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Prediction of conversion at equilibrium for lipase esterification in two-phase systems

Jose Scillipoti[a,b,c,e], Claudia Nioi[d], Alain Marty[a,b,c], Séverine Camy[d], Jean-Stephane Condoret[d,*]

[a] Université de Toulouse; INSA, UPS, INP; LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France
[b] INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse, France
[c] CNRS, UMR5504, F-31400 Toulouse, France
[d] Université de Toulouse; INPT, UPS; Laboratoire de Génie Chimique UMR CNRS 5503; 4, Allée Emile Monso, F-31030 Toulouse, France
[e] IDTQ–Grupo Vinculado PLAPIQUI–CONICET-FCEFyN, Universidad Nacional de Coírdoba, X5016GCA, Av. Veílez Sarsfield 1611, Coírdoba, Argentina

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Two-phase enzymatic catalysis

A B S T R A C T

In this work, a calculation procedure to predict reactant conversion at equilibrium in the case of lipase esterification in two-phase water-organic solvent system has been developed. The method is based on conventional thermodynamics using the UNIFAC predictive model to calculate the value of activity coefficients of compounds. Our approach was validated by comparison with experimental results in the case of the enzymatic esterification of oleic acid by *1*-butanol in a two-phase water/*n*-heptanesystem. In this configuration only *1*-butanol partitions significantly between the two phases. With this procedure, it was shown that oleic acid conversion at equilibrium was influenced by the initial mole fraction ratio of reactants, the volume ratio of the organic phase to aqueous phase, as well as the temperature and the nature of the organic solvent. The value of the equilibrium constant of the reaction, which is required for the calculations, was estimated from experiments at 298.15 K and found equal to 78 ± 3. The results emphasize the prominent role of the thermodynamic activity of *1*-butanol in the reactional mixture. In the case of different investigated solvents, it was shown that the thermodynamic activity of this compound has to be maximized to favor conversion of oleic acid at equilibrium.

1. Introduction

Enzymatic reactions in two-phase media are usually performed with hydrophobic reactants and water-soluble catalysts, as it is the case for enzymes. The non-aqueous phase may be an organic solvent, a supercritical solvent, an ionic liquid or a fluorinated solvent [1–4]. Use of such two-phase systems allows a possible shift of the reaction equilibrium that favors direct synthesis rather than hydrolysis [5,6]. Moreover, separation of reaction products is typically easier since the catalyst and products are present in distinct liquid phases. For these reasons, developing enzymatic catalysis in two-phase media is of great interest for fine chemical and pharmaceutical processes [7–10].

Maximizing the reaction conversion at equilibrium (which represents the maximum attainable conversion) is one of the most important criteria for reaction optimization, alongside as well as of catalyst activity, selectivity, and kinetics. The optimum operating conditions for the two-phase enzymatic reactor are a trade-off between all these criteria. For instance, global kinetics are often shown to greatly depend on the interfacial area between the liquid phases, which simultaneously depends on droplet size and phase ratio but the phase ratio also influences the conversion at equilibrium. This emphasizes that, among all these aspects, the prediction and understanding of how the operating parameters influence conversion at equilibrium is of great importance when optimizing the operation of a two-phase enzymatic reaction. However, understanding the evolution of equilibrium conversion in such non-ideal systems is not straightforward. Indeed, maximizing the equilibrium conversion requires a large number of experiments to be performed in order to screen all operating conditions, such as the nature of the solvent, the solvent phase volume ratio, the initial substrate concentration, the pH and temperature requires [11]. To overcome these difficulties several predictive approaches, which describe thermodynamics for reactions in two-phase media [12–15], have been developed in order to compute conversion at equilibrium. Eckestein et al. [16] proposed a method that requires the preliminary

* Corresponding author.
E-mail address: jeanstephane.condoret@ensiacet.fr (J.-S. Condoret).

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Nomenclature

a	Activity
ΔG°_{fi}	Standard state gibbs energy of formation of component *i* at 298.15 K (J/mol)
ΔG°_{R}	Standard gibbs energy of reaction (J/mol)
ΔH°_{fi}	Standard state enthalpy of formation of component *i* at 298.15 K (J/mol)
ΔH°_{R}	Standard enthalpy of reaction (J/mol)
K	Chemical equilibrium constant
m	Partition coefficient
R	Perfect gas constant (J/mol/K)
S	Ratio of initial molar fractions of 1-butanol/oleic acid
$S^{\circ}{}_{i}$	Standard state absolute entropy of component *i* at 298.15 K (J/mol/K)
ΔS°_{R}	Standard entropy of reaction (J/mol/K)
T	Temperature (K)
V	Ratio of organic phase to aqueous phase volumes
Vol	Volume
X	Oleic acid conversion yield
γ	Activity coefficient
ν	Stoechiometric coefficient

Superscripts

aq	Aqueous phase
calc	Calculated
exp	Experimental
org	Organic phase
α	α phase
β	β phase

Subscripts

Ac	Oleic acid
Al	Alcohol
calc	Calculated
Es	Ester
exp	Experimental
i	Component i
w	Water

experimental determination of the partition coefficients of reactants and products between organic and aqueous phase. In addition, they supposed that these coefficients are independent of mixture composition, which is a rough approximation in certain conditions. Martinek et al. [17] showed that the partition coefficients of reactants and the volume ratio of both phases affect conversion yield. They demonstrated this experimentally for the oxidation of isobutanol into isobutyraldehyde, catalyzed by alcohol dehydrogenase in water/hexane medium and for the α-chymotrypsin-catalyzed synthesis of ethyl ester of *N*-benzoyl-L-phenylalanine.

To compute partition coefficients of reactants and products in two-phase media, predictive activity coefficients models such as UNIFAC, which are based on group contributions, are of great help. They have often been used to describe phase equilibria in the field of biocatalysis [18–21]. For example, Janssen et al. [22] used the TREP computer program based on the UNIFAC group contribution method to predict composition at equilibrium for the esterification of fatty acids with glycerol, taking into account monoester, diester and triester synthesis. Based on a set of experimental equilibrium constants, their program was able to reproduce the composition at equilibrium of a non-diluted two-phase reaction system with quite a good accuracy and their results emphasized the prominent role of the nature of the solvent. More recently, Bucalá et al. [15], presented a procedure for the calculation of chemical equilibrium constants in two-phase systems, based on experimental equilibrium conversions, in the case of solvent-free (*i.e.*, with immobilized enzyme) enzymatic esterification of pure oleic acid and a mixture of fatty acid with ethanol. From the value of chemical equilibrium constants, both phase and reaction equilibria are simultaneously solved and this method proved to be able to correctly model solvent-free enzymatic esterification. In their work, the UNIFAC model was used to predict activity coefficients of the compounds.

In the present study, a similar procedure was developed in order to predict conversion at equilibrium in a two-phase aqueous/organic solvent system. The method was applied to the esterification of oleic acid by *1*-butanol using *n*-heptane as the organic solvent phase. The reaction was performed using the commercial *Rizhomucor Miehei* lipase as the catalyst. Indeed, the same model reaction was also chosen in our previous work on the use of Centrifugal Partition Chromatography as a novel two-phase enzyme reactor [23]. The method developed in this study was used to analyze the influence of operating parameters such as the initial organic to aqueous phase volume ratio, the initial substrate ratio and the nature of the solvent. The aim of the study is to improve the understanding of the influence of experimental operating parameters on the value of oleic acid conversion at equilibrium. Since the chemical equilibrium constant for this particular reaction was not available in the literature, it was experimentally determined prior to calculations.

2. Experimental section

2.1. Materials

For all experiments, the lipase of *Rizhomucor miehei* produced in *Aspergillus oryzae* (Sigma-Aldrich Chemie, Saint-Quentin Fallavier, France) was used. Oleic acid (purity 95%) was obtained from the same provider. All other chemicals were of analytical grade (*n*-heptane, *1*-butanol, *n*-decane, 5-methyl-2-hexanone and ethanol) and purchased from Fischer Scientific (Illkirch, France).

2.2. Experimental procedure

The biocatalyst was dissolved in the aqueous phase and the reactants (*1*-butanol and oleic acid) were solubilized in *n*-heptane, which was the organic phase.

Esterification of oleic acid was performed in a batch setup consisting of a 200 mL glass reactor, magnetically stirred at 600 rpm and thermo-regulated at suitable temperature with a thermostatic bath. In a typical experiment, the organic phase that contained reactants was introduced in the reactor and heated or cooled to the desired temperature. The reaction was considered to start when the aqueous phase containing the dissolved catalyst ($3\,gL^{-1}$) was added.

2 mL samples of the organic phase were taken after agitation was stopped and the system left to decant. Each sample was dissolved in 10 mL of ethanol and a few droplets of phenolphthalein (1% alcoholic solution) were added as an indicator. The sample was then titratedin order to determine the residual oleic acid content using a 0.01 M KOH solution (in ethanol). The substrate conversion was calculated by comparing the acidity value with that measured at the beginning of the reaction. This procedure was repeated until no change in conversion was observed in the samples. At this point, the final equilibrium conversion was considered to be reached. To confirm the results, all experiments were repeated three times. The duration of a typical experiment was around 180 min.

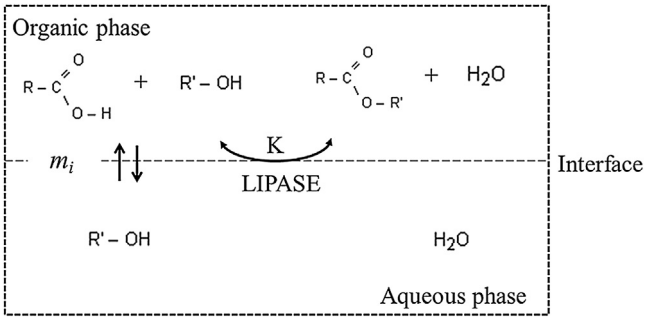


Fig. 1. Scheme of two-phase reaction system. K is the reaction equilibrium constant and m_i is the partition coefficient of component i (only 1-butanol and water are considered to partition).

3. Methodology

3.1. Description of the two-phase system

The esterification reaction considered in this study is written as:

$$Oleic\ \ acid\ \ (Ac) + Butanol\ \ (Al) \leftrightarrow Butyl\ Oleate\ \ \ (Es) + \ Water\ \ (Wa)$$

Due to the use of a lipase in a two-phase system, the reaction occurs at the interface between the aqueous phase, where enzyme is solubilized, and the *n*-heptane phase. The whole system can be represented as shown on Fig. 1 with the simplification that only *1*-butanol (R'-OH), water and lipase are significantly present in the aqueous phase, as will be justified later.

The reaction equilibrium constant is conventionally expressed as a function of the thermodynamic activities of reactants and products in the reaction mixture. For the stoichiometry of the esterification reaction considered here, the expression of the equilibrium constant in the α phase is:

$$K^{\alpha} = \frac{a_{Es}^{\alpha} a_{Wa}^{\alpha}}{a_{Ac}^{\alpha} a_{Al}^{\alpha}} \tag{1}$$

Moreover, at equilibrium, Eq. (2) relative to thermodynamic liquid–liquid equilibrium is satisfied:

$$a_i^{\alpha}\left(\bar{x}^{\alpha},\ \mathrm{T}\right) = a_i^{\beta}\left(\bar{x}^{\beta},\ \mathrm{T}\right) \tag{2}$$

The activity coefficient of compound *i* (γ_i) is a parameter accounting for the deviation from an ideal mixture and relates activity to the molar composition of the mixture, following Eq (3).

$$a_i^{\alpha}\left(\bar{x}^{\alpha},\ \mathrm{T}\right) = x_i^{\alpha} . \gamma_i^{\alpha}\left(\bar{x}^{\alpha},\ \mathrm{T}\right) \tag{3}$$

At equilibrium, Eq. (2) must be satisfied for all components *i* so:

$$\left(\gamma i\left(\bar{x}^{aq},\ \mathrm{T}\right) x_i\right) aq = \left(\gamma i\left(\bar{x}^{org},\ \mathrm{T}\right) x_i\right) org \tag{4}$$

At equilibrium, Eq. (1) should be satisfied too and thus K^{org} and K^{aq} have the same value, *K*, which only depends on temperature.

Thus, in order to predict oleic acid conversion at equilibrium, and thus the composition of both aqueous and organic phases, it is necessary to know the value of the reaction equilibrium constant *K* and to calculate the value of the activity coefficients of compounds.

3.2. Choice of the model for activity coefficients prediction

Liquid–liquid equilibria occurring in binary, ternary and quaternary mixtures involving *1*-butanol, oleic acid, water and *n*-heptane were previously studied by Winkelman et al. [24]. The authors aimed at predicting liquid–liquid equilibria involved in an aqueous-organic two-phase system for the esterification of oleic acid by *1*-butanol and demonstrated that the UNIQUAC activity coefficient model, which was extended with ternary interaction parameters, was able to predict these liquid–liquid equilibria with good accuracy. Winkelman et al.'s approach, however, cannot be directly used for the computation of the UNIQUAC model, which is basically defined with binary interaction parameters only, in commercial thermodynamic software. For this reason, in this study, the UNIFAC [25] model available in the Simulis® Thermodynamics software (ProSim SA, France) was employed. The main feature of this model lies in its predictive capabilities, provided that compounds can be decomposed into functional groups available in the UNIFAC database. The proposed approach is thus easily generalizable to other systems.

Table 1
Calculated Average Absolute Relative Error (%) between UNIFAC model and experimental data of Winkelman et al. [25].

	Organic phase				Aqueous phase	
	$x_{but}{}^{org}$	$x_{oleic}{}^{org}$	$x_{water}{}^{org}$	$x_{hept}{}^{org}$	$x_{but}{}^{aq}$	$x_{water}{}^{aq}$
AARE(%)	1.2	0.8	23.4	1.3	1.9	0.011

In order to assess the conventional modelling using UNIFAC, the calculations performed with Simulis® Thermodynamics software were compared with Winkelman et al.'s [24] experimental results for quaternary liquid–liquid equilibria from. Their data consist of a set of 42 experimental tie lines giving the global composition of the mixture, composition of aqueous phase (containing *1*-butanol and water) and organic phase (containing *1*-butanol, oleic acid, water and *n*-heptane) at 301, 308 and 313 K. Table 1 gives the Average Absolute Relative Error (*AARE%*) between the predicted and experimental data as calculated with Eq (5) for each experimental liquid composition x_i:

$$AAREz_i = \frac{100}{N_p}\sum_{i=1}^{Np}\left|\frac{x_i^{\exp} - x_i^{calc}}{x_i^{\exp}}\right| \tag{5}$$

Moreover, a visual appreciation of the agreement of the experimental data and UNIFAC predictions is provided in Fig. 2. Parity plots and *AARE* show that the UNIFAC model is globally able to predict the experimental behaviour of the system. It can be noticed that the solubility of water in the organic phase ($x_{water}{}^{org}$) seems to be overestimated (*AARE = 23.4%*). Observation of Fig. 2(b) and (c) shows that UNIFAC has a slight tendency to underestimate the *1*-butanol mole fraction in the aqueous phase at 301 K.

Although the fifth component involved in the reactional system, i.e. butyl oleate, is missing in this study, these results validate the predictive capability of the UNIFAC model to represent the thermodynamic behaviour of the actual reactional mixture in the range of temperature considered in this study. Obviously, use of a semi-predictive model for activity coefficients, such as the UNIQUAC or NRTL models, would improve the description of this liquid–liquid equilibrium, as shown for example by Winkelman et al. [24]. However, the identification procedure for binary interaction coefficients is a tricky and tedious task. Globally, the predictive UNIFAC model was chosen here because it provides sufficient accuracy.

3.3. Estimation of the value of the reaction equilibrium constant K

Theoretically, the value of *K* can be obtained from the standard Gibbs energy of reaction $\Delta G_R^{\circ}(T)$ according to the following expression:

$$K(T) = exp\left(\frac{-\Delta G_R^{\circ}(T)}{RT}\right) \tag{6}$$

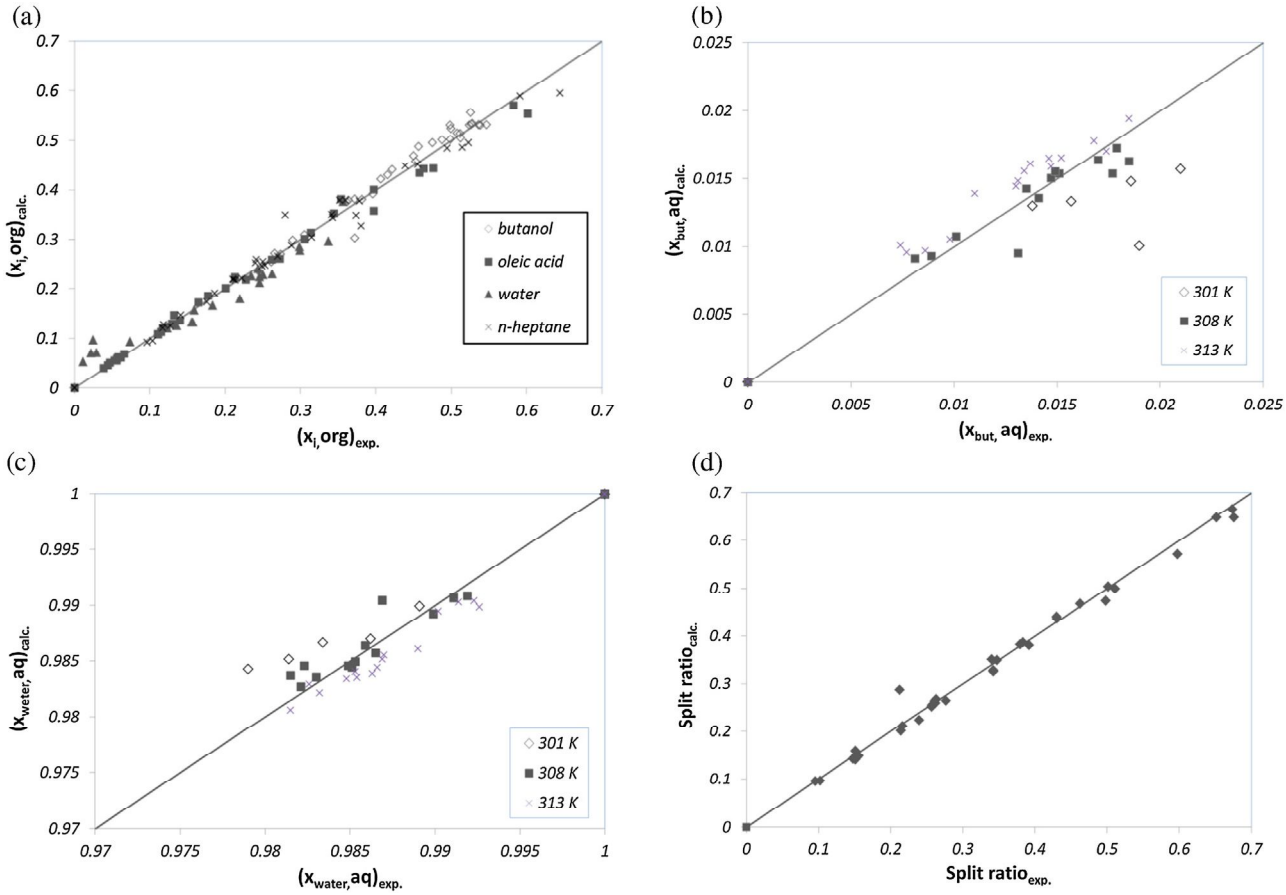


Fig. 2. Comparison of liquid–liquid equilibria of 1-butanol-oleic acid-water-*n*-heptane quaternary mixture calculated using the UNIFAC model developed in this study with experimental results given by Winkelman et al. [24]. (a) composition of organic phase at different temperatures (b) 1-butanol molar fraction in aqueous phase (c) water molar fraction in aqueous phase (d) organic to water phase split ratio.

Table 2
Standard state enthalpy of formation at 298.15 K (ΔH_{fi}°), Standard State absolute entropy ($S^{\circ}{}_i$) at 298.15 K and standard state Gibbs energy of formation (ΔG_{fi}°) at 298.15 K. Data from DIPPR database [27]. Calculated Standard State entropy of formation ΔS_{fi}° at 298.15 K.

i	ΔH_{fi}° kJ/mol	S_i° J/mol.K	ΔG_{fi}° kJ/mol	ΔS_{fi}° J/mol.K
1-butanol	−326.400	225.8	−161.700	−552.5365
oleic acid	−802.491	712.1	−261.100	−1815.97
water	−285.83	70.033	−237.214	−163.0595
butyl oleate	−816.88*	n.a.	n.a.	n.a.

* From NIST [28] n. a. not available.

Table 3
Experimental oleic acid conversion at equilibrium at different reaction conditions ($S = x_{BuOH}/x_{Ac}$; $V = Vol_{org}/Vol_{aq}$;). *K* is the equilibrium constant computed from the value of X_{exp}.

Run	T (K)	S	V	X_{exp}	K
1	298.15	1.54	0.13	0.825	82.11
2	298.15	1.54	1.02	0.867	82.16
3	298.15	1.54	2.37	0.877	75.76
4	298.15	1.54	9.07	0.895	74.39
5	298.15	0.51	2.39	0.459	77.62
6	298.15	1.03	2.39	0.794	77.75
7	298.15	1.54	2.39	0.913	81.45
8	298.15	2.57	2.38	0.942	74.02

where, by definition:

$$\Delta G_R^{\circ}(T) = \sum i \quad \nu_i \Delta G_{fi}^{\circ}(T) = \Delta H_R^{\circ}(T) - \mathrm{T}.\Delta S_R^{\circ}(T) \tag{7}$$

In Eq. (7), $\Delta H_R^{\circ}(T)$ and $\Delta S_R^{\circ}(T)$ are the standard enthalpy of reaction and standard entropy of reaction at temperature *T*, respectively. These values can be calculated from molar enthalpies of formation (ΔH_{fi}°) and molar entropies of formation (ΔS_{fi}°) of compounds in their standard state at temperature *T*, from which the corresponding standard Gibbs energy of formation of species *i* (ΔG_{fi}°) can be deduced. As a consequence of Eq. (7), the standard Gibbs energy of reaction at *T*, $\Delta G_R^{\circ}(T)$, and therefore *K(T)*, can be calculated provided that the values of ΔG_{fi}° for all compounds are known. Table 2 gives an overview of available thermodynamic properties of compounds involved in the esterification reaction of *1*-butanol. Unfortunately, it is observed in Table 2 that the molar entropy of formation of *n*-butyl oleate could not be calculated because the value of the absolute standard entropy (S°) of this compound is not available. Consequently, the reaction equilibrium constant of this esterification reaction could not be directly theoretically obtained by applying Eq. (6).

Since the *n*-butyl oleate data was missing, the value of the equilibrium constant *K* at 298.15 K (25 °C) was firstly deduced from experiments reported in Table 3. Eight runs corresponding to the esterification of *1*-butanol by oleic acid in two-phase water-*n*-heptane systems at 298.15 K with different phase volume ratios (runs 1–4) and different substrates molar ratios (runs 5–8) were performed. The overall oleic acid conversion yield (X_{exp}) was deduced from each experiment after measurement of the residual oleic acid in the organic phase, as described in Experimental section. The final overall composition of the mixture was calculated from the experimental oleic acid conversion at equilibrium.

UNIFAC model was then used to predict the values of the activity coefficients for all species in each phase at equilibrium. Finally, the K value for each experiment was calculated by applying Eq. (1). This procedure led to an average value of K equal to 78 ± 3 at 298.15 K.

3.4. Estimation of standard state gibbs energy and absolute entropy of butyl oleate

As previously mentioned, the available thermodynamic properties of compounds in Table 2 do not allow the direct calculation of the standard state Gibbs energy of reaction at 298.15 K. From the results of the preceding paragraph, it was useful to estimate this value, which can be easily deduced by Eq. (6) from the value of the experimental equilibrium constant determined in this study. This led to $\Delta G^{\circ}_{R} = -10.8$ kJ/mol. By combining this value and the standard state Gibbs energies of formation of *1*-butanol, oleic acid and water given in Table 2, the standard state Gibbs energy of formation of butyl oleate at 298.15 K can be computed as −196.307 kJ/mol. This value is consistent with data obtained by Bucalá et al. [15] for ethyl oleate (−136.2 kJ/mol) and data given in the literature [29] for methyl oleate (−117.0 kJ/mol).

The standard state enthalpy of reaction at 298.15 K was computed from enthalpies of formation reported in Table 2 ($\Delta H^{\circ}_{R} = 26.18$ kJ/mol) and allowed the value of the standard state entropy of reaction ($\Delta S^{\circ}_{R} = 124.0$ J/(mol.K)) to be determined. Finally, this yielded a value of the standard state absolute entropy of *n*-butyl oleate equal to 991.9 J/(mol.K) at 298.15 K. The value of standard state enthalpy of our reaction ($\Delta H^{\circ}_{R} = 26.18$ kJ/mol) is of the same order of magnitude of the standard enthalpy of esterification of oleic acid with ethanol (21.58 kJ/mol) [15]. Note that this value is quite low and corresponds to a slightly endothermic reaction, which ensures mild reaction conditions with no heat effects.

3.5. Methodology to compute oleic acid conversion at equilibrium

From the known value of K obtained in Section 3.3, the prediction of equilibrium conversion for any system for which the initial mixture composition is known can be done using the procedure described in Fig. 3. From an initial value of conversion at equilibrium of oleic acid (X_{guess}), the final overall composition of the mixture is calculated. The activity of each compound is then estimated by the UNIFAC model implemented in Simulis® Thermodynamics add-in and used in Microsoft Excel. The activities are used for calculation of value of K. This procedure is repeated until the difference between the target and calculated K value is minimized. The optimization was done using the non-linear Generalized Reduced Gradient (GRG) method implemented in the solver function of the Microsoft Excel software [26].

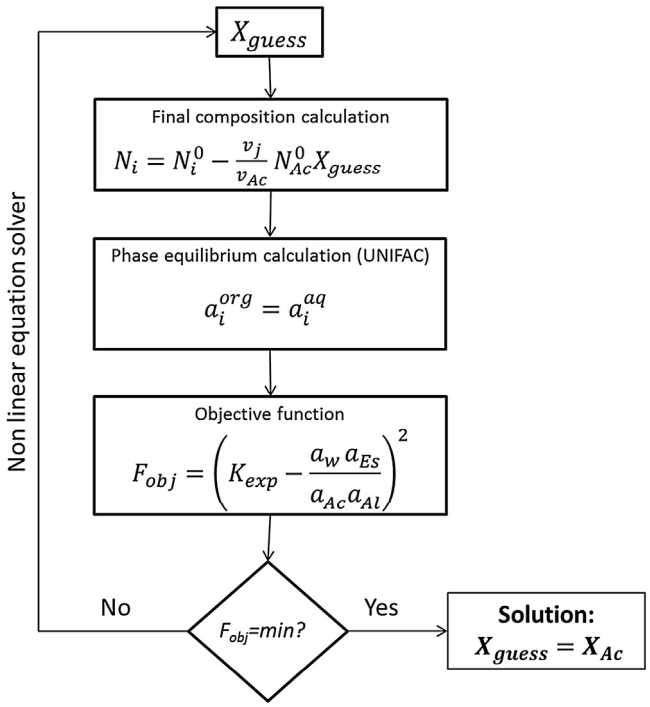


Fig. 3. Flow chart for calculation of equilibrium conversion of esterification reaction in two-phase media.

4. Results and discussion

4.1. Preliminary analysis of partition coefficients

As mentioned in the introduction, the method proposed by Eckstein et al. [16] for the calculation and prediction of equilibrium conversion in a two-phase reaction mixture required the value of partition coefficients of both products and substrates, which were assumed independent of the mixture composition. Partition coefficients m_i are defined as the ratio of the molar fraction of species i in the organic phase divided by its molar fraction in the aqueous phase. In this case, high partition coefficients correspond to hydrophobic compounds whereas low partition coefficients are related to compounds essentially present in the aqueous phase. To assess the partitioning of compounds in the two-phase medium, the partition coefficients were computed for initial mixtures of runs 1–4 (Table 4) for which the volume ratio of organic to aqueous phase (V) has been varied. These conditions correspond to the initial amounts of reactants and solvent, which are given in Table 4.

The partition coefficient of substrates and solvent calculated with UNIFAC for runs 1 to 4 values are reported in Fig. 4. The computations led to very high values for oleic acid (around 10^7) and hexane (around 10^4) indicating that these compounds are almost completely insoluble in the aqueous phase, and low values (around 10^{-3}) for water, indicating its low solubility in the organic phase. Finally, in this two-phase system, *1*-butanol is the only compound that significantly partitions between both phases (partition coefficient varying from 6 to 3.7). Fig. 4 shows, for a wide range of variation of V, that the partition coefficient of *1*-butanol does not vary greatly and its value can be taken around 3.7 at 298.15 K. However, it can be noted that at low V values (corresponding to low amount of organic solvent), its value increases significantly (to around 6 when V is equal to 0.13). This surprising increase of affinity of *1*-butanol for the organic phase can be attributed to the presence of oleic acid in this phase. Indeed, the initial amount of oleic acid is the same for all V values (2.2 mL) and when V becomes very low, the oleic acid concentrates significantly in the organic phase because it does not partition at all. Its presence in the organic phase increases the solubility of *1*-butanol because of the polar carboxylic group of the acid. From a practical point of view, this situation of very low V values is not interesting because in order to obtain good productivity of the esterification process (either in batch mode or in continuous mode), the quantity of organic phase (containing substrates) has to be maximized. Also, a very low organic phase volume would not allow the generation of sufficient interfacial area to ensure good kinetics. As a conclusion, it can be stated that in this system only *1*-butanol is present in a significant way in both phases and that the hypothesis of constant partition coefficients is finally quite valid for the study of such reactions in the range of initial compositions investigated here.

Table 4
Overall amounts and corresponding molar fractions of mixtures of oleic acid, *1*-butanol,water, and *n*-heptane at 298.15 K.

Run	V	Overall initial amount					Overall initial molar fraction		
		oleic acid [mL]	*1*-butanol [mL]	water [mL]	*n*-heptane [mL]	$z_{oleicacid}$	$z_{1\text{-butanol}}$	z_{water}	$z_{n\text{-heptane}}$
1	0.13	2.2	1	180	20	0.00070	0.00108	0.98477	0.01346
2	1.02	2.2	1	100	100	0.00113	0.00175	0.88792	0.10920
3	2.37	2.2	1	60	140	0.00165	0.00254	0.77378	0.22204
4	9.07	2.2	1	20	180	0.00300	0.00464	0.47102	0.52134

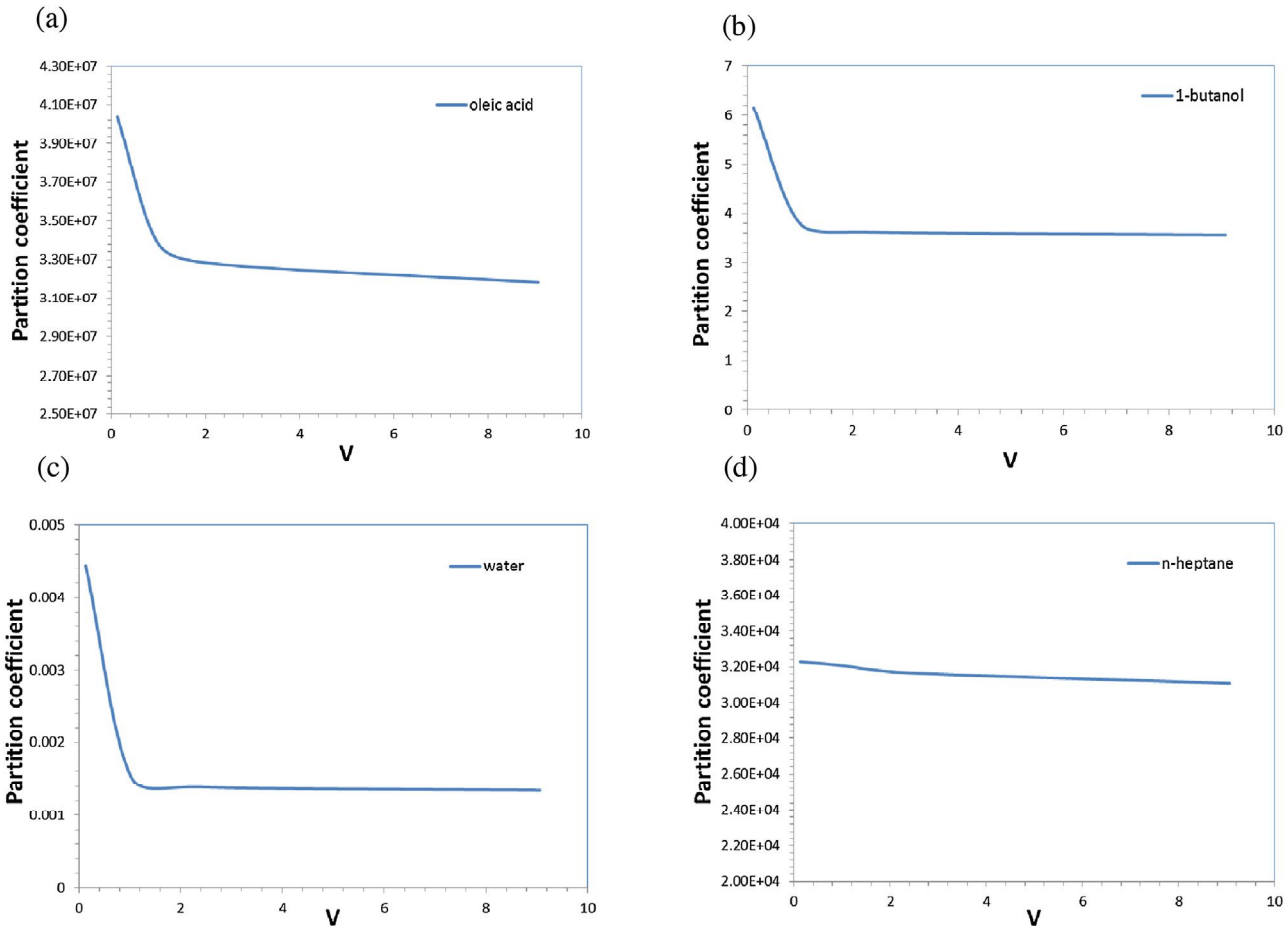


Fig. 4. Computed partition coefficients at 298.15 K obtained with UNIFAC model as a function of the organic and aqueous phase volume ratio (V): (a) oleic acid, (b) 1-butanol, (c) water and (d) *n*-heptane.

4.2. Influence of initial molar ratio of reactantson conversion at equilibrium

The initial ratio of reactants is an important parameter that affects the equilibrium conversion in accordance with the Le Chatelier–Braun principle. At 298.15 K, the initial molar ratios of *1*-butanol and oleic acid molar fractions, $S = x_{BuOH}/x_{Ac}$, were experimentally varied (runs 4–8 in Table 3) for a fixed organic phase to aqueous phase volume ratio *V* equal to 2.39. Note that the ratio *S* wasvaried by increasing the initial amount of *1*-butanol, whilst keeping the initial amount of oleic acid constant. This logically led to an increase of oleic acid conversion at equilibrium according to Le Chatelier's principle (applied to the case where sum of stoichiometric coefficients is equal to 0), as shown in Fig. 5.

The initial activities of compounds were also computed as a function of the ratio *S*. In this configuration, it is observed in Fig. 6 that when *S* is increased by increasing the initial amount of *1*-butanol in the mixture, the activity of this latter is increased, whilst the water and oleic acid activities only decrease slightly. This suggests that the increase of conversion at equilibrium of oleic acid with *S* is mainly related to the increase of the *1*-butanol activity in the initial mixture.

4.3. Influence of the water content on conversion at equilibrium

The global water content of the system is indeed inversely proportional to the organic to aqueous volume phase ratio, $V = Vol_{org}/Vol_{aq}$, which was used in paragraph 4.1. Indeed, for esterification reactions in two-phase media, this parameter was shown to affect the equilibrium conversion [15,25] but also the chemical kinetics because they are related to interfacial area [23]. Thus, the influence of the initial proportion of organic and aqueous phases on oleic acid conversion at equilibrium was studied. For practical reasons, the total volume of the mixture was kept constant and *V* was increased by increasing the volume of *n*-heptane (Vol_{org}) and simultaneously reducing the volume of water (Vol_{aq}), whilst keeping the initial amounts of reactants constant. Both the experimental

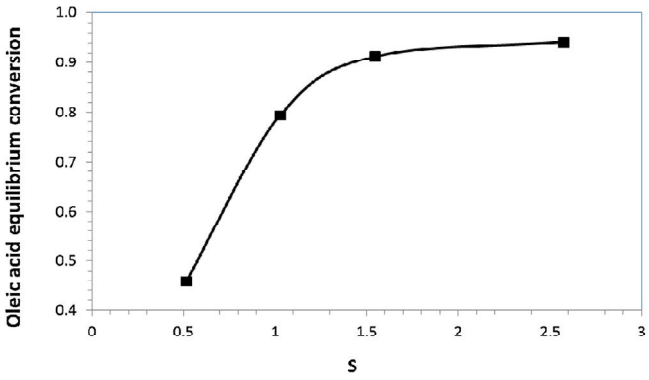


Fig. 5. Experimental (symbols) and predicted (line) oleic acid equilibrium conversion as a function of initial molar ratio of reactants S. Experimental conditions: V = 2.39 and T = 298.15 K. Each experimental data was analyzed in triplicate and estimated error was ± 1.05%.

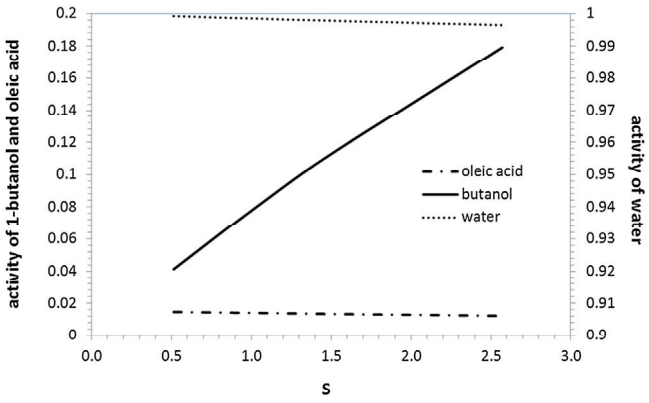


Fig. 6. Calculated initial activity of reactants and water when increasing the initial molar ratio of reactants, S. Experimental conditions: V = 2.39 and T = 298.15 K.

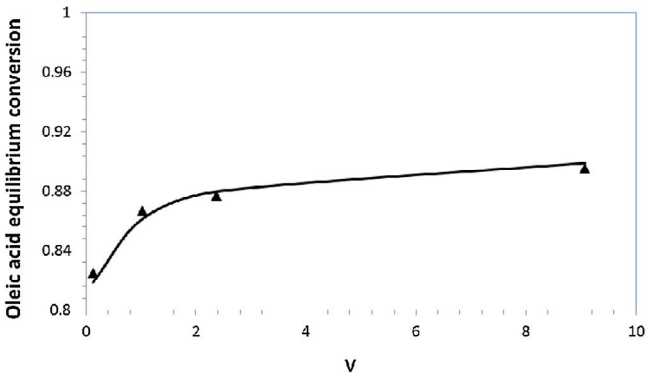


Fig. 7. Experimental and predicted conversions at equilibrium as a function of initial phase volume ratio $V = Vol_{org}/Vol_{aq}$. Each experimental data was analyzed in triplicate and estimated error was ± 1.12%.

and predicted results show an increase of oleic acid conversion at equilibrium when *V* is increased (Fig. 7).

Fig. 8 shows the computed initial activities of reactants and water; it can be seen that the *1*-butanol activity increases and the activity of oleic acid decreases with *V*. Indeed, a consequence of the operating procedure that increased *V V* was an increase in the mole fraction of *1*-butanol in the aqueous phase, thereby leading to an increase of the activity of this compound. On the other hand, y, due to the non-partitioning of oleic acid, its molar fraction in the organic phase is decreased when *V* is increased. For the same reason, a similar dilution effect is present for the ester and its activity decreases with increase of *V* (results not presented). This compensates the influence of oleic acid dilution upon the conversion value at equilibrium, because the activity value of oleic acid and ester appears in the denominator and the numerator of the equilibrium constant, respectively. The value of water activity in the aqueous phase remains very close to 1 because *1*-butanol is always diluted in this phase. In consequence, the activity of water in the organic phase in equilibrium is also always close to 1.

As a global conclusion, it can be stated that decreasing the global water content, at constant initial amount of substrates and constant total volume, positively affects equilibrium conversion, mainly because the activity of *1*-butanol increases, whilst the oleic acid and ester activities compensate one another and the water activity is more or less constant.

4.4. Effect of temperature on equilibrium conversion

Van't Hoff's equation (Eq. (8)) relates the variation of the equilibrium constant *K* with respect to the temperature. When the temperature range is such that the standard state enthalpy of reaction can be considered as a constant, Van't Hoff's equation can be used to deduce the value of the equilibrium constant at temperature *T* from the knowledge of *K* at a given temperature T_0, 298.15 K for example.

$$\ln\left[\frac{K(T)}{K(298.15\mathrm{K})}\right] = -\frac{\Delta H^{\circ}}{R}\left(\frac{1}{T} - \frac{1}{298.15\mathrm{K}}\right) \quad (8)$$

Esterification experiments were performed at several temperatures between 291.15 K and 315.15 K in order to estimate the influence of the temperature upon oleic acid equilibrium conversion. This temperature range was chosen such that the inhibition of enzyme activity at high temperature was prevented [30]. Experimental values of the equilibrium constant deduced from oleic acid conversion at equilibrium at different temperatures were compared with values of the equilibrium constant obtained from Van't Hoff's equation using the value of ΔH°_R previously calculated at 298.15 K ($\Delta H^{\circ}_R = 26.18$ *kJ/mol*, which was assumed to be independent of *T*). The comparison of the experimental and calculated values is represented by the parity plot in Fig. 9, which shows good agreement. Note that the computed and experimental values at 298.15 K are exactly the same because the equilibrium constant value was identified from the set of experiments at 298.15 K.

These results show that operating the reaction at high temperature is only of interest for increasing kinetics. Consequently, the "conversion at equilibrium" parameter is not pertinent for the choice of the optimum reaction temperature. Thus, a trade-off has to be made between favouring kinetics at high temperature or ensuring enzyme stability with lower temperatures.

4.5. Effect of the nature of the solvent on equilibrium conversion

In order to validate the generic character of the proposed calculation procedure, it has been applied to a two-phase system that uses *n*-decane as the solvent instead of *n*-heptane. The UNIFAC model is used in the same way to predict activity coefficients of compounds in the mixture. Table 5 summarizes the experimental and calculated data of oleic acid conversion at equilibrium for the reaction of esterification of oleic acid with *1*-butanol in the *n*-decane-water system. All data were obtained at 298.15 K with an organic to aqueous phase ratio *V* equal to 2.39. From Table 5, it can be seen that the predicted and experimental data are in good agreement. It can also be seen that the conversion at equilibrium obtained with *n*-decane and with *n*-heptane at the same operating conditions are very similar. This is not surprising since both *n*-heptane and *n*-decane are highly non polar solvents with a logP value of 4.5 and 6.25, respectively [32]. The logP is the logarithm

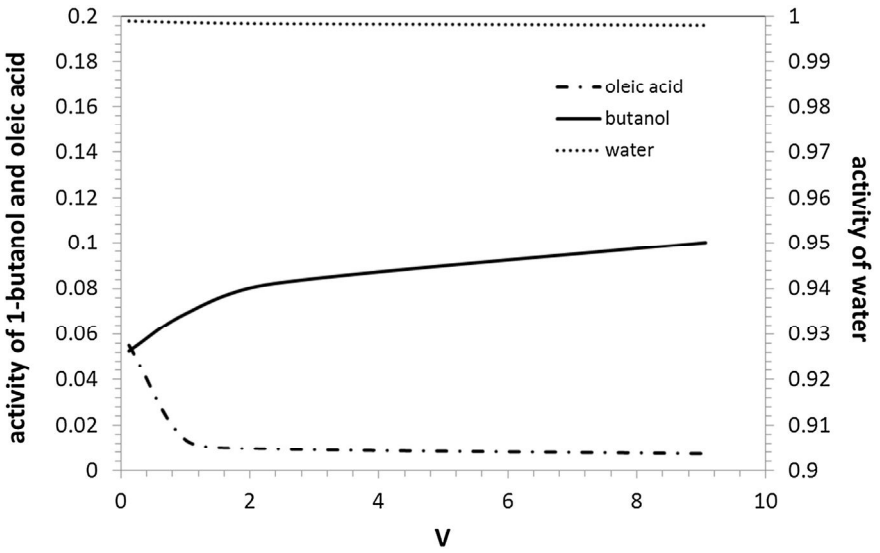


Fig. 8. Calculated initial activity of reactants and water with increasing initial phase volume ratio = Vol_{org}/Vol_{aq}. Experimental conditions: initial reactants molar ratio S = 1.54 and T = 298.15 K.

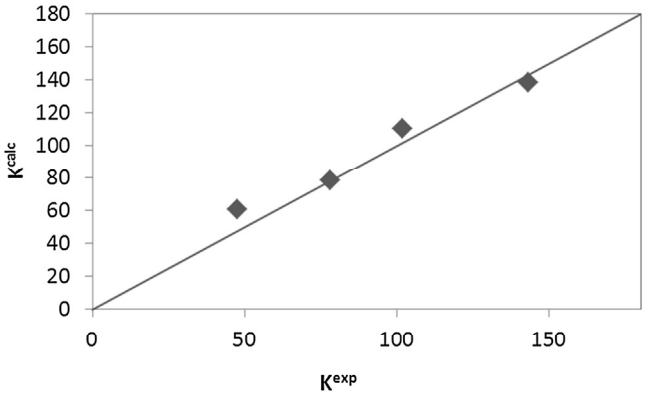


Fig. 9. Parity plot of the equilibrium constant of esterification of *1*-butanol and oleic acid at different temperatures in the range [291.15–315.15] K.

Table 5
Experimental and calculated oleic acid conversion at equilibrium in *n*-heptane and *n*-decane at 298.15 K and $V = 2.39$.

S	*n*-heptane		*n*-decane	
	X^{exp}	X^{calc}	X^{exp}	X^{calc}
0.51	0.459	0.462	0.510	0.461
1.03	0.794	0.794	0.780	0.791
1.54	0.913	0.909	0.890	0.907
2.57	0.942	0.958	0.940	0.957

of the partition coefficient of the compound in the octanol-water two-phase system and is commonly used to characterize the polarity of a compound [31]; high logP values correspond to low polarity. In our case, the polarity of the solvent affects the activities of reactants and products in the mixture and consequently is expected to affect the conversion at equilibrium [33]. For non-polar solvents like *n*-decane and *n*-heptane, the distribution coefficients for all compounds are very close and result in similar values of conversion at equilibrium.

In order to further evaluate the influence of the polarity of the solvent, oleic acid conversion at equilibrium was predicted using our procedure with solvents presenting logP values ranging from 0.99 to 4.69, as shown in Table 6. Data are computed for six two-phase systems at 298.15 K with an initial molar ratio of reactants

Table 6
Organic solvent logP values.

Solvent	LogP	Reference
2-methyloctane	4.69	Chao et al. [34]
n-heptane	4.50	Sangster [31]
2-methylpentane	3.21	Chao et al. [34]
3-nonanone	2.94	Bylaite et al. [35]
2-heptanone	1.98	Wasik et al. [36]
3-pentanone	0.99	Tewari et al. [37]

$S = 1.54$ and for different initial global water contents (phase volume ratio, V, equal to 0.13, 1.02, 2.37 and 9.07). In Fig. 10(a) it is observed that the polarity of the solvent significantly affects the oleic acid conversion at equilibrium. For non-polar solvents (logP > 3), the conversion at equilibrium gradually increases with the increase of the phase volume ratio V. On the other hand, for more polar solvents (logP < 3) lower conversion at equilibrium is obtained, and it decreases as the phase ratio V increases. The initial activity of *1*-butanol was computed in each case and is reported in Fig. 10(b). The activity of *1*-butanol is shown to follow the same trend as oleic acid conversion at equilibrium. In the case of polar solvents, like *3*-nonanone, *2*-heptanone and *3*-pentanone, a decrease in *1*-butanol activity and thus of oleic acid conversion at equilibrium is observed. This could be due to the presence of polar OH functional groups, which are more likely to develop interactions with the alcohol, therefore decreasing its activity. Again, high values of activity of *1*-butanol are shown to positively influence the oleic acid conversion at equilibrium.

A similar observation is made when the polarity of the organic phase is modified by adding different proportions of a polar solvent such as 5-methyl-2-hexanone (5M2H, logP = 1.88) in *n*-heptane. It is shown in Fig. 11(a) that when the proportion of 5M2H in *n*-heptane is increased, the conversion of oleic acid at equilibrium is decreased, which is expected. Fig. 11(b) indicates that the initial alcohol activity decreases in the same way. In addition, it can be observed that the medium polarity has a low influence on the activity value of the acid, which remains low whatever the 5M2H proportion. This is probably due to the fact that the long hydrophobic carbon chain of this fatty acid is the prominent parameter, which masks the influence of the interaction of the polar solvent with the carboxylic group of the acid. Once again, these results point out the

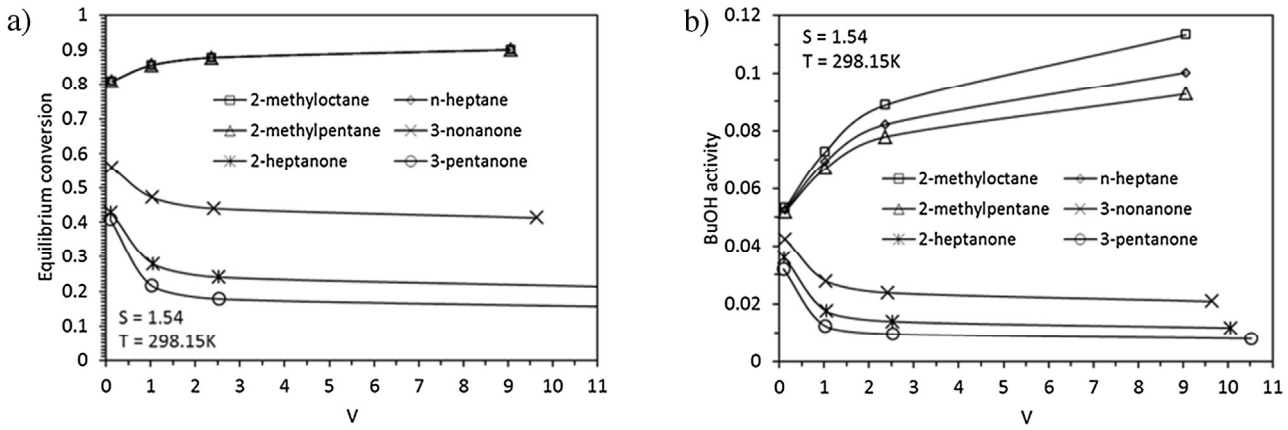


Fig. 10. (a) Influence of phase volume ratio on oleic acid conversion at equilibrium with different organic solvents. (b) Influence of phase volume ratio on *1*-butanol activity for different organic solvents. Calculated values were obtained at initial molar ratio of alcohol to acid of 1.54 and at 298.15 K.

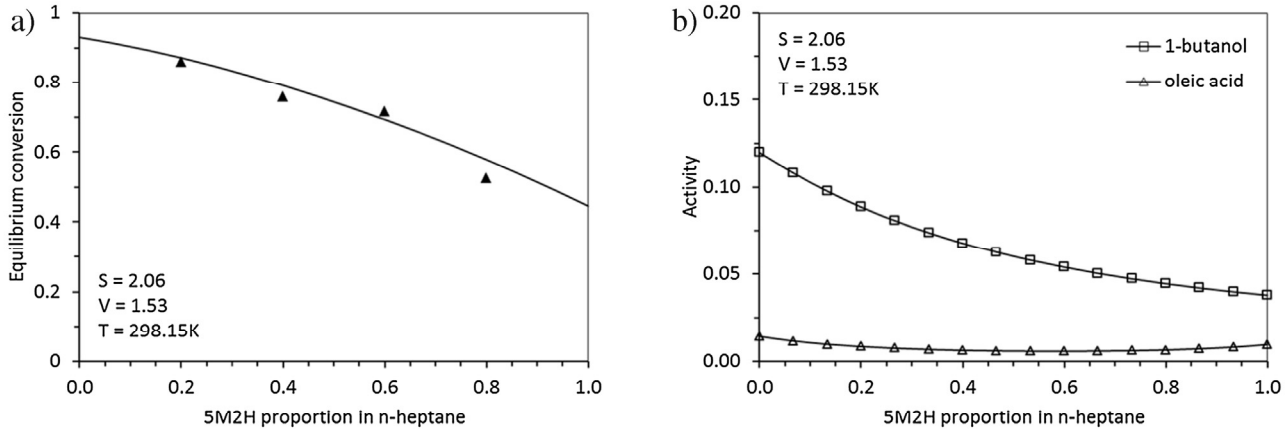


Fig. 11. (a) Influence of 5M2H proportion in *n*-heptane on oleic acid conversion at equilibrium. Experimental (▲) and predicted data (solid line). (b) Influence of 5M2H proportion in *n*-heptane on initial reactants activities.

prominent influence of the activity value of *1*-butanol on the value of oleic acid conversion at equilibrium.

5. Conclusion

In this study, a methodology based on computations using the UNIFAC model for the evaluation of activity coefficients was presented as a tool to estimate the conversion at equilibrium of a two-phase reaction system. The methodology was validated by comparing the calculated values with experimental data for the lipase catalyzed esterification of oleic acid with *1*-butanol. Following the experimental determination of the equilibrium constant *K* at 298.15 K, our approach allowed the operating conditions for maximum conversion at equilibrium to be predicted. Furthermore, the influence of the initial global water content, initial molar ratio of reactants, reaction temperature and polarity (logP) of the organic phase were studied. The value of the partition coefficients of all compounds were also able to be predicted using this method and it was shown that the value of the partition coefficient for *1*-butanol, which is the only constituent that effectively partitions between phases, more or less constant except at very low organic to aqueous phase ratios.It is worth noting that computation of the partition coefficients could also be useful to assess reaction rate in the two-phase reaction system using the enzyme kinetic equation and this could be the focus of future work.

In terms of best operating parameters for the reaction, it was found that the key parameter for maximizing equilibrium conversion is the activity value of the alcohol (*1*-butanol, in this case) in the initial mixture. The method presented here could be used to predict the best operating conditions for the esterification of long chain alcohols.

This work has provided knowledge concerning the final conversion in order to optimize operating conditions of enzymatic two-phasereaction systems where a trade-off has to be made between kinetic aspects and final conversion considerations.

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