an example of a model which considers the ionic interaction term, ion specific parameters and the molecular structure of the organic anion (e.g. citrate) [13]. The ePC-SAFT model showed quantitatively correct predictions of ATPS composed by polymers and salts [13,14].

FH model is composed of a combinatorial term (entropic

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contribution) and a short-range interaction term (enthalpic contribution). Some works [12] have also described the short-range interactions by replacing the enthalpic term of the FH equation (composed by the interchange energy or interaction parameter) for the so called Chen-NRTL. The addition of this enthalpic term to the already defined FH combinatorial contribution (entropic term) and to the PDH long-range interaction contribution improves the fitting of the experimental data, when applied to systems composed by polymer and citrate salts. However, a deep understanding of the influence of the input parameters would benefit the theoretical prediction of phase separation when a FH model composed only by the entropic and the short-range interaction terms are applied. Although there are many techniques to measure the interaction parameter experimentally, they do not correspond exactly to the proper parameters present in the FH theory [4]. In order to avoid this divergence, we propose mathematical ways to estimate the interchange energy using solely the FH theory in a reverse approach. Since the interaction parameters (χ_{ij}) are a direct correlation from the interchange energy (w_{ij}), χ_{ij} can also be inferred and interpreted based on this approach. Moreover, this work aims to present advances and limitations of existent calculations based on FH theory to predict phase separation in salt-polymer based ATPS. Different problem formulations and numerical methods were developed and evaluated for that purpose. Based on experimental data, two mathematical approaches to regress the interchange energy are presented. The developed framework is divided in two steps (regression of interchange energy and calculation of phase diagram) and provides qualitative prediction and understanding of enthalpic and entropic contributions to phase separation. Based on a useful thermodynamic modelling, the framework could identify trends to guide the screening of ATPS.

2. Methods

2.1. Research design

The implementation and analysis of FH theory were divided in two steps: step 1, estimation of interchange energy (w_{ij}); step 2, calculation of phase diagrams (Fig. 1). In step 2, the calculation of volume fractions for each component in top and bottom phases (ϕ_i^t and ϕ_i^b, respectively) through FH theory requires the following input information: interchange energy between the components (w_{ij}), degree of polymerization (M_i), molecular weight (Mw_i), and component charge (z_i).

The ATPS studied in this work were formed by polymers and salts. Phase diagrams were determined while varying polymer molecular size, salt type, concentration of these phase forming components (assessed by the tie line length - TLL) and ionic strength (evaluated here in terms of pH and ion charges). Parameters as temperature (T) and pressure (P) were assumed constant. The polymer type was kept as being polyethylene glycol (PEG), varying the molecular weight in 2000 g/mol, 4000 g/mol and 6000 g/mol. The salt types evaluated were sodium phosphate, magnesium sulphate and potassium citrate.

2.2. FH framework

The FH equation was derived by Flory [15] and Huggins [16] independently. The FH mean field theory is applied here in order to calculate the volume fraction of components in top and bottom phases. The influence of system variables, such as polymer size or salt type, on phase separation can be directly retrieved from the calculated volume fractions, which provides information to phase diagram construction.

The volume fraction of components is defined by $\phi_i = \frac{M_i n_i}{N}$, where M_i is the degree of polymerisation of component i, n_i the number of molecules of each component and N is the total number of lattice sites computed as $N = \sum_{i=1}^{m} M_i n_i$, for m being the number of components in the system. The volume fraction represents the fraction of sites occupied by the components in the total lattice. The concept of lattice models assumes that each site of the lattice is occupied by either a
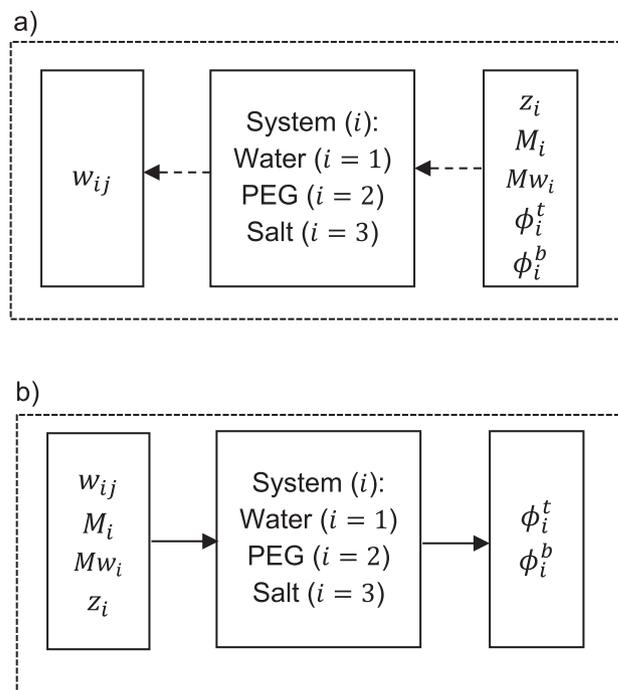

Fig. 1. Schematic display of the algorithm applied to calculate interchange energy and phase separation. a) Step 1 of the algorithm - the dashed arrows represent the inverse order of calculation, in which the volume fractions are used to estimate the interchange energy; b) step 2 of the algorithm, used to calculate the phase diagram.

solvent molecule or a polymer segment [17].

The calculation of the optimum volume fractions per component in both top and bottom phases should satisfy the condition that the sum of the Gibbs free energy in both phases is smaller than the Gibbs free energy calculated for the homogeneous system.

The Gibbs free energy of mixing combines an enthalpic (ΔH) and an entropic (ΔS) term and is represented by Equation (1). According to the definition of entropy (Equation (2)) and the short range interaction term for enthalpy (Equation (3)) [18], the Gibbs free energy of mixing can be rewritten as Equation (4).

$$\Delta G_{mix} = \Delta H_{mix} - T\Delta S_{mix} \tag{1}$$

$$\Delta S_{mix}^c = -NR \sum_{i=1}^{m} \frac{\phi_i}{M_i} \ln \phi_i \tag{2}$$

$$\Delta H_{mix} = N \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} \phi_i \phi_j w_{ij} \tag{3}$$

$$\Delta G_{mix} = N \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} \phi_i \phi_j w_{ij} + NRT \sum_{i=1}^{m} \frac{\phi_i}{M_i} \ln \phi_i \tag{4}$$

where R is the universal gas constant and has the value of 8.3144 J/K/mol, and T is the temperature.

The FH model is independent on the molecular shape, and because of that the model does not always give the presumably correct, quantitative combinatorial entropy of mixing. The entropy, however, is the dominant term for solutions of molecules of very different size [17].

The pair-wise interchange energy, w_{ij}, is often replaced with the FH interaction parameter χ_{ij}. They are related by $\chi_{ij} = \frac{w_{ij}}{RT}$ [17]. The dimensionless FH interaction parameter χ_{ij} is a measurable quantity. In the FH equation, although w_{ij} is used, χ_{ij} can also be inferred and interpreted from the application of such equations·

Table 1

Description of the methods used to determine the interaction parameters (which are derived from the interchange energies), given the compositions of the components in top and bottom phases.

Characteristics	Methods	
	Linear regression, A	Reconciliation, B
Overview	Volume fractions ($\phi_i^{t/b}$) are given experimentally. The interchange energies (w_{ij}) are calculated in order to minimize the difference in chemical equilibria of the components for each phase.	Postulated compositions of the phases are introduced for each experimental point. In the first optimization, the postulated compositions are fit under fixed interchange energy (calculated through linear regression). The second optimization follows by fitting both the postulated compositions and interchange energy.
Objective function	According to the chemical potential equality ($\mu_i^t = \mu_i^b$), and considering that μ can be expressed as $\mu_i^\alpha = C^\alpha w - D^\alpha$, for each phase α, the linear solution is written as $Cw = D$. The matrix C contains the coefficients of w, while the vector D is a function of the experimental volume fractions ($\phi_i^{t/b}$) and degree of polymerization (M_i).	The objective function searches for the reduction of the sum square of the residues between the postulated and experimental compositions, as well as interchange energy which satisfy the equilibrium between the phases ($\mu_i^t = \mu_i^b$), according to Equation (10).
Mathematical algorithm	w is regressed such as $w = (C^T C)^{-1} C^T D$	Postulated compositions are regressed through minimization of the sum of the squares of the residues between calculated and experimental compositions. w is determined considering the postulated volumes fractions and the chemical equilibrium conditions, though minimization of the difference of chemical potential in the phases for each component.
Constraints	No constraints are added to this approach.	The sum fraction is equal to 1 for top and bottom phases. The charge balance constraint is fulfilled in the determination of the interchange energy, once w can vary such that charge balance is respected.

2.3. Algorithm implementation

In this section, the problem formulation for each step is presented, in terms of objective functions, constraints and mathematical algorithm. The way of evaluating and comparing the different approaches for interaction parameters determination and phase diagrams calculation are also displayed here.

2.3.1. Step 1: Pair-wise interchange energy determination

The interaction parameters were determined through two different mathematical approaches (Table 1). In approach A, it was aimed to obtain a linear expression involving the interchange energy, in which the experimental volume fractions are fitted. Approach B accepts the fact that there might be a difference between experimental and modelled phase compositions. Then, the pair-wise interchange energies are calculated in order to reduce this difference as much as possible. For all the approaches, experimental fractions for each component in top and bottom phases are required.

In the regression of the interchange energy through a linear expression represented by method A, the objective function was written to satisfy the condition that the chemical potential in top (μ_i^t) and bottom (μ_i^b) phases for each component should be equal, according to:

$$\mu_i^t = \mu_i^b \tag{5}$$

Furthermore, the chemical potential consists of the summation of three terms given by:

$$\mu_i = \mu_i^\circ + RT \ln\phi_i + \mu_i^{ex} \tag{6}$$

where μ_i° and μ_i^{ex} are the reference equilibrium and excess chemical potential of component i, respectively, and ϕ_i the volume fraction of component i with respect to the total of components in that phase. Combining Equation (6) and the constraint imposed in Equation (5), the equilibrium partition coefficient is computed as:

$$\ln K_i^\circ = \frac{1}{RT}((\mu_i^{ex})^b - (\mu_i^{ex})^t) \tag{7}$$

being $K_i^\circ = \phi_i^t/\phi_i^b$ the partition coefficient where the component have no charge.

The expression for the equilibrium partition coefficient according to FH is given below:

$$
\begin{aligned}
\ln K_i^\circ &= M_i\left(\sum_j^m \left(\frac{\phi_j}{M_j}\right)^t - \sum_j^m \left(\frac{\phi_j}{M_j}\right)^b\right) - \frac{M_i}{RT}\left(\sum_{j\neq i}^m (\phi_j^t - \phi_j^t)w_{ji} - \sum_j^{m-1}\sum_{k=j+1}^m (\phi_j^t \right. \\
&\left. \phi_k^t - \phi_j^b\phi_k^b)w_{jk}\right)
\end{aligned}
\tag{8}
$$

where m is the number of phase forming components and M_i is the degree of polymerisation of component i. Also, the relation between the equilibrium partition coefficient ($\ln K_i^\circ$) and the actual partition coefficient (K_i), also considering the charge of component i, is given by:

$$\ln K_i = \ln K_i^\circ + \frac{z_i F \Delta\psi}{RT} \tag{9}$$

The Equations (8) and (9) can be rewritten such that on the left-hand side a linear equation as a function of all interchange energies is obtained and on the right-hand side a constant value composed of the volume fractions and degree of polymerisation (input variables that remain constant during the calculation) is computed ($constant = f(\phi_i^t, \phi_i^b, M_i, z_i)$):

$$
\begin{aligned}
&-\frac{M_i}{RT}\left(\sum_{j\neq i}^m (\phi_j^t - \phi_j^t)w_{ji} - \sum_j^{m-1}\sum_{k=j+1}^m (\phi_j^t\phi_k^t - \phi_j^b\phi_k^b)w_{jk}\right) \\
&= \ln K_i - \frac{z_i F \Delta\psi}{RT} - M_i\left(\sum_j^m \left(\frac{\phi_j}{M_j}\right)^t - \sum_j^m \left(\frac{\phi_j}{M_j}\right)^b\right)
\end{aligned}
\tag{10}
$$

This regression requires as input a matrix of volume fractions in top and bottom phases, where each column is a component and each row is a data point. Four variables are provided for each phase (volume fractions of water, polymer, anion and cation. However, not all the four variables were measured, even though they are considered as input data. For each data point, the linear term and the constant part of the equation (Equation (10)) are stored in vectors denominated C and D, respectively. The vector W, containing the interchange energy w_{ij}, is regressed in order to minimize the residual of the expression $CW - D$. This linear regression was performed considering two scenarios for the error: no errors in the chemical potentials (ordinary least square regression) and a constant relative error of 1% on the chemical potential. For the constant relative error, the error model consisted of a weighted

variation on the chemical potential, following the equation $error_\mu = 0.01 \frac{\mu_t + \mu_b}{2} \sqrt{2}$.

An alternative method to estimate the pair-wise interchange energy (Method B, Table 1) consists of setting compositions of the phases as close as possible to the compositions obtained experimentally, by fitting the w_{ij} according to Equation (10). The initial part of the *reconciliation* method consists on determining appropriated initial guesses for the w_{ij}. This is performed through the linear regression method considering a constant relative error. Subsequently, given the initial guessed w_{ij}, the calculated compositions are fit for each experimental point, under the constraint that the chemical potentials are equal in both phases for each component in the system. This constraint provides postulated compositions (and consequently binodal curves) which satisfy the equilibrium condition of the system. The next optimization routine involves the fit of both the compositions and the w_{ij}. Here, the objective function searches for the reduction of the sum square of the residues between the postulated and experimental volume fractions (difference between the volume fractions over the estimated experimental error), as well as w_{ij} which satisfy the equilibrium between the phases, according to Equation (10). The input experimental data considers the fractions in weight percentage. When using weight fractions, only two variables are provided for each phase (weight fractions of polymer and salt). These variables are obtained directly from experiments, propagating less errors in their values. In order to consider the weight fractions for the w_{ij} calculation, the results obtained via volume fractions were used as initial values. The error model for this approach consisted of a weighted variation on the weight fraction, with a minimum being 0.0001, or $error_{wt} = 0.02\sqrt{wt(1 - wt)}$.

In order to evaluate the coherence of both the mathematical algorithm and numerical methods suggested in each of the two approaches used to calculated w_{ij} (Table 1), volume fractions determined through FH theory were used as input to calculate the w_{ij}. The volume fractions, used as input to estimate the w_{ij}, were calculated according to model parameters described in Table 2. The w_{ij} were applied to the recalculation of volume fractions, according again to the FH theory. The phase diagrams generated through FH theory using reported w_{ij} (Johansson et al., 1998) and the calculated w_{ij} were compared. This test was described in Fig. 2 a.

Finally, the two approaches to estimate w_{ij} (Linear regression, A; Reconciliation, B) were compared in terms of reproducibility of the experimental phase diagram (Fig. 2 b). The same input compositions experimentally determined were used to calculate the w_{ij} according to each method (Table 1). The resulting w_{ij} were applied to the calculation of the phase compositions according to FH theory. In order to obtain the binodal curve, the calculated phase composition data (in weight fraction) was regressed according to the Merchuk equation [19] presented below:

$$c_{polymer} = A \exp\left[(Bc_{salt}^{0.5}) + (Cc_{salt}^3)\right] \tag{11}$$

where $c_{polymer}$ and c_{salt} are the polymer and the salt concentrations, respectively, expressed in weight percentage. A, B and C are the fitting parameters obtained by least squares regression. This non-linear regression was performed with the *fmincon* routine in Matlab® R2018b (The Mathworks, Natick, ME, USA).

Table 2
Model parameter values for polymer-salt ATPS. PEG-phosphate system parameters was retrieved from Johansson et al. (1998) [9].

System	PEG-phosphate
System composition	(1) water, (2) PEG, (3) sodium, (4) phosphate
Interchange energy (J/mol)	$w_{12} = 100$, $w_{13} = -10000$, $w_{14} = -30000$, $w_{23} = -1000$, $w_{24} = -3000$, $w_{34} = -50000$
Degree of polymerisation	$M_1 = M_3 = M_4 = 1$, $M_2 = 100$
Charge	$z_1 = z_2 = 0$, $z_3 = 1$, $z_4 = -1.5$
Molecular weight (g/mol)	$Mw_1 = 18$, $Mw_2 = 6000$, $Mw_3 = 23$, $Mw_4 = 94$

The model parameters for each experimental system are presented in Table 3. The values for the degree of polymerization (M) varies for the same polymer molecular weight between Tables 2 and 3. These values were extracted as reported in the cited references. A sensitivity analysis on the M, performed at this work, brings an indication of the most appropriate value for M.

Although the thermodynamic model considers four components (water, polymer, cation and anion), the experimental systems present a fifth components, which is the salt not dissociated into ions. In the model, this molecule is being considered under the charge and molecular weight of component four (z_4 and Mw_4). The charge (z_4) defines the percentage of salt molecule and salt anion in the system, which is connected to the calculation of the molecular weight of this fourth component (Table 3).

2.3.2. Step 2: Phase diagram calculation

For the estimation of phase diagrams, the compositions of top and bottom phases should be determined, given a certain range of total composition of the system. This calculation was based on Johansson et al. (2011) [20] and implemented in Matlab R2018b. Other input variables are the interchange energies (w_{ij}), the degree of polymerization (M_i), the molecular weight (Mw_i), and the charge of each component (z_i). The optimization routine calculates the number of lattice sites of each component in one of the phases that minimizes the Gibbs Comment (Equation (4)). The calculation of the phase distribution of each component is restricted to satisfy the chemical equilibrium and the electroneutrality of the system (Equation (5)). In this work, we overview this approach, in terms of the thermodynamic basis, and bring improvements in terms of the numerical implementation and structure of the mathematical algorithm.

2.4. Monte Carlo sensitivity analysis

The uncertainty on the w_{ij} determination, and consequently on the interaction parameters, due to system composition variation was assessed via Monte Carlo sensitivity analysis. The volume fractions of phase forming components (PEG 6000 and sodium phosphate) were calculated through the model input parameters published by Johansson et al. (1998). Weighted errors from 0.01 to 5%, at nine levels, were added to the data, considering the composition of 50% volume fraction the largest point of error. The errors correspondent to other compositional data (ϕ_i^α) follow the equation $error_{\phi_i^\alpha} = 2\sqrt{\phi_i^\alpha(1 - \phi_i^\alpha)} error_{50\%v/v}$. The w_{ij} were calculated 4000 and 400 times for each case, through linear regression and reconciliation approach (Table 1), respectively.

3. Results and discussion

3.1. Step 1: FH parameter estimation

In the FH theory [7], the entropic term assumes an ideal mixture and represents the mixing of dissimilar components. The enthalpic term accounts for the deviation from ideal mixing due to differences in molecular sizes and energy between the monomeric units and solvent molecules. The key point of the enthalpic part is represented by the interaction energy parameter (w_{ij}). This input parameter is influenced by the absolute temperature and pressure, compressibility, degree of polymerization (chain structure), and relative concentrations of phase forming components [7].

The implementation of FH equation considers a set of straightforward input parameters (degree of polymerization, charge and molecular weight of each component), with the exception of the interchange energy (w_{ij}) or interaction parameters (χ_{ij}). In order to overcome the extensive experiments to determine these parameters, let alone the fact that they do not correspond exactly to the proper parameter present in the FH theory [4], the FH equation could be used in a reverse way in which interchange energy, and consequently interaction parameters,
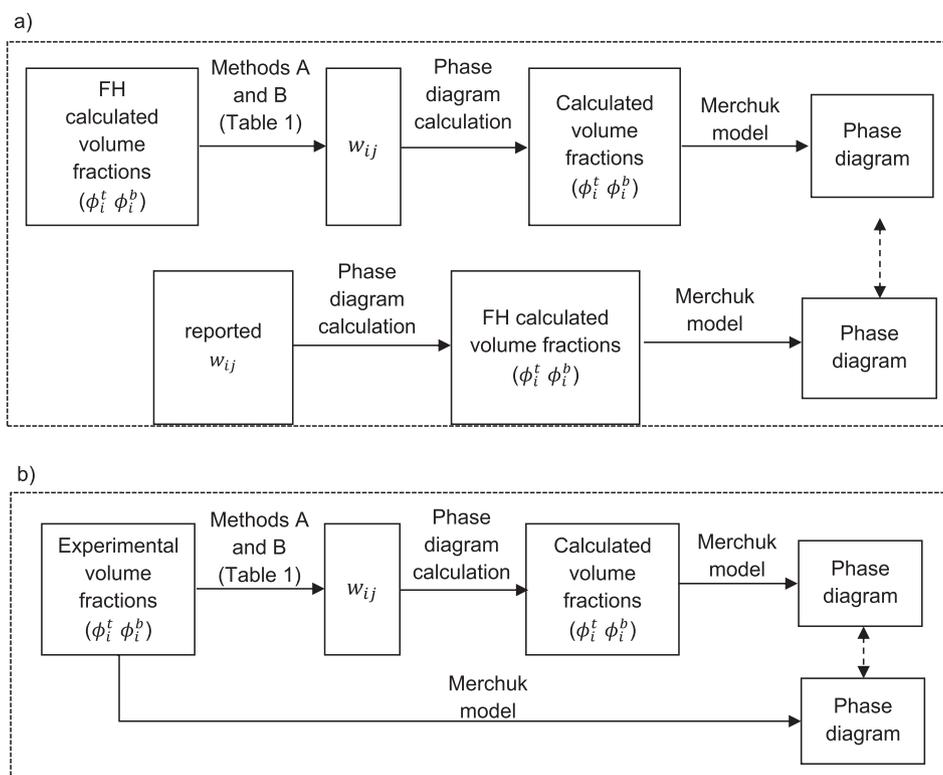
a)

b)

Fig. 2. Description of the algorithms to test the mathematical approaches used to determine the pair-wise interchange energies w_{ij} (a) and the use of experimental data to calculate w_{ij} and phase diagram according to FH theory (b).

Table 3
Model parameter values for polymer-salt ATPS, based on Johansson et al. 2011 [20].The charge (z_4) selection depends on the pH of the system and the speciation of the salts.

System	PEG-sulphate	PEG-citrate
System composition	(1) water, (2) PEG, (3) magnesium, (4) sulphate	(1) water, (2) PEG, (3) potassium, (4) citrate
Degree of polymerisation	$M_1 = M_3 = M_4 = 1$, $M_2 = 101$ (PEG2000), $M_2 = 202$ (PEG4000), $M_2 = 303$ (PEG6000),	$M_1 = M_3 = M_4 = 1$, $M_2 = 101$ (PEG2000), $M_2 = 202$ (PEG4000), $M_2 = 303$ (PEG6000),
Charge	$z_1 = z_2 = 0$, $z_3 = 2$, $-2 \leq z_4 < 0$	$z_1 = z_2 = 0$, $z_3 = 1$, $-3 \leq z_4 < 0$
Molecular weight (g/mol)	$Mw_1 = 18$, $Mw_2 = 2000$ (PEG2000), $Mw_2 = 4000$ (PEG4000), $Mw_2 = 6000$ (PEG6000), $Mw_3 = 24$, $Mw_4 = 96 + (1 + {}^{z_4}/_{z_3})Mw_3$	$Mw_1 = 18$, $Mw_2 = 2000$ (PEG2000), $Mw_2 = 4000$ (PEG4000), $Mw_2 = 6000$ (PEG6000), $Mw_3 = 39$, $Mw_4 = 189 + (3 + z_4)Mw_3$

Fig. 3. Influence of the mathematical approach to regress the w_{ij}, given as input the same FH calculated compositions, according to parameters published by Johansson et al. (1998) [9]. The system is composed by PEG 6000 and sodium phosphate. R and T have the values of 8.3144 J/K/mol and 298.12 K, respectively.

are determined from experimental data points.

In the first approach to estimate w_{ij} (Linear Regression, A) given the volume fractions of phase forming components in the top and bottom phases, the equations for the partition coefficient of the phase components were rewritten to a linear format (Equation (10)). The problem is solved such as the residue of the difference in chemical potential for each component at top and bottom phases is minimized. However, a consistent variation (at the same time and summing a constant factor) in all the w_{ij} does not influence the calculated volume fraction of the components in the system. This can be explained through the solution space of the linear regression. The solution vector w_{ij} presents a null space (N) for the linear problem $Cw_{ij} = D$. This means that there is not a single solution for w_{ij} where the equality $Cw_{ij} = D$ is satisfied. The null space reflects the ambiguity of the solution and is a cause of interdepende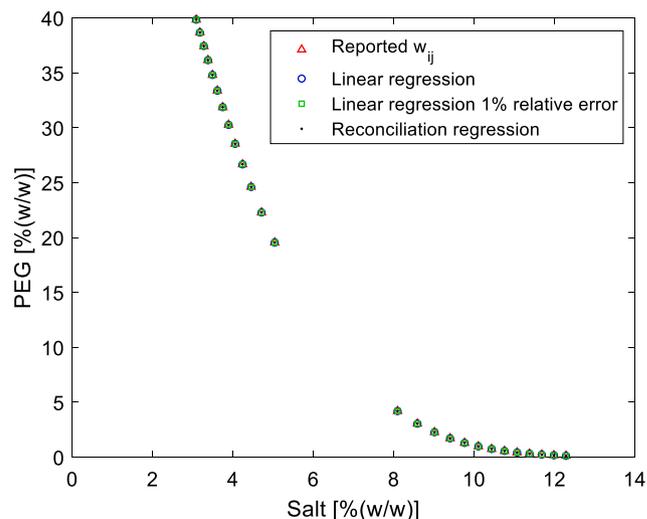nce of the equations defined in C. This interdependence is generated by the constraint of electroneutrality and the fact that the charge of the ions are interconnected.

Alternatively to the Linear Regression approach, the w_{ij} can be estimated through the 'Reconciliation' approach. In this method, postulated volume fractions are introduced for each experimental composition. The objective function searches for the reduction of the sum square of the residues between the postulated and experimental compositions, and the w_{ij} are determined considering the postulated volume fractions and the chemical equilibrium condition (chemical potential is equal for each phase forming component in both phases).
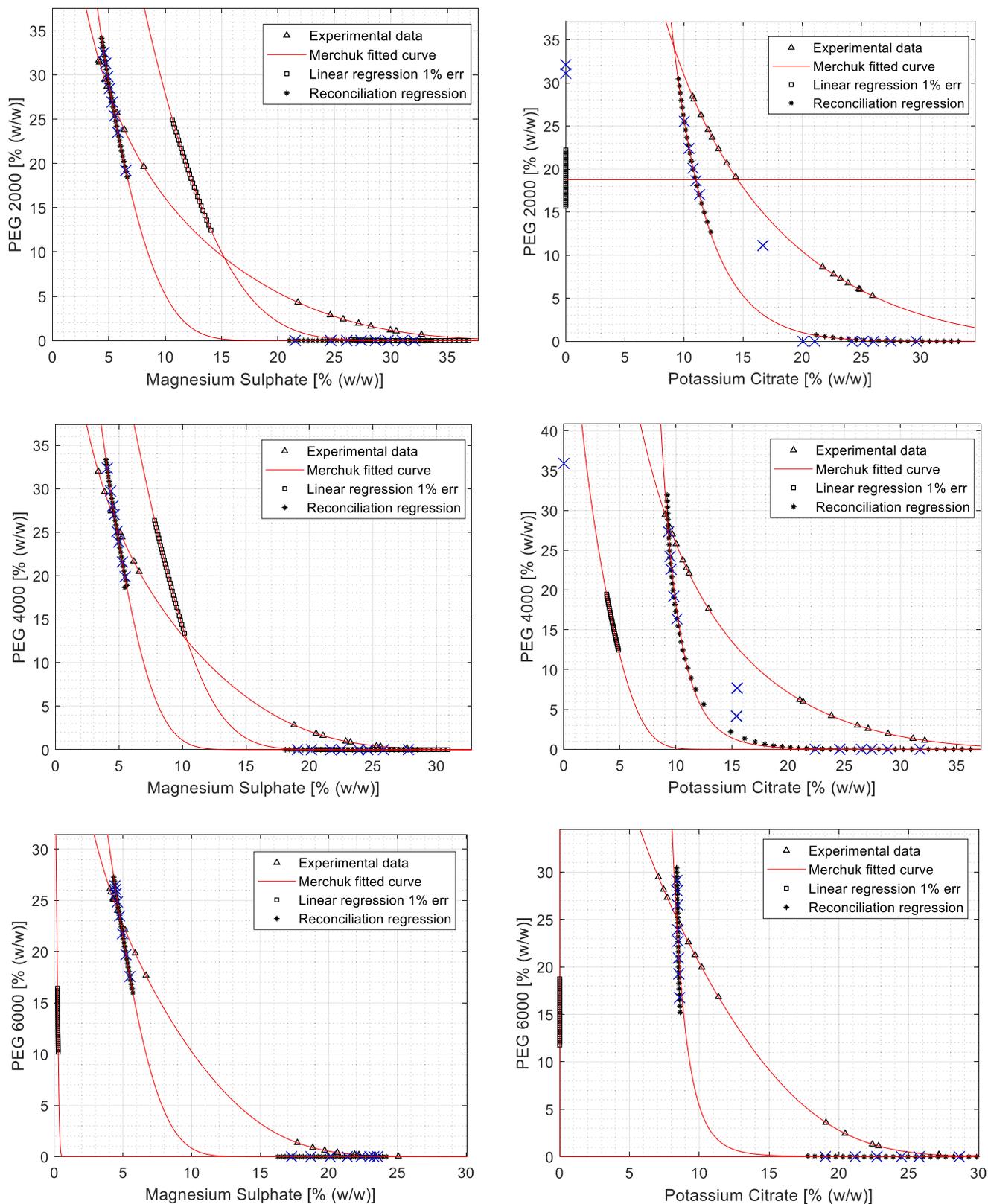
Fig. 4. Comparison between experimental and model determined composition of ATPS (represented by binodal curves). The w_{ij} were calculated using the experimental data. The blue crosses represent the postulated volume fractions calculated by the reconciliation approach. $z_4 = -1$ for all magnesium sulphate-based systems, $z_4 = -1.5$ for potassium citrate and PEG 2000 system, and $z_4 = -0.5$ for potassium citrate and PEG 4000 or PEG 6000 systems. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.
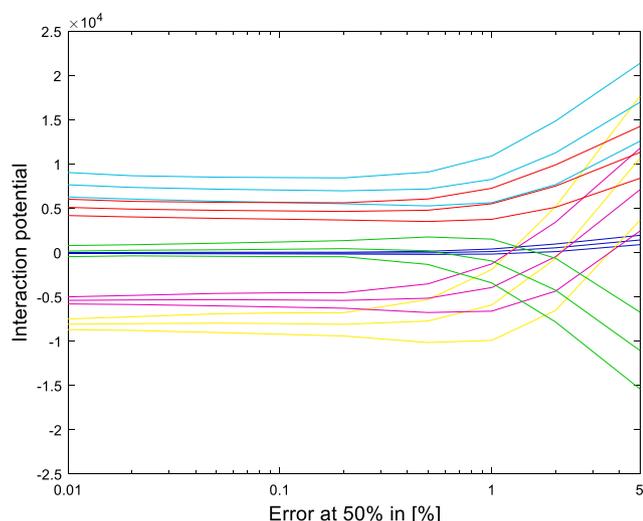
Fig. 5. Influence of uncertainty in the data on the estimation of interaction potentials (w_{ij}), according to linear regression method. Errors of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, and 5% were applied. R and T assume the values of 8.3144 J/K/mol and 298.12 K, respectively. For each w_{ij}, the mean and the mean $\pm$ 1 σ were plotted, according to the legend: w_{12} = *blue*, w_{13} = *yellow*, w_{14} = *magenta*, w_{23} = *cyan*, w_{24} = *green* and w_{34} = *red*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
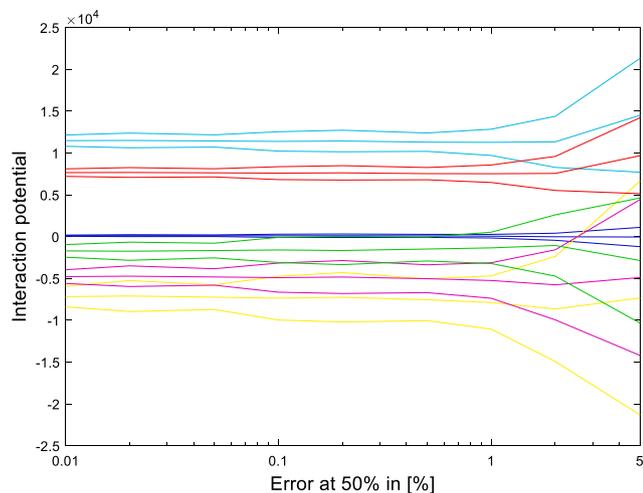
Fig. 6. Influence of uncertainty in the data on the estimation of interaction potentials (w_{ij}), according to the reconciliation method. Errors of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, and 5% were applied. R and T assume the values of 8.3144 J/K/mol and 298.12 K, respectively. For each w_{ij}, the mean and the mean $\pm$ 1 σ were plotted, according to the legend: w_{12} = *blue*, w_{13} = *yellow*, w_{14} = *magenta*, w_{23} = *cyan*, w_{24} = *green* and w_{34} = *red*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Both mathematical approaches were validated in terms of estimating coherent results that reproduce the input data. The interaction parameters calculated using both mathematical approaches (Table 1), when applied to phase separation calculation according to FH theory, provided binodal curves nearly equivalent (Fig. 3). Moreover, these binodal curves also reproduced the phase compositions used as input data to estimate the w_{ij} in both approaches. The input data for phase composition was calculated via FH theory for a set of w_{ij} reported by Johansson et al. (1998) [9].

For phases to separate, the interaction between polymer segments are more important than the polymer shape, size or excluded volumes
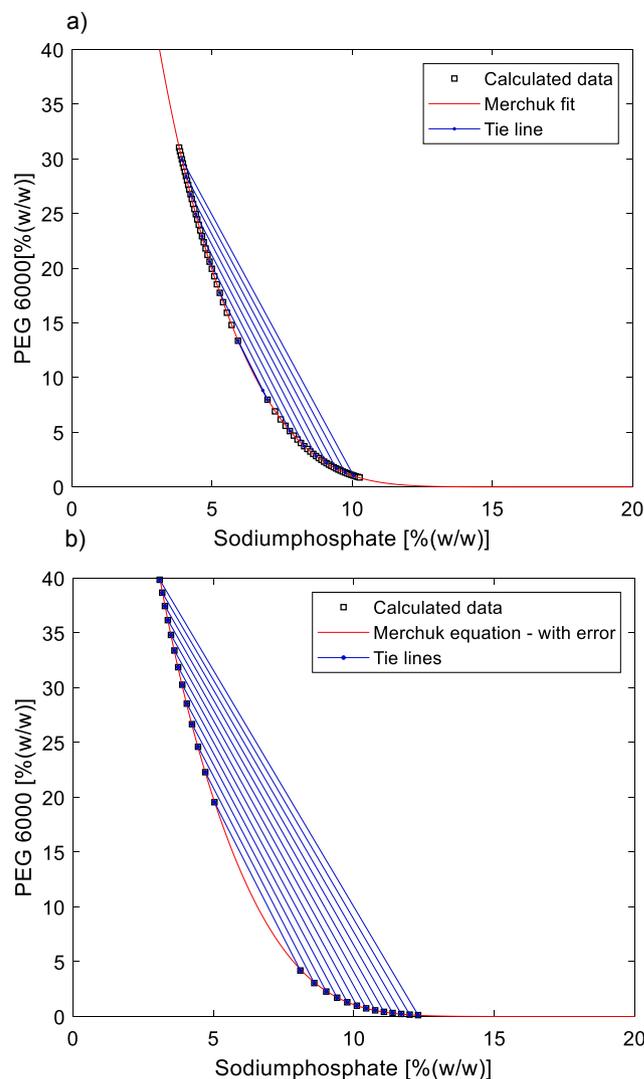
Fig. 7. Comparison of binodal curves generated using FH mean-filed model and parameters reported by Johansson et al. (1998) [9]. a) Model considering the salt as separated species and not full filling the chemical balance for all the components [20] ($c_{polymer} = 2.672 \cdot exp\,[(-10.3577 \cdot c_{salt}^{0.5}) - (2267.187 \cdot c_{salt}^{3})]$). b) Model considering the salt as one molecule and providing chemical balance for all the components ($c_{polymer} = 2.5201 \cdot exp\,[(-10.0877 \cdot c_{salt}^{0.5}) - (2262.3493 \cdot c_{salt}^{3})]$). R and T assume the values of 8.3144 J/K/mol and 298.12 K, respectively.

[21]. This interaction between phase forming components is presented by the FH interaction parameters. The ambiguity in estimating the interaction parameters, evidenced by the null space, suggests that the volume fraction of the components as the sole source of data in this determination is not enough. In the mathematical approaches compared in Fig. 3, all the six w_{ij} were determined for a system containing four phase forming components.

Because of the charge conservation, the interaction parameters present a fixed relation between each other, for a specific salt (charged molecules of the system) and charge. This relation can be beneficial to determine a set of w_{ij}, in case a specific one is known or obtained not via the mathematical approaches presented here. However, reducing the number of w_{ij} to be estimated in order to apply these relations does not improve or alter the results obtained via the linear regression or reconciliation approach. Interaction parameters can also be set to a fixed value in line with physical support. Foroutan and Zarrabi (2008) [11], for instance, fixed the interaction parameter between polymer and salt to zero, while fitting the others according to FH thermodynamic equations and based on experimental values.
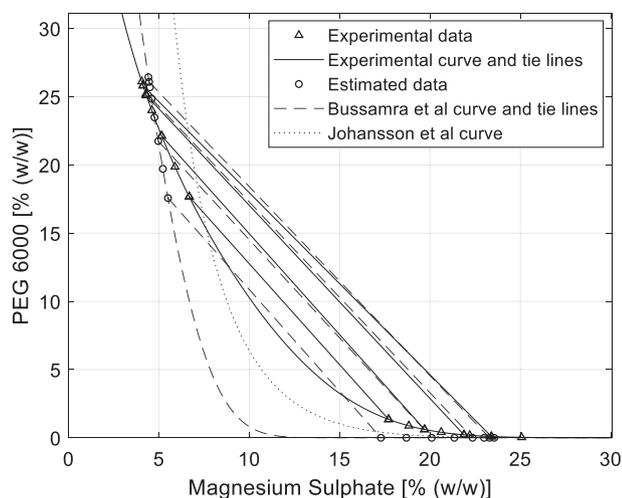
Fig. 8. Phase separation calculated via FH theory implemented by Johansson et al. (2011) [20] and by this work. The interchange energies were estimated from the experimental data for the system PEG 6000 and magnesium sulphate. The experimental tie lines and the estimated tie lines (according to the FH model implemented by this work) are also plotted. The binodal curves were fitted according to Merchuk equation. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.

When the methods to estimate the w_{ij} consider as input experimental data of system compositions, one can expect errors associated to it. The source of these systematic deviations relies on the parameters determination of the binodal curve, according to Merchuk equation, and experimental deviation on the mixing point formulation and on the determination of top and bottom volume ratios through a calibration curve [22]. Moreover, the experimentally calculated top and bottom phase compositions used by this work and published before [22] relies on the extrapolated part of the Merchuk curve, contributing to a certain inaccuracy of the experimental data. Fig. 4 shows the phase compositions generated through experimental determination [22] in comparison to compositions calculated by the FH theory. For the later approach, the w_{ij} were fitted using the experimental data. In contrast to w_{ij} fitted using system compositions calculated by FH theory (Fig. 3), when the experimental data composition is used instead, the binodal curves are not similar between each other.

The w_{ij} estimated by the linear regression could not always generate a binodal curve. The reconciliation approach determines, for each experimental data, a postulated point, being the w_{ij} fitted in order to reduce the sum square difference between the residues of these two points. The graphs (Fig. 4) show these calculated points as blue crosses. The similarity between the experimental and postulated volume fractions does not lead to a set of interaction parameters able to generate similar phase diagrams. For calculating binodal curves via FH model, the optimizer searches for phase compositions that globally minimize the Gibbs energy. For both salt-forming systems studied (potassium citrate and magnesium sulphate) and applying the w_{ij} estimated by the reconciliation approach, the global minimum output of phase composition differed from the experimental values, for the range of salt and polymer evaluated in the binodal curves. The Gibbs energy surface presents more than two inflection points, leading to false local minima when estimating the interaction parameters [21]. Different sets of estimated w_{ij} were tried, by randomly varying the initial values of w_{ij} and/or by applying bigger steps in the optimizer process. However, the calculated binodal curve differs significantly from the experimental one. Based on that, we could infer that the FH model is not an exact description of phase separation in ATPS composed by salt and polymer.

FH model does not present predictive value, being the model only useful for representing the data in a simplified molecular thermodynamic framework [17]. Some physical discrepancies of the FH theory

have already been reported, such as loss of the chemical structure of the polymer segment when represented as a sphere; the requirement by the geometry of the lattice site for the conformations of the polymer chain and the solvent to be identical; and the assumption of no volume change upon mixing [21]. Moreover, this theory was developed for polymer solutions, and it is mostly applied to polymer blends, block copolymers [8] and mixture of polymers presenting similar degree of polymerization (chain length) [7]. Regarding the molecular structure of the ions, the model does not differentiate the organic anion (citrate) from the inorganic sulphate anion. When the non-spherical shape of organic anions is taken into consideration, the thermodynamic model can improve to predict quantitatively polymer/salt ATPS [13].

The measurement errors significantly affect the interaction parameters estimation. Consequently, when experimental data is used to regress the w_{ij}, the reverse calculation of phase separation differs from the original (experimental) phase compositions. In order to evaluate how errors in the phase compositions would impact the determination of the w_{ij}, a Monte Carlo sensitivity analysis was performed on the FH calculated compositions. Given the null space found, the variability was assessed on the w_{ij} values belonging to a vector perpendicular to the null space. As demonstrated in Fig. 3, the use of this data (with no error associated to it) to regress the w_{ij} provides model-based binodal curves very similar to the original data.

As expected, an increased uncertainty in the data resulted in increasing uncertainty in the w_{ij}, and consequently in the interaction parameters (Figs. 5 and 6). The variability is larger for the reconciliation approach method of estimating these parameters, even though this method (Reconciliation, B) expects error in both weight percentages (salt and polymer), excluding the premise that FH equations should predict experimental compositions. From two experimental observations (weight fractions of salt and polymer) per phase and tie line, the linear regression (method A, Table 1) implies four observations — volume fractions of water, polymer and both ions —, while the reconciliation approach remains exclusively to the two observed data. The variation of the w_{ij} might also be connected to the composition dependence of this parameter [4], which is represented in this sensitivity analysis by the 'uncertainty' in the data.

The linear regression method of estimating the w_{ij} is sensitive to errors in the data above 1%. As depicted in Fig. 5, a large inaccuracy is associated to the determination of w_{ij}, when an error of 1% or more is applied to the phase composition data. This can be explained by the fact that the non-linearity of the FH theory is evidenced above 1% errors associated to the experimental data, being the linear regression approach only applicable below this threshold. However, different set of interaction parameters can still generate the same binodal curve, as exemplified by the null space case. The influence of the uncertainty in the data should be checked in terms of binodal curve generated, instead of simply the variability on the interaction parameters.

3.2. Step 2: determination of phase diagrams

The thermodynamic prediction of phase separation in ATPS composed by polymer and salts, according to the FH theory, was implemented through a mean-field model developed by Johansson et al. (1998) [9]. Within that set of relatively simple and analytical equations, it was intended to qualitatively interpret the general nature of the system and the influence of experimental variables on phase behaviour and partitioning of solutes.

In order to use the set of equations based on FH theory, the interchange energy w_{ij} or interaction parameter χ_{ij} are fundamental and not straight forward obtained. Here, we presented forms of estimating this parameter from experimental data of phase compositions. However, the application of the estimated parameters to the mean-field theory based on FH results in calculated phase separation different to the experimental one (Fig. 4). This limitation can be explained by: FH not being an appropriate theory to describe phase separation in ATPS composed
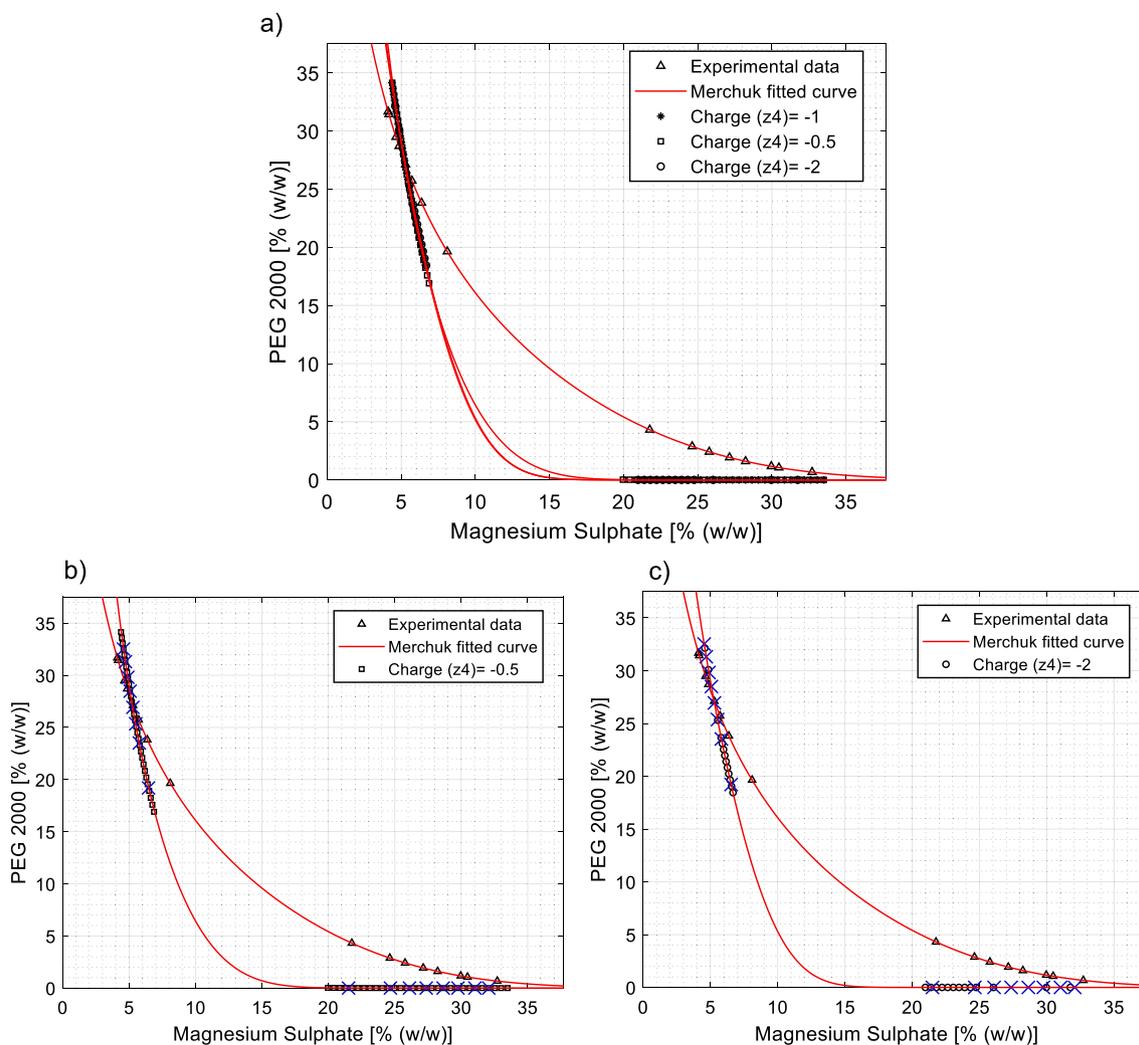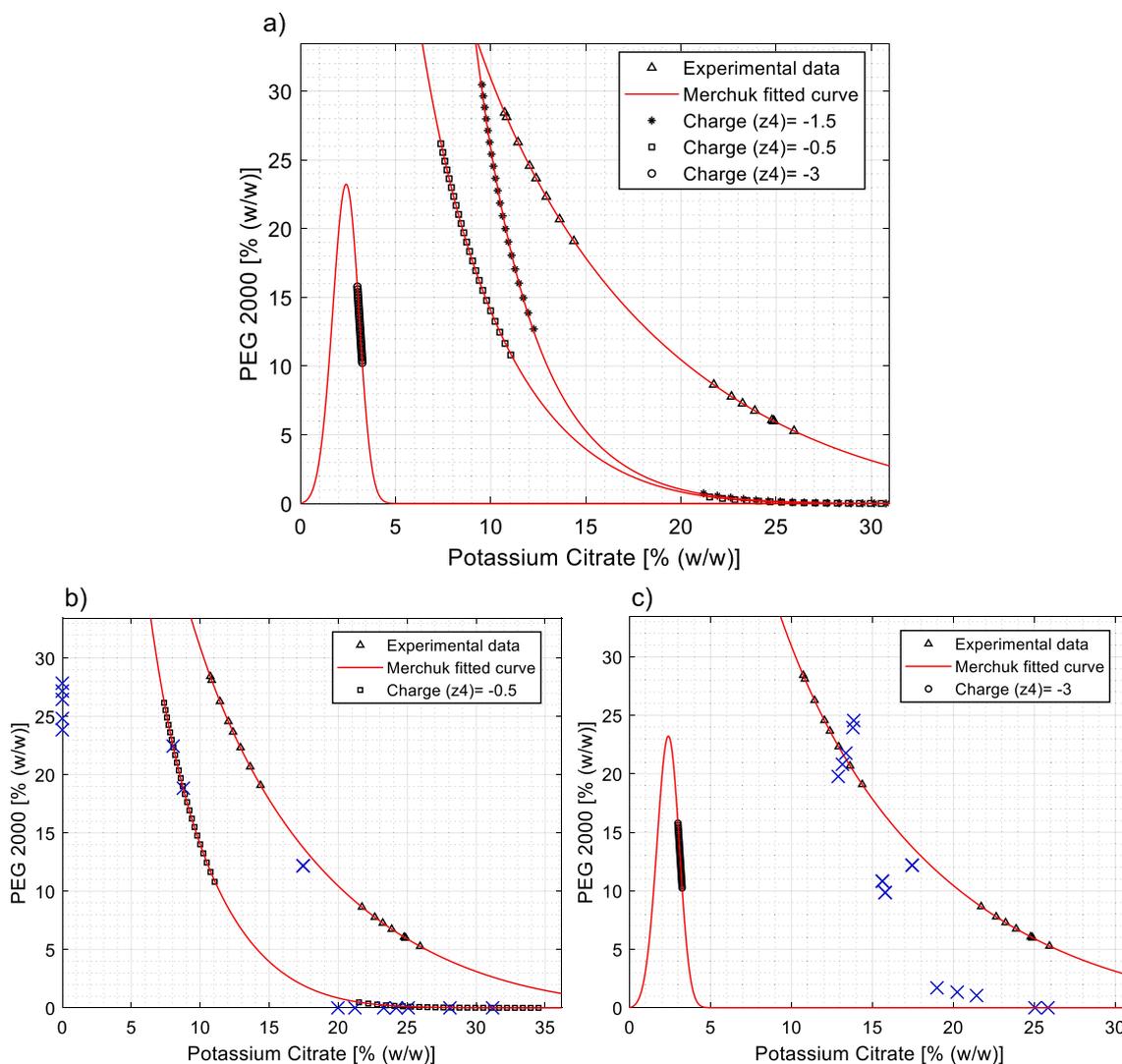
Fig. 9. Influence of the charge on the phase separation according to FH theory, for the system composed by Magnesium sulphate and PEG 2000. For each charge selection (z_4), the interchange energies were fitted according to the reconciliation approach (Method B, Table 1). The subplots b and c show the fitted points of the model ('postulated' fractions), in blue crosses. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.

by polymer-salt; errors associated to the experimental data used to retrieve the w_{ij} [22]; reduced set of experimental data exclusively involving the liquid-liquid equilibrium region [23]; not proper numerical implementation of the theory; and poor description of the phase separation when binodal curve is described by Merchuk equation [19]. Moreover, it has been reported the strong dependence of the interaction parameters to the composition of the system [4,24]. Here, we used a range of experimental data (composed by different phase compositions) to regress one set of w_{ij} aimed to represent to whole phase separation area. The assumption of interaction parameters being independent on composition has already been assumed by other authors [11], and still a reasonable agreement between modelled and experimental data was observed. Yan and Cao (2014) also estimated the FH interaction parameters by fitting experimental data. The calculation of phase diagrams using the regressed interaction parameters presented a good agreement with the experimental phase diagram. The solution for the Gibbs energy minimization was achieved by assuming a value for the composition of one of the solutes, and retrieving the composition of the other solute via the model equation at system chemical equilibrium [25].

The optimization routine developed and presented by Johansson et al. (2011) [20] to predict phase separation calculates the fraction of each component when the Gibbs free energy is minimized. Considering the chemical equilibrium as a constraint in the problem formulation implies ambiguity, since the derivative of Gibbs energy is the proper

chemical potential, and minimizing the Gibbs energy suggests to equalize the chemical potentials. Moreover, the Gibbs energy difference surface is at the optimum very flat, leading to irreproducible results and inefficient search for the global minimum [21]. An alternative to overcome the local minimum is to relieve the restrictions of chemical equilibrium for all the components, except for the water, the uncharged and dominant component of the system [20]. However, this results in volume fractions which do not fulfil the requirement of equal chemical potential between the phases for all the components. It has been already reported that the FH model cannot solve simultaneously the chemical potential for all the species of the system [21]. In order to overcome the ambiguity of having such a constraint and the local minimum of the Gibbs energy surface, we suggest the optimization routine to be divided in two steps: first, the fraction of components in a specific phase is determined such that the Gibbs free energy is minimized, followed by a fine tuning of the solutions, where the chemical equilibrium for all the components are solved. Moreover, the single initial guess of phase separation was replaced by a search routine through the whole grid of salt and polymer. Fifty positions (concentrations) of salt, polymer and water were evaluated (resulting in 125,000 calculations) in order to obtain the combination which provided the minimum Gibbs energy. This was the initial value used by the optimizer. Apart from the points above, the robustness of the minimization of the Gibbs energy can be affected by scaling issues related to the wide range of compositions and input

Fig. 10. Influence of the charge on the phase separation according to FH theory, for the system composed by Potassium citrate and PEG 2000. For each charge selection (z_4), the interchange energies were fitted according to the reconciliation approach (Method B, Table 1). The subplots b and c show the fitted points of the model ('postulated' fractions), in blue crosses. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.

parameters such as the degree of polymerization and FH interaction parameters [21].

When the salt is considered as an undissociated molecule, the minimization of Gibbs energy fulfils the condition that chemical potentials are in balance for all the components is the system. This approach of connecting the partition of both ions as one molecule between the phases, in order to guarantee charge equilibrium, results in balanced chemical equilibrium, one of the main thermodynamic requirements for calculating phase separation in two-phase systems. Other studies regarding the modelling of salt-polymer ATPS according to FH theory also supports the consideration of the salt as one molecule for the Gibbs free energy minimization [11]. However, extra terms expressed by the DH and PDH were included in order to consider the electrostatic interaction between the ions [11], [12]. The reference [10] evaluated the influence of the DH term added to both the osmotic virial and UNIQUAC equation for the prediction of phase behaviour in polymer-salt ATPS. For both models, the long-range contribution term improved the quality of fitting the liquid-liquid equilibrium data. In the approach provided here, these electrostatic terms are thought to reduce the ambiguity in determining the interaction parameters, although their contribution were not evaluated at this work.

Both approaches (proposed by Johansson et al 2011 [20] and by this work) of implementing the FH mean-field theory were compared in

terms of phase separation for the system PEG 6000 and sodium phosphate, according to input parameters published by Johansson et al. (1998) [9]. The mean of the absolute difference (MAD) between the binodal curves generated by both approaches was 0.71%[1] (Fig. 7). This analysis measures the deterministic difference between the curves, representing the real distance between the two models.

By analysing the residuals of the calculated data fitted to the Merchuk equation, in Fig. 7 b, the deviation of 0.7% (data not shown) implies the Merchuk is a good approximation of the binodal curve derived from FH calculated data, for this specific system of salt and PEG. Merchuk also provides a good description of the binodal curves for ionic liquid based systems [26].This empirical equation could also have the exponents adjusted in order to optimize the fitting [26]. Certainly, other experiential equations could be used to correlate the binodal data. Lu et al. [12] compared three different equations to fit experimental data from polymer and potassium salts. In general, the Merchuk-like equation provided the best fit, even though the suitability of the equation seems to be dependent on the constituents of the system.

However, when the interchange energies (w_{ij}) were estimated from

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[1] $MAD = \frac{1}{K}\sum_{l=1}^{k} |c_{PEG,Johansson} - c_{PEG,Bussamra}|$, for the independent variable C_{salt} being 4%, 5%, 6%, 7%, 8%, 9% and 10% (w/w).
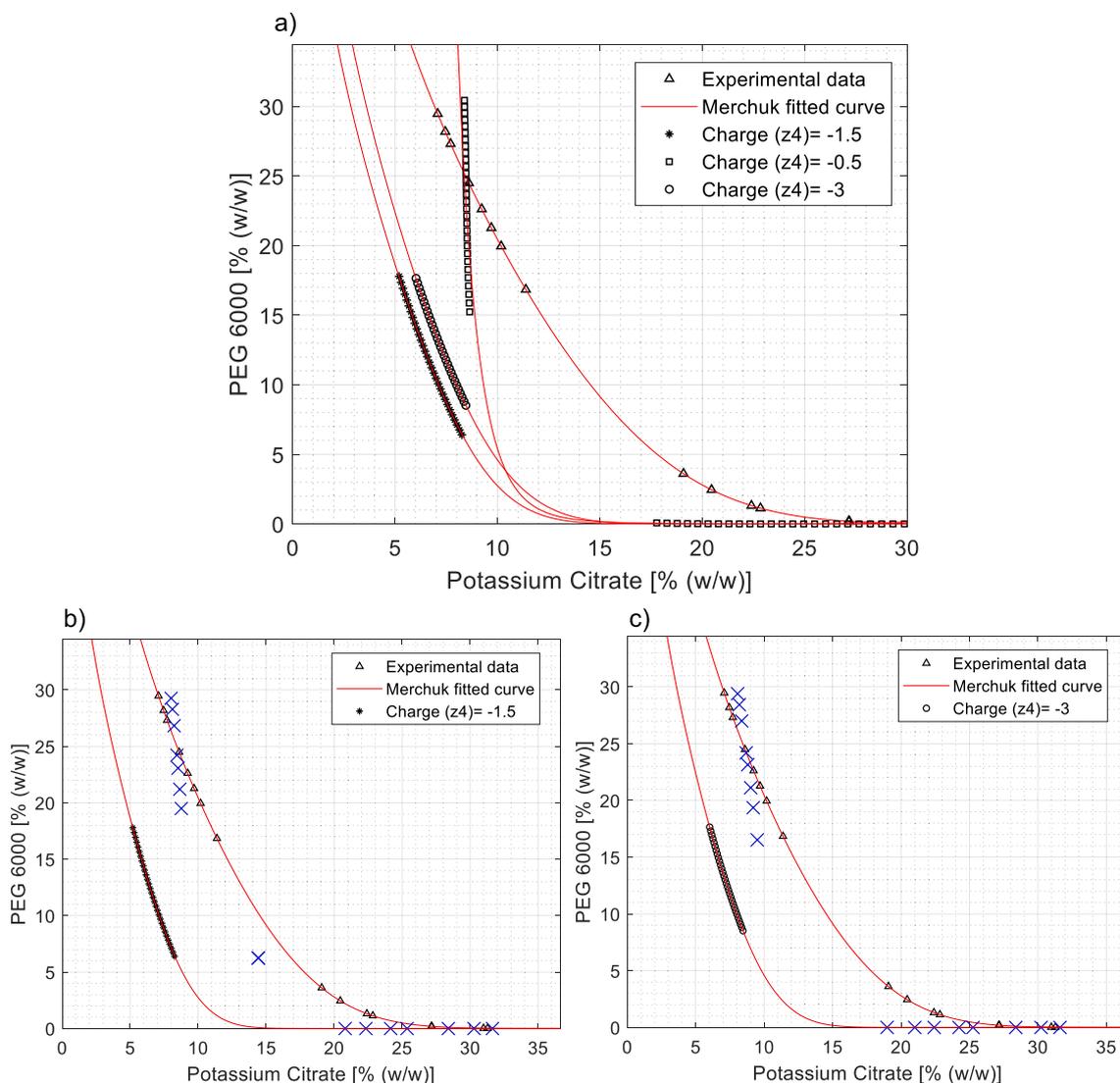
a)

b)

c)
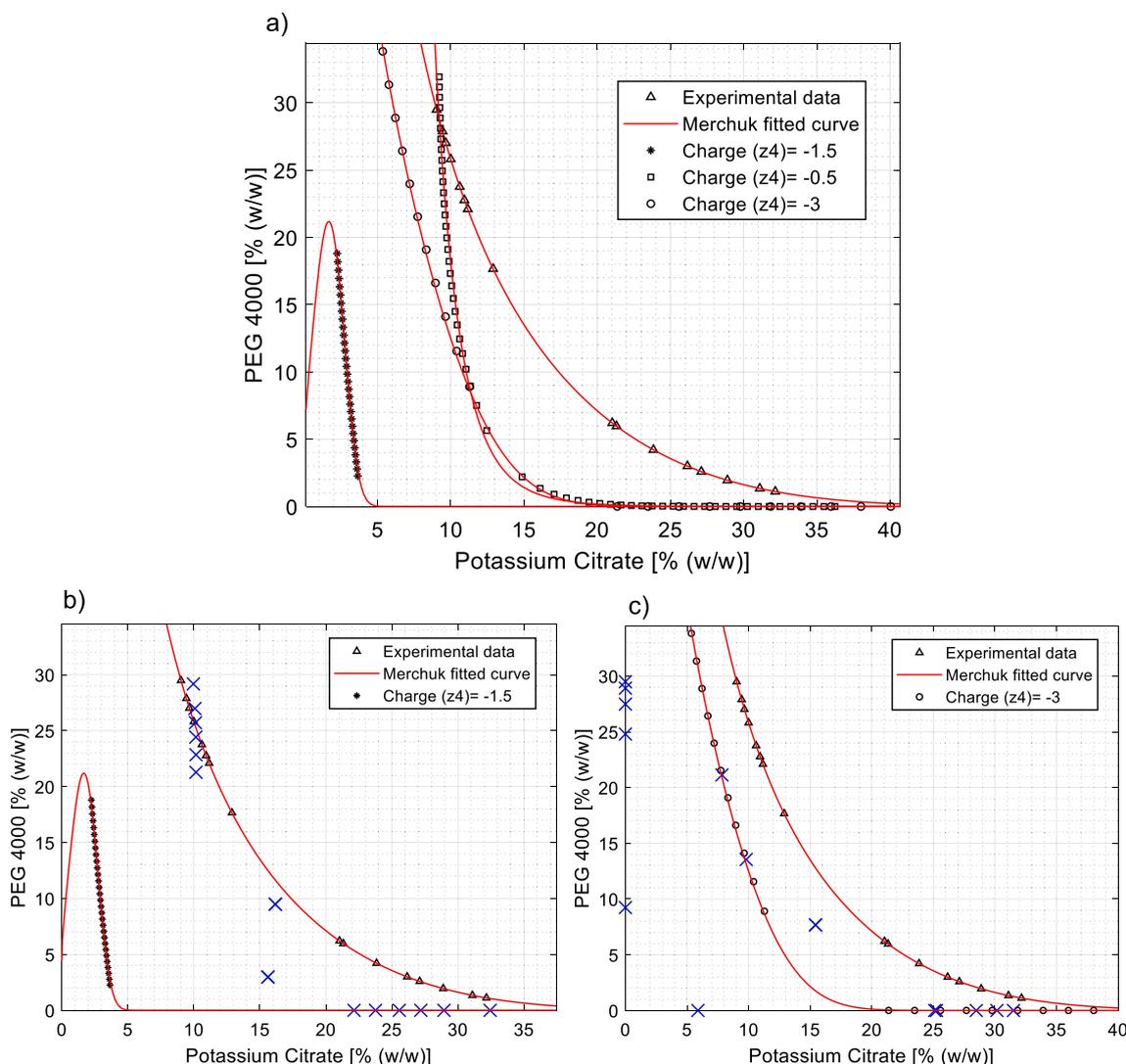
Fig. 11. Influence of the charge on the phase separation according to FH theory, for the system composed by Potassium citrate and PEG 4000. For each charge selection (z_4), the interchange energies were fitted according to the reconciliation approach (Method B, Table 1). The subplots b and c show the fitted points of the model ('postulated' fractions), in blue crosses. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.

experimental data (via the reconciliation method), both approaches to calculate phase separation resulted in binodal curves far apart (Fig. 8). The absolute deviation was larger than when the phase separation was calculated using input parameters published by Johansson et al. (1998) [9]. Two likely causes mentioned before of interaction parameters estimated from experimental data do not generate phase separation similar to experimental one are maintained: FH theory does describe properly phase separation of polymer-salt systems, and the errors associated to the experimental data influence the estimation of χ_{ij}. When both curves were compared in relation to the experimental binodal curve, Johansson method presented a MAD of 5.1%,[2] while the method suggested by this work showed a MAD of 4%[2]. However, these absolute differences do not indicate a preferable curve, since this measurement is influenced by the salt concentration range applied. At low concentration of salt, the calculated concentration of polymer via Merchuk equation varies considerably due to the exponential shape of the binodal curves. Interaction parameters have a great influence over the phase separation calculation, and their choices and estimation procedures should be carefully taken into consideration.

The interchange energy can be scaled to RT units, and there is a limited range for the interaction parameters to generate a binodal curve. When the factor w_{ij}/RT is large, the energy (entalpic) part is more important for the Gibbs free energy calculation. On the other hand, given a small w_{ij}/RT factor, the entropic part becomes more important to this calculation. The enthalpy part is a linear term and numerically simpler to be solved. The entropic part involves logarithmic terms and the numerically solution is more complex. The use of scaled interchange energy, or the direct use of the interaction parameter χ_{ij}, can also improve the robustness of the Gibbs energy minimization routine [21].

Apart from the interaction potential, the degree of polymerization (M_i) and charge of the species (z_i) can influence the calculation of phase separation. The later parameter, z_i, reflects the amount of dissociated ion molecules. The salt dissociation varies according to the pH of the system. In our model, the component 4 (negative charged species) counts for the anion and the ions present in the salt form. For the system composed by magnesium sulphate salt (Fig. 9), the charge of component 4 seems to not influence the phase equilibria calculation, neither in the fitting of the model (represented by the blue crosses in figures sub-items b and c). This behaviour was observed for all polymer molecular weights in combination with magnesium sulphate. In contrast to what was pointed out by the magnesium sulphate systems, the

[2] $MAD = \frac{1}{K}\sum_{i=1}^{k}|c_{PEG,model} - c_{PEG,experimental}|$, for the C_{salt} range from 4% to 24% (w/w).

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Fig. 12. Influence of the charge on the phase separation according to FH theory, for the system composed by Potassium citrate and PEG 6000. For each charge selection (z_4), the interchange energies were fitted according to the reconciliation approach (Method B, Table 1). The subplots b and c show the fitted points of the model ('postulated' fractions), in blue crosses. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.

potassium citrate systems confirm a clear influence of the ions charge on the phase separation. Moreover, the charge of the anion and the polymer molecular weight seem to be related (Figs. 10, 11 and 12). For the experimental data provided and the FH model applied, at higher polymer molecular weights (PEG 4000 and PEG 6000), lower is the solubility of the salt. This is demonstrated by a fitted curve closer to the experimental one when $z_4 = -0.5$, in comparison to fitted curves at $z_4 = -1.5$ or $z_4 = -3$. When PEG 2000 is considered, a charge of $z_4 = -1.5$ represents better the experimental data, implying that more ions are being considered as dissociated in the system. This suggests that the ions influence the estimation of interaction parameters for this system, affecting the calculation of the phase separation according to the speciation of the salt and molecular weight of the polymer.

The degree of polymerization is a measure of the molecular size expressed in units of molar volume of water (18 cm^3/mol). The molar volume of the polymer considers the molecular weight of the molecule, molar volume of every monomer calculated by software material studios and the ratio of monomers [25]. Due to the theoretical assessment of the degree of polymerization, we based our selection on literature indication [20]. Sensitivity analysis showed that the indicated degree of polymerization should be reduced by approximately 60% (Fig. 13). This applies for both salts studied. Applying a group contribution method to

defined the molar volume of PEG 4000, its degree of polymerization would be approximately 104 [11]. The correction on the degree of polymerization produced binodal curves based on FH theory closer to the experimental ones. However, the shape of these curves still differs between each other, corroborating the hypothesis that FH model is not a proper quantitative description of phase separation in polymer-based ATPS, being restricted to predict trends (qualitative application).

Even though the FH mean-field theory cannot qualitatively predict the phase behaviour in ATPS, trends can be predicted by evaluating the contribution of the dominant energetics (entropy and enthalpy) to the system. By analysing the Gibbs free energy after mixing, it is possible to estimate whether the system separated to top and bottom phases. In comparison to a monophasic system, a two-phase system presents lower values for the Gibbs free energy (the repulsive intermolecular interaction overcompensates the favourable entropy of mixing). Although the combinatorial entropy ΔS_{mix}, defined by Equation (2), predicts only positive values, ΔS_{mix} decreases with increasing degree of polymerization. This means that longer molecules favours demixing (aqueous two-phase system formation). This behaviour is demonstrated by the experimental data and calculated curves in Fig. 4. Moreover, the model developed here can also predict the same trends when fixing the w_{ij} and varying the size component of the polymer (represented by the
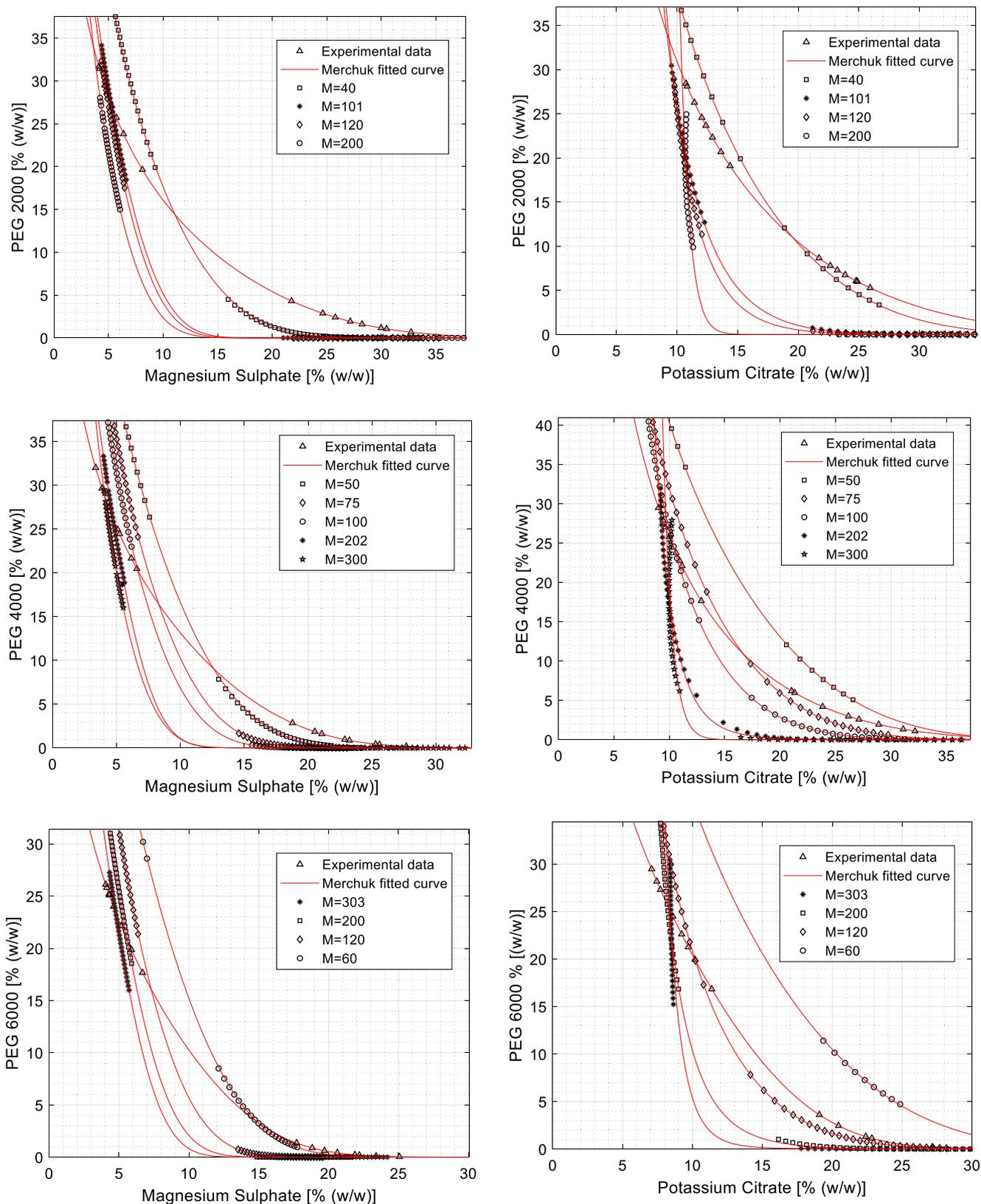
Fig. 13. Influence of the degree of polymerization (M) on the phase separation according to FH theory, for the systems composed by PEG 2000, 4000 or 6000, in combination with each of the salts magnesium sulphate or potassium citrate. The interchange energies (w_{ij}) were fitted considering the M presented in Table 3 and according to the reconciliation approach (Method B, Table 1). The simulations were performed by varying exclusively the M and calculating the phase separation. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.
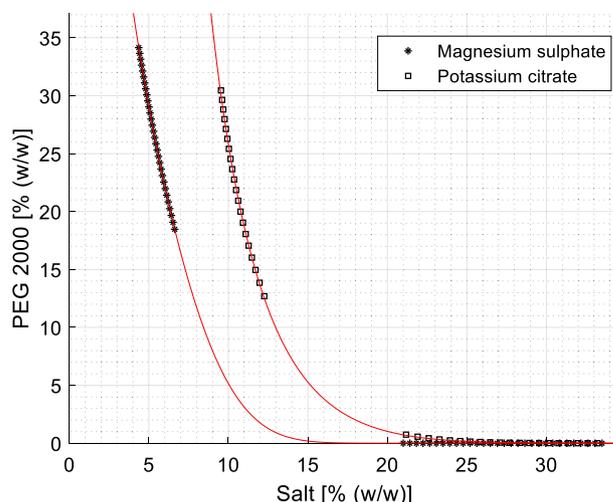
Fig. 15. Influence of the salt type on the phase separation. Binodal curves calculated considering the interchange energies (w_{ij}) fitted according to the reconciliation approach (Method B, Table 1). R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.
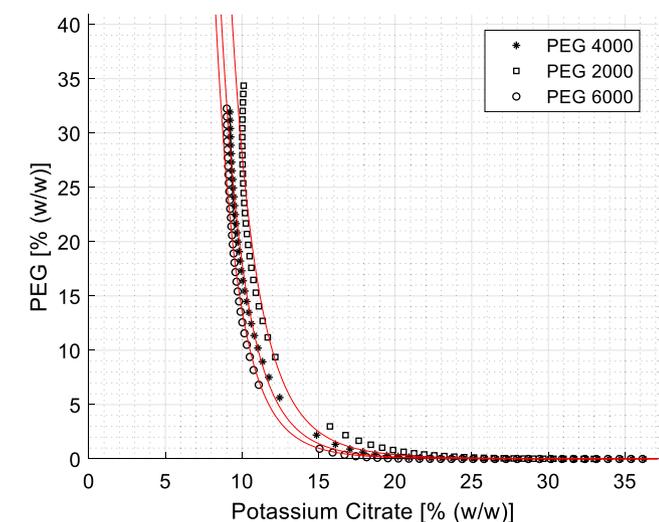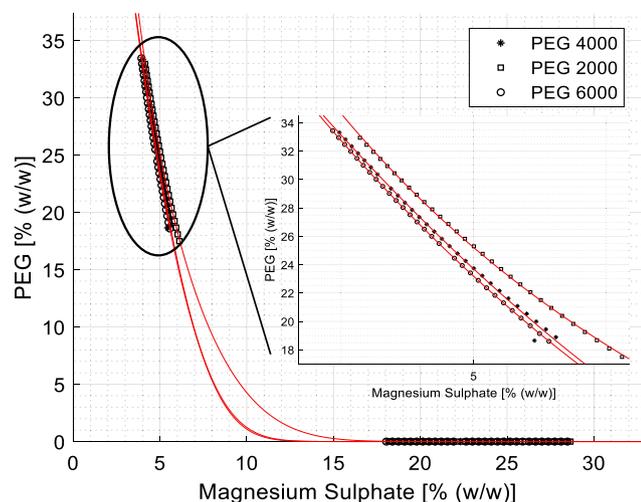
Fig. 14. FH model predicts the trend of decrease in monophasic region with increasing the polymer size. The w_{ij} (w_{ij}) were fitted considering the system composed by PEG 4000 and according to the reconciliation approach (Method B, Table 1). The simulations were performed by varying the component size (M and Mw) of the polymer. R and T have the values of 8.3144 J/K/mol and 313.12 K, respectively.

molecular weight and the degree of polymerization) (Fig. 14).

The size of the monophasic region of the ATPS is influenced by the hydrophobicity of the anion. An increase in the hydrophobicity of the anion reduces the monophasic region [20]. This trend is observed experimentally, and also by the FH calculated curves. In Fig. 15, this is demonstrated for systems composed by both salts and PEG 2000. The same pattern follows for systems containing PEG 4000 and PEG 6000 (data not shown). However, this trend prediction could be bias, once the interaction parameters were regressed considering the experimental data already presenting such a trend. ePC-SAFT can predict ATPS for which no experimental data is given [14]. This capability is mainly assigned to the ion specific parameters. Even though FH applies ion specific interaction parameters, the transferability to other systems were not assessed at this work.

Not only the hydrophobicity of the anion, but also the salting-out power can determine the effectiveness of the ion in forming an aqueous two-phase system. When salts formed by the same cation are compared, the ability to form two phase systems rises with the increase in the valence of the anion [12], because higher valence anions are less hydrated. The salting-out characteristic of the salt is related to the

effective volume this component can exclude. However, among the salts analysed here (magnesium sulphate and potassium citrate), the salting-out effect is higher for the sulphate-based salt, even though its valence is lower than the citrate anion. Contrary to the experimental observation, Sadeghi and Jahani [27] reported a higher effectiveness in salting-out the polymer for the anion citrate than sulphate. In terms of the cation, magnesium showed in our experiment to be more effective than potassium to promote phase separation. This salting-out ability of Mg^{2+} in comparison to K^+ follows the Hofmeister series and is also observed for ATPS composed by other components than polymer and salts (e.g. ionic liquids and salts) [26]. This suggests that the cations of these salts also contribute to the phase separation, let alone the anion hydrophobicity mentioned above.

4. Conclusion

The ATPS formation is susceptible to parameters definition, such as polymer molecular weight and salt type. The mathematical framework suggested by this work, based on the FH thermodynamic model, can be used to perform sensitivity analysis on these parameters, based on predictable trends of phase separation. The algorithm is composed of two steps: estimation of the pair-wise interchange energies and calculation of phase compositions. The thermodynamic modelling of phase separation according to FH theory and applied to polymer-salt ATPS should consider the phase-forming salt as a single (undissociated) molecule in order to satisfy the chemical equilibrium for all the components in the system. Here, we also disclosed a mathematical way of determining the FH interaction parameters based on experimental data. These parameters are not straightforward obtained in literature or by experiments, and this computational approach can boost the application of FH mean-field model to predict phase separation. However, FH interaction parameters should not be determined purely based on experimental binodal curves, since our results show a non-uniqueness solution. Moreover, the uncertainty in data strongly influences the estimation of the interchange energies. Our analysis on published models and the poor approximation to experimental results obtained by the algorithm sustains the statement that FH model is not an exact description of phase separation in salt based ATPS.

CRediT authorship contribution statement

Bianca Consorti Bussamra: Writing - original draft, Software,

Validation, Investigation, Project administration. **Devi Sietaram:** Methodology, Software. **Peter Verheijen:** Methodology, Software, Validation, Formal analysis, Writing - review & editing. **Solange I. Mussatto:** Writing - review & editing, Supervision. **Aline Carvalho da Costa:** Conceptualization, Supervision. **Luuk van der Wielen:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition. **Marcel Ottens:** Conceptualization, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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