Partitioning of Porphyrin Compounds in An Aqueous Two-phase System of Cationic-anionic Surfactant Mixture

Aijun TONG, Ying Wu*, Longdi Li, Yoshifumi AKAMA** and Shigeyuki TANAKA**

Department of Chemistry, Tsinghua University, Beijing 100084, China
*Department of Fundamental Science, Xinjiang Araer Talimu Agriculture University, Xinjiang Araer 843300, China
**Department of Chemistry, Meisei University, Hino, Tokyo 191, Japan

A new aqueous two-phase system of cationic-anionic surfactant mixture (ATP-CAS) is proposed for the extractions of porphyrin compounds. ATP-CAS is formed when 0.05 mol/l aqueous solutions of dodecyl triethylammonium bromide (C\textsubscript{12}NE) and sodium dodecyl sulfate (SDS) are mixed with a molar ratio of 1.62:1. The two phases are of mixed surfactant solutions but the concentration in the upper phase is much higher than that of the lower phase. Effects of strong acid, base solutions on the two-phase system are investigated. The results show that negatively charged porphyrins such as meso-tetraphenyl porphine tetrasulfonic acid (TPPS\textsubscript{4}) and protoporphyrin (proto) are extracted into the upper phase efficiently while other porphyrins like meso-tetraphenyl porphine (TPP) and tetrakis(4-trimethylammoniophenyl)porphine (T-4(TAP)P) and vitamin B\textsubscript{12} distributed in the two phases almost uniformly.

Keywords Aqueous two-phase, ionic surfactant, extraction, porphyrin compounds

Recently, aqueous two-phase separation\textsuperscript{1, 2} has been received great attention for their favorable properties when used in separation, purification and preconcentration of biomaterials\textsuperscript{3}, organic compounds\textsuperscript{4} as well as metal chelates\textsuperscript{3}. Compared with the conventional organic solvent extraction, aqueous two-phase separation performed in a milder system which is more like a physiological environment and, correspondingly, prevented labile biomaterials from denaturation. The material needed is much less and usually could be easy recovered or disposal while large volumes of organic solvent are required in the conventional extraction, thus the aqueous two-phase extraction greatly reduced volatility, flammability and cost. Such aqueous two-phase system included the well known aqueous polymer two-phase separation\textsuperscript{6} and surfactant mediated liquid-liquid phase separation such as cloud-point extraction with nonionic\textsuperscript{7} or zwitterionic surfactant\textsuperscript{8}, coacervation\textsuperscript{9} induced in an ionic surfactant solution by the addition of an electrolyte or another amphiphile.

In this paper, a new aqueous two-phase system of cationic-anionic surfactant mixture (ATP-CAS) is proposed for the partitioning of porphyrin compounds. It had been generally considered that precipitation would occur when a cationic and an anionic surfactant are mixed above their CMC for strong electrostatic interaction present. However, Zhao and Xiao's work\textsuperscript{10, 11} indicated that homogeneous or even aqueous two-phase could be obtained at concentrations higher than CMC. When alkyl groups bigger than methyl were introduced into the "head group" (alkyl ammonia group of a quarternary ammonium salt) and such a cationic surfactant was mixed with an anionic surfactant, turbid solution, aqueous two-phase system or homogeneous solution could be obtained in different surfactant concentration and mixing ratio regions for weaker electrostatic attraction. For example, aqueous two-phase formed for dodecyltriethylammonium bromide (C\textsubscript{12}NE) and sodium dodecyl sulfate (SDS) mixture when the surfactant concentrations and C\textsubscript{12}NE/SDS ratios are in certain regions. The system needs much less surfactants (total surfactant concentration is less than 1%, w/w) compared either to the aqueous polymer two-phase or to the cloud-point extraction, indicating it would be milder and more economic when used in separation. Furthermore, the system is homogeneous and thus
phase separation is much easier than the cloud-point extraction. Extractions of BSA\textsuperscript{11}, tryptophan and its derivatives\textsuperscript{12} as well as trypsin\textsuperscript{13} have been practiced. This paper reports the extraction results of proto, an essential precursor of heme, the prosthetic group of hemoglobin, and three porphyrin reagents of analytical interest TPPS\textsubscript{4}, T-4(TAP)P and TPP, as well as vitamin B\textsubscript{12} with the C\textsubscript{12}NE-SDS two-phase system.

Experimental

Reagents and Apparatus

C\textsubscript{12}NE was synthesized by mixing 1-bromododecane with excess amounts of triethylamine under refluxing for 48h. The crude product was recrystallized five times from acetone-ethyl ether. The obtained C\textsubscript{12}NE were white slice crystals confirmed by IR and NMR spectroscopy. SDS are Sigma product (purity higher than 99%) provided by Baitai Co. Ltd., Beijing, China, it was used without further purification. Proto are Sigma product (purity higher than 95%). TPPS\textsubscript{4}, TPP and T-4(TAP)P were kindly provided by the professors of Beijing university. Vitamin B\textsubscript{12} are product of Beijing Fang Cao Chemical Company (purity higher than 96%). All water used was redistilled from deionized water by a sub-boiling quartz distillator.

UV detections were made on a Shimazu UV-2100 type ultraviolet and visible spectrometer, bandwidth was set at 2.0 nm.

Procedure

Stock solution of ATP-CAS was prepared by mixing 0.05 mol/l C\textsubscript{12}NE and SDS with a molar ratio of 1.62:1. Then the top and bottom phases were separated carefully. Calibration curves of the top and bottom phase solutions were made respectively by adding certain amounts of porphyrin solution to the appropriate solutions. Certain amounts of a porphyrin solution at a given pH were transformed into the stocked two-phase solution, the system was shaken for about 2 min. and then allowed to stand for phase separation. Extraction efficiencies were calculated by determining the concentrations of a porphyrin distributed in the two phases of ATP-CAS by UV detection.

Results and Discussion

Properties of the C\textsubscript{12}NE-SDS ATP-CAS System

Phase diagram of C\textsubscript{12}NE-SDS system when C\textsubscript{12}NE are in excess is shown in Fig.1. Homogeneous solution, aqueous two-phase and turbid solution could be obtained at certain surfactant concentrations and molar ratios. At the center of the two solid lines the formation speed of the two-phase is fastest (about 3-5 min) and the phase ratio is nearly 1. The top phase is slightly opalescent like color while the bottom phase is colorless; the surfactant concentration in the top phase is about ten times higher than that of the bottom phase\textsuperscript{10, 11}. The two-phase system is stable for at least one year, but the addition of a chemical reagent or water will cause phase change of the system. For example, phase ratio decreased as the concentration and amounts of a reagent added increased for the examined hydrochloric acid, sulfuric acid, sodium hydroxide and sodium sulfate. The addition of redistilled water also cause phase ratio decreasing. When the concentration and amounts of a reagent added or the amounts of water added are much enough aqueous two-phase system would be damaged. Thus attention should be paid when ATP-CAS are used in separation.

Fig. 1 Phase diagram of C\textsubscript{12}NE-SDS system when C\textsubscript{12}NE are in excess

Effects of acid solution on the C\textsubscript{12}NE-SDS two-phase system

As can be seen in Table 1, the volume of the top phase decreased and that of the bottom phase increased as increasing the amounts of 0.1 mol/l hydrochloric acid. It is interesting that the pH of the top phase remained almost unchanged while that of the bottom phase decreased dramatically according to the addition of the acid. This indicates that the top phase is more hydrophobic than the bottom phase and thus aqueous ions like H\textsuperscript{+} and Cl\textsuperscript{-} could not be extracted into the top phase. When the top phase was
separated and allowed to stand for a while, phase separation occurred once more and the properties of the newly formed two phases were just like the original two phases. It is considered that the newly formed bottom phase contained aqueous H⁺ and Cl⁻ which were excluded by the net work of the micelles of the top phase.

Effects of base solution on the C₁₂NE-SDS two-phase system

Table 2 showed the pH and volume changes of the two-phase system when 0.1 mol/l sodium hydroxide were added. It could be seen that the volume changes of the two phases were almost the same as the case that hydrochloric acid were added, but the pH changes were more complex. When the volumes of 0.1 mol/l sodium hydroxide were added is smaller than 0.09 ml, the pH in the top phase is higher than that of the bottom phase; when the volumes of sodium hydroxide added is bigger than 0.09 ml, the pH in the top phase is lower than that of the bottom phase. It is considered that in the ATP-CAS system in which cationic surfactant C₁₂NE were in excess, negatively charged chemical species would be easy extracted into the top phase. When little amounts of 0.1 mol/l sodium hydroxide were added to the ATP-CAS system OW were extracted into the top phase for the charge interaction and thus its pH values higher than the bottom phase. When the amounts of sodium hydroxide increased hydrophobicity played an important role and thus some OH⁻ were excluded from the bottom phase. As in the case of hydrochloric acid added, the top phase could also separate into two immiscible phases again on addition of 0.1 mol/l sodium hydroxide.

Partitioning of porphyrin compounds in the C₁₂NE-SDS two-phase system

Porphyrin compounds are very important in life process for their ability of redox, electron and oxygen transfer as well as charge separation etc. Some porphyrin compounds are also well known as sensitive reagents for photometric determination of metals. In this paper, the partition behavior of protoporphyrin and three porphyrin chelating agents TPP₄, TPP, T-4(TAP)P as well as vitamin B₁₂ in the C₁₂NE-SDS two-phase system were studied. Table 3 showed the extraction results of these porphyrin compounds. Chemical structures of proto, TPP₄, TPP and T-4(TAP)P are also given below.

Chemical Structures of Porphyrin Compounds

Table 3 Degrees of extraction of porphyrin compounds by the C₁₂NE-SDS two-phase system

<table>
<thead>
<tr>
<th>Porphyrin Compounds</th>
<th>Added, mmol</th>
<th>E%</th>
<th>R%</th>
</tr>
</thead>
<tbody>
<tr>
<td>proto*</td>
<td>1.90×10⁻⁴</td>
<td>98.2±0.2</td>
<td>98.1±4.1</td>
</tr>
<tr>
<td></td>
<td>1.90×10⁻⁵</td>
<td>97.4±0.7</td>
<td>95.9±1.6</td>
</tr>
<tr>
<td></td>
<td>1.90×10⁻⁶</td>
<td>94.5±0.5</td>
<td>99.0±2.1</td>
</tr>
<tr>
<td>TPP</td>
<td>4.99×10⁻⁴</td>
<td>93.4±0.4</td>
<td>92.2±3.4</td>
</tr>
<tr>
<td>TPP**</td>
<td>1.17×10⁻⁵</td>
<td>39.8±4.2</td>
<td>94.2±5.5</td>
</tr>
<tr>
<td>T-4(TAP)P*</td>
<td>7.09×10⁻⁵</td>
<td>33.8±2.0</td>
<td>91.6±2.0</td>
</tr>
<tr>
<td>VB₁₂</td>
<td>1.17×10⁻⁴</td>
<td>48.4±3.1</td>
<td>101.4±1.9</td>
</tr>
</tbody>
</table>

E and R refer to the extraction efficiency and recovery, respectively;

E%=(C₁/C₀)×100%,
R%=(C₂/C₀)×100%,
t, b and a refer to the top, bottom phase and the total amounts added, respectively.

As to be expected, carboxylic or sulfonic acid group substituted porphyrins proto and TPP₄ were extracted into the top phase with extraction efficiencies higher than 90%, while phenyl or
trimethylammonio group substituted porphyrins TPP and T-4(TAP)P as well as VB12 were extracted into the bottom phase with extraction efficiencies about 60%. This is not surprising because proto and TPPS4 could dissociate as negatively charged porphyrin species and thus extracted into the top phase effectively; while TPP has no net charge and T-4(TAP)P being positively charged compared with proto and TPPS4 and thus not be extracted efficiently.

Table 4 Degrees of extraction of various amounts of TPPS4 at different pH* by the C12NE-SDS two-phase system

<table>
<thead>
<tr>
<th>TPPS4 added, nmol</th>
<th>pH</th>
<th>E%</th>
<th>R%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.14x10^3</td>
<td>5.5</td>
<td>94.0±0.9</td>
<td>94.9±5.4</td>
</tr>
<tr>
<td>4.99x10^4</td>
<td>5.5</td>
<td>94.3±0.4</td>
<td>92.2±3.4</td>
</tr>
<tr>
<td>9.24x10^5</td>
<td>5.5</td>
<td>92.9±0.9</td>
<td>97.2±3.4</td>
</tr>
<tr>
<td>1.53x10^6</td>
<td>1.1</td>
<td>96.9±0.1**</td>
<td>—</td>
</tr>
<tr>
<td>9.60x10^6</td>
<td>4.0</td>
<td>94.5±1.0</td>
<td>93.5±3.0</td>
</tr>
<tr>
<td>9.06x10^6</td>
<td>8.8</td>
<td>98.8±0.0</td>
<td>97.8±0.6</td>
</tr>
<tr>
<td>1.53x10^5</td>
<td>12.8</td>
<td>95.5±0.2</td>
<td>98.7±0.5</td>
</tr>
</tbody>
</table>

* pH value of the TPPS4 solution added
** E% was calculated as (1-Cw/Vs/Cw) x100% because the top phase was separated into two phases spontaneously in strong acid solution.

Table 4 showed the extraction results of TPPS4 with the C12NE-SDS two-phase system at different pH and different TPPS4 concentrations. Although wide pH range and different amounts of TPPS4 solution were studied, higher than 92% extraction efficiencies were obtained in all cases, indicating the effective extraction ability of the system. Another interesting phenomenon is that the color change of TPPS4 in the extraction process. The color of TPPS4 in the top phase at different pH solutions (note that the pH is the value in TPPS4 solution not in the top phase) are always pink, the color of negatively ion of TPPS4, while the interfacial boundary at high TPPS4 concentrations and low pH values showed greenish, the color of the acid type of TPPS4, and the bottom phase is always colorless. This is coincide with our hypothesis that negatively charged porphyrins could be well extracted into the top phase. If the TPPS4 in the interfacial boundary is included the extractions would be perfect.

In conclusion, we proposed a new and effective aqueous two-phase extraction system for porphyrin compounds. The system composed of different charged surfactant mixture, charge interaction would be the predominant factor that governing the extraction. Although the two phases are of mixed cationic-anionic micellar solutions the top phase is more hydrophobic, hence, hydrophobic force also play an important role in the extraction.

This work was supported by the National Natural Science Foundation of China (Grant No. 29509037).

References