EFFECT OF H_20-CO_2 ORGANIZATION ON OVALBUMIN ADSORPTION AT THE SUPERCRITICAL CO_2-WATER INTERFACE

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ABSTRACT

We have previously investigated the formation of water-CO_2 interface by means of a drop tensiometer [1]. In presence of CO_2 , the interaction between the two adjacent phases leads to an organization of interfacial cluster network which controls the tension and the mechanical properties of the interface.

The formation of those clusters at CO_2 pressure lower than 80 bar inhibits the adsorption of protein (ovalbumin (OVA)) dissolved in water at low concentration (0.002 g/L) whereas higher concentration (0.047 g/L) led to a protein film. We noticed a peculiar behavior at 90 bar where penetration of protein into the interface modified the kinetics and elasticity of the interface whatever the protein concentration.

1. INTRODUCTION

It is known that the quality of surfactant layers plays an important role in emulsion stability, mainly when electrical and/or sterical repulsion between droplets is difficult to obtain as it is the case for H_2O/CO_2 emulsions. On the other hand, very few studies concern the adsorption of macromolecular surfactants like proteins at H_2O-CO_2 interface.

We have previously investigated the formation of water-CO_2 interface by means of a drop tensiometer [1]. We can describe it as a two step phenomena. Firstly, the CO_2 molecules quickly adsorb onto the water surface for equilibrating their chemical potential between the bulk CO_2 and the water surface. This physisorption leads to the interaction of one CO_2 molecule with one H_2O molecule. From the calculated CO_2 molecular area at saturation and the thermodynamical parameters, we have found that this H_20-CO_2 complex would be of H type, as it was found by Da Rocha et al.[2] by Monte Carlos simulation. The amount of adsorbed CO_2 and the relative orientation of water-CO_2 molecules in the H type complex depend on temperature and pressure.

Secondly, after this adsorption, CO_2 molecules diffuse into the water subsurface and then modify the water organization. This reorganization of the interface creates a network H_20-CO_2

solid clusters and leads to a decrease of the interfacial tension (γ) until an equilibrium state. These H_20-CO_2 clusters are formed at temperature upper (20 – 40°C) to the one usually described as the limit (10°C) for the so-called clathrate hydrate formation.

Elasticity measurements and macroscopic visualization suggest that the growing of clusters is driven by assembly of many small blocks and accelerates with the CO_2 pressure (Figure 1). Their interfacial concentration and their size increase with time until the saturation of the interface.

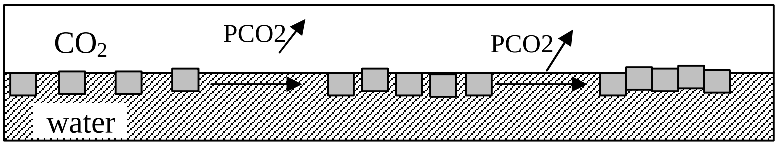

Figure 1: Schematic representation of the interfacial organization of H_2O-CO_2 clusters upon the effect of pCO_2

This organization of the pure H_2O-CO_2 interface raises an important question. How does the interface react in presence of usual or polymeric surfactant molecules like protein? To answer this question, we have studied by means of tensiometry measurements the H_2O-CO_2 interface at 40°C, in presence of OVA, and for various CO_2 pressures.

2. Materials and Methods

Materials. The vessel was filled with CO_2 (purity 0.99) (Air liquide, France). Ultrapure water was produced by MilliQ plus 188 apparatus (Millipore$^{®}$ St Quentin en Yveline, France) and then saturated 12 hours with pure CO_2. Ovalbumin of grade V was purchased by SIGMA, Germany.

Pendant Drop Tensiometer. The drop tensiometer (Tracker, IT Concept, Longessaigne, France) allows the determination of the interfacial tension by analyzing the axial symmetric shape (Laplacian profile) of the pendant drop of CO_2 saturated water in pressurized CO_2. The apparatus has been described in a previous study [1]. Pendant drops were formed at the end of a stainless steel tube, 1 mm inside diameter, connected to a syringe. Drop volume and area were controlled during all experiment by a step by step motor. Therefore, it was possible to maintain constant the area of the drop during the time of experience, so that the surface tension variation was related only to the interfacial phenomenon.

The interfacial tension was determined by digitizing and analyzing the profile of a droplet using a CCD camera coupled to a video image profile digitizer board connects to a computer. The drop profile was processed according to the fundamental Laplace equation (Eq 1) applied to the drop profile:

$$\frac{1}{x}\frac{d}{dx}\left(x\sin\Theta\right)=\frac{2}{b}-cz \quad \textbf{(Eq: 1)}$$

Where x and z are the Cartesian coordinates at any point of the drop profile. b is the radius of the curvature of the drop apex.q is the angle of the tangent to the drop profile. In addition, c is the capillarity constant, equal to "(gρ)/γ", where ρ is the difference between the densities of the two liquids and g is the acceleration due to gravity. Five times per second, the computer calculates the characteristic parameters of the drop (area, volume and interfacial tension).

Elasticity measurement. The common idea of all elasticity measurements is to apply a controlled perturbation to the surface in order to follow simultaneously the related surface pressure variations. We have measured the dynamical response of a surface film (interfacial tension) when a dilational mechanical strain (drop compression) was applied. A slow compression $\frac{1}{Ai}\frac{d(\Delta A_t)}{dt}$, typically lower than 0.003 s^{-1} was performed in order to reach a total relative strain ($\Delta A(t)/Ai$) of 40-50% of the initial area. From the variation of interfacial tension ($d\gamma$) versus relative strain, we calculated the interfacial dynamic elasticity as follow.

$$E_a = -Ai\frac{dg}{dA} \qquad \textbf{(Eq: 2)}$$

Where *Ai* is the initial drop area and dA is the deformation.

This dynamic elasticity which is assimilated as an apparent elasticity (*Ea*), involves the contribution of the elastic properties of the monolayer. It is linked to the number of intermolecular interaction in the interface.

3. Results and Discussion

1. Kinetics

We have measured the adsorption kinetics of OVA (0.002 g/L) at the H_2O-CO_2 interface at 40°C, and at several CO_2 pressures (50 – 90 bar).

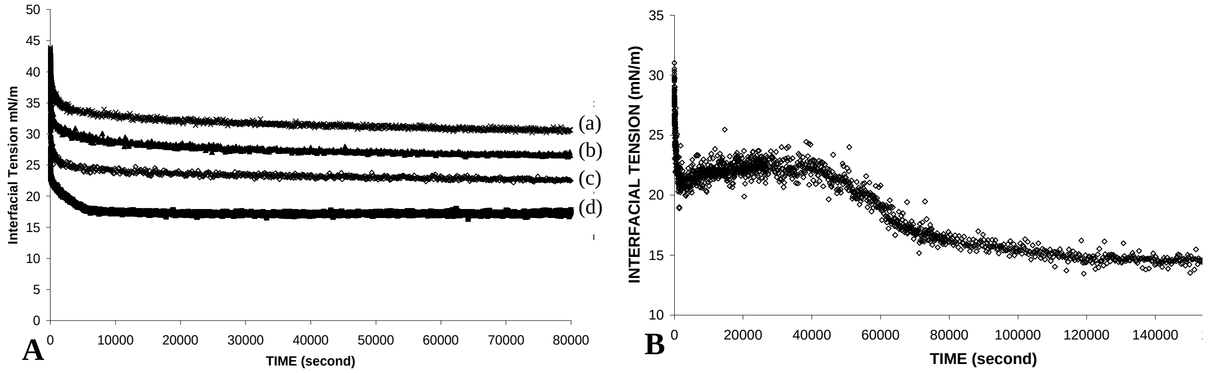

Figure 2: Kinetics of OVA adsorption for a concentration of 0.002 g/L at 40°C and for A) pCO_2 was: 50 bar (a); 60 bar (b); 70 bar (c); and 80 bar (d) B) pCO_2 was 90 bar.

For the lowest pCO_2 (Figure 2-A), the evolution of γ in presence of OVA resembles the kinetics we obtained previously for pure CO_2-H_2O interface [1]. However, for 90 bar, the shape of the kinetics differs from those obtained at pure interface except for the initial rapid decrease of γ, which is attributed to the H_2O-CO_2 organization. In particuliar, we can notice the presence of a plateau during about 40 000 sec, with γ higher than the equilibrium value obtained for the pure interface. This plateau is followed by a decrease of γ, what indicates a new organiztion of the interface.

For dilute protein solutions, the “classical” shape of kinetics at fluid interfaces is generally described as the resulting of phenomenon modeled with three characteristics times[3-6]. The first time is attributed to the protein adsorption controlled by the diffusion from the bulk. The two next steps are generally related to the protein film reorganization via molecular unfolding and intermolecular interaction. In our case, until 80 bar, the superimposition of the kinetics in presence of OVA with those obtain at pure CO_2-H_2O interface suggests the possibility of inhibition of OVA adsorption by the concomitant formation of a network composed of H_2O-CO_2 clusters.

For 90 bar, the peculiar shape of the kinetic could be due to the contribution of both the CO_2 hydrate formation and the OVA adsorption on the interfacial tension. This could indicate some interaction between OVA and H_2O-CO_2 clusters.

It should be mentioned (results not shown) that equilibrium values of γ in presence of OVA at higher concentration (0.0477 g/L) was systematically lower than those obtained for the pure CO_2-H_2O interface, whatever the pCO_2. This indicates the formation of an OVA interfacial film in these situations.

2. *Kinetics*

In order to precise the interfacial organization in the different conditions, we performed interfacial elasticity measurement at the different pCO_2 (Figure 3).

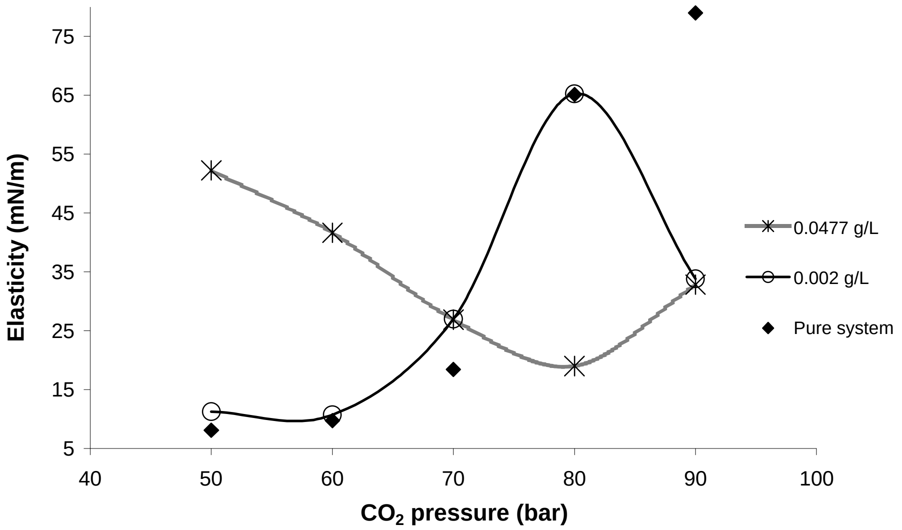

Figure 3: Apparent elasticity versus pCO_2

For an OVA concentration equal to 0.0477 g/L, Ea values differs from those of the pure interface, and decrease with pCO_2 until 80 bar. These results indicate the formation of an OVA interfacial film, for which the organization depends on the pCO_2.

For an OVA concentration equal to 0.002 g/L, Ea increases with the pCO_2 until 80 bar, like in the case of the pure H_2O-CO_2 interface [1]. This confirms that for this concentration, the interface is composed of H_2O-CO_2 clusters, inhibiting the OVA adsorption. At 90 bar in presence of OVA, Ea decreases contrarily to the pure H_2O-CO_2 interface for which an increase of Ea was still measured at this pressure. Since elasticity is related to the cohesiveness of the interfacial film, this result could be interpreted by the penetration of the OVA inside the H_2O-CO_2 network which reduces the interaction between the H_2O-CO_2 cluster blocks. Several hypotesis can be made concerning the origin and consequences of this phenomenon which appears pCO_2 dependant. Firstly, a change in the protein conformation may occur at highest pCO_2 because of the pressure and of the pH decrease of the aqueous solution [7]. This new conformation would favor adsorption. Another explanation could be linked to the increase of the density of the CO_2 which in turn could increase the affinity of the CO_2 with specific protein segments (CO_2-phile segments). Consequently, after 90 bar, the question remain wether the interface is composed of a mixture of H_2O-CO_2 clusters and OVA or just of modified OVA. Experiments are in course to answer those important questions. In particuliar we carry out some IR-ATR and IR RAMAN microscopy on protein sample treated at different pressures.

4. Conclusion

Tensiometry and elasticity measurements show that OVA adsorption is inhibited by H_2O-CO_2 cluster formation for a bulk concentrations of 0.002 g/L for pCO_2 comprised between 50 and 80 bar. For higher concentration, and in these ranges of pCO_2, OVA is able to adsorb to form an interfacial film with an organization pCO_2 dependent.

Moreover, for a pCO_2 of 90 bar, and for all OVA concentration, we obtained peculiar effects probably related to the formation of mixed films made of protein and cluster.

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