Experimental Investigation and Thermodynamic Assessment of Phase Equilibria in the PLLA/Dioxane/Water Ternary System for Applications in the Biomedical Field
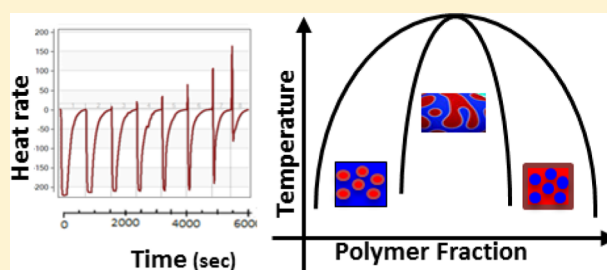
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S *Supporting Information*

ABSTRACT: Fundamental understanding of thermodynamic of phase separation plays a key role in tuning the desired features of biomedical devices. In particular, phase separation of ternary solution is of remarkable interest in processes to obtain biodegradable and biocompatible architectures applied as artificial devices to repair, replace, or support damaged tissues or organs. In these perspectives, thermally induced phase separation (TIPS) is the most widely used technique to obtained porous morphologies and, in addition, among different ternary systems, polylactic acid (PLLA)/dioxane/water has given promising results and has been largely studied. However, to increase the control of TIPS-based processes and architectures, an investigation of the basic energetic phenomena occurring during phase separation is still required. Here we propose an experimental investigation of the selected ternary system by using isothermal titration calorimetric approach at different solvent/antisolvent ratio and a thermodynamic explanation related to the polymer−solvents interactions in terms of energetic contribution to the phase separation process. Furthermore, relevant information about the phase diagrams and interaction parameters of the studied systems are furnished in terms of liquid−liquid miscibility gap. Indeed, polymer−solvents interactions are responsible for the mechanism of the phase separation process and, therefore, of the final features of the morphologies; the knowledge of such data is fundamental to control processes for the production of membranes, scaffolds and several nanostructures. The behavior of the polymer at different solvent/nonsolvent ratios is discussed in terms of solvation mechanism and a preliminary contribution to the understanding of the role of the hydrogen bonding in the interface phenomena is also reported. It is the first time that thermodynamic data of a ternary system are collected by mean of nano-isothermal titration calorimetry (nano-ITC). Supporting Information is available.

INTRODUCTION

Phase separation processes of ternary solutions, made up of an organic compound and a binary mixture of a solvent and a nonsolvent, are of remarkable interest in a wide range of application fields. In general, these systems can experience two different types of phase separation, liquid−liquid demixing and crystallization, but in many cases both liquid demixing and crystallization occur. Phase separation processes have been also studied in order to optimize the production of polymeric membranes for a variety of industrial applications, such as microfiltration, reverse osmosis, and gas separation;[1] frequently liquid−liquid demixing and crystallization are combined in order to control the final morphologies of the porous media. An in-depth knowledge of phase diagrams showing boundaries for these two phase separations can be very useful for tuning the desired features of the final products.

In the biomedical application field, porous media obtained as biodegradable and biocompatible scaffolds play a crucial role as

artificial devices for tissue engineering, with the aim of providing a favorable microenvironment for neo-tissue generation, in order to repair and replace damaged tissues or organs.[2] Scaffolds are biodegradable 3D supports, with an interconnected porous structure, emulating the extracellular matrix, guiding cell adhesion, proliferation, and differentiation.[3] The scaffolds must provide (1) a proper combination of physical properties, such as strength, stiffness, and toughness; (2) adequate porosity, interconnectivity, and permeability for nutrient delivery and metabolite removal; and (3) a controllable degradation rate in nontoxic products.[4]

Several synthetic polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), and their copolymers have been widely used to fabricate scaffolds, not

only because of their characteristics of biodegradability and biocompatibility,[5] but also for their availability, ease of processing and adjustable degradation.[6]

There are several ways to fabricate porous systems from polymer-containing systems, such as expansion in high pressure gas,[7] emulsion freeze-drying,[8] salt leaching,[9] or 3D printing;[10] among all, the most successful is the Thermally Induced Phase Separation (TIPS) technique.[11] Indeed, TIPS offers a reliable, flexible, and cost-effective process to control microstructures.

TIPS technique is based on the controlled thermodynamic demixing of a homogeneous ternary polymer solution in two liquid phases with different composition, a *polymer-rich phase* and a *polymer-lean phase*. This step is followed by a stabilization of the polymer-rich phase as a porous structure by quenching the system until it reaches the thermodynamic instability for the monophasic state.

Among the several advantages of the TIPS technique, the possibility to vary several process parameters allows one to obtain systems with a very large variety of pore size or pore interconnectivity.[4] Therefore, the TIPS process is more flexible than other techniques, such as wet or dry-casting,[12,13] since it depends primarily on heat transfer rather than on more complex multicomponent mass transfer.

The TIPS can follow two different mechanisms: nucleation and growth of droplets or spinodal decomposition, giving rise to a co-continuous morphology.

The final characteristics of the porous scaffold depend on several parameters of the TIPS process, such as quenching speed, initial solution composition and final temperature.

In order to produce a scaffold with the desired final characteristics, phase diagrams can be used to acquire reliable information about critical conditions for the control of phase transitions in ternary or binary polymer solutions, in terms of binodal and spinodal curves.

A widely used method to determine liquid–liquid phase boundary in polymer solutions is the cloud point measurement:[14] it is the point at which, by slowly cooling the polymer solution, it becomes cloudy due to the formation of two different phases, characterized by different optical properties. Generally, thermo-optical analysis provides a simple, rapid, and reliable experimental method to determine cloud-point curves of binary or ternary polymer solutions, together with their upper or lower critical solution temperature. Although it is a widely used technique, it provides only qualitative, not quantitative, information.

Among other techniques used to determine the thermodynamic parameters of interactions in solution, isothermal titration calorimetry (ITC) has been employed in the past decade for the study of partial molar enthalpies and excess enthalpies for liquid mixtures.[15] This type of thermodynamic study has been extended to polymer solutions in a recent work by Mohite et al.[16] where a correlation based on the generalized Flory–Huggins model, which relates the activity data of aqueous solution of poly(ethylene glycol) solution to the binodal curve, was developed estimating the enthalpy of aqueous PEG solution by means of ITC.

ITC experiments have also been used by Hamedi et al. to determine the solid–liquid equilibrium line in ternary systems containing the solute to precipitate and an aqueous mixed solvent.[17]

In this work, thermodynamic investigations on the ternary system polylactic acid/dioxane/water, widely used for scaffold fabrication, are carried out for the first time by means of ITC, in order to study the interactions between polymer and water as nonsolvent. Indeed, the mechanism of the polymer–solvents interactions have been extensively hypothesized as reported by Su et al.[18] and Tsuji and co-workers;[19−21] however, our intent is to add some explanations in terms of energetic contributions to the above ternary system. In these perspectives, our study aims mainly to investigate the thermodynamics of the interaction among the species of the considered ternary system, leading to a better understanding of the basic energetic phenomena of the phase separation, used to control the structure of biomaterials. In particular, the goal is to monitor, by means of titration at a fixed temperature, the behavior of the polymer at different solvent/nonsolvent ratios, focusing on the study of its solvation process.. In conclusion, considerations about phase diagrams are made starting from these results, to support the classic optical methods for thermodynamic studies on polymer solutions. Further, the calorimetry explains, in terms of thermodynamics of the ternary system, the choice of the solvent/nonsolvent ratios most commonly used for scaffold fabrication by means of controlled TIPS processes of polylactic acid/dioxane/water solutions.

■ MATERIALS AND METHODS

Materials. Poly-L-lactic acid (PLLA or Resomer L 209 S), purchased from Evonik Industries, with an average molecular weight of 125 000 Da is used. Diethylene dioxide (1,4-dioxane) purchased from Sigma-Aldrich), of analytical quality and without any further purification, is used as a solvent for the chosen polymer; Milli-Q water is used as a nonsolvent.

Polymer Solution Preparation. PLLA in solid pellets is added in weight/volume (w/v) percentages (0.5% w/v, 1% w/v and 1.5% w/v) to pure dioxane in an Erlenmeyer bulb, immersed in a silicone oil bath on a stirring hot plate. A thermocouple is used to control the oil bath temperature, set at 116 °C, in order to allow the polymer pellets to dissolve. The solution is maintained at this Temperature for at least 2 h. The system is kept in continuous stirring (300−350 rpm). Temperature of 116 °C is higher than the dioxane boiling point (101 °C); thus, during the dissolution of the polymer, evaporation of dioxane is expected, with consequent undesired changes in system composition. To avoid this drawback, a glass Liebig condenser is installed on the top of the Erlenmeyer bulb. When the solution appears transparent because of the dissolution of the polymer, without stopping the stirrer, the system temperature is finally lowered to 80 °C and maintained at this value for at least 1 h before starting the experiment

Experimental Details. Titration experiments of PLLA/dioxane solutions with pure water are carried out by means of a Nano ITC Low Volume calorimeter (TA Instruments); the principles of operation have been exhaustively described previously in literature.[22] Before starting the experiment, the instrument idle temperature is set to the desired value and several minutes are needed to reach the heat flow stability corresponding to the new temperature. When such stability is reached, the syringe holder is removed and the sample cell (its volume of reaction 170 μL) is filled with the PLLA/dioxane solution previously prepared and degassed, while the reference cell is filled with pure dioxane. The titration syringe (maximum volume 50 μL) is charged with degassed pure water and restored in the buret; at this point the buret in inserted in its accommodation and the calorimeter is closed.

Before starting the experiment, the stirring rate is set to 250 rpm, and several minutes must elapse again so that the heat flow stability is restored, then the experiment can definitively start.

All the experiments were carried out with an equilibration time of 600 s, initial and final baselines of 120 s, and a titration program made up of 25 injections, each of 2 μL of pure water (the instrument automatically sets an actual volume of 1.96 μL). The stirring rate is fixed at 250 rpm; the time elapsed between two successive injections is
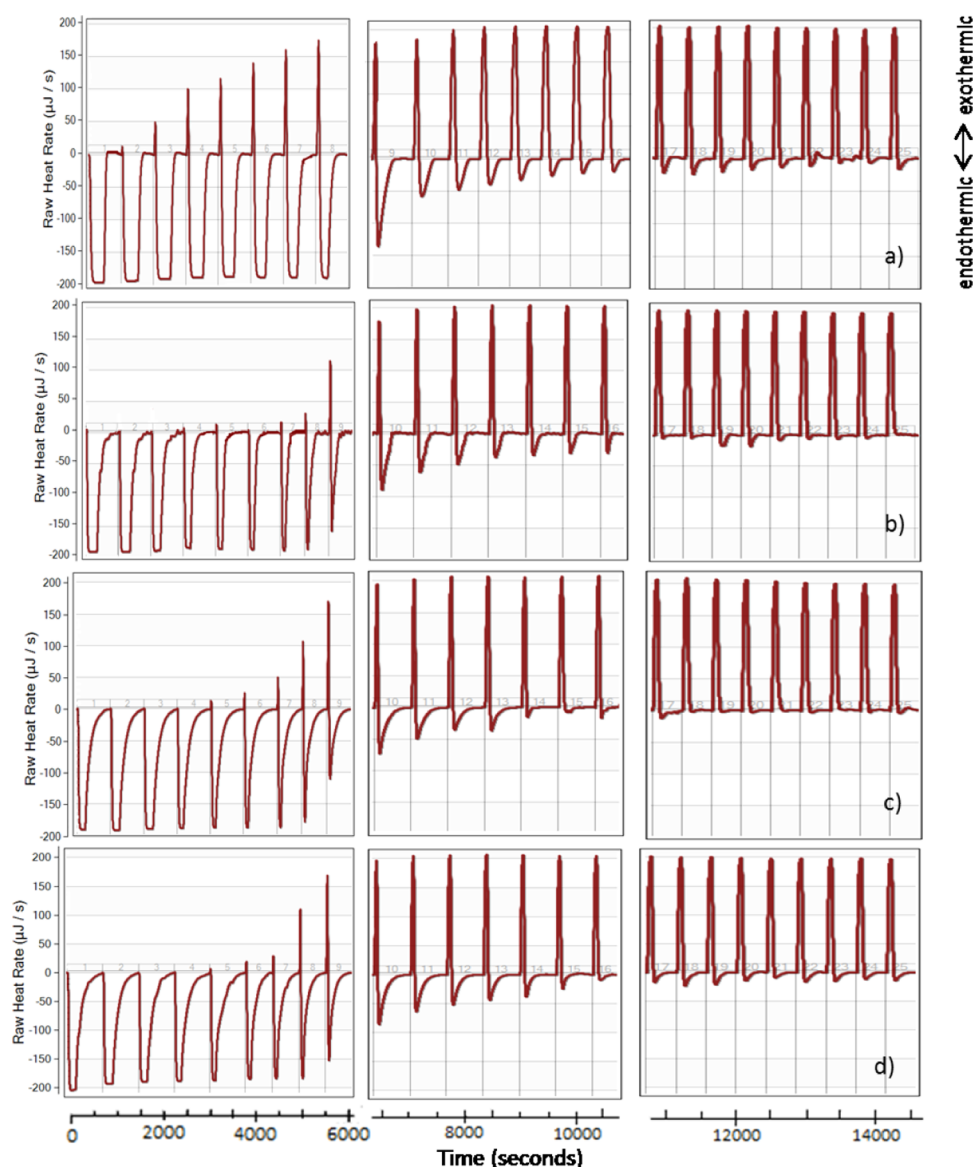
Figure 1. Titration experiments results at 333.15 K. Titration of pure dioxane with pure water (a); titration, with pure water as titrant, of PLLA/dioxane solutions, at different polymer percentages: 0.5 wt %/v (b), 1 wt %/v (c), 1.5 wt %/v (d).

set to 800 s for the first six injections, to 600 s for the following 10 injections (the ones going from the seventh to the 15th), and to 420 s for the remaining ones. The injection volume was chosen after some optimization experiments, in order to avoid the drawback linked to the instrument full scale; some preliminary experiments were needed to identify the time for the heat flow signal to return to the baseline in order to determine the time between two successive injections.

The polymer percentages of the starting solution were chosen in order to the avoid heat flow signal reaching the full scale.

For all the experiments, the starting solution is made up of PLLA in pure dioxane, and the final solvent/nonsolvent ratio is equal to 74.8/25.2. This final value was chosen in order to cover the practical interest range, according to the typical ratios used for scaffolds fabrication in previous literature studies[23] (Table 1, Supporting Information).

■ RESULTS

The aims of our measurements are to study and to thermodynamically characterize the interaction between PLLA and water. Referring to blank test water/dioxane, first results are obtained by carrying out titrations of pure dioxane with pure water, both at 333.15 K and at 313,15 K, until a final

dioxane (D) to water (W) ratio of about 75/25 is reached. Then, the same titrations are carried out for initial solutions prepared by dissolving PLLA in pure dioxane, which is a good solvent for the selected polymer. Three different initial polymer percentages are used, 0.5/1/1.5 w/v%, but in all the cases the dilution due to the titration gives a reduced final polymer percentage.

Figure 1 shows the results of the titration experiments carried out at 333.15 K in terms of power released or adsorbed from the reaction cell of the calorimeter as a function of the time (peaks above the baseline represent exothermic phenomena while peaks under the baseline represent endothermic phenomena); to better understand the differences between the different cases, the diagrams are organized in a matrix form. In detail, Figure 1a represents the result of the titration of pure dioxane with pure water (blank test) while, Figure 1b−d shows the diagrams of the titration of a PLLA/dioxane solution (at different w/v% of PLLA) with pure water. The differences among the first diagram (Figure 1a) and the following figures (Figure 1b−d) are due to the contribution of the heat involved

in the interactions PLLA/water, which overlaps the interaction dioxane/water. The PLLA/water contribution is then isolated and quantified in Figure 2. This particular aspect is highlighted in the Supporting Information section.
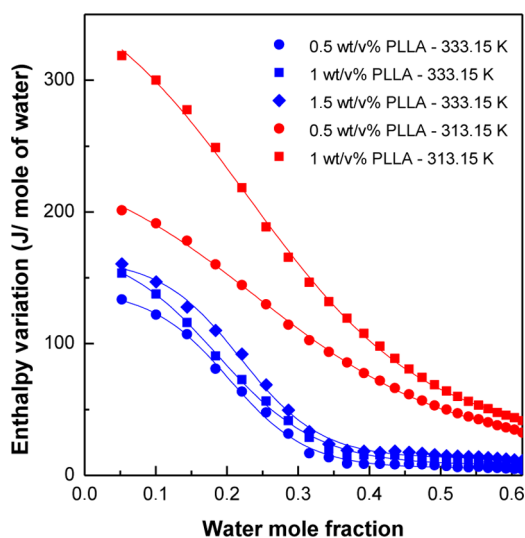

Figure 2. Enthalpy variation for PLLA−water interaction as a function of the water mole fraction in the binary solvent. The values for the specific enthalpy variation were obtained by subtracting the blank test results from the results of the polymer solutions titrations.

Generally, in each of these result diagrams, it is possible to recognize three different regions (not strictly corresponding to the diagram matrix cells): each region represents a different state of the interaction phenomena developing in the sample cell between the species in solution. In particular, in the first region, it is possible to notice that the initial injections give rise to purely endothermic peaks, whose area is approximately the same, showing that all the injected titrant interacts with the solution in the cell and such interaction has the same intensity for all initial injections.

The second region is characterized by the evolution of the interaction phenomena involved in the mixing process between the water and the PLLA/dioxane solution: the peaks show the overlap of an endothermic phenomenon and an exothermic one, whose intensity changes with the increase in the number of injections. This competition makes the shape of the peaks changing at each injection, showing the evolution of the solvation phenomena for the species involved in the system: it appears clear as a growing number of the polymer sites, available for the solvation, become saturated.

The third region shows peaks the same height: they represent the saturation of the interactive phenomenon; the addition of further titrant determines only a dilution of the system.

The "boundaries" of each region, in terms of number of injections or in terms of dioxane/water ratio, change with the polymer content of the initial solution and/or the temperature of the titration experiment.

The outputs obtained for the titration experiments of PLLA/dioxane solutions are the overlapping of several phenomena and do not represent the pure heat contribution of the interaction polymer/water: they include also the heat of dilution of water in dioxane. In order to evaluate the only contribution deriving from PLLA/water interactions, the

output of a blank test (consisting of a titration experiment of pure dioxane with pure water as titrant) must be subtracted from the titration results of the polymer solution. After this subtraction, it is possible to evaluate the only heat involved in the interaction between PLLA and water. To this aim, the blank tests were carried out at 313.1K and 333.15 K.

From the comparison between the strips of Figure 1, it appears clear as all the results are qualitatively similar: they differ for the number of injections needed for the saturation of the power signal and for the relative importance of the endothermic and exothermic phenomena, that is, for the relative height of the peaks, under and above the baseline. For the studied polymer percentages, these differences are not very relevant; this evidence can be attributed to the high thermal energy content of the system: the polymer chains have enough mobility in solution to arrange themselves in new conformations by adding water to the system. In the case of lower temperature, the differences in terms of saturation of the interaction phenomena are more relevant, due to the lower temperature (Figure 2, Supporting Information).

In order to evaluate the heat involved in the mixing phenomena between the PLLA target molecules and the water, the power signal is integrated in time for each peak, until the baseline is reached again after each injection; this way, the heat generated or absorbed at each water injection is calculated (Figure 3, Supporting Information).

Figure 2 shows results for the enthalpy variation, reduced to the only contribution due to the interaction phenomena occurring between the target molecules (PLLA chains) and the water used as titrant, to focus on the interaction between the polymer and its nonsolvent. These results were obtained by subtracting the blank test results from the titration results obtained for the polymer solution. For all the investigated conditions, in terms of both temperature and polymer initial percentage, the trend is almost monotonically decreasing and, in particular, it is well fitted by a Boltzmann sigmoid. Furthermore, the enthalpy variation is always positive, that is, the process of mixing between PLLA and water is always endothermic, in accordance with the hydrophobicity of the polymer. Focusing the attention on a single temperature, and in particular on the results obtained at 333.15 K, the changing in the initial polymer percentage does not greatly affect the enthalpy variation. Indeed, by increasing the PLLA content, only small increments in the enthalpy variation are observed for the whole binary solvent composition range studied. Switching on the study of the results obtained at 313.15 K, it is possible to notice that the same increment in the polymer percentages determines an increase in enthalpy variation (at a fixed binary solvent composition) higher than the one determined at 333.15 K. By comparing the curves obtained at 313.15 and 333.15 K for the same initial PLLA percentage, it is possible to notice at a fixed water mole fraction the enthalpy variation is higher at 313.15 K; that is, the process of mixing is more endothermic.[1,24−26] Experiments at 313.15 K are performed for polymer percentages until 1 w/v% PLLA, instead of 1.5, to avoid the occurring of potential phase separation phenomena (as for example the reaching of cloud point) or PLLA precipitation within the ITC sample cell. Indeed, in that case, measurements could be affected by different undesired heat contributions. In fact, the aim of the work is to estimate the interaction parameters of the species in solution, and not the thermodynamic parameters during phase separation processes.
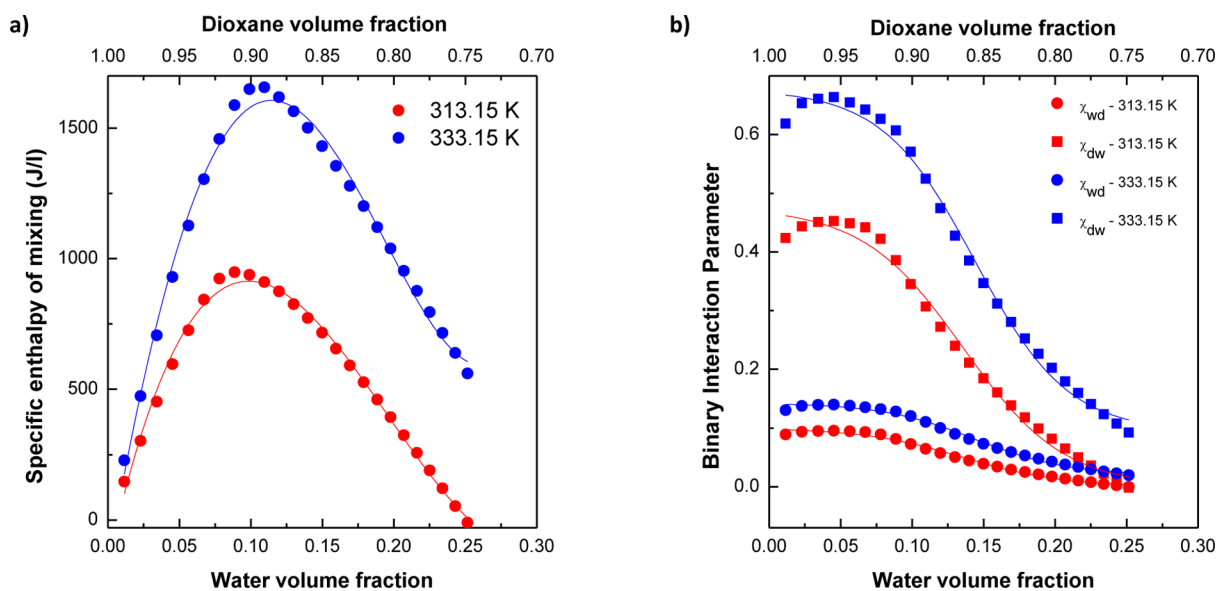
Figure 3. Specific enthalpy of mixing (a) and binary interaction parameter (b) as functions of the water volume fraction in the dioxane/water binary solution. (a) Enthalpy variation is shown for both the studied temperatures. (b) the figure shows the interaction parameter of dioxane in water and that of water in dioxane, at the two studied temperatures; symbols represent the experimental data and lines represent the fitting curves.

The interactions between PLLA and water can be explained in terms of hydrogen bonding phenomena, in accordance to the molecular structures of the considered species. There are several different types of hydrogen bonding involved in the mixing process: the PLLA–water hydrogen bonds, the intramolecular PLLA hydrogen bonds and the water–water hydrogen bonds. From literature,[27] it is well-known that PLLA is a hydrophobic polymer, and that its interaction with water is characterized by a positive Gibbs free energy variation; but it is important to keep in mind that the Gibbs free energy variation of a mixing process is made of an enthalpic contribution and an entropic one, in accordance to the following law:

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

As shown in Figure 2 for the studied systems, the enthalpy variation is always positive; by increasing the water content, the fraction of PLLA–water hydrogen bonds increases, giving an endothermic contribution. On the other hand, the enthalpy variation decreases during the titration because of the intramolecular PLLA hydrogen bonding that gives the enthalpy variation a contribution that is opposite to the previous one. At the end, the asymptotic value is reached when the polymer hydrogen bonding sites are saturated and only water–water hydrogen bonding occurs. The intramolecular PLLA interactions occur because the polymer chains are "forced", by the increasing water content and because of their hydrophobicity, to take a conformation characterized by a reduced radius of gyration in solution, thus encouraging the intramolecular PLLA interactions.

Figure 3a shows the excess enthalpies of mixing for the binary solution made of solvent and nonsolvent, at 313.15 and 333.15 K, as a function of the solution composition. The excess is always positive in the studied composition and temperature conditions, showing that the mixing process is endothermic: the trend passes through a maximum and then begins to decrease. The number and strength of hydrogen-bonding between water and 1,4-dioxane might be affected on enthalpy behavior very strongly, as also reported by Suzuki et al.[28] This behavior can be explained in terms of hydrogen bonding networks between

the species. In detail, at the beginning of the experiment, we have a large amount of 1,4-dioxane, and therefore, hydrogen bonding between 1,4-dioxane and water mostly occurs. On the contrary, for higher water contents, the water–water hydrogen bonding becomes relevant. In this concentration region, the solution might consist of a cluster of water and 1,4-dioxane within the solution. The Flory–Huggins lattice model for the enthalpy of mixing[29] is then employed in order to calculate the binary interaction parameters for the couple dioxane/water, further referred to as χ_{wd} and χ_{dw} starting for the excess enthalpy values. Parameter values can be calculated: their evolution as a function of the water volume fraction is shown in Figure 3b (Table 2b, Supporting Information).

In Figure 4a, the excess enthalpy values for the mixing curves for the ternary solutions are shown. The curves for the binary solutions are also reported, in order to make comparisons. For all studied ternary solutions the enthalpy of mixing is positive; that is, in the range of temperatures and compositions investigated, the mixing process is endothermic. At 333.15 K, the enthalpy values for the ternary solutions are not very different from the binary mixtures ones; at 313.15 K, at a fixed value for the water volume fraction, the enthalpy of mixing increases by increasing the PLLA volume fraction in solution, according to the polymer hydrophobicity. Starting from these results for the enthalpy variation of mixing, by means of the extension to the Flory lattice model for ternary systems,[29] the water–PLLA interaction parameters are estimated starting from the following expression for the enthalpy of mixing of the ternary solution made of the species i, j, and z:

$$\frac{\Delta H_{M}}{RT} = \chi_{Ij} n_{i}\phi_{j} + \chi_{iz} n_{i}\phi_{z} + \chi_{zj} n_{z}\phi_{j}$$

The values obtained for the interaction parameters are shown in Figure 4b. For the solvent/nonsolvent interaction parameter, the results shown in Figure 3b are used, while for the PLLA–dioxane interaction parameter a value from literature[14] is used, assumed to be constant both with temperature and solution composition (it was chosen equal to 0.2). The numerical values are summarized in Table 1. For each temperature value studied,
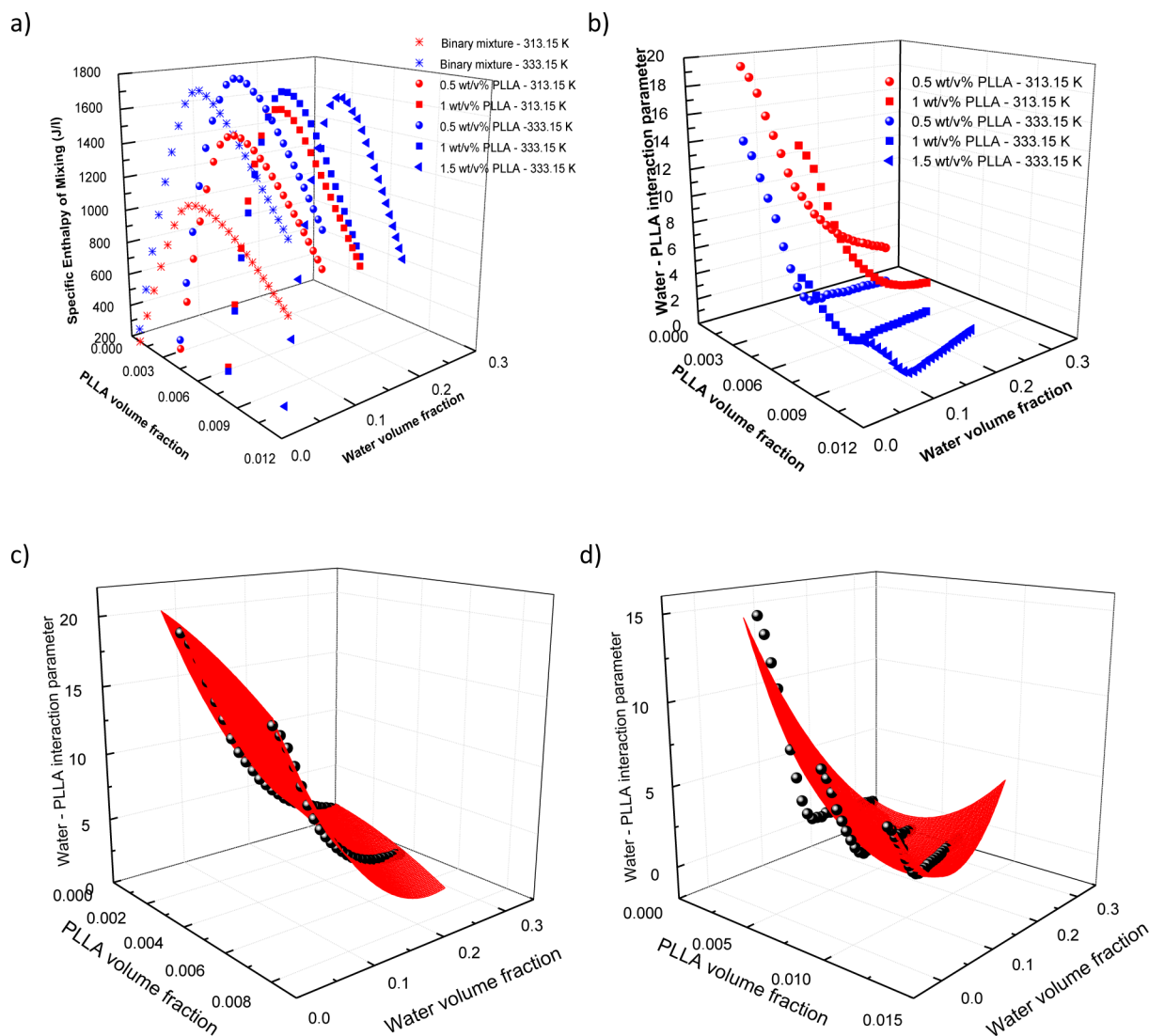
Figure 4. Specific enthalpy of mixing (a) and water−PLLA interaction parameter (b) as a function of both water and PLLA volume fraction in the ternary solution; fitting surfaces for water−PLLA interaction parameters: (c) data at 313.15 K and (d) data at 333.15 K.

Table 1. Water−PLLA Interaction Parameters in the TernarySystem

water volume fraction	PLLA volume fraction	χ_{wp} (313.15 K)	χ_{wp} (333.15 K)
0.05633	0.0037	15.05627	9.10295
	0.00741	11.40259	3.67066
	0.01111		3.12585
0.09911	0.00354	10.25006	2.53203
	0.00707	7.22581	1.12687
	0.01061		0.9237
0.12991	0.00341	8.11505	1.92621
	0.00683	5.553	0.90421
	0.01024		0.70184
0.14986	0.00334	7.10514	1.97179
	0.00667	4.67684	0.85316
	0.01001		0.70589
0.207	0.00311	5.25411	1.67607
	0.00622	3.30029	0.56214
	0.00934		0.58366

the data of water−PLLA interaction parameters are fitted by means of a two independent variable functional form, in order to model its dependence from the ternary solution composition, that is, PLLA and water volume fractions. In particular, the data are fitted by means of the following equation:

$$\chi_{wp} = \chi_0 + A\phi_p + B\phi_w + C\phi_p^2 + D\phi_w^2 + E\phi_p\phi_w \quad (2)$$

The surfaces fitting the data and the model parameters obtained from the fitting are summarized in Figure 4c,d and Table 2, respectively. In Table 2, the values for the adjusted coefficient of determination are also reported: they reveal that the model (2) gives a very good fitting of data at 313.15 K, while for data at 333.15 K the fitting is less accurate, but still satisfactory.

■ CONCLUSIONS

Several important advances in terms of thermodynamics of ternary solutions are reported by using isothermal titration calorimetric approach with the aim to overcome the limits of control the architecture obtained by TIPS. In particular, titration experiments are used in order to quantify the energies linked to the polymer−solvent interactions: information about

Table 2. Fitting Parameters for the Model (2)[a]

T (K)	χ_0	A	B	C	D	E	$\overline{R}^2$
313.15	24.14	−224.97	−155.03	−74659.09	311.90	1510.36	0.996
333.15	22.95	−2501.47	−158.78	95713.26	326.63	5982.96	0.917

[a]$\overline{R}^2$ represents the adjusted squared coefficient of determination for the considered model.

the phase diagrams of the studied systems are obtained, in terms of liquid−liquid miscibility gap. Despite the mechanism of polymer−solvent interactions in ternary systems similar to those under investigation has already been reported in previous studies, explanations in terms of energetic contribution are still lacking. It has been reported that when the content of water is low, it is moderately bound to PLLA, forming hydrogen bonds with C=O groups. In contrast, when water content is high, it interacts with the polymer, forming both water clusters and strong hydrogen bonds with the polymers inside the polymer network. In this work, we have clearly highlighted the energetic contributions involved in this mechanism showing how these energetic variations about polymer−solvent interactions are responsible for phase separation process and, therefore, for final morphology features. The knowledge and understanding of such data is fundamental to control processes for the production of membranes, scaffolds, and several other structures.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.5b02460.

> Water volume fraction and polymer percentage as functions of the number of injections; titration experiments at 25 °C; heat developed or adsorbed at each titrant injection; results reported in terms of entropy variations and mathematical derivations (PDF)

■ AUTHOR INFORMATION

Notes
The authors declare no competing financial interest.

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