EFFECT OF H$_2$O-CO$_2$ ORGANIZATION ON OVALBUMIN ADSORPTION AT THE SUPERCRITICAL CO$_2$-WATER INTERFACE

Frederic Tewes and Frank Boury*
Ingénierie de la Vectorisation Particulaire, INSERM unité 646, Bat. IBT, 10 rue A. Boquel, 49100 Angers, France.

Alain Cagna
IT Concept, Parc de chancolan 69770 Longessaigne
E-mail: boury@ibt.univ-angers.fr
Phone number: +332 41 73 58 48
Fax number: +332 41 73 58 03

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$_2$O/CO$_2$ emulsions. On the other hand, very few studies concern the adsorption of macromolecular surfactants like proteins at H$_2$O-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$_2$O molecule. From the calculated CO$_2$ molecular area at saturation and the thermodynamical parameters, we have found that this H$_2$O-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$_2$O-CO$_2$
solid clusters and leads to a decrease of the interfacial tension ($\gamma$) until an equilibrium state. These H$_2$O-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$_2$O-CO$_2$ clusters upon the effect of pCO$_2$](image)

This organization of the pure H$_2$O-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$_2$O-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:

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

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, $\Theta$ is the angle of the tangent to the drop profile. In addition, $c$ is the capillarity constant, equal to “($g \rho$)/$\gamma$”, where $\rho$ 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 $\frac{1}{A_i} \frac{d(\Delta A_i)}{dt}$, typically lower than 0.003 s$^{-1}$ was performed in order to reach a total relative strain ($\Delta A(t)/A_i$) 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.

$$E_a = -A_i \frac{d\gamma}{dA} \quad (Eq: 2)$$

Where $A_i$ is the initial drop area and $dA$ is the deformation.

This dynamic elasticity which is assimilated as an apparent elasticity ($E_a$), 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$_2$O-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.](image)

For the lowest pCO$_2$ (Figure 2-A), the evolution of $\gamma$ in presence of OVA resembles the kinetics we obtained previously for pure CO$_2$-H$_2$O 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 $\gamma$, which is attributed to the H$_2$O-CO$_2$ organization. In particular, we can notice the presence of a plateau during about 40 000 sec, with $\gamma$ higher than the equilibrium value obtained for the pure
interface. This plateau is followed by a decrease of $\gamma$, what indicates a new organization 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\(_2\)O interface suggests the possibility of inhibition of OVA adsorption by the concomitant formation of a network composed of H\(_2\)O-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\(_2\)O-CO\(_2\) clusters.

It should be mentioned (results not shown) that equilibrium values of $\gamma$ in presence of OVA at higher concentration (0.0477 g/L) was systematically lower than those obtained for the pure CO\(_2\)-H\(_2\)O 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\(_2\)](image)

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 pCO₂ until 80 bar, like in the case of the pure H₂O-CO₂ interface [1]. This confirms that for this concentration, the interface is composed of H₂O-CO₂ clusters, inhibiting the OVA adsorption. At 90 bar in presence of OVA, Ea decreases contrarily to the pure H₂O-CO₂ 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 H₂O-CO₂ network which reduces the interaction between the H₂O-CO₂ cluster blocks. Several hypotesis can be made concerning the origin and consequences of this phenomenon which appears pCO₂ dependant. Firstly, a change in the protein conformation may occur at highest pCO₂ 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 CO₂ which in turn could increase the affinity of the CO₂ with specific protein segments (CO₂-phile segments). Consequently, after 90 bar, the question remain wether the interface is composed of a mixture of H₂O-CO₂ clusters and OVA or just of modified OVA. Experiments are in course to answer those important questions. In particular 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 H₂O-CO₂ cluster formation for a bulk concentrations of 0.002 g/L for pCO₂ comprised between 50 and 80 bar. For higher concentration, and in these ranges of pCO₂, OVA is able to adsorb to form an interfacial film with an organization pCO₂ dependent.

Moreover, for a pCO₂ 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.