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COMMUNICATION

Interaction of Iodine with 2-Hydroxypropyl-α-cyclodextrin and Its Bactericidal Activity

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ABSTRACT

To obtain an effective iodine solution, the use of 2-hydroxypropyl-α-cyclodextrin (2-HP-α-CD) as solubilizer was examined in comparison with α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), potassium iodide (KI), and polyvinylpyrrolidone (PVP). The stability constants for inclusion of iodine with cyclodextrin and KI were ascertained by the solubility method. The apparent stability constants increased in the following order: KI < β-CD < α-CD < 2-HP-α-CD. This order was nearly in accordance with that of the stabilization ability. The largest volatile depression effect was exhibited by 2HP-α-CD. The measurement of the minimum inhibitory concentration (MC) using Escherichia coli NIH-J-2 and Staphylococcus aureus FDA209P suggested that the bactericidal activity of the iodine/2-HP-α-CD system was the same as that of the iodine/α-CD, iodine/β-CD, and iodine/PVP systems. The present results suggest that the combination of 2-HP-α-CD and iodine is useful for a stable and effective iodine solution.

Key Words: *Antimicrobial activity; 2-Hydroxypropyl-α-cyclodextrin; Inclusion complex; Iodine; Solubilization; Volatility*

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INTRODUCTION

In recent years, the methicillin-resistant Staphylococcus aureus (MRSA) infection problem has become worse in the clinical field, and iodine is receiving considerable attention as an important bactericidal drug again. However, iodine has some impediments, such as strong odor, irritation to the wound, low water solubility, and high volatility. α-Cyclodextrin (α-CD) and β-cyclodextrin (β-CD) have been studied for the improvement of the pharmaceutical properties of iodine, e.g., solubility stability and bactericidal activity. A gargle containing iodine and β-CD is already commercially available in Japan.[1,2] However, the wide practical use of iodine with α-CD and β-CD in clinical fields has been limited because of their low solubility in water.[3,4] In the present study, the interactions of iodine with 2-hydroxypropyl-α-cyclodextrin (2-HP-α-CD) were studied in detail by the solubility method and nuclear magnetic resonance (NMR) spectroscopy. Furthermore, the volatile depression effect by 2-HP-α-CD and the resulting bactericidal activity were investigated in order to ultimately obtain an effective iodine solution.

METHODS

Materials

The 2-HP-α-CD (DS = 3.0), β-CD, and α-CD were supplied by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Polyvinylpyrrolidone K-30 (PVP) was purchased from Yoneyama Yakuhin Kogyo Ltd. (Osaka, Japan). A PVP–iodine solution, marketed as "Isojine® solution" by Meiji Seika Ltd. (Tokyo, Japan) was used without further treatment. Iodine and potassium iodide were of Japanese Pharmacopoea (JP XIII) grade. All other chemicals and solvents were of analytical reagent grade from various sources. Distilled water was used throughout the experiments.

Solubility Measurements

Iodine (3 mg, about 12×10^{-3} M) was added to water, α-CD solution (0.2 to 7.3×10^{-3} M), β-CD solution (0.5 to 4.0×10^{-3} M), 2-HP-α-CD solution (0.8 to 4.2×10^{-3} M), potassium iodide solution (1.8 to 7.6×10^{-1} M), or PVP solution (1.9 to 7.8×10^{-1} M) in a 1-mL measuring flask, respectively. They were sealed and shaken at 5°C. To achieve equilibrium, the sample solutions were ultrasonicated occasionally (power: 150 W). After equilibration (seven days), an aliquot was filtered with a membrane filter (0.45 μm). The concentration of iodine in the filtrate was determined by the titrimetric method using 0.02 mol/L sodium thiosulfate.

Determination of Stability Constants

The phase solubility diagrams were prepared by the method of Higuchi and Connors,[5] and an apparent 1:1 stability constant (K', M^{-1}) was calculated from the slope and intercept values of the initial straight-line portion of the solubility diagram, according to the following equation:

$$K' = \text{slope}/[\text{intercept} \times (1 - \text{slope})]$$

^{13}C-NMR Spectroscopic Study

This was done at 30°C using a JEOL GX-400 (JEOL, Tokyo, Japan); ^{13}C-chemical shifts were measured relative to external tetramethylsilane (TMS), within±0.008 ppm. Preparation of samples for the ^{13}C-NMR study: 3 mg iodine and 20 mg 2-HP-α-CD or α-CD were dissolved in 1 mL D_2O and filtered through a 0.4-μm membrane filter.

Evaluating the Prevention of Iodine Volatility

The α-CD, β-CD, 2-HP-α-CD, KI, or PVP were dissolved in a saturated solution of iodine (0.017 w/v %) at a molar ratio of 1:2 (I_2:additive), respectively. The mixtures were maintained under atmospheric pressure at 40°C, 50°C, or 60°C. At appropriate intervals, the remaining concentrations of iodine in the solution were determined according to the ultraviolet absorption method, using a Hitachi-U2000 Spectrophotometer (Tokyo, Japan) at 210 nm. Fresh air was circulated in the cuvette chamber through the silica gel layer.

Bactericidal Activities

At first, the iodine solution containing various CDs (α-CD, β-CD, 2-HP-α-CD) at a molar ratio of 1:2 was prepared at an active iodine concentration of 167.5 μg/mL. The active iodine concentration of Isojine® solution was also adjusted to 167.5 μg/mL

with sterile water. The diluted disinfectants (167.5–5 μg/mL) were prepared by serial twofold dilution with sterile water. Bactericidal activities were evaluated by *Escherichia coli* NIH-J-2 and *Staphylococcus aureus* FDA209P. The bacterial strains were cultured for 18 hr at 35°C on Brain Heart Infusion agar (Difco Lab., Detroit, MI). One microliter of bacterial suspension was mixed in each 100 μg/mL of disinfectant solution and then each 1 μL of the mixture after a contact time of 15 sec or 60 sec was inoculated on Brain Heart Infusion agar. The number of inoculated *E. coli* or *S. aureus* was 7.8×10^5 or 4.0×10^5, respectively. The bactericidal activity was defined as the lowest concentration preventing visible growth after incubation for 18 hr at 35°C.

RESULTS AND DISCUSSION

Solubility Studies

The formation of a complex between iodine and 2-HP-α-CD in aqueous solution was studied by the solubility method, and the solubility compared to those of iodine, iodine/α-CD, iodine/β-CD, iodine/KI, and iodine/PVP. Figure 1 shows the equilibrium phase solubility diagrams obtained for each system in distilled water at 5°C. The plots of the iodine/α-CD system and iodine/β-CD system revealed B_S-type solubility diagrams. A fine-dispersed precipitate was observed in both iodine/α-CD and iodine/β-CD systems. From the initial straight-line portion of the solubility diagrams, the K' values of the iodine/α-CD complex and the iodine/β-CD complex were estimated to be $1.36 \times 10^{-4}\,M^{-1}$ and $1.08 \times 10^{-3}\,M^{-1}$, respectively. In these calculations, we shall assume that I_2 is one molecule. It was reported that the hexakis(2,6-di-*o*-methyl)-α-cyclodextrin (DM-α-CD) included two iodines in its cavity.[6] Stability constants of iodine/α-CD and iodine/β-CD systems were also measured by Sanemasa et al.[7] Their results are a little lower than our results. This difference may be explained by the experimental error caused by the high volatility of iodine. The plot of the iodine/KI system shows an A_L-type solubility diagram. Its apparent stability constant K' was estimated to be $1.39 \times 10^2\,M^{-1}$ from the initial straight-line portion between 0 and 2×10^{-1} M of KI. The plot of the iodine/PVP system showed an A_N-type solubi-

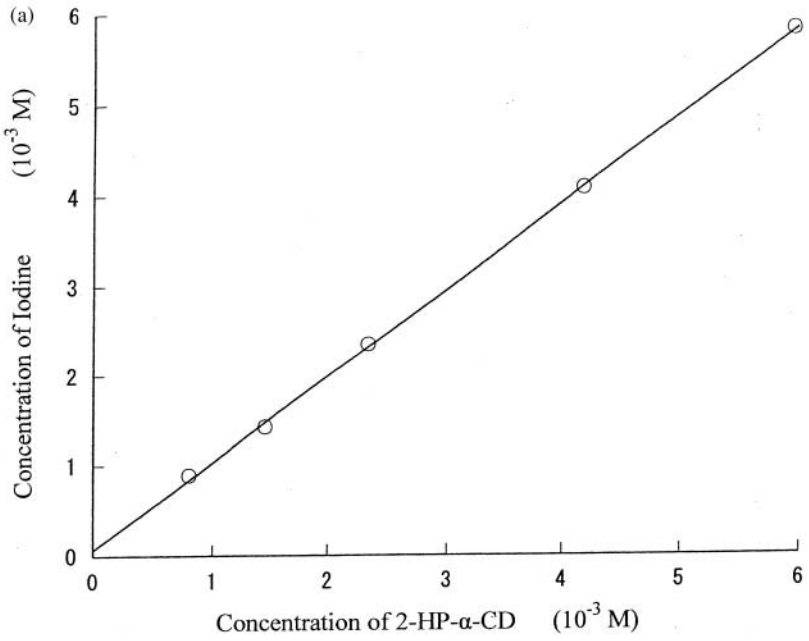


Figure 1. (a) Phase solubility diagram of iodine/2-HP-α-CD system in water at 5°C. ○: 2-HP-α-CD. (b) Phase solubility diagram of iodine:CD systems in water at 5°C. ▲: α-CD, ●: β-CD. (c) Phase solubility diagrams of iodine:KI and PVP systems in water at 5°C. △: KI, □: PVP.

(continued)

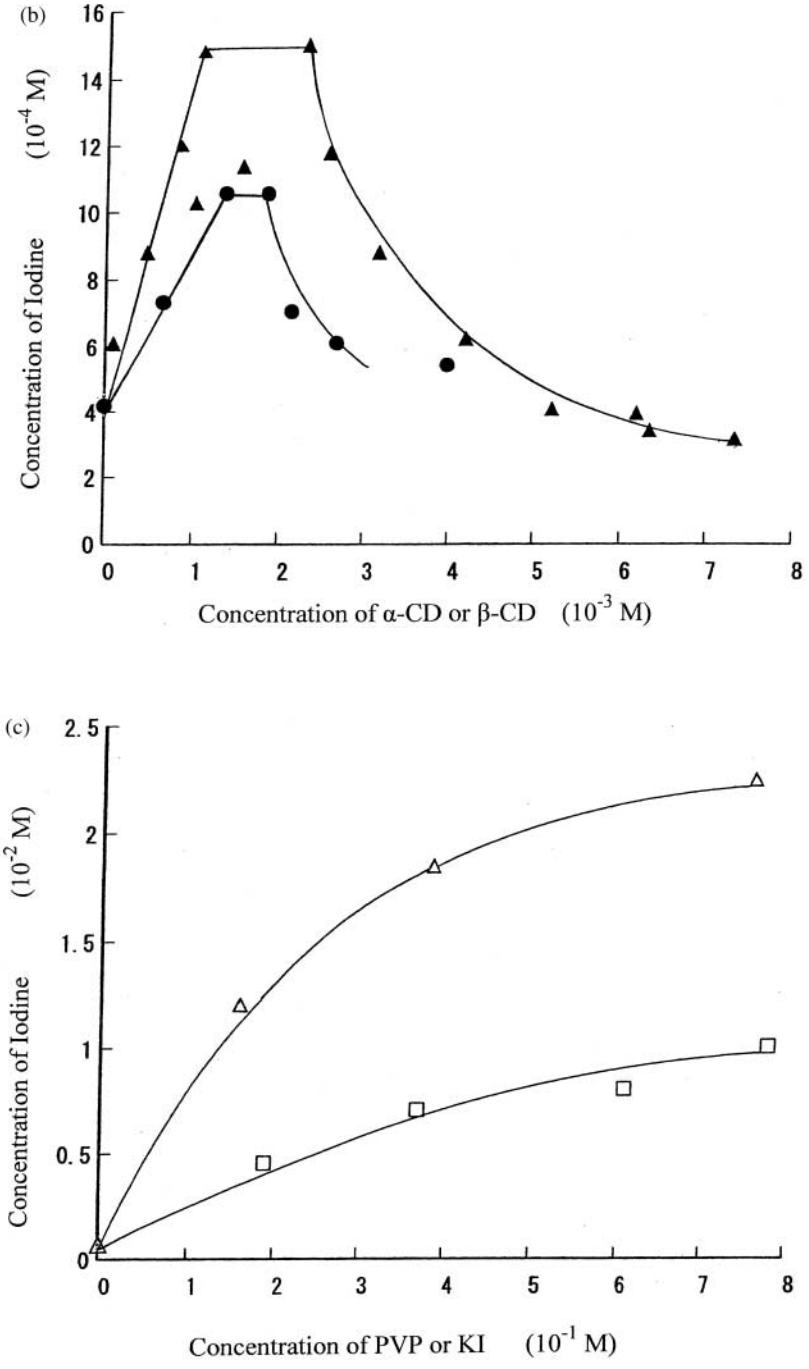


Figure 1. Continued.

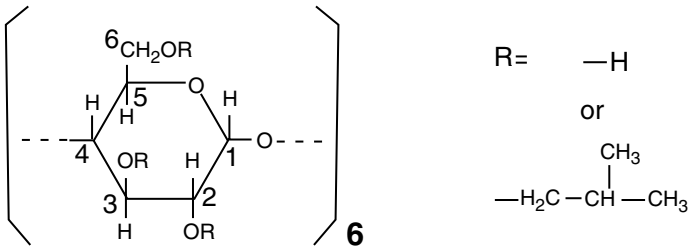
lity diagram. Its apparent stability constant K' was estimated to be $35.5\,M^{-1}$ from the initial straight-line portion between 0 and 2×10^{-1} M of PVP. However, the plot of the iodine/2-HP-α-CD system was a typical A_L-type solubility diagram. The slope gave an apparent stability constant of $7.70 \times 10^4\,M^{-1}$. This value showed the strong interaction between iodine and iodine/2-HP-α-CD. The saturated

Table 1

^{13}C-NMR Chemical Shifts of CDs in the Presence or Absence of Iodine

	α-CD		2-HP-α-CD	
Carbon	Without Iodine δ	With Iodine Δδ[a]	Without Iodine δ	With Iodine Δδ[a]
1	101.001	0.061	100.971	0.197
2	71.654	−0.009	71.630	0.015
3	72.920	0.076	72.920	0.182
4	80.828	−0.030	79.857	0.911
5	71.311	0.046	71.290	−0.009
6	60.049	−0.031	60.033	−0.091

[a]Chemical shift changes (ppm) expressed as $\Delta\delta=\delta_{complex}-\delta_0$.



concentration of iodine in distilled water was 0.08 mg/mL at room temperature. As shown in Fig. 1b, the aqueous saturated concentrations of iodine in the iodine/β-CD system and the iodine/α-CD system were 0.26 and 0.36 mg/mL at 5°C, respectively. The aqueous saturated concentration of iodine in the iodine/2-HP-α-CD system was 50 mg/mL with about 450 mg/mL 2-HP-α-CD at 5°C. The viscosity of the solution increased with increasing concentrations of 2-HP-α-CD. The preparation of a 200 mg/mL solution is possible laboratories, but rather impractical in the clinical field because of its high viscosity, like paste. However, the Isojine® solution with 7.5 mg/mL (0.075%) I_2 concentration has been widely used in the clinical field. This concentration is easily attained using about 35 mg/mL 2-HP-α-CD, and its viscosity becomes almost trivial for practical use.

^{13}C-NMR Study

Carbon-13 NMR chemical shifts of CDs in the absence and presence of iodine are summarized in Table 1. The 2-HP-α-CD (MS = 3) used was a mixture of α-CD randomly substituted by the 2-hydroxypropyl group. Here MS is the number of substitutes per α-CD molecule. The exact assignment of all ^{13}C-NMR spectra of 2-HP-α-CD was difficult, so the main spectra of 2-HP-α-CD (C_1–C_6) corresponding to the cyclic structure of α-CD could be assigned to discuss the interaction between 2-HP-α-CD and iodine. Table 1 shows the relatively large chemical shifts of 2-HP-α-CD at C_1 and C_4, which were used for binding to two glucopyranose units, in comparison with the chemical shift of other carbons. These differences might be caused by the difference in the state of α-(1,4)glucosidic linkage between intact 2-HP-α-CD and 2-HP-α-CD complexing with iodine. The ^{13}C-NMR measurements suggest that interaction of the iodine/2-HP-α-CD system was stronger than that of the iodine/α-CD system.

Evaluating the Prevention of Iodine Volatility

The volatilities of iodine from iodine/2-HP-α-CD, iodine/β-CD, iodine/KI, and iodine/PVP systems were measured at 60°C, 50°C, and 40°C,

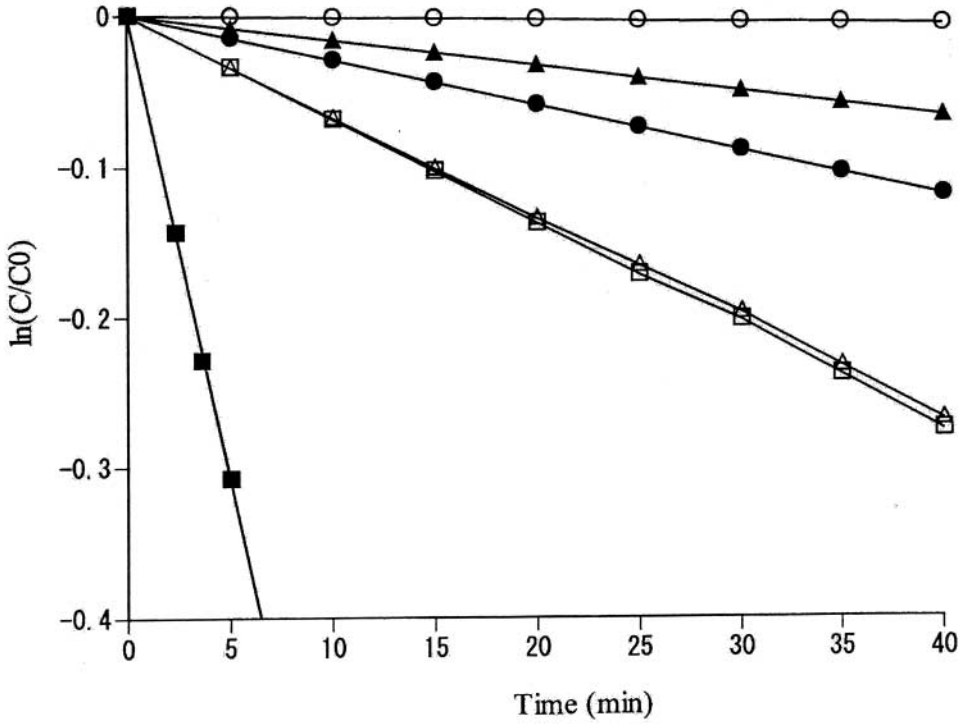


Figure 2. Loss of iodine at 40°C: alone (■) and in the presence of 2-HP-α-CD (○), α-CD (▲), β-CD (●), KI (△), and PVP (□).

respectively. Figure 2 shows that the decrease of iodine concentration by vaporization follows a first-order kinetic reaction, and the rate constants of iodine decrease from solution reduced in the order of iodine alone > PVP > KI > β-CD > α-CD > 2-HP-α-CD. This order was correlated with the degree of the stability constant. The iodine/2-HP-α-CD system was the most protected against loss of iodine from aqueous solution. Activation energies of iodine volatility were calculated from Arrhenius plots. The results are summarized in Table 2. The activation energy of iodine volatility of the iodine/2-HP-α-CD system was the largest in comparison with those of other systems. This large activation energy was consistent with the large volatile suppression of iodine from the solution.

Table 2

Comparison of Activation Energy of Iodine Evaporated from Each System at Solution and Apparent Stability Constant with CDs

System	Evaporate Energy (kJ/mol)	Apparent Stability Constant (M^{-1})
2-HP-α-CD	141.11	7.70×10^4
α-CD	33.44	1.36×10^4
β-CD	26.47	1.08×10^3
PVP	10.25	35.5
KI	25.87	1.39×10^2
Intact	4.51	—

Bactericidal Activities

Iodine and its other complex solution with 15-sec contact time did not show any bactericidal activities at all concentrations examined (even at the highest 167.5 μg/mL), so both *E. coli* NIHJ JC-2 and *S. aureus* FDA 209P colonies were visibly observed on the agar plate.

In 60-sec contact time, all test solutions contacting the lowest active iodic concentration 5 μg/mL inhibited *E. coli* NIHJ JC-2 growth. On the other hand, the bactericidal activities on *S. aureus* FDA 209P by test solutions showed some differences, as seen in

Table 3

Evaluation of Disinfectants on S. aureus *FDA 209P at 6-sec Contact Time*

	Concetration of Iodine (μg/mL)					
System	167.5	83	41	20	10	5
Iodine/α-CD	○	○	○	○	×	×
Iodine/2-HP-α-CD	○	○	○	○	○	×
Iodine/β-CD	○	○	○	○	○	×
Iodine/PVP	○	○	○	○	×	×

○: Colony not found visibly.
×: Colony found visibly.

Table 3. This result suggests that the bactericidal activities of iodine/2-HP-α-CD and iodine/α-CD were slightly superior to those of iodine/PVP (Isojine®) and iodine/α-CD. Approximately, iodine/PVP, iodine/α-CD, iodine/α-CD, and iodine/2-HP-α-CD appear to have almost the same bactericidal activities.

CONCLUSION

2-Hydroxypropyl-α-cyclodextrin forms a stable inclusion complex with iodine, and its aqueous solubility is about 100 times higher than those of other cyclodextrins with iodine. The iodine/2-HP-α-CD complex strongly prevents iodine volatility in the aqueous solution. Its ability was about 14 times larger than that of PVP. The sterilizing ability of the iodine/2-HP-α-CD complex is nearly equal to that of Isojine® liquid, by our bactericidal activities test. This property of 2-HP-α-CD is expected to give a better therapeutic effect. Therefore, the iodine/2-HP-α-CD complex should be useful as a disinfectant in pharmaceutical forms.

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