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# PRESSURE-ANESTHETIC ANTAGONISM ON THE PHASE SEPARATION OF NON-IONIC SURFACTANT MICELLES

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## Summary

An aqueous solution of non-ionic surfactants becomes suddenly turbid when heated to a critical temperature, known as the cloud point, and concomitantly expands the volume. The volume expansion is caused by release of structured water molecules from the hydrophilic polyoxyethyelene moieties. Inhalation anesthetics decreased the cloud-point temperature of hexaoxyethylene dodecyl ether micelles. The concentrations of methoxyflurane, halothane and enflurane causing a 1°C depression of the cloud-point temperature were 0.51, 0.71 and 0.78 mmolal, respectively. Hydrostatic pressure increased the cloud-point temperature in the absence and presence of the anesthetics. The change of the apparent molal volume at the cloud point was estimated to be 2.2 $cm^3$/mol in the absence of anesthetics. This value decreased in the presence of the anesthetics, dose dependently. The results indicate that the anesthetics favor dehydration of the hydrophilic surface of the non-ionic surfactant micelles.

## Introduction

It is generally recognized that anesthetics dilate, fluidize and disorder cell membranes [1]. It is also known that the actions of anesthetics are usually antagonized by a pressure of 100—150 bar [2]. Recently, several investigations have been reported about the combined effects of pressure and anesthetics

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upon the phase transition of phospholipid bilayers [3–6]. Anesthetics decrease and pressure increases the phase-transition temperature.

These observations lead to the conclusion that anesthetics fluidize the lipid core of the phospholipid bilayers by their direct action on the lipid tail of the phospholipid molecules to form *gauche* rotations, and concomitantly increase the volume, i.e., the volume expansion and fluidization are indivisible properties.

Although the change of the partial molal volume of anesthetics may contribute to the volume expansion, it is generally ignored because the concentration of anesthetics is smaller than that of the membrane molecules. The slope of the lines between the phase-transition temperature vs. pressure plot reported by Kamaya et al. [5] among different concentrations of the anesthetics was almost the same within the limits of the experiment. This suggests that the contribution of the change of the partial molal volume of anesthetic molecules may be minor in the membrane expansion.

The interfacial actions of anesthetic molecules have been grossly ignored except by Shieh et al. [7] who reported that the inhalation anesthetics did not penetrate deep into the core of phospholipid bilayers at clinical concentrations. Eyring et al. [8] and Ueda et al. [9] emphasized that the change of the interfacial water structure should be the main cause of the volume expansion related to the pressure reversal of anesthesia.

The present study deals with the interaction between the non-ionic surfactant micelles and inhalation anesthetics. The antagonizing effect of high pressure upon the anesthetic action is also studied. An aqueous solution of non-ionic surfactants becomes suddenly turbid on being heated to a critical temperature, known as the cloud point, and the solution separates into two phases. This phase separation is caused by the partial release of interfacial water molecules, hydrogen-bonded to the ether oxygens of the hydrophilic polyoxyethylene group [10,11]. The dehydration is accompanied by an increase in the partial molar volume of the surfactants [12], because the volume of the structured water molecules hydrogen-bonded to a surface is smaller than that of the bulk water (see, for instance, Ref. 14). It has been reported [12,13] that high pressures increase the cloud-point temperature.

It will be shown that inhalation anesthetics (halothane, methoxyflurane and enflurane) decrease the cloud-point temperature of an aqueous solution of the homogeneous non-ionic surfactant, hexaoxyethylene dodecyl ether, and increase the apparent molal volume of the surfactant.

## Materials and Methods

Homogeneous hexaoxyethylene dodecyl ether was obtained from Nikko Chemicals (Tokyo, Japan). Its purity was checked by gas chromatography and found to show a single peak. Inhalation anesthetics, halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether), and enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) were gifts from Ayerst Laboratories (New York, NY), Abbott Laboratories (North Chicago, IL), and Ohio Medical Products (Madison, WI), respectively. Water was purified by triple distillation, once from alkaline $KMnO_4$

solution. The surfactant and anesthetics were dissolved in water and their concentrations were checked by weighing.

The cloud points of non-ionic surfactant solutions under high pressure were determined by a Hitachi 139 ultraviolet-visible spectrophotometer with the high-pressure cell with sapphire windows, which was described in the previous study [5]. The cuvette temperature was monitored by a thermistor probe inserted into the body of the cell block close to the cuvette compartment. The calibration curves for the difference between the temperature inside the cuvette compartment and that of the thermistor probe were constructed during heating and cooling, and the observed temperature was corrected for the difference.

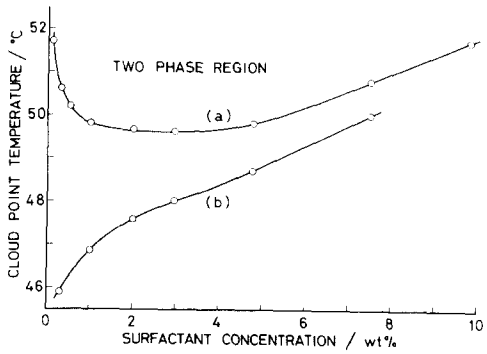
The solutions were heated or cooled slowly (0.5°C per min). The rates of heating and cooling did not affect the cloud-point temperature. The cloud points were measured by observing the appearance and disappearance of turbidity during the course of heating and cooling at a given pressure. The transmittance at 528 nm was recorded together with the temperature on an $x$-$y$ recorder. The differences between the temperatures of the cloud point determined on heating and on cooling were within 0.5°C.

The densities of non-ionic surfactant solutions without or with anesthetics were measured as a function of temperature by using Pyrex glass pycnometers with a capacity of about 10 $cm^3$. The pycnometers were immersed in a water bath to equilibrate at the desired temperature. The four sides and the bottom of the water bath were covered by thick (5 cm) Styrofoam panel and the water surface was covered by floating Styrofoam beads. The temperature of the bath was controlled by a Haake temperature controller-mixer to an accuracy of ±0.01°C. An additional mixer was inserted into the water bath. Heavy insulation of the water bath with a floating surface insulator and vigorous mixing were essential for the maintenance of constant temperatures. After equilibration for 30 min at the desired temperature, the pycnometers were fished out of the water bath, wiped dry and the weight was measured by a Mettler analytical balance. The error in measurements was within $\pm 2 \cdot 10^{-5}$ $g/cm^3$.

## Results

The cloud points of aqueous hexaoxyethylene dodecyl ether solutions are shown as a function of surfactant concentration in Fig. 1. The cloud-point temperature of a 1.0% (w/w) solution was 49.8°C, which is in good agreement with the value in the literature [11]. Note that curve a in Fig. 1 is a part of the phase diagram representing the mutual solubility which has a lower (minimum) critical solution temperature. In the region above the curve of the cloud points, the surfactant solution no longer exists as micelles, and the surfactant molecules are segregated from the aqueous phase and the whole system separates into two phases; one phase is an aqueous phase containing a small amount of singly dispersed surfactant monomer and the other is a surfactant phase containing dissolved water and floating upon the aqueous phase [10—13].

In the presence of methoxyflurane at $1.5 \cdot 10^{-3}$ molal, the cloud points are depressed (Fig. 1). The depression of cloud-point temperature depends on the



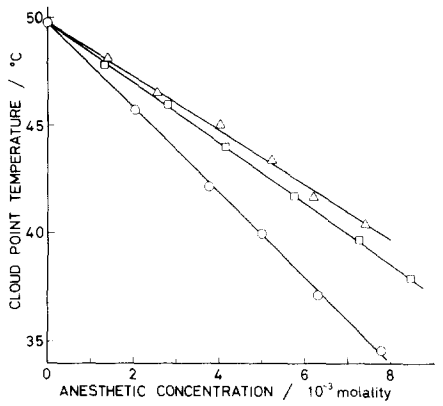




Fig. 1. The cloud points of aqueous hexaoxyethylene dodecyl ether solutions as a function of surfactant concentration. (a) Without anesthetic; (b) with methoxyflurane, $1.5 \cdot 10^{-3}$ molal.

Fig. 2. The depression of cloud-point temperature by various anesthetics. The concentration of non-ionic surfactant solutions remains constant at 1.0% (w/w). ○, methoxyflurane; □, halothane; △, enflurane.

concentration of the surfactant. The minimum temperature in the cloud-point diagram is shifted towards the lower or higher concentration of the surfactant in the presence of the third component, depending upon its physical property [15]. In the present case of anesthetics, the minimum temperature in the cloud-point diagram is shifted to the lower concentration (Fig. 1, curve b) but the upward turn of curve b in the lower concentration range was not detectable due to the difficulty of observing the turbidity change at extremely low concentrations of the surfactant.

Further experiments with the anesthetics were performed with the surfactant concentration at 1.0% (w/w). This concentration was arbitrarily chosen because most of the reports on experiments with non-ionic surfactants were performed at this concentration.

Effects of the anesthetics on the cloud point are shown in Fig. 2. The cloud-point temperature decreased linearly with the increase in the concentration of each anesthetic in the range studied. The depression of the cloud-point temperature is in the order: methoxyflurane > halothane > enflurane.

The application of hydrostatic pressure non-linearly elevated the cloud-point temperature both in the absence and the presence of the anesthetics (Fig. 3). The slopes of the cloud-point temperature vs. pressure curves were dependent upon the anesthetic concentration; the higher the concentration of anesthetics, the steeper the slope at atmospheric pressure. The cloud-point temperature was depressed linearly with the increase in the concentration of anesthetics at any pressure studied (Fig. 4).

We determined the apparent molal volumes of surfactant at temperatures below and above the cloud point. The apparent molal volume, $V_\phi$, of the solute was calculated from the expression:

$$V_\phi = \frac{1000}{m}\left(\frac{1}{d} - \frac{1}{d_0}\right) + \frac{M}{d} \qquad (1)$$

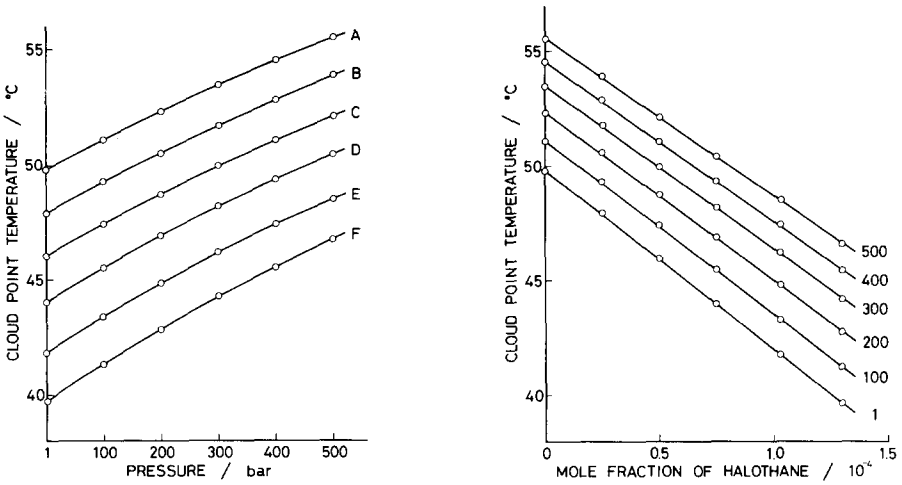



Fig. 3. The effect of pressure on the cloud-point temperature of non-ionic surfactant solution (1.0%, w/w) containing various amounts of halothane. Concentration of halothane: (A) without halothane; (B) with halothane at $1.41 \cdot 10^{-3}$, (C) $2.79 \cdot 10^{-3}$, (D) $4.15 \cdot 10^{-3}$, (E) $5.73 \cdot 10^{-3}$ and (F) $7.24 \cdot 10^{-3}$ molal.

Fig. 4. The depression of cloud-point temperature as a function of the concentration of halothane at various pressures, replotted from Fig. 3. The concentration of halothane is expressed as the mole fraction in order to fit the data to Eqns. 2 and 3. Numerical values refer to the pressure in bar.

where $m$ is the molality, $M$ is the molecular weight of the solute, $d$ and $d_0$ are the densities of the solution and solvent, respectively. The density of water at various temperatures was taken from the literature [16]. The apparent molal volumes of the surfactant without anesthetic are plotted against temperature in Fig. 5. $V_\phi$ increased linearly with temperature both below and above the cloud point. The reported value of $V_\phi$ for hexaoxyethylene dodecyl

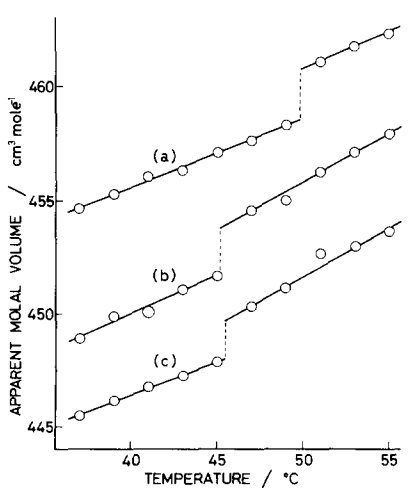



Fig. 5. The apparent molal volume of non-ionic surfactant as a function of temperature. (A) Without anesthetic; (B) with methoxyflurane at $2.4 \cdot 10^{-3}$ molal; (C) with enflurane at $3.5 \cdot 10^{-3}$ molal.

ether at 25°C is 451.4 $cm^3$/mol [17], which is in agreement with the value extrapolated in the present result. $V_\phi$ changes abruptly at the cloud-point temperature. The change of the apparent molal volume at the cloud point was estimated to be 2.2 $cm^3$/mol.

In order to obtain the apparent molal volume of surfactant in the presence of anesthetics, it is necessary to use the density of the micellar solution with solubilized anesthetics and that of the aqueous anesthetic solution for $d$ and $d_o$, respectively, in Eqn. 1. Since anesthetics are dissolved mainly in the micelle domain and are sparingly soluble in water, it is impossible to obtain density data on the aqueous anesthetic solutions with the desired concentration. Therefore, we used the density of water instead of the aqueous anesthetic solution for $d_o$. In Fig. 5, the absolute values of $V_\phi$ in the presence of anesthetics are not correct, but the change of apparent molal volumes at the cloud-point temperature represents the true value.

## Discussion

At the cloud point of surfactant solution with solubilized anesthetics, two phases are in equilibrium. In the three-component two-phase system. the Clausius-Clapeyron equation is written as follows:

$$\left(\frac{\partial T}{\partial P}\right)_{x_2,x_3} = \frac{y_1 \Delta\bar{V}_1 + y_2 \Delta\bar{V}_2 + y_3 \Delta\bar{V}_3}{y_1 \Delta\bar{S}_1 + y_2 \Delta\bar{S}_2 + y_3 \Delta\bar{S}_3} \tag{2}$$

where the subscripts refer to the components, and $x$ and $y$ are the mole fraction in phases $\alpha$ and $\beta$, respectively. $\Delta\bar{V}$ and $\Delta\bar{S}$ are the changes of partial molal volume and entropy, respectively, on the transfer of the solute from phase $\alpha$ to $\beta$. In the present system, we define surfactant as component 2, anesthetics as component 3, and the micellar solution as phase $\alpha$.

Without anesthetics, Eqn. 2 becomes:

$$\left(\frac{\partial T}{\partial P}\right)_{x_2} = \frac{y_1 \Delta\bar{V}_1 + y_2 \Delta\bar{V}_2}{y_1 \Delta\bar{S}_1 + y_2 \Delta\bar{S}_2} \tag{3}$$

The left-hand side of Eqns. 2 and 3 can be determined from the slopes in Fig. 3 and is shown in Fig. 6 as a function of concentration of the anesthetics. The elevation of the cloud-point temperature by pressure becomes linearly greater as the concentration of anesthetics increases. The increment of $(\partial T/\partial P)$ at 1 bar with anesthetics is in the order: enflurane > halothane > methoxyflurane, which is in the reverse order compared to the depression of cloud-point temperature by anesthetics. That is, the presence of the anesthetic, which lowers most effectively the cloud-point temperature, retards most effectively the elevation of the cloud-point temperature by pressure.

The numerator and denominator in the right-hand side of Eqns. 2 and 3 indicate the overall changes of volume ($\Delta V$) and entropy ($\Delta S$), respectively, at the cloud point. It is not usually possible to determine the partial molal quantities for each component. One can determine, however, the overall change of volume ($\Delta V$) from the apparent molal volume change at the cloud point, which is shown in Fig. 5. The volume changes, $\Delta V$, are listed in Table I

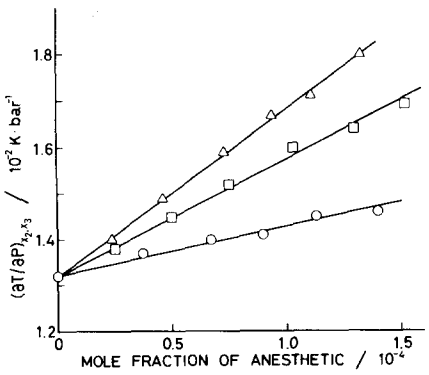



Fig. 6. Increment of cloud-point temperature increased by pressure, $(\partial T/\partial P)_{x_2,x_3}$, as a function of anesthetic concentration. ○, methoxyflurane; □, halothane; △, enflurane.

together with $(\partial T/\partial P)_{x_2}$ or $(\partial T/\partial P)_{x_2,x_3}$ taken from Fig. 6, and $\Delta S$ calculated from Eqn. 2 or 3. Both $\Delta V$ and $\Delta S$ are positive.

In the micelle, the hydrophilic oxyethylene groups are oriented outwards and the hydrocarbon tail groups are oriented inwards in aqueous solution. The partial molal volume of the hydrated polyoxyethylene group is known to be lower than that of pure polyoxyethylene in the liquid state by 2.0–2.5 cm$^3$ per oxyethylene unit [17]. The negative volume change for the transfer of the oxyethylene chain from its liquid state into water is attributable mainly to hydrogen bond formation between ether oxygens in the poly(oxyethylene) chain and water molecules [10—13]. The positive volume change shown in Table I, therefore, is mainly caused by the dehydration of the polyoxyethylene chain due to the hydrogen bond breaking. The positive volume change is also caused by the elimination of hydrocarbon-water contact. But the contribution of the hydrophobic hydration to the volume change at the cloud point is considered to be minor [12,15].

It is obvious from Table I that the volume change is reduced by the presence of anesthetics. Since the partial dehydration of the hydrophilic group is the major change which takes place at the cloud point, the reduction of $\Delta V$ and the depression of the cloud-point temperature by anesthetics indicate that the association of the anesthetics to the surfactant micelles favors the release of bound water at the interface. That is, the hydrogen-bonded water, which should be released at the cloud point, is likely to be partly released in advance by the presence of anesthetic.

TABLE I

THERMODYNAMIC PARAMETERS ON THE CLOUD POINT AND ANESTHETIC EFFECT

| Anesthetic concentration (molal)($\times 10^{-3}$) | $(\partial T/\partial P)_{x_2}$ or $(\partial T/\partial P)_{x_2,x_3}$ (K · bar$^{-1}$) ($\times 10^{-2}$) | $\Delta V$ (cm$^3$ · mol$^{-1}$) | $\Delta S$ (J · K$^{-1}$ mol$^{-1}$) |
|---|---|---|---|
| Without anesthetic | 1.32 | 2.2 | 16.9 |
| Methoxyflurane (2.4) | 1.37 | 2.0 | 14.8 |
| Enflurane (3.5) | 1.53 | 1.7 | 11.3 |

The concentrations of methoxyflurane, halothane and enflurane which depressed the cloud-point temperature by 1°C were 0.51, 0.71 and 0.78 mmolal, respectively. The partition coefficients of these anesthetics between the micelles and water are not available and an accurate comparison of these values with their clinical potency is not feasible at present. However, by assuming that the micelle/water partition coefficients are not too different from the partition coefficients between phospholipid vesicles and water, which are 57.9, 63.3 and 41.4 on the molality basis for methoxyflurane, halothane and enflurance, respectively (unpublished results), and using water/gas partition coefficients for methoxyflurane [18], halothane [18] and enflurane [19] of 4.8, 0.9 and 0.8, respectively, the corresponding partial pressures are calculated to be $0.15 \cdot 10^{-2}$, $1.08 \cdot 10^{-2}$ and $1.54 \cdot 10^{-2}$ bar, respectively. The clinical potencies (minimal alveolar tensions to induce anesthesia) for these anesthetics are listed [20] as $0.16 \cdot 10^{-2}$, $0.77 \cdot 10^{-2}$ and $1.68 \cdot 10^{-2}$ bar, respectively, for humans and $0.23 \cdot 10^{-2}$, $0.87 \cdot 10^{-2}$ and $2.2 \cdot 10^{-2}$ bar, respectively for dogs. The values for the depression of the cloud-point temperature are in reasonable agreement with these values.

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