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Study of the binding between lysozyme and C_{10}-TAB: Determination and interpretation of the partial properties of protein and surfactant at infinite dilution

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ABSTRACT

This work examines the binding in aqueous solution, through the experimental determination of specific volumes and specific adiabatic compressibility coefficients, of decyltrimethylammonium bromide to lysozyme and to non-charged polymeric particles (which have been specially synthesized by emulsion polymerization). A method was developed to calculate the specific partial properties at infinite dilution and it was shown that a Gibbs–Duhem type equation holds at this limit for two solutes. With this equation, it is possible to relate the behavior of the partial properties along different binding types at a constant temperature. It was found that the first binding type, specific with high affinity, is related to a significant reduction of surfactant compressibility. The second binding type is accompanied by the unfolding of the protein and the third one is qualitatively identical to the binding of the surfactant to non-charged polymeric particles.

1. Introduction

Elucidation of the thermodynamic nature and relative importance of the forces that govern the cooperativity of the folding/unfolding transitions of proteins is a key to understanding and, ultimately, addressing the protein folding problem [1]. These transitions are highly cooperative and of the all-or-none type, and they can be induced by changes in pH, temperature, pressure, cosolvent composition and presence of ligands. In this respect, interactions between surfactants and proteins are of major importance since they involve different types of binding [2]. The important features of the binding isotherms are the combination of a low degree of non-co-operative binding at low surfactant concentrations and of a massive co-operative binding at higher concentrations [3]. Often binding is described in terms of a low amount of high affinity binding to specific sites, followed in the second stage by co-operative binding. The first binding type is electrostatic in origin, and the second is accompanied by the unfolding of the protein.

The volumetric thermodynamic properties (volume and its derivatives with respect to temperature, expansibility, and with respect to pressure, compressibility) have been widely employed in the study of folding/unfolding transitions due to changes in temperature [4–7], pressure [6,8], pH [7,9–13], cosolvent composition [14,15], oxidation/reduction reactions [16] and binding of ligand [17–23] because these properties are sensitive to the solute–solvent interaction (hydration) and to the intrinsic packing. In this respect, it has been suggested that the "efficacy of the use of volumetric measurements for solving problems of biological relevance ultimately depends on our ability to rationalize measured volumetric observables in terms of various volumetric inter- and intramolecular interactions including, but not limited to, hydration and intrinsic packing" [24].

There are two methods for the study of the binding to macromolecules by volumetric properties. The most frequently used method [17–22] is based on the calculation of the variation of apparent properties when the ligand (or macromolecule) is transferred from a solution with a solvent to another solution with this solvent in admixture with the macromolecule (or ligand). The other method was

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proposed by Bernhardt and Pauly [23] in 1977, and is based on the consideration of a "complex solute" composed of protein and ligand. In this way, it is possible to calculate the partial volume of protein and ligand at infinite dilution. In a previous work [25], we employed this method to study the swelling process of functionalized polymer particles. However the narrow interval of ligand concentrations limits the possibility to study the binding process. In order to employ this method over a broad interval of ligand concentrations, it was necessary to establish the thermodynamic basis of the method. Therefore, this paper demonstrates that a Gibbs–Duhem type equation holds at infinite dilution for two solutes and two different interaction thermodynamic patterns. The first pattern concerns two solutes interacting at infinite dilution. The second one serves to locate where the maximum (saturation) of interaction between two solutes takes place at infinite dilution.

Molecular interactions between surfactants and hydrophobic surfaces were used to develop an interaction model between surfactants and proteins. For this purpose, we have synthesized for the present work non-charged polymer particles, and investigated their interactions with a surfactant. This permitted to show that partial properties behave in these systems like partial properties associated to a particular binding type between a protein and a surfactant. Such similarity would confirm that, in both cases, the binding is of hydrophobic nature, co-operative and takes place on the surface of the particle or of the macromolecule neither involving specific sites nor protein unfolding.

1.1. Thermodynamics

In a three-component system, an extensive thermodynamic property such as the volume is expressed as $V=V(m_1, m_2, m_3)$, where m_1, m_2 and m_3 respectively represent the masses of the components 1, 2 and 3. Defining a fraction of the system [25] as a thermodynamic entity with an internal composition that groups several components, the volume of the system can be written as $V=V(m_1, m_F, t_{f3})$, where $m_F=m_2+m_3$ is the mass of the fraction F composed of components 2 and 3, and t_{f3} is the composition of the fraction ($t_{f3}=m_3/(m_2+m_3)$). With this variable change, the partial property of the fraction F in the presence of component 1 is [25]:

$$v_{F;1}(t_F, t_{f3}) = \left(\frac{\partial V(m_1, m_F, t_{f3})}{\partial m_F}\right)_{m_1, t_{f3}} = t_{f2} v_{2;1,3} + t_{f3} v_{3;1,2} \tag{1}$$

where t_F is the mass fraction of F in the system ($t_F=m_F/(m_1+m_2+m_3)$), $t_{f2}=1-t_{f3}$; and $v_{2;1,3}$ and $v_{3;1,2}$ are the specific partial volume of component 2 in the presence of components 1 and 3 and the specific partial volume of 3 in the presence of 1 and 2, respectively.

The limit at infinite dilution for fraction F is defined as [25]:

$$\lim_{\substack{t_F \to 0 \\ t_{f3} = t_{f3}^c}} v_{F;1}(t_F, t_{f3}) = v_{F;1}\left(0, t_{f3}^c\right) \equiv v_{F;1}^o\left(t_{f3}^c\right) \tag{2}$$

For components 2 and 3, the limits are defined as [25]:

$$\lim_{\substack{t_F \to 0 \\ t_{f3} = t_{f3}^c}} v_{2;1,3}(t_F, t_{f3}) = v_{2;1,3}\left(0, t_{f3}^c\right) \equiv v_{2;1,3}^{\Delta}\left(t_{f3}^c\right) \tag{3}$$

$$\lim_{\substack{t_F \to 0 \\ t_{f3} = t_{f3}^c}} v_{3;1,2}(t_F, t_{f3}) = v_{3;1,2}\left(0, t_{f3}^c\right) \equiv v_{3;1,2}^{\Delta}\left(t_{f3}^c\right). \tag{4}$$

The superindex "Δ" indicates the mathematical conditions under which the limit is considered (that is, t_F tends to zero while t_{f3} is kept constant). Taking the limits at infinite dilution in Eq. (1), and using the Eqs. (2)–(4) [25]:

$$v_{F;1}^o = t_{f2} v_{2;1,3}^{\Delta} + t_{f3} v_{3;1,2}^{\Delta}. \tag{5}$$

Appendix 1 demonstrates that the following equation holds at infinite dilution:

$$t_{f2} \frac{dv_{2;1,3}^{\Delta}}{dt_{f3}} + t_{f3} \frac{dv_{3;1,2}^{\Delta}}{dt_{f3}} = 0. \tag{6}$$

Eq. (6) cannot be obtained from the Gibbs–Duhem equation for a three-component system, for this reason it is referred to in this work as the Gibbs–Duhem type equation. Differentiating now with respect to t_{f3} in Eq. (5) and assuming that relation (6) holds:

$$\frac{dv_{F;1}^o}{dt_{f3}} = v_{3;1,2}^{\Delta} - v_{2;1,3}^{\Delta}. \tag{7}$$

Considering the system of two Eqs. (5) and (7) and solving it, yields:

$$v_{2;1,3}^{\Delta} = v_{F;1}^o - \frac{dv_{F;1}^o}{dt_{f3}} \times t_{f3} \tag{8}$$

$$v_{3;1,2}^{\Delta} = v_{F;1}^o + \frac{dv_{F;1}^o}{dt_{f3}} \times (1 - t_{f3}). \tag{9}$$

From Eqs. (8) and (9) it is possible to calculate the partial properties of 2 and 3 using experimental measurements of the specific partial property of the fraction F at infinite dilution and its derivative with respect to its composition.

The specific partial volume of component 2, and similarly for component 3, will be expressed as follows [24,26–29]:

$$v_{2;1,3}^{\Delta} \approx v_{2;1,3/M}^{\Delta} + \Delta v_{2;1,3/h}^{\Delta}, \tag{10}$$

where $v^{\Delta}_{2;1,3/\mathrm{M}}$ is the specific intrinsic volume of component 2 and $\Delta v^{\Delta}_{2;1,3/\mathrm{h}}$ the specific volume of solvation. The specific intrinsic volume corresponds to the solute domain into which the solvent molecules cannot penetrate. The specific volume of solvation is the solute-induced change in the solvent volume. The contribution $v^{\Delta}_{2;1,3/M}$ is positive, and the contribution $\Delta v^{\Delta}_{2;1,3/h}$ is negative. The specific intrinsic volume has two contributions:

$$v_{2;1,3/M}^{\Delta} \approx v_{2;1,3/c}^{\Delta} + v_{2;1,3/cav}^{\Delta}, \tag{11}$$

where $v^{\Delta}_{2;1,3/c}$ is the specific constitutive atomic volume and $v^{\Delta}_{2;1,3/cav}$ results from imperfect atomic packing. Defining the isothermal compressibility of the solvent as $\kappa_{T0}=-1/V\,(\partial V_1/\partial P)_T$ where V_1 is the volume of the solvent in pure state, the specific volume of solvation can be expressed as:

$$\Delta v_{2;1,3/h}^{\Delta} \approx v_{2;1,3/I}^{\Delta} + v_{2;1,3/T}^{\Delta} + \kappa_{T0} RT \tag{12}$$

where $v^{\Delta}_{2;1,3/I}$ is the interaction volume which is a consequence from the solvent contraction in the vicinity of charged and polar groups of the solute, $v^{\Delta}_{2;1,3/T}$ is the thermal volume which is a void volume around the solute that results from thermally activated mutual vibrations of contacting solute and solvent molecules, and $\kappa_{T0}RT$ is the ideal term that describes the volume effect related to the kinetic contribution to the pressure of a solute molecule due to the translational degrees of freedom, R being the gas constant and T the temperature.

In this work the coefficient of isothermal compressibility, K_T, is defined as:

$$K_T = -\left(\frac{\partial V}{\partial P}\right)_T \tag{13}$$

In this way, for example for the component 2, the specific partial coefficient of isothermal compressibility, $k_{T\,2;1,3,}$ is calculated as:

$$k_{T\,2;1,3} = \left(\frac{\partial K_T}{\partial m_2}\right)_{T,P,m_1,m_3}, \tag{14}$$

Subsisting Eq. (13) in Eq. (14), $k_{T\,2;1,3}$ can be obtained as:

$$k_{T\,2;1,3} = \frac{\partial^2 V}{\partial P \partial m_2} = \left(\frac{\partial v_{2;1,3}}{\partial P}\right)_T \tag{15}$$

The specific partial coefficient of isothermal compressibility of the component 2 at infinite dilution, $k^{\Delta}_{T\,2;1,3}$, can be interpreted substituting Eqs. (10) and (11) into Eq. (15) [30–32]:

$$k^{\Delta}_{T\,2;1,3} = \left(\frac{\partial v^{\Delta}_{2;1,3}}{\partial P}\right)_T \approx k^{\Delta}_{T\,2;1,3/cav} + \Delta k^{\Delta}_{T\,2;1,3/h} \tag{16}$$

where the effect of the pressure on $v^{\Delta}_{2;1,3/c}$ was neglected, $k^{\Delta}_{T\,2;1,3/cav} = (\partial v^{\Delta}_{2;1,3/cav}/\partial P)_T$ is the cavity contribution to $k^{\Delta}_{T\,2;1,3}$, and $\Delta k^{\Delta}_{T\,2;1,3/h} = (\partial \Delta v^{\Delta}_{2;1,3/h}/\partial P)_T$ is the hydration contribution. As in the case of $v^{\Delta}_{2;1,3/cav}$, the contribution $k^{\Delta}_{T\,2;1,3/cav}$ is positive, and the contribution $\Delta k^{\Delta}_{T\,2;1,3/h}$ is negative.

2. Materials and methods

2.1. Chemicals and solvents

Commercial grade methyl methacrylate (MMA) was provided by National Starch & Chemical, and was used as received. Reactive grade potassium persulfate (Aldrich) was employed as an initiator and was used without further purification for the synthesis of latex. Decyltrimethylammonium bromide, C_{10}-TAB (98% purity, Fluka) and Lysozyme, (hen egg, 95% purity, Sigma), were used as received. Solutions were prepared using double distilled water, which was degassed by boiling.

2.2. Latex synthesis

The Polymethyl methacrylate (PMMA) was prepared via emulsion polymerization without surfactant. The reaction was carried out in a semi-continuous reactor, consisting of a jacketed principal reactor and a feeding tank. A continuous pre-emulsion flow was ensured by a dosing pump. The reactor consisted of a 1 L stirred glass reactor under a dynamic flow of N_2 maintained at 75 °C by a thermal bath. The stirring rate was adjusted to 250 rpm. The final solid content of latex was 2.0 wt.% The formulation used to prepare 600 g of latex is shown in Table 1.

2.3. Measurements of density and sound speed

Measurements of density (ρ) and sound speed (u) were carried out using an Anton Paar DSA 5000 (Density and Sound Analyzer), and the working temperature was 303.150±0.005 K (30.000±0.005 °C).

2.4. Sample preparation

The first system studied in this work was composed of water, uncharged polymeric particles and surfactant and the other was composed of water, protein and surfactant. In this way, water was considered as component 1, uncharged polymeric particles or protein as component 2, and surfactant as component 3. The fraction F was considered to be composed of the components 2 and 3, and the variables t_F and t_{f3} are used as defined for Eq. (1). The mass fraction of water, t_1, is defined as $t_1 = 1 - t_F$. For each system, a series of measurements of the density and sound speed were carried out in such a way as to maintain a constant composition of fraction F (t_{f3}) while t_F was varied.

The experimental range of t_F concentrations was established so that the measured specific properties with respect to t_F could be described by Taylor's expansions of first order. This region wherein the measured properties as functions of t_F are linear is the so-called high dilution region [25]. In addition to this, the amount of C_{10}-TAB was chosen in order to obtain values of t_F below its critical micelle concentration [33] (66.1 mol m^{-3}). For the polymeric particles system, eight concentrations were selected at regular intervals from $t_F = 0$ to 0.0035 and for the protein system seven concentrations were chosen from $t_F = 0$ to 0.003, also at regular intervals. After this, all samples were heated at 303.1±0.1 K (30.0±0.1 °C) for 24 h in a water bath.

Table 1
Polymerization recipe

Components	Reactor (g)	Feeding tank (g)
Methyl methacrylate	0.0	12.0
Potassium persulfate	0.14	0.7
Distilled water	199.86	387.3

2.5. Calculation of partial properties

The specific volume v is calculated by:

$$v = \frac{1}{\rho} \tag{17}$$

The coefficient of adiabatic compressibility K_S is defined as:

$$K_S = -\left(\frac{\partial V}{\partial P}\right)_S \tag{18}$$

and the specific adiabatic compressibility coefficient k_S is:

$$k_S = \frac{K_S}{m} \tag{19}$$

where m is the total mass of the system. By definition the adiabatic compressibility κ_S is:

$$\kappa_S = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_S \tag{20}$$

and κ_S can be obtained experimentally by the Laplace equation ($\kappa_S = 1/(\rho u^2)$ where ρ is the density and u is the sound speed). Then substituting Eqs. (18) and (19) in Eq. (20) yields:

$$k_S = \left(\frac{1}{\rho u}\right)^2 \tag{21}$$

In this work it has been assumed that $K_T \approx K_S$, as consequence it is possible to demonstrate that there is not difference between the partial properties of K_T and K_S.

Fig. 1 shows an example of the specific volume and specific adiabatic compressibility coefficient of the system composed of polymeric particles, surfactant, and water as a function of the mass fraction of the water for several compositions of the fraction F (for clarity in the plot, some t_{f3} values were omitted). From these plots, the specific partial property at infinite dilution for the fraction ($j^o_{F;1}$) was calculated at each composition t_{f3} as the intercept at $t_1 = 0$ of the following fit function:

$$j = j^o_{F;1} + (j_1 - j^o_{F;1})t_1 \tag{22}$$

where j_1 is the specific property of 1 in its pure state, and j stands for v or k_S. In order to calculate the partial properties of components 2 and 3, Eqs. (8) and (9) were used. The values of the derivatives in Eqs. (8)

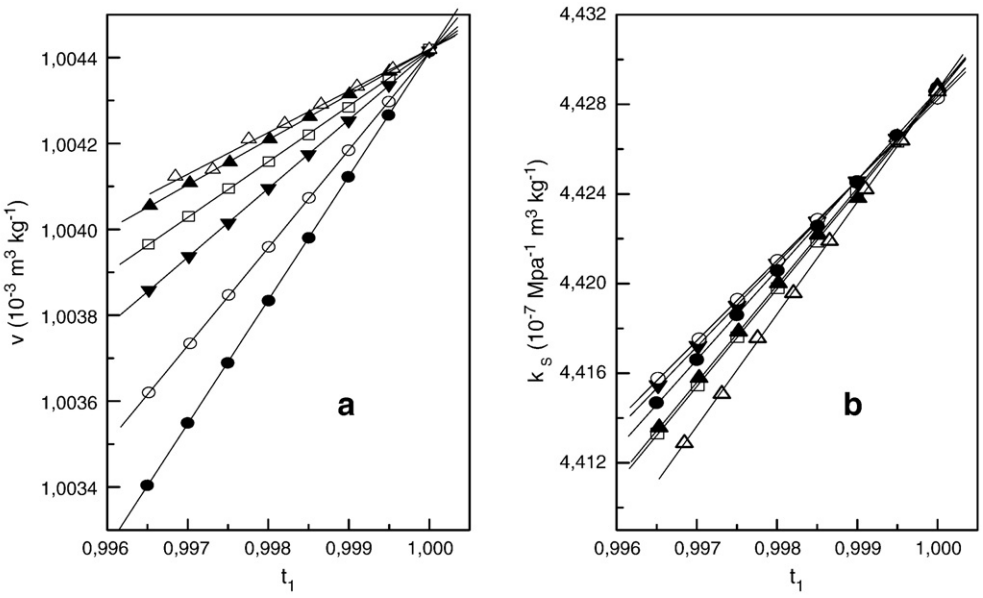

Fig. 1. (a and b). (a), Specific volume at 303.150 K (30.000 °C) of the system composed of uncharged polymeric particles, surfactant and water as a function of the mass fraction of water (t_1) at several ratios of surfactant and polymeric particles (t_{f3}=0.1040 ●, 0.3003 ○, 0.5999 ▼, 0.8002 □, 0.8998 ▲, 1.0000 △). (b), Specific adiabatic compressibility coefficient of the same system and temperature as a function of t_1. Symbols are defined a.

and (9) were estimated from polynomial or straight line fits of plots of $j^o_{F;1}$ against t_{f3}.

3. Results and discussion

In the investigated systems, the surfactant is adsorbed on polymeric particles or protein obeying a particular binding isotherm. In this way, surfactant can be considered as a fraction composed of adsorbed and free surfactant:

$$v^{\Delta}_{3;1,2}(t_{f3}) = t_{\text{ads}}(t_{f3})v^{\Delta}_{3a;1,2}(t_{f3}) + t_{\text{free}}(t_{f3})v^{o}_{3;1} \tag{23}$$

where $v^{\Delta}_{3a;1,2}$ is the specific partial volume of the surfactant adsorbed on the protein, $v^o_{3;1}$ the specific partial volume of the surfactant free in solution, and t_{ads} and t_{free} the relative amounts of the two species where $t_{\text{ads}}+t_{\text{free}}=1$. Substituting Eq. (23) in the Gibbs–Duhem type Eq. (6) yields:

$$t_{f2}\frac{dv^{\Delta}_{2;1,3}}{dt_{f3}} + t_{f3}\left(v^{\Delta}_{3a;1,2} - v^{o}_{3;1}\right)\frac{dt_{\text{ads}}}{dt_{f3}} + t_{f3}t_{\text{ads}}\frac{dv^{\Delta}_{3a;1,2}}{dt_{f3}} = 0 \tag{24}$$

The binding isotherm is a representation of the number of ligand molecules capable of binding to a single protein molecule. As stressed before, the shape of the binding isotherm depends on the binding type [2]. Information about the binding isotherm is introduced in Eq. (24) through the equality $t_{\text{ads}}=t_{\text{ads}}(t_{f3})$. Eq. (24) is thus essential since it correlates the behavior of partial properties to a particular binding type. As a consequence of this fact, the behavior of the partial properties can be employed in order to identify the binding type.

3.1. Surfactant and uncharged polymer particles

In Fig. 2 (a and b), $v^o_{F;1}$ and $k^o_{SF;1}$ are plotted as functions of the composition t_{f3} of the fraction *F*, which is composed of uncharged polymeric particles (component 2) and surfactant (component 3). In both cases, two different regions were obtained. The first region is characterized by an initial increase in the partial properties (region A), while the second (region B) shows a linear behavior. The transition from the first region to the second occurs around t_{f3}=0.45. If the specific partial property at infinite dilution of a fraction *F* is linear with respect to t_{f3} over the whole interval [0,1], then (Appendix 2) the components of this fraction are not interacting. When considering a fraction with constant composition as a "pseudo-component", it can be established (Appendix 3) that when the specific partial property of a fraction *F* is linear only in an interval $[t^c_{f3},1]$, then the linearity is due to the non-interaction between the pseudo-component (composed of component 2 and a part of component 3) and the rest of component 3.

The interaction between surfactants and uncharged polymeric particles is driven by a particular adsorption isotherm, where a part of the surfactant is adsorbed on the surface of the particles, and the other part remains in solution. In this configuration, one can suppose that, in region A, part of the surfactant molecules are adsorbed on the polymeric particles and that the dependence of the partial properties is not linear due to this interaction. The linear behavior of region B can be interpreted as a consequence of the saturation of the interaction in terms of the presence of a pseudo-component. This pseudo-component can only be the aggregate composed of polymeric particles with their surfaces fully occupied by surfactant molecules. Since no more surfactant molecules can reach the surface of the particles, saturation occurs. The linear dependence results from the non-interaction between aggregates and the other part of the surfactant which remains free in solution.

The specific partial properties of 2 and 3 at infinite dilution were calculated using Eqs. (8) and (9). In order to obtain the derivatives of $v^o_{F;1}$ and $k^o_{SF;1}$ with respect to t_{f3}, they were fitted to a fourth order polynomial in the region A and to a straight line in the region B. In Fig. 2 (c and d), $v^{\Delta}_{2;1,3}$ and $v^{\Delta}_{3;1,2}$, respectively, are shown as functions of the composition t_{f3} of the fraction *F*. The specific partial property of the polymeric particle ($v^{\Delta}_{2;1,3}$) increases until a limiting constant value. Because the polymeric particles were synthesized without charge, the interactions with the surfactant can only be hydrophobic. For this reason, the increase of $v^{\Delta}_{2;1,3}$ is interpreted as a loss of hydration due to the adhesion of surfactant molecules to the surface of the particles. When the surface of the particles is saturated by surfactant molecules, a constant value is expected since no more water molecules can be removed from the particles surface. This is experimentally observed because the specific partial volume of the particles becomes constant at saturation.

The specific partial volume of the surfactant ($v^{\Delta}_{3;1,2}$) decreases until a limiting constant value, which is the value of the specific partial volume of the free surfactant ($v^o_{3;1}$). The decrease in $v^{\Delta}_{3;1,2}$ can be explained in terms of Eq. (23). At low values of t_{f3}, the partial volume

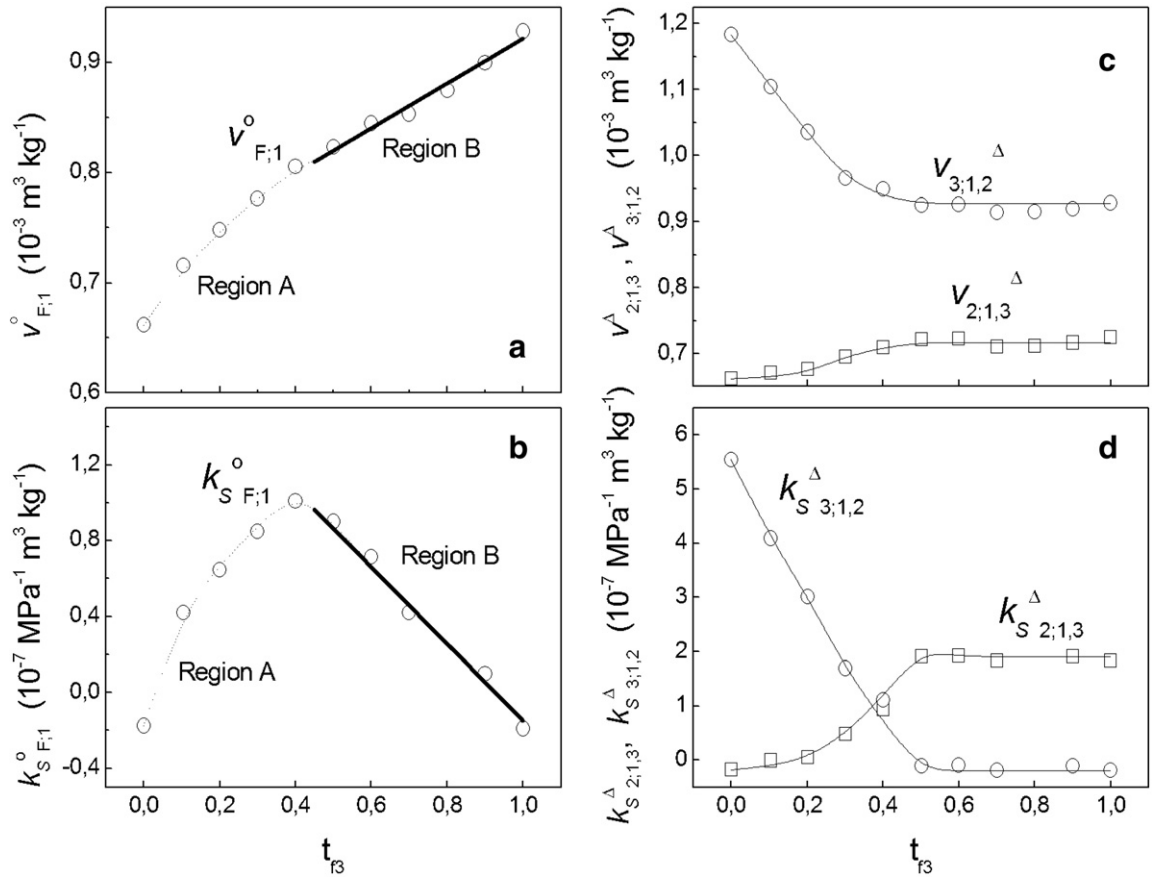

Fig. 2. (a, b, c and d). System at 30 °C composed of uncharged polymeric particles of PMMA (component 2), surfactant C_{10}-TAB (component 3) and water (component 1). (a), Specific partial volume at infinite dilution of the fraction *F* composed of polymeric particles and surfactant ($v^o_{F;1}$) as a function of the composition of the fraction (t_{f3}). The dotted line indicates the region A and the solid line indicates the region B where the interaction is saturated. (b), Specific partial adiabatic compressibility coefficient ($k^o_{S\,F;1}$) at infinite dilution of the fraction F as a function of the composition of the fraction. (c), Specific partial volumes of the non-charged polymer particles (□ component 2) and surfactant (○ component 3) as a function of t_{f3}. (d), Specific partial adiabatic compressibility coefficient of the uncharged polymeric particles and surfactant as a function of t_{f3} (symbols have the same meaning as in 2c).

of the adsorbed surfactant molecules ($v^{\Delta}_{3a;1,2}$) can be considered as constant. Differentiating Eq. (23) with respect to t_{f3} leads to:

$$\frac{dv^{\Delta}_{3;1.2}}{dt_{f3}} = -\left(v^{\Delta}_{3a;1.2} - v^{o}_{3;1}\right)\frac{dt_{\text{free}}}{dt_{f3}} \tag{25}$$

The term $v^{\Delta}_{3a;1,2} - v^{o}_{3;1}$ must be positive because the adsorbed surfactant is less hydrated than the free surfactant, accordingly as shown on Fig. 2c, $dv^{\Delta}_{3;1,2}/dt_{f3}$ is negative. In this way, dt_{free}/dt_{f3} must be positive, which means that the diminution of $v^{\Delta}_{3;1,2}$ can be interpreted as a consequence of the presence of free surfactant in solution which is more hydrated than the adsorbed surfactant.

Fig. 2d shows $k^{\Delta}_{S\ 2;1,3}$ and $k^{\Delta}_{S\ 3;1,2}$ as functions of the composition of the fraction *F*, and their behaviors are similar to that of volume.

3.2. Surfactant and protein

Fig. 3 (a and b) shows $v^o_{F;1}$ and $k^o_{S\ F;1}$, respectively, as functions of the composition of the fraction *F* which is composed of protein (component 2) and surfactant (component 3). Interestingly one observes that $v^o_{F;1}$ seems linear with respect to t_{f3} over the whole interval [0,1]. According to the mathematical development in Appendix 2, the protein and surfactant are not interacting. On the other hand, $k^o_{S\ F;1}$ is not linear, indicating some type of interaction between the protein and the surfactant. In previous work [34], the interaction between lysozyme and a strong denaturant (guanidinium chloride) was studied. In that case it was found that the specific partial volume of protein is independent of the concentration of guanidinium chloride, suggesting a non-interaction, while the specific adiabatic compressibility shows some type of dependence. As a general rule [24], in all cases of protein recognition, including folding and binding, measured changes in volume are very small and do not exceed 1 to 2% of the absolute value of the protein partial volume, and the sign of these changes can be either positive or negative. In addition, the small changes in partial volume accompanying the protein denaturation are not consistent with the expectation of large negative changes in volume based on the elimination of intraglobular voids and an increase in hydration of protein groups. In order to solve this inconsistency [35], a model [24] was proposed where the change in the partial volume is explained in terms of the intrinsic, thermal and hydration contributions. This model reveals that near zero values of the change in the partial volume of protein folding and binding reflect compensation between significant changes in the intrinsic, thermal and hydration contributions. Accordingly the "linearity" observed on Fig. 3a, is interpreted as a lack of precision in the estimation of the interaction effects on the specific partial volume of the fraction *F*.

Fig. 3b shows that $k^o_{S\ F;1}$ is non-linear from t_{f3}=0 until around 0.4 (region A), and linear from about 0.4 to 1 (region B). As in the case of the interaction between polymeric particles and surfactant, following the mathematical development of Appendix 3, the linearity in region B is due to the saturation of the interaction between the protein and the surfactant.

The specific partial compressibility coefficients of the protein and surfactant were calculated using Eqs. (8) and (9), and the values of $dk^o_{S\ F;1}/dt_{f3}$ were estimated from the derivation of a polynomial fit function in region A and from a linear fit function in region B. Fig. 3c and d shows the partial compressibilities of protein and surfactant, respectively, at infinite dilution.

From t_{f3}=0 to around 0.05, the partial adiabatic compressibility coefficient of the surfactant $k^{\Delta}_{S\ 3;1,2}$ is negative (Fig. 3d) and it is smaller

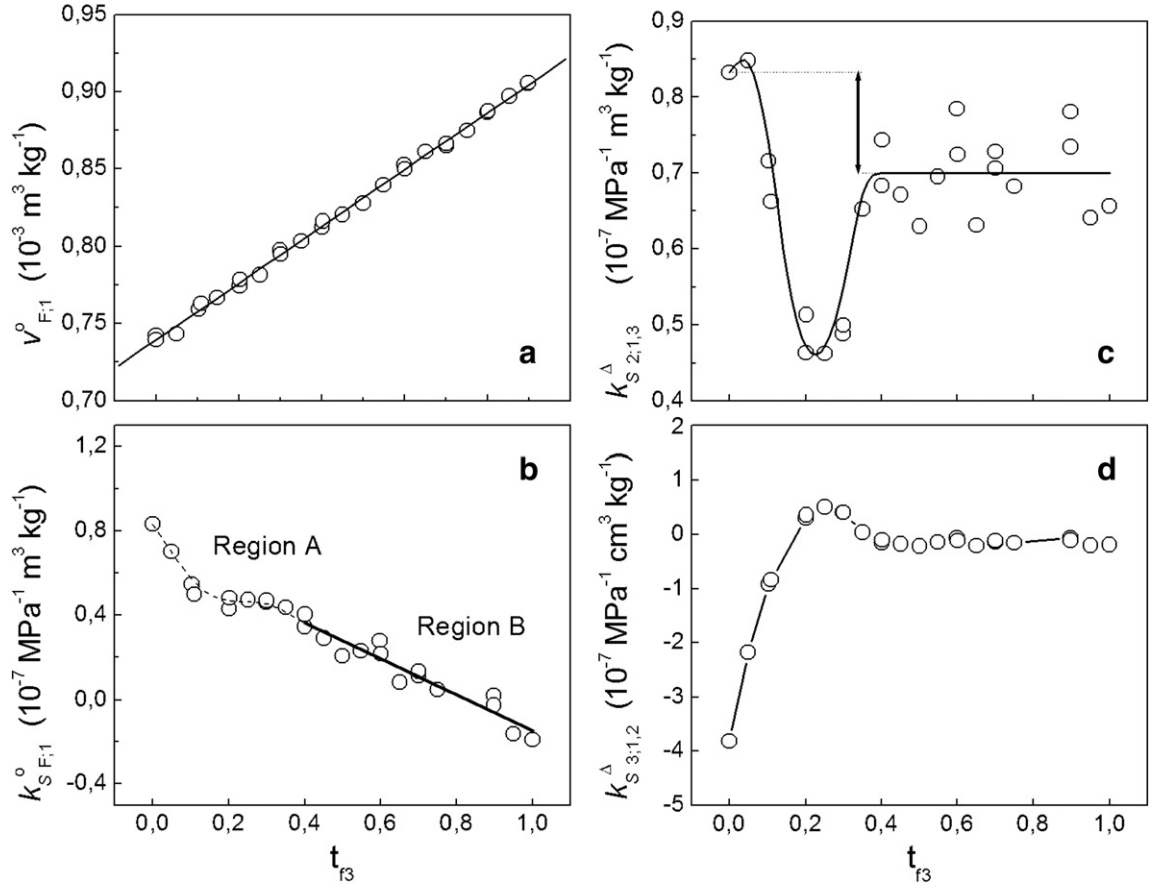

Fig. 3. (a, b, c, and d). System at 30 °C composed of water, protein (lysozyme), surfactant (C_{10}-TAB) and water (components 1, 2 and 3 respectively). (a), Specific partial volume at infinite dilution of the fraction *F* composed of protein and surfactant ($v^o_{F;1}$) as a function of the composition of the fraction. (b), Specific partial adiabatic compressibility coefficient ($k^o_{S\,F;1}$) at infinite dilution of the fraction *F*. The dotted line indicates the region A and the solid line indicates the region B where adsorption saturation occurs. (c), Specific partial adiabatic compressibility coefficient of the protein at infinite dilution (component 2) as a function of t_{f3}. (d), Specific partial adiabatic compressibility coefficient of the surfactant at infinite dilution (component 3) as a function of t_{f3}.

than when the surfactant is alone in water solution ($k^o_{S\,3;1}$). It is not possible then to explain this low value in partial adiabatic compressibility coefficient by a loss of hydration. In a previous work [36], where the denaturation in alkaline solution of lysozyme by C_n-TAB with n=8 to 16 was studied, it was found that the shortest surfactant, C_8-TAB, does not interact with lysozyme. This result supports the assumption that this specific binding which takes place at low concentrations of ligand, involves the surfactant alkyl chain in addition to the cationic head [37]. Since the specific compressibility coefficient is proportional to the mean square volume fluctuation [1], the decrease in specific partial adiabatic compressibility coefficient can be interpreted in terms of a loss of flexibility of the surfactant molecule due to the interaction with a binding site of the protein with high affinity. Using a similar equation to (25) for the specific partial adiabatic compressibility coefficient and similar arguments to those for interaction between polymeric particles and surfactant, it is possible to conclude that $k^{\Delta}_{S\,3;1,2}$ increases in this region due to the presence of free surfactant in solution. In this region the binding isotherm increases v0ery slowly with the surfactant concentration [2]. For this reason our finding is in agreement with the shape of the binding isotherm.

In this domain of concentration a slight increase in the specific partial adiabatic compressibility coefficient of the protein is observed (Fig. 3c). According to the Gibbs–Duhem type Eq. (6), if $k^{\Delta}_{S\,3;1,2}$ increases then $k^{\Delta}_{S\,2;1,3}$ must decrease. It might then be possible that the slight increase in the adiabatic compressibility coefficient of the protein, which should decrease, is within the overall experimental error.

Accordingly, one can conclude that, initially, at $t_{f3}=0$, $k^{\Delta}_{S\,3;1,2}$ is smaller than the specific partial adiabatic compressibility when the surfactant is alone in solution ($k^o_{S\,3;1}$). This fact can be interpreted as the effect of a specific interaction with high affinity and strength. As consequence the specific partial adiabatic compressibility coefficient of the protein slightly decreases due to a loss in the cavity terms and/or to a slight gain in hydration.

In the following region of compositions (from around t_{f3}=0.05 to 0.2), $k^{\Delta}_{S\,2;1,3}$ drops after a sharp transition. This decrease in the specific partial adiabatic compressibility coefficient can be caused by a decrease of the cavity term and/or a gain of hydration. It is not possible to consider the second effect by itself, because it would be first necessary to increase the surface of contact with the solvent, and this could only be possible through a conformational transition that would change the cavity term. This means that both factors must contribute simultaneously: the conformational change caused by the protein unfolding with a reduction of the cavity term and a gain of solvation caused by buried groups in the native state now exposed to the solvent by protein unfolding [4]. Fig. 3d shows the change in the behavior of surfactant in this composition region. It is interesting to observe that $k^{\Delta}_{S\,3;1,2}$ progresses from a negative to a positive value, and that its slope is smaller than that of the region above. Most likely this is possible, if another type of binding occurs in the system.

In this case, it is possible to conclude that the decrease of the compressibility coefficient of the macromolecule while that of the ligand increases, is characteristic of a second binding type which involves the unfolding of the protein.

From around t_{f3}=0.2 to around t_{f3}=0.4, the specific partial adiabatic compressibility coefficient of the protein levels off until a limiting constant value, while in a mirror image the specific partial adiabatic compressibility coefficient of the surfactant decreases from a positive value to a limiting constant value. This pattern of behavior is

similar to that of the interaction between the same surfactant with uncharged polymeric particles (Fig. 2d). This indicates that the protein unfolding process is completed and that the binding is hydrophobic in origin, involving only the surface of the particle. It was observed that the variation of specific partial compressibility coefficient between the native and unfolded states is $-0.13\times10^{-7}\pm0.05\times10^{-7}$ MPa m^3 kg^{-1}.

In this case, it is possible to conclude that the increase towards a limiting value of the compressibility coefficient of the macromolecule while the compressibility coefficient of the ligand decreases down to a constant value, is characteristic of a third binding type which is hydrophobic and where the ligand covers the surface of the macromolecule.

4. Conclusions

In the investigated systems, the contributions to $v^o_{F;1}$ and $k^o_{S\,F;1}$ are consequence of the balance between the "free" surfactant in the solution, and the "interacting" surfactant involved in clusters with either polymeric particles or protein. Because the specific partial properties are at infinite dilution, they do not show contributions due to the interactions between clusters, between the surfactant molecules free in solution or between clusters and free surfactant molecules.

The derivatives of $v^{\Delta}_{2;1,3}$ and $v^{\Delta}_{3;1,2}$ with respect to t_{f3} in the Gibbs–Duhem type Eq. (6) are mathematically coupled, indicating that the behavior of one affects that of the other. The behavior of component 1 is not affected by the interaction between 2 and 3 because the term $dv^{\Delta}_{1;2,3}/dt_{f3}$ does not appear in the Gibbs–Duhem type Eq. (6). However, the presence of 1 affects the behavior of 2 and 3. Since the interaction occurs at infinite dilution, component 1 is in its pure state. Accordingly, the Gibbs–Duhem type Eq. (6) translates the scenario in which components 2 and 3 are interacting together while being immersed in pure component 1 which acts as a solution medium.

Equation (24) relates the partial properties of surfactant and protein (or polymeric particles) to the binding isotherm. Three types of binding were found from the behavior of the partial properties. The first binding type is characterized by a high affinity and is related to a slight dehydration of the protein and a large reduction of the specific partial adiabatic compressibility coefficient of the surfactant. The second binding type is related to a drop of the specific partial adiabatic compressibility coefficient of the protein. The transition between the first and second binding types is sharp. The last binding type was found to be qualitatively identical to that of the interaction between uncharged polymeric particles and surfactant.

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Appendix 1

In this appendix, Eq. (6) will be demonstrated. By partial derivation of Eq. (1) with respect to t_{f3}:

$$\left(\frac{\partial v_{F;1}}{\partial t_{f3}}\right)_{t_F} = \left(v_{3;1,2}-v_{2;1,3}\right) + t_{f2}\left(\frac{\partial v_{2;1,3}}{\partial t_{f3}}\right)_{t_F} + t_{f3}\left(\frac{\partial v_{3;1,2}}{\partial t_{f3}}\right)_{t_F} \tag{26}$$

On the other hand, because $V=V(m_1, m_F, t_{f3})$ and using Eq. (1):

$$\left(\frac{\partial v_{F;1}}{\partial t_{f3}}\right)_{t_F} = \frac{\partial}{\partial t_{f3}}\left\{\left(\frac{\partial V}{\partial m_F}\right)_{m_1,t_{f3}}\right\}_{m_1,m_F} = \frac{\partial}{\partial m_F}\left\{\left(\frac{\partial V}{\partial t_{f3}}\right)_{m_1,m_F}\right\}_{m_1,t_{f3}} \tag{27}$$

In a previous work, it was demonstrated that:

$$\left(\frac{\partial V}{\partial t_{f3}}\right)_{m_1,m_F} = \left(v_{3;1,2}-v_{2;1,3}\right)m_F \tag{28}$$

Substituting Eqs. (28) in Eq. (27) and deriving with respect to m_F:

$$\begin{aligned}\left(\frac{\partial v_{F;1}}{\partial t_{f3}}\right)_{t_F} &= \left(v_{3;1,2}-v_{2;1,3}\right) + m_F\left(\frac{\partial\left(v_{3;1,2}-v_{2;1,3}\right)}{\partial m_F}\right)_{m_1,t_{f3}} \\ &= \left(v_{3;1,2}-v_{2;1,3}\right) + t_F(1-t_F)\left(\frac{\partial\left(v_{3;1,2}-v_{2;1,3}\right)}{\partial t_F}\right)_{t_{f3}}\end{aligned} \tag{29}$$

In this way, we set (26) equal to (29):

$$t_{f2}\left(\frac{\partial v_{2;1,3}}{\partial t_{f3}}\right)_{t_F} + t_{f3}\left(\frac{\partial v_{3;1,2}}{\partial t_{f3}}\right)_{t_F} = t_F(1-t_F)\left(\frac{\partial\left(v_{3;1,2}-v_{2;1,3}\right)}{\partial t_F}\right)_{t_{f3}}. \tag{30}$$

Taking the limits on the left hand side of Eq. (30):

$$\begin{aligned}&\lim_{t_F\to0}\left\{t'_{f2}\left(\frac{\partial v_{2;1,3}\left(t_F,t'_{f3}\right)}{\partial t'_{f3}}\right)_{t_F} + t'_{f3}\left(\frac{\partial v_{3;1,2}\left(t_F,t'_{f3}\right)}{\partial t'_{f3}}\right)_{t_F}\right\} = \\ &t'_{f3}=t_{f3} \\ &\qquad = t_{f2}\left(\frac{\partial v^{\Delta}_{2;1,3}}{\partial t_{f3}}\right)_{t_F=0} + t_{f3}\left(\frac{\partial v^{\Delta}_{3;1,2}}{\partial t_{f3}}\right)_{t_F=0}.\end{aligned} \tag{31}$$

In order to take the same limit on the right-hand side of Eq. (30), it is necessary to bear in mind that:

$$\begin{aligned}&\lim_{\substack{t_F\to0\\ t'_{f3}=t_{f3}}}\left(\frac{\partial\left(v_{3;1,2}\left(t_F,t'_{f3}\right)-v_{2;1,3}\left(t_F,t'_{f3}\right)\right)}{\partial t_F}\right)_{t'_{f3}} = \\ &\qquad = \left(\frac{\partial v_{3;1,2}\left(0,t_{f3}\right)}{\partial t_F}\right)_{t_{f3}} - \left(\frac{\partial v_{2;1,3}\left(0,t_{f3}\right)}{\partial t_F}\right)_{t_{f3}} = f\left(t_{f3}\right)\end{aligned} \tag{32}$$

In Eq. (32), $v_{2;1,3}(0,t_{f3})$ and $v_{3;1,2}(0,t_{f3})$ are well-defined (see Eqs. (3) and (4)), and $(\partial v_{2;1,3}(0,t_{f3})/\partial t_F)$ and $(\partial v_{3;1,2}(0,t_{f3})/\partial t_F)$ physically represent the evolution of $v_{2;1,3}(t_F,t_{f3})$ and $v_{3;1,2}(t_F,t_{f3})$ with respect to the amount of fraction F at $t_F=0$. The result of Eq. (32) is a function that only depends on the composition of the fraction F. In this way, when the type II limit is taken for the right-hand side of Eq. (30):

$$\lim_{\substack{t_F\to0\\ t'_{f3}=t_{f3}}} t_F(1-t_F)f\left(t'_{f3}\right) = 0 \tag{33}$$

and then, setting Eq. (31) equal to Eq. (33) yields Eq. (6).

Appendix 2

In this appendix the following theorem will be demonstrated: "the plot of $v^o_{F;1}$ against t_{f3} is linear over the interval [0,1] if and only if 2 and 3 are not interacting."

"⇐") if 2 and 3 are not interacting, $v^{\Delta}_{2;1,3}$ and $v^{\Delta}_{3;1,2}$ can be written as $v^o_{2;1}$ and $v^o_{3;1}$. That is, 2 is only in the presence of 1, and in the same way 3 is only in the presence of 1; then, $v^o_{F;1}$ takes the linear form $v^o_{F;1}=v^o_{2;1}+(v^o_{2;1}-v^o_{3;1})\,t_{f3}$.

"⇒") Differentiating Eq. (5) and bearing in mind that $v^o_{F;1}$ is linear, we determine that $dv^o_{F;1}/dt_{f3}=v^{\Delta}_{3;1,2}-v^{\Delta}_{2;1,3}$ is constant. Differentiating again and combining the result with the Gibbs–Duhem type Eq. (6), we obtain the solution $(dv^{\Delta}_{2;1,3}/dt_{f3})=(dv^{\Delta}_{3;1,2}/dt_{f3})=0$. That is, $v^{\Delta}_{2;1,3}$ is constant, and then $v^{\Delta}_{2;1,3}(t_{f3})=v^{\Delta}_{2;1,3}(0)=v^o_{2;1}$. In the same way, $v^{\Delta}_{3;1,2}$ is equal to $v^o_{3;1}$.

Appendix 3

Let A be the region where $v^o_{F;1}$ is not linear and B be the region where $v^o_{F;1}$ is linear. The non-linear part of $v^o_{F;1}$ will be represented by $v^o_{F;1/A}$, and the linear part will be represented by $v^o_{F;1/B}$. Between

these two behaviors, the composition t_{f3}^c is defined as $v^o_{F;1/A}(t_{f3}^c) = v^o_{F;1/B}(t_{f3}^c)$, where $v^o_{F;1}(t_{f3}^c) = v^{\Delta}_{2;1,3}(t_{f3}^c)\, t_{f2}^c + v^{\Delta}_{3;1,2}(t_{f3}^c)\, t_{f3}^c$. In region 1:

$$\lim_{t_{f3}\to t_{f3}^c} v^o_{F;1/B}\left(t_{f3}^c\right) = v^o_{F;1}\left(t_{f3}^c\right) \tag{34}$$

In addition, in a previous work [25] it was demonstrated that:

$$\lim_{t_{f3}\to 1} v^o_{F;1/B} = \lim_{t_{f3}\to 1} v^o_{F;1} = v^o_{3;1} \tag{35}$$

With this, in the linear region (region B) it is possible to consider the change in variable $T_{f3} = (t_{f3} - t_{f3}^c)/t_{f2}^c$ with $T_{f2} = 1 - T_{f3}$. In this way, it is possible to describe the behavior of $v^o_{F;1/B}$ as the straight line $v^o_{F;1/B}(T_{f3}) = v^o_{F;1}(t_{f3}^c) T_{f2} + v^o_{3;1}\, T_{f3}$ when T_{f3} goes from 0 to 1. It is now possible to interpret $v^o_{F;1}(t_{f3}^c)$ as the specific partial volume of a pseudo-component because its composition is constant in region B and to consider that the total amount of 3 is composed of two types of 3. The first type of 3 forms part of the pseudo-component, and the other type does not form part of the pseudo-component. Because $v^o_{F;1/B}$ is linear over the interval [0,1], by the above theorem (Appendix 2), the pseudo-component does not interact with the second type of 3.

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