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Relationship between precipitation in aqueous solution of glutaraldehyde for chemosterilization and impurities detected by gas chromatography

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Summary

Glutaraldehyde (GA) has been used widely for chemosterilization of hospital instruments as a weakly alkaline aqueous solution (pH about 8). However, stains on instruments caused by white precipitates produced from some impurities in commercially available GA solution is a defect of this sterilant. The purity of GA solution has been expressed by the purification index (P.I.) which is defined as A_{235}/A_{280}, where the absorbances at 280 nm (A_{280}) and at 235 nm (A_{235}), are ascribed to GA and impurities such as α,β-unsaturated aldehyde polymers, respectively. The lower P.I. value implies, therefore, the higher purity. However, the formation of precipitates was found in a certain GA solution of low P.I. value. Using gas chromatography, we found a particular compound which may be responsible for the precipitation. This compound did not show a UV-absorption maximum at 235 nm. Accordingly, the conventional P.I. value does not always offer a reliable indication of the purity of GA solution. Gas chromatographic analysis of GA solutions showed the existence of some impurities which may be precursors of the precipitate.

Introduction

Over the past two decades, glutaraldehyde (GA) has been used for various purposes, such as the tanning of leather, fixation of living cells and tissues, cross-linking of proteins, immobilization of enzymes, and chemosterilization of hospital instruments (Hopwood, 1972; Gorman et al., 1980).

As a sterilant for hospital instruments, it has been recommended to use a 2% aqueous GA solution buffered at about pH 8 with sodium bicarbonate, since Stonehill et al. (1963) pointed out that an aqueous GA solution exhibited strong antimicrobial activity in a weakly alkaline condition. Under this condition, however, white precipitates frequently appear in commercially available GA solutions stored for a few days at room temperature. The stain caused by the precipitate on instruments is an impediment for its use, and GA solution has to be frequently renewed even before the loss of its sterilizing activity. The pre-

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cipitation is also disadvantageous in the other uses of GA solution mentioned above (Franke et al., 1969; Webster et al., 1964). However, GA solution purified by distillation or treated with activated carbon does not produce a precipitate. This suggests that the precipitation is caused by certain impurities, including polymers of GA in the aqueous GA solution.

Purification index (P.I.) defined as the ratio (A_{235}/A_{280}) of the absorbance of GA (at 280 nm) and that of impurities, α,β-unsaturated aldehydes (at 235 nm) (Anderson, 1967) has been an indication used for quality control (Dijk, et al., 1985). Accordingly, product of low P.I. value is regarded as GA solution of high purity. However, in our investigation of the impurities in the GA solution, we found that a commercial product of low P.I. value yielded white precipitate. Consequently, a need has arisen to elucidate the impurities responsible for the precipitation.

This paper deals with the search by gas chromatography for the impurity which correlates to the formation of the precipitation in a 2% weakly alkaline GA solution.

Materials and Methods

Materials

Aqueous stock solutions of GA were purchased from Union Carbide Co. (U.S.A., technical grade (50% w/v)), Ishidzu Pharmaceutical Co. Ltd. (Osaka, Japan, EM grade (25%)), Wako Pure Chemicals Ltd. (Osaka, Japan, reagent grade (25%) and EM grade (20%)), and Nakarai Chemicals Ltd. (Kyoto, Japan, reagent grade (25%)). These solutions were stored in a refrigerator. Other chemicals used were of analytical reagent grade. Alkaline aqueous 2% solutions of GA were prepared by diluting the stock solutions with 0.3% sodium bicarbonate followed by adjusting the pH of the solution to 8 by adding 1 N NaOH.

Gas chromatography

GC analysis of GA solution was performed with a Shimadzu (Kyoto, Japan) model GC-9A gas chromatograph equipped with a flame ionization detector and a cross-linked flexible capillary column (Ultra no. 2, 25 m × 0.31 mm i.d., film thickness d_f 0.52 μm) in a split mode. The operating conditions were as follows: injection temperature, 260 °C; carrier gas, N_2; average linear velocity, 23 cm/s; splitting ratio, ca. 1/50; chart speed, 5 mm/min; and oven temperature, 80 °C to 250 °C at 5 °C/min.

Determination of impurity was carried out using a Shimadzu GC, model 4CM under the following conditions: injection temperature, 240 °C; oven temperature, 210 °C; carrier gas, N_2; flow rate, 18 ml/min; internal standard, benzyl butyl phthalate (BBP); and column, a chemically bonded wide bore capillary column SE-54 (15 m × 0.53 mm i.d., d_f 2 μm) purchased from Gaskuro Kogyo (Tokyo, Japan).

Measurement of absorption spectra

Approximately 0.5% GA solutions were prepared by the dilution of stock solutions with distilled water, and their UV absorption spectra were recorded at 25 °C with a Hitachi (Tokyo, Japan) model 200-20 spectrophotometer by using a 1 cm quartz cell. Distilled water was used as a reference.

Measurement of turbidity

After the alkaline GA solution was kept at 40 °C for 8 days, the precipitate formed was crushed with a glass rod and dispersed by an ultrasonicator for 10 min. The turbidity of the solution was measured using a Nihon Seimitu Kogaku (Tokyo, Japan) model SET-PT-501D-S turbidimeter with an integrating sphere.

Measurement of metal contents

Contents of various metals were obtained using a Nippon Jarrel-Ash model ICAP-575 II inductively-coupled plasma emission spectrophotometer after dilution of commercial GA solutions to 2% level with distilled water.

Results and Discussion

Fig. 1 shows the time courses of the formation of the turbidity observed in different grades of commercial GA solutions. The turbidity values

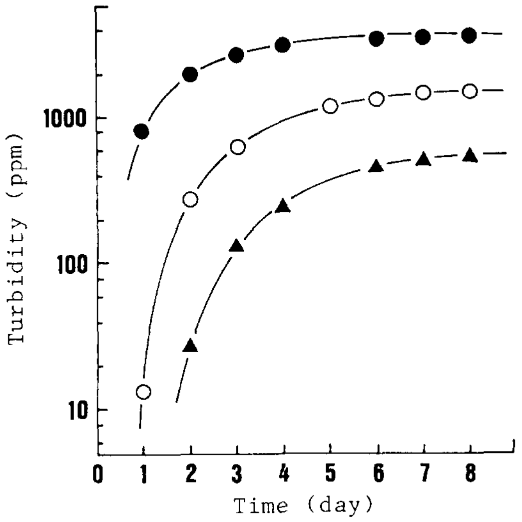

Fig. 1. Turbidity of various 2% alkaline glutaraldehyde solutions (pH 8) stored at 40 ° C for 8 days. ●, electron microscopy grade (Ishidzu); ○ and ▲, reagent grade (Nakarai and Wako).

increased rapidly during storage at pH 8 and 40 ° C for the first 3–4 days, followed by gradual approach to the individual maximum levels. The maximum turbidity observed after 8 days showed good linear correlation with the amount of the precipitates (Fig. 2) collected on a membrane filter of 0.45 μm pore size. In a 25% aqueous GA solution of pH 11.5, an aldol condensation reaction takes place to yield insoluble polymers called polyglutaraldehyde (poly-GA) (Rembaum et al., 1978). However, the present results suggest that certain impurities originally present in GA solution (e.g. polymers) rather than poly-GA formed in the solution are responsible for the formation of the precipitates, because the purified GA solution obtained by vacuum distillation (Hardy et al., 1972) did not produce any precipitates during storage in sterilizing condition (2% GA and pH 8) for 8 days and the rate of increase of turbidity found in Fig. 1 varied, depending on the quality of GA solution.

On the other hand, in a commercially available GA solution, impurities such as polymers of α,β-unsaturated aldehyde type are usually detected (Gillet et al., 1972). Fig. 3 shows the UV absorption spectra of two different grades of GA solutions, indicating that a reagent grade solution showed two absorption maxima at 280 and 235 nm, while a solution for electron microscopy (EM grade) showed one maximum at 280 nm. The

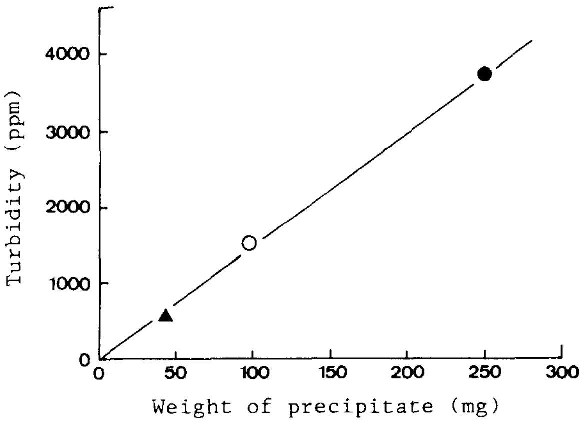

Fig. 2. The relationship between turbidity and amount of precipitate in 200 ml of the 2% alkaline aqueous glutaraldehyde solutions stored for 8 days at 40 ° C. The symbols (●, ○, ▲) correspond to those symbols in Fig. 1, respectively.

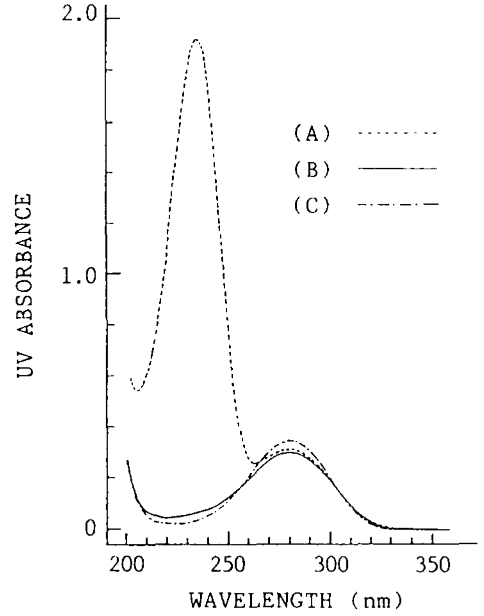

Fig. 3. Ultraviolet absorption spectra of commercially available aqueous solution of glutaraldehyde. A: reagent grade (Nakarai), B and C: electron microscopy grade (Wako and Ishidzu).

TABLE 1

P.I. value and turbidity of aqueous glutaraldehyde under alkaline condition (pH about 8)

Sample no. *	Turbidity (ppm)	P.I. value (A_{235}/A_{280})
1	2900	7.58
2	1525	6.06
3	462	4.79
4	1	0.21
5	3730	0.11

* Sources of glutaraldehyde solutions are as follows: No. 1, UCC (technical grade); No. 2, Nakarai (reagent grade); No. 3, Wako (reagent grade); No. 4, Wako (EM grade); No. 5, Ishidzu (EM grade).

maxima at 280 and 235 nm have been ascribed to GA monomer and α,β-unsaturated aldehydes, respectively (Robertson et al., 1970). Thus, the absorbance ratio A_{235}/A_{280} called the purification index (P.I.) has been regarded as an indication of purity of GA solution (Anderson, 1967). The GA solution of high P.I. value caused serious trouble on the fixation of tissues in histochemistry (Fahimi et al., 1968). These facts prompted us to investigate the relationship between turbidity and P.I. value of GA solution.

Table 1 shows the P.I. values of several stock solutions of GA and the turbidity of GA solutions diluted with carbonate buffer solution of pH 8. In samples No. 1–4, the turbidity decreased with the decrease of P.I. value, suggesting that the impurities showing UV absorption at 235 nm are responsible for the precipitation. On the contrary, sample No. 5 which exhibited the lowest P.I. showed the highest turbidity. In addition, this solution exhibited only one UV absorption maximum at 280 nm (curve C in Fig. 3). Therefore, sample No. 5 may contain the impurities having either absorption at around 280 nm or no UV absorption. Even if the impurities may have an absorption at 235 nm, their absorption coefficient should be very small. It follows, therefore, that the P.I. value does not always give proper information about the precipitation in an alkaline solution of GA. We attempted to elucidate the factors which cause precipitation.

The effect of metal impurities was investigated. Table 2 shows the contents of various metals in the GA solutions of samples No. 2, 4 and 5 in Table 1. The results in Table 1 exhibited the degree of turbidity in the order of sample No. 4 < 2 < 5. However, no apparent relation to the turbidity was found with metal elements. This result was further confirmed by the fact that the turbidity values were not changed by addition of EDTA to the solutions.

Then, we tried to detect the impurities by means of chromatography. The literature survey indicated that fractionation of GA solution by Sephadex G-10 column chromatography (Hopwood,

TABLE 2

Content of various metals in aqueous glutaraldehyde solution [a]

Metal species	A [b]	B [b]	C [b]	Metal species	A [b]	B [b]	C [b]
V	< 0.01	< 0.01	< 0.01	Mo	< 0.005	0.8	0.7
Ti	< 0.005	< 0.005	< 0.005	P	< 0.05	300	100
Al	0.07	< 0.05	0.2	Zn	< 0.002	0.09	0.07
Ca	0.2	50	2	Cd	0.05	0.04	< 0.002
Sr	< 0.0005	< 0.4	0.07	Ni	< 0.01	< 0.01	< 0.01
Ba	< 0.0005	< 0.03	0.07	Cu	< 0.005	< 0.005	< 0.005
Fe	0.04	< 0.002	0.05	Be	0.03	< 0.0005	< 0.0005
B	0.1	0.4	0.2	Co	< 0.01	0.3	< 0.01
Mn	< 0.001	0.02	< 0.001	Na	20	200	40
Cr	< 0.01	< 0.01	< 0.01	K	2	9	2
Mg	0.03	30	0.8	Pb	< 0.05	1	2

[a] Metal concentration was represented as ppm unit.

[b] Samples of A, B and C correspond to the samples No. 2, 4 and 5 in Table 1, respectively.

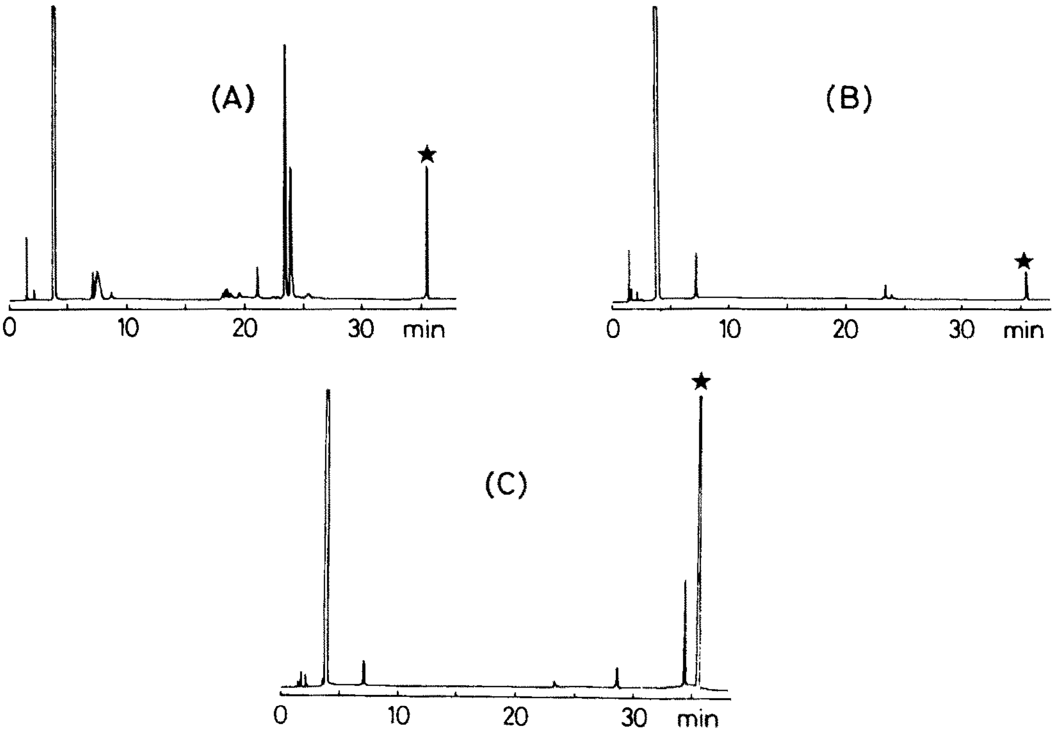

Fig. 4. Gas chromatograms of aqueous solutions of glutaraldehyde. A 0.5 μl of 20–25% GA solutions were directly injected into an injection port. A, B and C correspond to samples No. 2, 4 and 5 in Table 1, respectively.

1967) gave two fractions exhibiting absorption maxima at 280 and 235 nm. Robertson et al. (1970) carried out preparative GC analysis and found an impurity which was yellow and absorbed at 235 nm. By Blass et al. (1975), a GA solution was chromatographed on silica gel TLC and a few spots of impurities sensitive to aldehyde coloring test were detected. These chromatographic analyses seem insufficient for separation and specification of the impurities responsible for the precipitation. Thus, in order to detect as many impurities as possible, we performed capillary GC analysis giving high resolution and inertness. The gas chromatograms of samples No. 2, 4 and 5 in Table 1 are shown in Figs. 4A, B and C, respectively, where a peak with a retention time of 4 min is based on GA monomer because of its intense relative area and identical mass spectrum with published data (Stenhagen et al., 1974). Fig. 4 indicates that sample No. 2 in Table 1 contains more impurities than No. 4 and 5. However, the results in Table 1 indicate that sample No. 2 shows lower turbidity than sample No. 5. These observations of the chromatograms suggest that the turbidity correlates with the amount of the particular compound which exhibited an asterisked GC peak in Fig. 4 rather than the total amount of impurities.

We carried out a GC determination of the compound which showed a peak asterisked in Fig. 4 to confirm the correlation between the turbidity and its amount. Fig. 5 shows the resulting chromatogram, where the asterisked compound in Fig. 4 and the internal standard were eluted at retention times of 5 min (peak A) and 8 min (peak I.S.), respectively. The relative intensity of peak A was estimated as the peak area ratio (A/I.S.).

Fig. 6 shows that logarithmic value of the relative intensity and turbidity are correlated linearly. The good correlation implies that this particular compound may be responsible for the precipitation. However, it still remained uncertain that

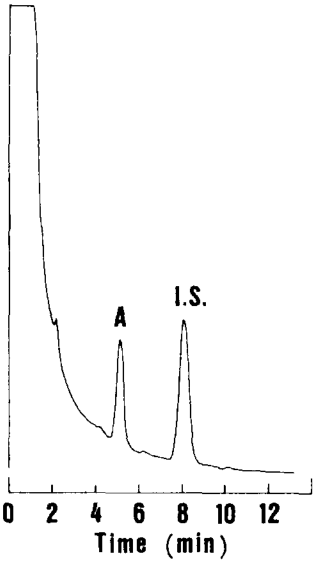

Fig. 5. The typical determination gas chromatogram of the asterisked compound in Fig. 4. Sample solution for determination was prepared by mixing an equal volume of a GA solution diluted to 5% level with distilled water with a 0.05% BBP methanol solution, and a 0.2 μl was injected into an injection port. This chromatogram was obtained by using the GA solution of reagent grade (Nakarai). A, the asterisked compound in Fig. 4; I.S., benzyl butyl phthalate as an internal standard.

only this component may be responsible for the formation of precipitates, since undetected species present in the same relative amounts as this compound may be responsible for the precipitation.

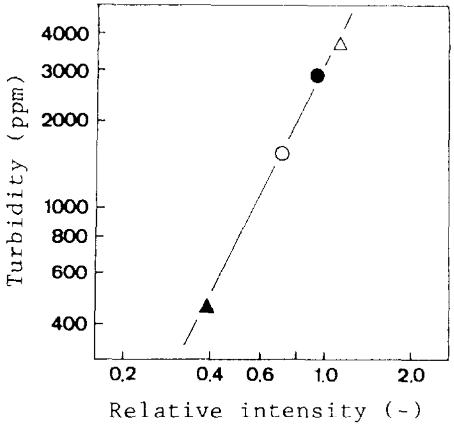

Fig. 6. Turbidity of aqueous solution vs relative intensity of asterisked compound in Fig. 4. The symbols (●, ○, ▲, △) correspond to samples No. 1, 2, 3 and 5 in Table 1, respectively.

Thus, we obtained the gas chromatograms of 2% alkaline GA solutions just after dissolution and after 8 days storage, which are shown in Fig. 7A and B, respectively. These chromatograms were obtained in a splitless mode (splitless time, 60 s) under the same GC conditions as those in Fig. 4. The samples were prepared by mixing the alkaline solutions of GA with the mixture of dioxane and acetone (1 : 1) in a ratio of 1 : 9, followed by filtration through a 0.45 μm membrane filter. The asterisked compound in Fig. 7A responsible for the precipitation was not found on the chromatogram after storage for 8 days at 40 ° C (Fig. 7B),

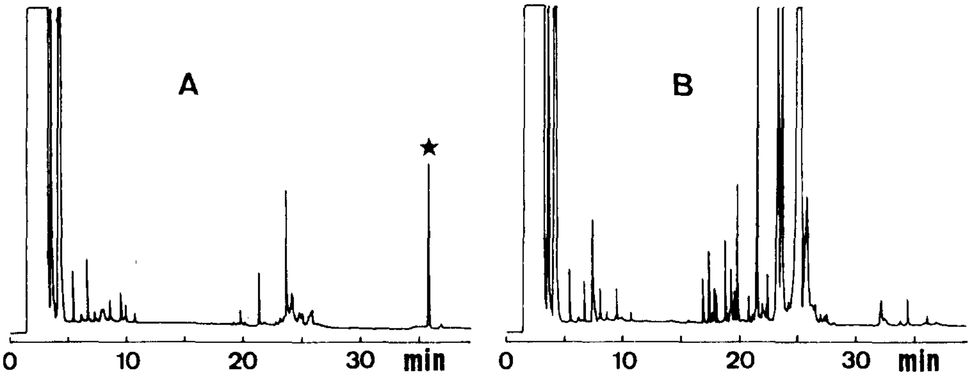

Fig. 7. Gas chromatograms of fresh and stored 2% alkaline glutaraldehyde solutions. A: immediately after preparation. B: stored for 8 days at 40 ° C. The GA solution of reagent grade (Nakarai) was used and a 0.8 μl of mixture sample solution was injected into an injection port.

whereas the disappearance of the asterisked peak was not observed in the original acidic GA solution under the same storage conditions. Thus, the compound detected as an impurity by GC analysis may be responsible for the precipitation in 2% alkaline aqueous GA solution. Preliminary investigatiion using chemical ionization GC-MS indicated molecular weight of this compound to be 300, suggesting the possibility of GA trimer. Isolation and detailed structural elucidation of this compound are now in progress.

The white precipitates were presumed to be composed of some polymers of α,β-unsatured aldehyde, because the infrared spectrum of the precipitate was similar to that of poly-GA (Margel et al., 1980). The precipitate mentioned above is presumed eventually to be the polymer formed by aldol condensation from the compound which showed a peak asterisked in Figs. 4 and 7.

The results presented here indicate that P.I. value obtained by UV absorption measurement does not always guarantee the quality of GA solution, but chromatography is a reliable alternative. Further investigation on other impurities of GA solution is now in progress.

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