Hydrodynamics of Rising Air Bubbles in Mixture of Proteins with Non-Ionic Surfactants to Declare their Interaction at the Air-Water Interface

Abstract

Evaluation of behavior of adsorbed layers of protein-surfactant mixture at the air-water interfaces have recently received great attention due to their wide applications in food and pharmaceutical industries. In this research, qualitative study of surface activity of two kinds of proteins, Beta-Lactoglobulin (BLG) and Beta-Casein (BCS), non-ionic surfactant of C_{10}DMPO, and the mixtures of them in different concentrations at the air-water interface is investigated using rising bubble method. The similarity between measured local velocity profiles can be due to the fact that all mentioned materials are surface-active one and could create the Marangoni effect and develop a dynamic adsorption layer. As there is no significant interaction between the protein and surfactant molecules, stable complex structures cannot be formed in the protein-C10DMPO mixture. The mixture velocity profile is more similar to that of the surfactant which is a result of replacement of protein molecules or complexes with free surfactant at the bubble interface. It is found that the mixture of non-ionic surfactant with BCS has a bit synergetic effect while for its mixture with BLG, a negative synergy is observed which is resulted from the shape of protein.

Keywords: dynamic of adsorption, rising bubble, bubble velocity profile, protein, ionic and non-ionic surfactant

1 Introduction

Proteins and surfactant mixtures are extensively a point of interest in recent works because of their use in different industries such as food processing, pharmaceuticals, etc. [1-3]. The interfacial characteristics of these mixtures play an important role in creation and stabilization of foams [4] and emulsions. In another word, the change of interfacial tension, the rheological performance of the interface, and the dynamic of adsorption, can be significantly different in comparison to those of the individual components [5-8]. It must be noted that the interaction among proteins which exist at liquid bulk volume or interfaces depends on their folded polypeptide chains structure [2]. While absorption of protein-surfactant mixture has got a great attention, even basic aspects of adsorbed protein at air/water interface are yet to be cleared for academia and industry.

The adsorption of surfactants and proteins at the interface, will decrease the interfacial tension. Whereas surfactants with low molecular weight cause the phenomena of foam/emulsion creation due to rapid adsorption at the interface. Those with higher molecular weights make a stable foam in extended time span, due to generating interfacial networks with elastically and electrically charged properties [9, 10]. Various techniques including equilibrium and dynamic measurements of surface tension, were utilized to evaluate the adsorbed proteins’ layers in mixtures with surfactants [1, 11, 12]. The interfacial behaviour of proteins and surfactants mixtures have been discussed extensively in literature [12-18].

Kragel et al [19] have investigated BLG/Tween 20 adsorption at air/water interface and noticed that at short time of adsorption, interface contains significant amount of BLG. Proteins are natural amino-acid made polymers with different chain sizes, so when concentration of proteins is low, smaller molecules with lower chain size have more chance to be absorbed quickly. Increasing the concentration of the surfactant, and also the time of adsorption, finally at a ratio of 5:1, the surface
would be completely occupied by surfactant molecules. However, it seems that non-ionic surfactants have a weak interaction with proteins such as BLG and BSC [20]. Miller et al [21] have experimentally studied the adsorption phenomena of HSA- C₁₀DMPO mixture. They have seen an induction time of \( \sim 200 \) s for HSA adsorption in lower concentration. Only after a competitive adsorption of HSA, C₁₀DMPO starts to be adsorbed. They reported that at higher concentration of C₁₀DMPO, adsorption of HSA is almost absent, while for lower concentration of C₁₀DMPO and in the equilibrium state, only the adsorption of protein occurs. Kotsmar et al [22] have studied adsorption behaviour of three mixtures (BLG- C₁₀DMPO, BSC- C₁₀DMPO and BSC- C₁₂DMPO) at air/water interface. They found that the adsorption of mixtures is of competitive nature and has a gradual replacement of the protein molecules at the interface with increasing surfactant concentration. Also, they have reported that the more rigid structure of BLG forces a positioning of protein at the surface, which leads to a thinner but optically denser adsorption layer as compared with that of the random coil structured milk protein BCS. C₁₂DMPO molecules can be replaced more effectively with proteins on the adsorption layer rather than C₁₀DMPO. Mainly, the complexes between non-ionic surfactants and proteins are formed during hydrophobic interactions. Also, the polarizable head of the used surfactants could cause weak interactions with the ionic amino acid (AA) side chains of proteins. Nonetheless, this interaction is assumed as a rather minor effect [23-27]. Wustneck et al [28] have studied the surface tension isotherms of mixture of BLG and BSC with anionic SDS and cationic CTAB surfactants and noticed the formation of surface-active complexes. The surface tension isotherms for the SDS-protein mixtures illustrate the marked plateau region. This surface saturation is indicated by proteins at specific concentrations. These concentrations are lower than the saturation concentration (at the CMC in phosphate buffer) of SDS. They have assumed the complexes of surface active SDS protein control the behaviour of mixtures surface in the plateau. Wustneck et al [28] also have found that for rare quantities of ionic mixtures in which the concentration of surfactant is 100 times less than the protein concentration, the results showed a minor growth in surface tension. Kragel et al [3] have focused on the adsorption behaviour of BLG/SDS at the water/air and water/hexane interfaces, and hence have analysed the interfacial structure of adsorbed layers. They have understood that after the formation of complexes in higher SDS concentrations, the loss of surfactant is insignificant. In addition, with the adsorption of protein molecules at the interface between air and water, the adsorbed molecules spread and unfold on the interface which can be correlated with slight variations in interfacial pressure. Also, their result recommends that the adsorption is basically a competition between SDS and BLG/SDS complexes at the interface region (water/air interface).

As it is shown in Figure 1 (a, b), the interaction of proteins with surfactants results in creation of complexes via electrostatic and hydrophobic interactions between protein and surfactants. For ionic surfactants, at the first stage the adsorption of molecules leads the formed complex to be charged neutral and more hydrophobic and consequently, this process results a higher surface-active complexes. In the next stage, increasing surfactant concentration results in the interaction of complexes of protein and surfactant complexes with more surfactant molecules mainly through hydrophobic interaction leading again to a more hydrophilic and therefore less surface-active complex as compared with the original protein. In case of non-ionic surfactants, the hydrophobic interactions result in creation of a complex which is more hydrophilic and therefore less surface active than the original protein.

(Figure 1)

Kostmar et al [29] have investigated interfacial behaviour of different mixtures of protein/surfactant by two methods of sequential adsorption or simultaneous adsorption. They have reported that mixed adsorption layers formed by both methods are the same in equilibrium properties but different in the dynamics. They showed that depending on whether the surfactant is ionic or non-ionic, surfactant molecules co-adsorb at the interface and modify the protein. At slight quantities, ionic surfactants can attach the protein stronger to the interface. At higher quantities, it must be noted that when the amount of surfactants (ionic or non-ionic) increases, a hydrophobic interaction will set in the resulting hydrophilization of the complex gradually. At the same time, with increasing the number of free surfactants a stronger competition at the interface will occur. Finally, both effects result in a progressive reduction in protein attachment on the interface [29]. The movement of bubbles in a liquid is significantly influenced by the adsorption of surface-active agents at the interface of bubbles. This hydrodynamic of a single rising bubble in surfactant solution has been considered as a significant evaluation for the creation of the adsorption layer in solutions containing surfactants in dynamic environments [30, 31]. Since aggregation and adsorption of proteins takes place at the interface of bubbles, it is useful to measure the velocity of rising bubbles in solutions containing protein and surfactants because it gives beneficial insights about their effect on the creation of layers of adsorption. Overall, the adsorbed layer limits the motion pattern of a bubble interface and, hence, a reduction of maximum 50% can occur in the rising velocity of a bubble [32-36]. As a bubble forms at the end of a capillary surrounded by a solution containing surfactant a layer of adsorbed surfactant is created through the whole surface of the bubble. The adsorption coverage in lower concentrations of surfactant is lower than the equilibrium conditions, however, the coverage occurs in a uniform pattern. After the separation of the bubble, surfactants begin to adsorb non-uniformly across the surface of the
bubble which is referred to as the dynamic structure of the adsorption layer (DAL) [37]. DAL formation can be traced by measuring the rising velocity of an air bubble in the surfactant solution. In general, the motion of the bubble can be divided into three to four steps, based on the surfactant concentration which are: (1) acceleration, (2) maximum velocity, (3) deceleration and (4) terminal velocity. At maximum velocity, the DAL formation process is going to be started. In the deceleration step, the surface tension and shear forces come to an equilibrium. Finally, in the terminal velocity step, the equilibrium is established and DAL is completely developed [38, 39]. This will minimize the amount of coverage at the upper of the mobile bubble, however at the lower part its value exceeds the equilibrium. This concentration gradient will result in a difference in surface tension which results in a decrease of the fluidity feature of the bubble (Marangoni effect). Hence, the hydrodynamic drag applied from the liquid to the bubble surface will increase due to the slowness of the bubble, which causes a decrease in the bubble velocity. In conventional surfactant solutions, the required time for forming of the DAL on the rising bubble surface is influenced by total surfactant concentration and the type of surfactant [40-43]. It was reported that the adsorption of protein over the bubble surface can considerably decrease the bubble rise velocity [13, 44, 45]. To the best of our knowledge, there are few studies reported on the motion of bubbles in mixtures containing protein and surfactant. Hence, the aim of this paper is to further investigate the bubble motion and the dynamic behaviour of adsorption layers in solution of surfactant and protein. The present work investigates the behaviour of rising air bubbles in aqueous solutions of BLG and BCS as proteins and C_{10}DMPO as a non-ionic surfactant, and the mixtures of these proteins and surfactant. The local velocity of air bubbles in solutions with different concentrations of surfactant and proteins, or their mixtures was measured with respect to the distance from the capillary tip. The observed profiles were evaluated qualitatively for pure surfactant and protein solutions as well as their mixtures.

2 Materials and Experimental Procedure

In order to perform the experiments, one non-ionic surfactant and two types of proteins were used. The non-ionic surfactant (C_{10}DMPO) with 98% purity and the BLG protein with 90% purity were purchased from Sigma Aldrich. The BCS protein with 98% purity was also supplied by Serva Company. Deionized water was used to prepare the solutions of pure surfactant and proteins as well as the surfactant/protein mixture.

Prior to each experiment, all laboratory equipment containing glass sections were cleaned with a commercial laboratory equipment cleaning liquid supplied by Sigma Aldrich followed by further rinsing with deionized water to ensure avoidance of any chemical residues. The bubble formation glass was also cleaned with a diluted chromic solution followed by rinsing with deionized water.

The experimental setup which is used for determining the velocity profile of rising bubble composed of: square glass column (with a 4 cm×4 cm cross section and 50 cm height) with a capillary at the bottom, bubble generator nozzle, syringe pump for providing air for the nozzle, camera for capturing the bubble motion, light source. The schematic representation of the experimental setup is shown in Figure 2. The aqueous phase was used to fill the glass column. Bubbles with diameters of 10 μm were created at the bottom of the column using the syringe pump and passed to the aqueous phase through the capillary. The bubble motion was recorded by using the camera. The vertical movement of the rising bubble across the glass column was identified by a stroboscope and Image processing. The detailed of setup and procedure could be find in previous publications [33, 37, 40, 46].

Obviously, the error in measurements are unavoidable and leads to in inaccuracies. Therefore, the calculation of uncertainty is vital to check the reliability of results. The uncertainty for the bubble diameter is estimated by dividing the accuracy of measurement by the lowest measured value of that variable, which is around 3%.

(Figure 2)

3 Results and Discussion

The rising bubble velocity is achieved by measuring the distance from the tip of the capillary to the upper point of the bubble at specific position intervals divided by the spent time. Then the local velocity profile (LVP) can be obtained by plotting the measured velocity versus the distance. Results could be explained in two parts, LVPs of single substance solutions and binary solutions.

3.1 LVPs of rising bubble in single substance solutions
There are a number of investigations on rising bubbles in a single substance solution of surfactants, alkanes or salts in water [13, 33-35, 37, 47] and fewer investigations on watery solution of protein [34]. Here, one could see LVPS measurements for two kinds of protein (BCS and BLG) and the non-ionic surfactant of C_{10}DMPO. For better understanding of surface activity power, adsorption isotherms of these three substances are shown in Figure 3 which is extracted from [2, 48].

(Figure 3)

The local velocity profile of rising air bubbles in deionized water and solution of BCS protein is shown in Figure 4(a) which is measured for the first time. As it is shown in Figure 4(a), the velocity profile of deionized water reaches the terminal velocity after a sharp acceleration. As is clear in this Figure, the bubble starts its motion rapidly in solutions containing BCS and also, the obtained velocity profiles are significantly influenced by BCS concentration. The velocity profile of the bubble in the solution with lowest BCS concentration of 1e-7 M, is similar to the profile of deionized water in which the terminal velocity is established immediately after the acceleration step. This is due to the fact that at very low BCS concentrations, the amount of adsorbed BCS at the bubble interface is small, and the surface tension drag forces on bubbles are not large enough to decrease the bubble velocity.

The velocity profiles show un-similar trends as the BCS concentration increases. In other words, in higher BCS concentrations, after the acceleration section, a steady decrease occurs in the velocity profile before reaching the terminal velocity. According to Figure 4(a), the velocity, position, and length of the maximum velocity region varies with the concentration of BCS. The increase in BCS concentration decreases the length of the region of maximum velocity and moves the maximum point to lower velocities and smaller distances. However, the value in which the curves level off at terminal velocity is nearly the same in higher BCS concentrations.

(Figure 4)

The velocity profile of rising air bubbles in deionized water and solution of BLG protein is shown in Figure 4(b). This Figure shows that the velocity profile of BLG protein exhibits identical trend and behaviour to those of BCS protein. To compare, for the same concentration, rising bubbles in BLG solutions shows faster DAL development and lower maximum and terminal velocity which means that BLG is more surface active than BSC. These results are almost in accordance with the isotherm shown in Figure 3, where at the same concentration, surface pressure of BLG is a little bit higher than BCS. Based on the fact that isotherms are obtained after a large time, but here rising bubble is highly dynamics so, it seems that kinetic of BLG adsorption at air/water interface is faster than BCS [48] because of difference in their structures in bulk and at the interface as it is shown in Figure 5.

On the other hand, at 5e-6 M, both BLG and BSC cause the same LVPS, which indicates that at higher concentration, the presence of protein at the interface alters the LVP and increases the drag more than that of DAL formation. However, when the concentration is high enough, the DAL becomes fully developed and the minimum terminal velocity of the bubble in BLG solution is lower than in BCS and as it is shown in case of BCS, maximum velocity is close to terminal velocity.

Also rising bubbles in BLG solution was investigated by [33] and later they have investigated about the effect of pH.

(Figure 5)

The local velocity profile of the rising bubble in C_{10}DMPO solution is shown in Figure 6. Overall, the velocity profiles in different surfactant concentrations are similar to those of BLG and BCS proteins. The similarity between velocity profiles can be due to the fact that at specific concentrations, the protein remains in spherical shape and the unfolding process does not occur, and hence the protein behaves similar to the C_{10}DMPO molecules.

Figure 6 shows that for concentration around 5e-7 M, the effect of C_{10}DMPO on LVP is less than BLG but more than BCS. But increasing the concentrations is more effective for proteins than C_{10}DMPO. After concentration of around 1e-4 M, the LVP does not show the decreasing stage, and reaches terminal velocity immediately after the acceleration stage. For more information about interfacial properties of C_{10}DMPO [49-52].

(Figure 6)

3.2 LVPS of rising bubble in binary solutions
Ulaganthan et al [13] in 2014 have investigated rising bubble hydrodynamics in a mixture of BLG as a protein with SDS, CTAB and C_{10}DMPO as surfactant for the first time. Here a mixture of BCS and BLG as proteins with C_{10}DMPO is presented to clear their interactions.

The LVPs of the rising bubble in the mixture of BCS and BLG with non-ionic C_{10}DMPO surfactant are depicted in Figure 7(a) and Figure 7(b), respectively. According to these Figures the mixture of protein and non-ionic surfactants exhibit an identical pattern of rising bubble velocity to those obtained in solutions of pure proteins or surfactants. In another word, the velocity increases in the acceleration step and after reaching a maximum point follows a steady decrease until levelling off at terminal velocity. As it is shown in Figure 7(b), for mixture of 5e-7 M BCS with 5e-7 M C_{10}DMPO, a complex is formed which is in competition with free surfactants to adsorb at the interface, so the LVP of mixture is almost the same as 1e-6 M C10DMPO. In another word, the bubble velocity values for pure BCS solution are higher than those of pure C10DMPO surfactant, and the final velocity profile of their mixture is nearer to the pure C10DMPO surfactant.

By addition of the C_{10}DMPO surfactant to pure BCS solution, the protein molecules at the bubble interface are gradually replaced by the surfactant molecules which causes the behaviour of the bubble velocity in the mixture to be more identical to the pure surfactant solution. However, a bit difference in the maximum velocity and DAL development of mixture against 1e-6 M C_{10}DMPO is because of the slow adsorption kinetic of the formed complex. The LVPs of pure C_{10}DMPO and BCS solutions with their mixture is depicted in Figure 7(b) and reveals that mixture of 5e-7 M BCS + 5e-7 M C_{10}DMPO is overall similar to those of pure solutions with 1e-6 M concentration. As it has been reported in [22], that surface tension of mixture BCS or BLG with C_{10}DMPO are the same and interestingly, here they have shown the same effect on rising bubble hydrodynamics.

(Figure 7)

For better comparison, the distance from nozzle which bubble reaches to its terminal velocity is considered as characteristic distance, which is shown in Figure 8. Form this figure; it is clear that for mixture of non-ionic surfactant with BCS has a bit synergetic effect while for its mixture with BLG, a negative synergy is observed.

(Figure 8)

4 Conclusion

Knowledge about dynamic interfacial properties play a significant role in surface engineering for study of multiphase flows of gas-liquid or liquid-liquid which involves in many processes in different industries. However, in some cases of dynamic and unsteady conditions of two-phase flow (e.g. rising bubble columns), the investigation methods are not enough to qualify the interface properties.

In current work, a new laboratory tool named “rising bubble method” is applied for investigating the highly dynamic two-phase condition, in order to collect some data for the behaviour of surfactant-protein mixture at interface. In this research, the rising of air bubbles in pure solutions of BLG or BCS proteins and their mixtures with non-ionic C_{10}DMPO was investigated. Overall, in pure solutions of BLG, BCS, and C_{10}DMPO, the bubble velocity profile shows a rapid acceleration stage followed by a steady decrease until levelling off at terminal velocity.

It is concluded that for a mixture of 5e-7 M BCS with 5e-7 M C_{10}DMPO, a complex is formed which is more surface active than 1e-6 M C_{10}DMPO or 1e-6 M BCS. The bubble velocity values for pure BCS solution are higher than those of pure C_{10}DMPO surfactant, and the final velocity profile of their mixture is close to the pure C_{10}DMPO surfactant. By the way, some small synergetic occurs.

The LVPs of pure C_{10}DMPO and BLG solutions with their mixture of 5e-7 M BLG + 5e-7 M C_{10}DMPO reveals that there is a no positive synergetic and the behaviour of the formed complex is similar to those of pure solutions with 1e-6 M concentration. The obtained results of this study can be used as a pioneer method for extraction and separation of surfactant and protein processes in food and pharmaceutical industries or diagnose of diseases caused by adsorption or coagulation of proteins.

Declarations

Funding
Conflicts of interest/Competing interests
Not applicable

Availability of data and material
Not applicable

Code availability
Not applicable

Authors' contributions
M. Lotfi: Supervision, Methodology, Writing-Review & Editing, Funding acquisition

A. Bahmani: Writing-Original Draft, Investigation

D. Bastani: Supervision, Writing-Review & Editing, Funding acquisition

Ethics approval
Not applicable

Consent to participate
Not applicable

Consent for publication
Not applicable

References


Marzieh Lotfi received her BSc and MSc degrees in 2008 and 2010 from Amir Kabir University of Technology, Department of Chemical Engineering, Tehran, Iran, all in Chemical Engineering, and received her PhD in 2015 from Sharif University of Technology and Petroleum Department, Tehran, Iran, in Chemical Engineering joint with Max Plank Institute of Colloids and Interfaces, Golm, Potsdam, Germany. Dr. Lotfi is currently an Assistant Professor and Head of Chemical Engineering Department at Jundi-Shapur University of Technology, Dezful, Iran. Her research interests are dynamic interfacial phenomena, wastewater treatment and energy optimization.

Alireza Bahmani received his BSc in 2014 from University of Tehran and MSc degree in and 2017 from Sharif University of Technology, Chemical and Petroleum Department, Tehran, Iran, all in Chemical Engineering, respectively. He is currently working as an Adsorbent & Catalyst Expert at Behdash Chemical Co. His research interest is adsorption processes, Interfacial phenomena & advanced materials.

Dariush Bastani received his BSc degree from Sharif University of Technology in Chemical Engineering. He also received his MSc and PhD degrees from University of Manchester Institute of Science and Technology, Manchester, United Kingdom, all in Chemical Engineering. Dr. Bastani is currently a Professor of Chemical Engineering at Chemical and Petroleum Department of Sharif University of Technology, Tehran, Iran. His research interests are separation processes, liquid-liquid extraction, and interfacial phenomena.

**Figures caption**

Figure 1. Scheme of protein-surfactant interactions and forming complexes at different surfactant concentrations (a) non-ionic surfactants, (b) ionic surfactants. 2

Figure 2. Schematic of the experimental setup. 3

Figure 3. Adsorption isotherm of proteins [2] and surfactant [48]. 4
Figure 4. The local velocity profile of rising bubble in (a) BCS solutions, (b) BLG solutions.

Figure 5. Schematics of Proteins structure and thickness of adsorbed layer at air/water interface at equilibrium with help of ref[53].

Figure 6. The local velocity profile of rising bubble in C_{10}DMPO solutions.

Figure 7. The velocity profile of rising bubble in mixture of (a) BCS with C_{10}DMPO.

Figure 8. Characteristic distance from the nozzle for single and binary solution of BLG, BCS and C_{10}DMPO.

**Figures**

![Scheme of protein-surfactant interactions and forming complexes.](image)

**Figure 1:** Scheme of protein-surfactant interactions and forming complexes at different surfactant concentrations (a) non-ionic surfactants, (b) ionic surfactants

![Schematic of the experimental setup.](image)

**Figure 2:** Schematic of the experimental setup
**Figure 3:** Adsorption isotherm of proteins [2] and surfactant [48]

**Figure 4:** The local velocity profile of rising bubble in (a) BCS solutions, (b) BLG solutions
**Figure 5:** Schematics of Proteins structure and thickness of adsorbed layer at air/water interface at equilibrium with help of ref[53]

**Figure 6:** The local velocity profile of rising bubble in C10DMPO solutions
Figure 7: The velocity profile of rising bubble in mixture of (a) BCS with C₁₀DMPO

Figure 8: Characteristic distance from the nozzle for single and binary solution of BLG, BCS and C₁₀DMPO