# Investigation on the Relation between Water Content and Microstructure of Several Different Reverse Micellar Systems

[1,2]JUNTING ZHAO, [2]DALIANG LIU, [2]FUSHENG CHEN AND [1]GUOJI LIU*
[1]*School of Chemical Engineering and Energy*, *ZhengZhou University*, *Zhengzhou*, *China*
[2]*School of Chemistry and Chemical Engineering*, *Henan University of Technology*, *Zhengzhou*, *China.*
guojiliu@zzu.edu.cn*

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**Summary**: The relation between water content and microstructure of reverse micellar systems of anionic surfactant sodium di-2-ethylhexyl sulfosuccinate (AOT) and sodium dodecyl sulfate (SDS) and nonionic surfactant octyl polyethylene glycol phenyl ether (OP) was investigated using Zeta Potential Analyzer□conductivity and so on. Additionally, the change reasons of extraction rate and particle size when reverse micelles extracted the sesame protein were explained. The study was prepared to explore forward extraction process mechanism of extracting protein from sesame seeds by reverse micelles.

## Introduction

Reverse micelles are water content aggregates which are contrast with normal micelles in structure. When the surfactant disperses in non-polar organic solvents (the dispersion medium) and its concontration exceeds the critical micelle concentration (CMC), reverse micelles will be self organized. The surfactant molecules of reverse micelles are composed of the hydrophilic (polar head) and hydrophobic (non-polar tail). When hydrocarbon chains face the organic solvent and polar head-groups point inwards, a polar water core forms which has solubilizing properties with respect to water. When water is added into the polar core, a "water pool" has formed. The reversed micelle of "water pool" is applied in extracting proteins, nucleic acids and amino acids and other biologically active substances by dissolving these substances in the "water pool".

The molar ratio of water to surfactant ($W_0$) of reversed micelle is an important physical parameter for the structure and physical properties of reverse micelle. The relationship between $W_0$ and particle size of reversed micelle can be described by "water shell-model" [1–6]. The relationship between particle size and $W_0$ is approximated as $r = \frac{3\upsilon_{H_2O}}{\alpha_{SURF}} \times W_0$,

where $\upsilon_{H_2O}$ is the volume of molecule; $\alpha_{SURF}$ is the area of the polar head in surfactant molecule. As a significant physical parameter, the particle size of reverse micelles is usually determined by viscometry, light scattering, small angle neutron scattering, nuclear magnetic resonance (NMR), ultracentrifugation, fluorescence spectroscopy, ultrasonics method and so on [7–13].

The behavior of conduction is another important property of reverse micelle [14]. Liu *et al*. [15] analyzed the relationship between conductivity and microstructure of reversed micelles of anionic surfactant AOT alone and mixed reversed micelles of anionic surfactant AOT and nonionic surfactant in n-heptane, then they interpreted that the conductivity of reverse micelles mainly depended on the charged state and particle size of droplets. Xue *et al*. [16] determined the conductivities of the microemulsion system fatty alcohol polyoxyethylene ether □$AEO_9$□ /kerosene/hexanol/water (salt) at the different volume ratios of water to oil, and then they thought that the changes of the droplet particle size and type were the main reasons for the conductivity rising.

In this paper□the sesame seeds were crushed and extracted with reverse micelles solution to obtain protein. The relation between water content ($W_0$) and microstructure of several reversed micelles systems of anionic surfactant AOT and SDS and nonionic surfactant OP is investigated using Zeta Potential Analyzer□Conductivity and so on□and then the change reasons of extraction rate and particle size when reverse micelles systems extracted the sesame protein are explained. The study is prepared to explore forward extraction process mechanism of extracting protein from sesame seeds by reverse micelles.

## Results and Discussion

Table-1 shows the physical properties of reverse micelles. All the physical properties of solvent were measured at 30°C. The density of distilled water was taken as 0.99567 g/cm$^3$.

*To whom all correspondence should be addressed.

Table-1: The physical properties of reverse micelles.

| Properties System | Surfactant concentration (g/mL) | Buffer pH | Solvent properties | | | |
|---|---|---|---|---|---|---|
| | | | Solvent to cosolvent | Density ($g/cm^3$) | Viscosity (mPa·S) | Refractive index |
| AOT/isooctane | 0.1 | 7 | 1□0 | 0.6839 | 0.439 | 1.3867 |
| SDS/isooctane/ octanol | 0.1 | 7 | 4□1 | 0.7114 | 0.656 | 1.3951 |
| OP/iso-octane/ n-octanol | 0.1 | 7 | 5□1 | 0.7069 | 0.610 | 1.3945 |

In the AOT/isooctane reverse micelle system, the reverse micelle diameter (D) increases with $W_0$ before and after the extraction of protein [1, 5–8]; in the same $W_0$, the reverse micelle has smaller diameter after extraction of proteins; protein extraction rate increases and then decreases with the $W_0$ (Fig. 1).

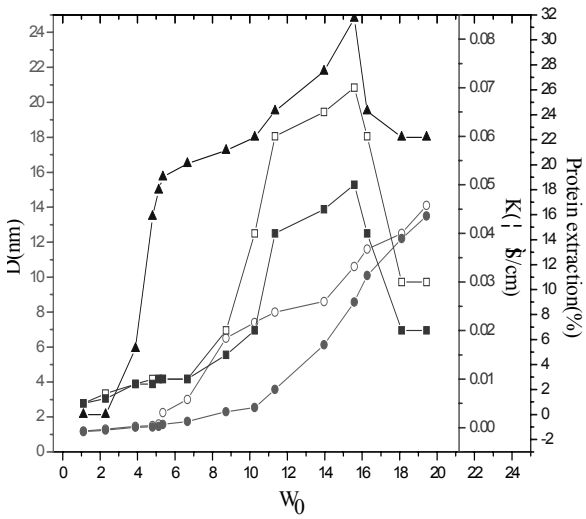


Fig. 1: The relationship between $W_0$ and D, K and protein extraction of AOT/isooctane/water reverse micelles. Protein extraction (″); the conductivity of reverse micelle before extraction (ϒ) and after extraction (□); the diameter of reverse micelle without protein (⊄); and with protein (∉).

Smaller in the D and $W_0$, changes in conductivity is also small and stable. With the increase of $W_0$, conductivity (K) sharply increases. When $W_0$ reaches a certain degree, K drastically declines with $W_0$ further increasing. There are some possible reasons for the phenomenon in this paper. The first water molecules which involve in the formation of reverse micelles would hydrate with $Na^+$. At the same time, the mobility of water molecules would reduce, conductive particles in reverse micelles solution are very fewer, the distance among the conductive particles becomes so far that conductive particles can't contact with each other, conductivity is lower [17]. When $W_0$ increases to 5, the conductivity of reverse micelles grows slowly, W/O microemulsion begins to take shape. With further increasing $W_0$, reverse micelles particle size gradually increases and the conductivity also gradually increases because of easily being charged of the droplet. On the other hand, the increase of droplet also slows down the transfer rate of droplet. When $W_0$ increases to a certain extent, the reducing transfer rate caused by the increase of droplet radius plays an important role in the changing of conductivity, therefore, conductivity decreases [15].

The particles size of reverse micelles significantly reduces after extraction. When the crushed sesame seed powder containing sesame oil is added, the system is still not clear after being ultrasounded. Water phase is separated at the bottom of the solution through centrifugation. We can speculate that it is the sesame oil in reverse micelles that leads to lower water content and smaller particle size. Ionic surfactants solubilize water molecules by combining with the ionic groups and counter ion of surfactant through the ion-dipole and hydrogen bonding interaction, more solubilizing water molecules loosely bind to the core of reverse micelles. The participation of sesame oil will result in the increase of polarity of reverse micelle solution and decrease of ion-dipole interaction. So, the aggregation number of reverse micelle will decrease. Surfactant will rearrange to form reverse micelles and excess water will separate out (Surfactant AOT dissolves in isooctane).

Protein extraction rate increases and then decreases with the $W_0$ and D. When $W_0$ is smaller, the first water molecules which involves in the formation of reverse micelles would hydrate with $Na^+$ but reverse micelles have not been formed. So protein extraction rate is almost zero at this time. With the increase of $W_0$, reverse micelles begin to form, extraction rate also increases dramatically [18]; when $W_0$ further increases, reverse micelle size is not the main factor affecting on the extraction rate, extraction rate slowly increases; when $W_0$ increases to a certain extent, the size of reverse micelles is relatively large, it is more difficult to enterer the capillary pores of oilseeds for reverse micelles. Therefore, the reverse

micelle size is too large to extract protein.

Fig. 1 also shows that D and $W_0$ approximately meet linear relationship in some ranges of water content. Fitting results are plotted in Fig.2. It is different mechanism of solubilization of water that results in different linear relationships in different ranges of water content.

As for SDS/isooctane/n-octanol reversed micelle, shown in Fig. 3, when $W_0$ in the rage of 4.59~10.21, D increases with the $W_0$; when $W_0$=11, D decreases rapidly; D increases with further increasing the $W_0$ value in the rage of 12~18. However, the particles size of reverse micelles is much smaller than that in $W_0$ value rage of 4.59~10.21. The particles size of reverse micelles of the entire system significantly reduces after extraction. Protein extraction rate increases with the $W_0$.

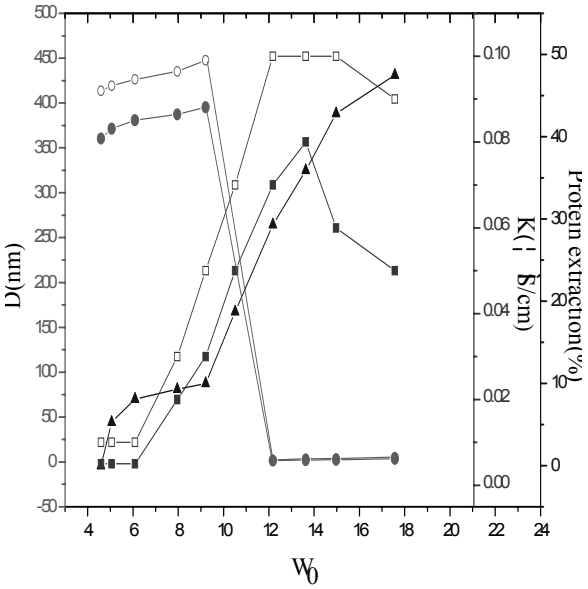


Fig. 3: The relationship between $W_0$ and D, K and protein extraction of SDS/ isooctane/ *n*-octanol reversed micelles. Protein extraction (▲); the conductivity of reverse micelle before extraction (□) and after extraction (■); the diameter of reverse micelle without protein (○); and with protein (●).

The relationship between $W_0$ and D in SDS/isooctane/n-octanol reversed micelle system could be interpreted as follows. When $W_0$ □ 4.59, SDS/isooctane/octanol reversed micelle and the solution is turbid. The similar results are in ref. [14]: when $W_0$ is small, the first water molecules which involves in the formation of reverse micelles can't associate with SDS, because each $Na^+$ will associate with 6 water molecules, considering S=O bond and alcohol and $H_2O$ in interface, an SDS molecules will associate with about 10 $H_2O$ molecules, so when $W_0$ is smaller, SDS/isooctane/ octanol reversed micelle can't formed. When $W_0$=4.59~10.21, SDS molecules will associate with $H_2O$ molecules, as the number of $H_2O$ molecules can't meet the full needs of association, number of SDS molecules may share a $H_2O$ molecule and form complex aggregation of reverse micelles whose shape may be crowded to layer or rod. With the increasing $W_0$, SDS is completely in association with the water. At the same time, free water appears. The radius $R_W$ of water core and the micelle aggregation number increase with $W_0$ [19,20]. When $W_0$ in the rage of 10.51~17.57, smaller spherical reverse micelles form by splitting crowded layer or rod complex aggregation, and particle size and aggregation number increase with the $W_0$ [21]. Therefore, in the second case, the particle size of reverse micelles is larger and there is almost no free water, SDS anion and $Na^+$ are the main charge carrier, conductivity K is lower. In the third case, the particle size of reverse micelles is smaller and there is more free water, the droplet easily charges, conductivity K is higher.

The mechanism of changes of diameter of SDS reverse micelles is similar to which of AOT reverse micelles. The difference is that not only water phase is separated but also SDS molecular. Because the solubility of SDS molecular in the isooctane/n-octanol and water solubility are both very small, SDS molecules dissociated from reverse micelles can't exist in free form in solution.

Protein extraction rate increases with the $W_0$ and D. When $W_0$ is smaller, the first water molecules which involve in the formation of reverse micelles will hydrate with $Na^+$, reverse micelles haven't formed. Protein extraction rate is very small at this time. However, the particle size of reverse micelles is very large. With the increasing of $W_0$, reverse micelles begin to form, extraction rate also increases dramatically.

As can be seen from Fig. 4, in OP/isooctane /n-octanol reversed micelle system, the diameter (D) increases with $W_0$ before and after the extraction of protein; in the same $W_0$ phase, the diameter is almost unchanged after protein extraction; Protein extraction rate doesn't change with the increase of $W_0$, and is almost zero.

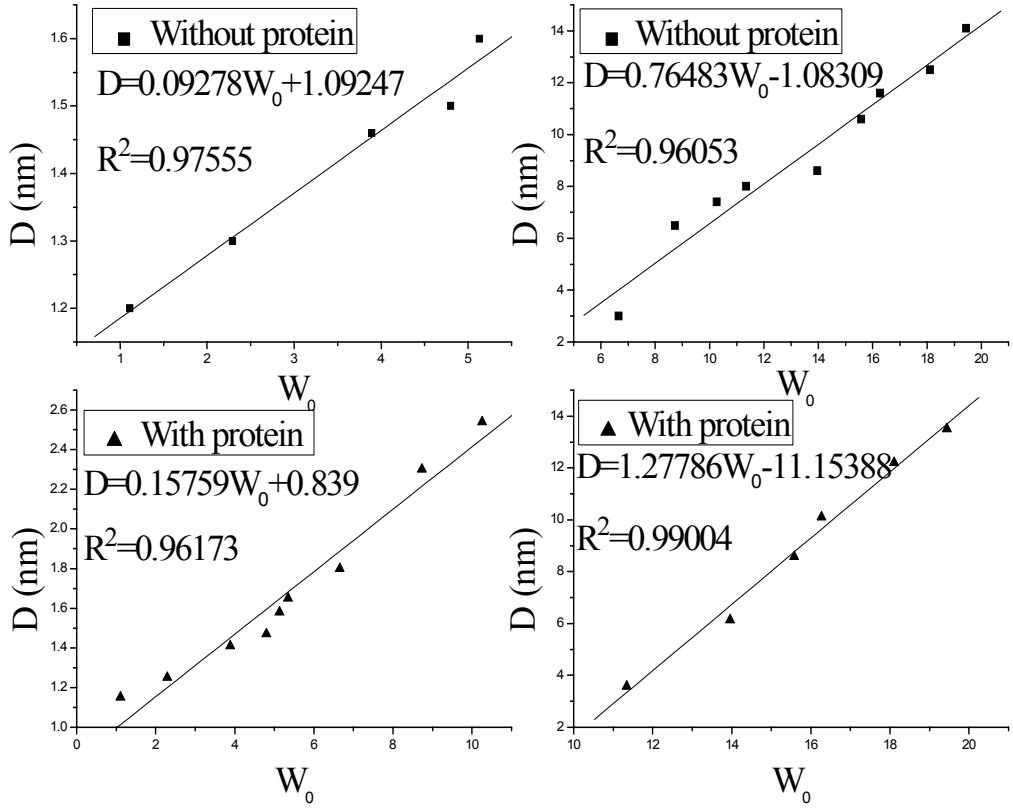


Fig. 2: The fitting results of D and $W_0$ of AOT/isooctane/water reverse micelle.

The diameter (D) and conductivity (K) both increase with $W_0$. When $W_0$=5.5, there is a turning point in the conductivity curve. Explaining this phenomenon are as follows: when $W_0$□5.5, water is in the form of bound water, there is almost no free water, hence the conductivity K increases slowly with $W_0$ and is smaller; When $W_0$□5.5, the increasing free water results in sharp increasing of conductivity K with $W_0$.

It is found that when only adding sesame seed powder to the OP/isooctane/octanol reversed micelle, the solution is still clear and transparent and there is no precipitated water even if the oil/solvent ratio is high. This shows that the participation of sesame oil will not affect the stability of OP/isooctane/octanol reverse micelles and there is no reorganization behavior such as AOT and other systems'.

Compared to the above two reversed micelles at the same concentration, OP/isooctane/ octanol reversed micelles being in lower protein extraction rate can be explained from two aspects. On one hand, ionic surfactants and nonionic surfactants have different mechanisms of water solubilization. Non-ionic surfactant solubilizes water by ether oxygen of polyoxyethylene chain of interaction with the solubilization of water [22], water may closely integrate around the ether oxygen of the polyoxyethylene chain, less is able to flow freely, and however, bound water doesn't have the capacity of secondary solubilizing protein. On the other hand, some nonionic surfactants themselves have the negative potential, the surface of proteins is negatively charged within pH values of the extraction. Generally considering, protein can be effectively extracted by electrostatic interaction between the protein surface charge and surface charge within the reverse micelle [23]. So nonionic surfactant reverse micelle system is not conducive to the extraction of protein.

As can be seen in Fig. 5, a strong positive relationship was found between D and $W_0$ when the value of $W_0$ was in the rage of 1~8, indicating that the linear relationships in low water content rage could take place in the OP/isooctane/n-octanol reversed micelle system.

## Experimental

Crude AOT was purred [8]. Reverse micelle solution was prepared through injection [24]. The water content $W_0$ (The mole fraction of water in reverse micelle solution/the mole fraction of surfactant in reverse micelle solution) in the reverse micelle phase was determined by Karl-Fischer titration [25]. The conductivity of reverse micelle was measured by DDS-11A conductivity meter at 30□.

The pre-treatment and some physical properties (these values are listed in Table-1) , such as density, viscosity, refractive index, of reverse micelle solution were required before measuring the particle size of reverse micelle by Zeta Potential Analyzer (Brookhaven instruments corporation, USA). The reverse micelle solution was centrifuged at 4000 rpm for about 20 minutes and then filtered with 0.22μm hydrophobic membrane. The viscosity measurements were performed using an ubbelohde viscometer (Φ0.6~0.7mm) [26-30] mounting inside a thermostatic bath regulating temperature to an accuracy of ±0.5°C of the set temperature. The efflux time was recorded. The conversion of efflux time to viscosity requires the a priori knowledge of liquid density. This was measured by taking the weight of 25 mL solvent with a gravity bottle. Refractive index of solvent was determined by Abbe refractometer calibrated against acetone.

The forward extraction process of sesame protein was performed in the water bath oscillator at 200r/min for about 1h and the forward extraction solution was centrifuged to remove debris at 3500 r/min. Protein content is determinated by Kjeldahl titration (the reverse micelle solution before extraction as a blank). Forward extraction of protein (%)=Protein content in reverse micelle solution/Protein content of sesame seeds×100. In addition, all the reagents were analytical reagent grade and as received.

## Conclusions

For different reverse micelles, the diameter (D) increases (or part of the increase) with the $W_0$; reverse micelle structure changes with the increase of $W_0$; for AOT and SDS reverse micelles, the diameter of reverse micelle becomes smaller, for OP reverse micelles, diameter almost has no change after extraction of protein; protein extraction rate increases and then decreases with the $W_0$.

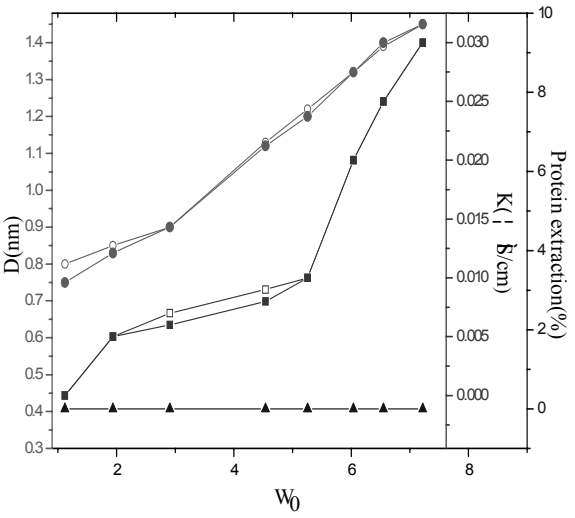


Fig. 4: The relationship between $W_0$ and D, K and protein extraction of OP/isooctane/n-octanol reversed micelles. Protein extraction (″); the conductivity of reverse micelle before extraction (ϒ) and after extraction (□); the diameter of reverse micelle without protein (⊄); and with protein (∉).

Based on these conclusions, we speculate the mechanism of extraction of protein as follows: reverse micelles without protein in bulk solution spread to the solution-seeds interface and enter the capillary pores of seeds. Reverse micelles encapsulate protein in the seeds by the hydrophobicity and electrostatic attraction between surfactant and protein. Reverse micelles with protein spread to the sesame seeds-solution interface and enter the bulk solution

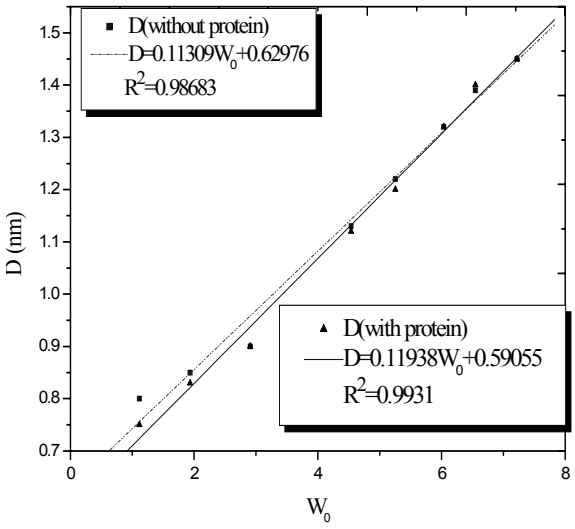


Fig. 5: The fitting results of D and $W_0$ of OP/isooctane/ octanol reversed micelle

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