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GAS CHROMATOGRAPHIC DETERMINATION OF GLUTARALDEHYDE

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SUMMARY

A specific, rapid, and precise gas chromatographic method is described for the analysis of glutaraldehyde. Solutions of glutaraldehyde are diluted to an appropriate level, internal standard is added, and the aliquots are injected directly onto a Carbowax 20M column. The procedure has been utilized on market products containing glutaraldehyde, and results have been compared for some samples with values obtained by published titrimetric methods. The assay is considered stability-indicating and a number of solutions subjected to stress conditions have been successfully analyzed.

INTRODUCTION

Over the past two decades glutaraldehyde has become an important compound in a number of different areas. Based on work by investigators such as Sabatini *et al.*[1], glutaraldehyde has become commonly used as a fixative agent for tissues being analyzed by electron microscopy. Its bifunctional nature has also been utilized successfully to cross-link various enzymes and proteins[2,3]. More recently, due to its excellent bactericidal and sporicidal properties, it has been marketed as a 2% aqueous solution (Cidex® and Sonacide®)** for use in the sterilization of hospital apparatus.

A need has arisen for specific analytical procedures for the determination of glutaraldehyde in bactericidal solutions since these products are now subject to tight controls on potency.

Presently, the most commonly used procedures involve titrimetric analysis. The method most frequently cited is a hydroxylamine titration[5]. Another assay uses the ability of glutaraldehyde to form an addition compound with bisulfite[6]. Both procedures have proved satisfactory, although somewhat time-consuming, for most samples encountered. However, with increased emphasis on stability-indicating methods and more interferences being discovered due to impurities, decomposition prod-

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** Cidex is marketed by Arbrook, Arlington, Texas, U.S.A. Sonacide is marketed by Ayerst Laboratories, New York, N.Y., U.S.A.

ucts and various additives, the requirement for a more specific method is obvious. The gas chromatographic (GC) procedure described offers a rapid and precise analysis for glutaraldehyde which is coupled with the inherent specificity of this technique.

EXPERIMENTAL

Equipment and assay conditions

A Bendix Model 2500 gas chromatograph equipped with an FID and an Autolab Systems IV peak integrator is used. The column is a 6 ft. × 4 mm I.D. glass U-tube packed with 7% Carbowax 20M coated on Chromosorb W HP, 80–100 mesh. Operation parameters: column temperature, 125°; inlet temperature, 150°; detector temperature, 200°; carrier gas, helium, 45 ml/min; sensitivity, $1 \cdot 10^{-9}$ A f.s.

Reagents and standards

The internal standard used is 2-(2-ethoxyethoxy)ethanol, also named diethylene glycol monoethyl ether, and is available from Aldrich (Milwaukee, Wisc., U.S.A.).

A commercial solution of glutaraldehyde may be used directly as a standard if it is fresh and there is reasonable confidence in the label claim. Dilute, if necessary, this solution quantitatively to a level of about 2% with distilled water.

Alternatively, a modification of the procedure described by Anderson[5] can also be used to obtain a more accurate reference solution. Place about 400 ml of a commercial 25% glutaraldehyde solution into a 1000-ml beaker, and with stirring, add sodium chloride until the solution is saturated. Allow excess salt to settle and decant the liquid into a 1000-ml separatory funnel. Extract with four 200-ml portions of diethyl ether, combine extracts, and dry with anhydrous sodium sulfate. Transfer the liquid to a 1000-ml round-bottomed flask and evaporate off the diethyl ether on a rotary evaporator at room temperature.

The light yellow viscous liquid is then distilled on a Vigreux column using mechanical vacuum (approximately 8 mm Hg and a distillate temperature of 55–60°). The middle third fraction only is collected in a small flask immersed in an ice bath.

An accurately weighed 20-g sample of the collected fraction is then immediately transferred to a 1000-ml round-bottomed flask. (Weighing is done as soon as possible after collection since the pure glutaraldehyde polymerizes quite rapidly and forms a glass within a few days.) Distilled water (500 ml) is added to dissolve the glutaraldehyde. If a white precipitate is noticed, attach a condenser and reflux for a short period until the solution is clear. Cool and transfer the contents quantitatively to a 1000-ml volumetric flask and dilute to volume with distilled water. Refrigerate when not in use. Standards prepared in this manner should last at least 3–6 months.

Procedure

Samples with a claim of 2% are used as is. Higher-level samples are quantitatively diluted to the 2% level.

Pipet 1-, 2-, and 3-ml aliquots of glutaraldehyde reference standard solution and 2 ml of each sample solution into separate 10-ml volumetric flasks. Pipet 5 ml of a 1% aqueous solution of 2-(2-ethoxyethoxy)ethanol into each flask and then dilute to volume with distilled water.

Inject 2-μl aliquots of each standard solution and determine the ratio of glutaraldehyde (elutes first) area to internal standard area for each injection. Construct a standard curve by plotting the glutaraldehyde concentration (mg/ml) *versus* ratio of areas. Inject 2 μl of sample solution and determine ratios of the peaks. Determine the glutaraldehyde concentration (mg/ml) from the graph and multiply by appropriate dilution factors to obtain sample potency. *Note*: Samples with lower concentrations can also be analyzed as long as the standard curve brackets the sample concentrations.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of glutaraldehyde with internal standard added. The standard curve (Fig. 2) has been found to be linear over a broad range of concentrations. However, it does not generally pass through the origin, indicating a small amount of column adsorbtion. To minimize the effects of variations in adsorbtion and instrumental parameters, a standard curve should be determined daily.

It is generally agreed that the chemistry of aqueous glutaraldehyde is very complex. Whipple and Ruta[7] indicated that the structure of aqueous glutaraldehyde is primarily a cyclic hemihydrate. Others, such as Robertson and Schultz[8] and Frigerio and Shaw[6], indicate the presence of various polymers in commercial solutions. Despite the large number of papers on this subject, however, the absolute composition of aqueous glutaraldehyde has yet to be determined. In an effort to ascertain the species present and detected in our GC system, we subjected a commercial sample, which had assayed near claim, to GC–mass spectrometry. The normalized spectrum obtained from the glutaraldehyde peak (Fig. 3) showed a molecular ion of *m/e* 100, corresponding to the monomeric species. This might be expected, as any hydrates or low-molecular-weight polymers present would probably revert back to their dehydrated or monomeric form in the heated inlet. Although one might expect that the titrimetric procedure would not detect these non-monomeric species in undecomposed

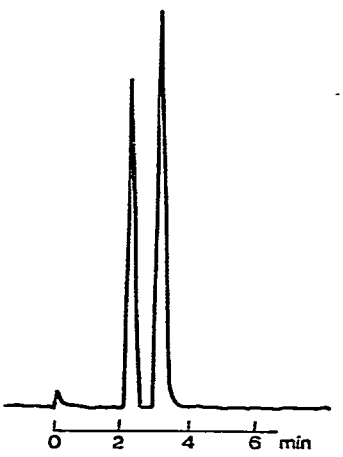

Fig. 1. Resolution of glutaraldehyde (elutes first) and internal standard on a Carbowax 20M column at 125° with a helium flow-rate of 45 ml/min.

Fig. 2. Typical standard curve plotting peak area ratio of glutaraldehyde to internal standard *vs.* glutaraldehyde concentration (mg/ml).

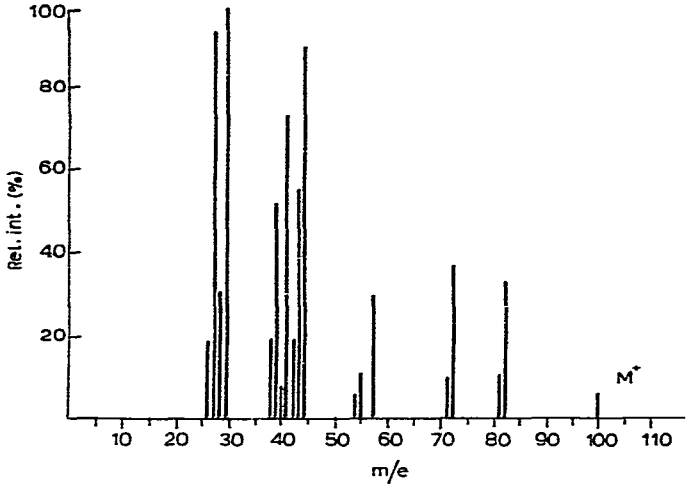

Fig. 3. Normalized mass spectrometric scan of glutaraldehyde peak showing weak M^+ ion at *m/e* 100.

solution, this was found not to be the case. The three procedures gave essentially equal results on the intact commercial solutions, as can be seen in Table I for the initial sample.

A comparison of method specificity was also made for decomposed commercial solutions. Several 25% samples containing glutaraldehyde were placed in sealed ampules and heated for 5, 21, 45 or 141 h in a steam bath. The samples were visibly decomposed. The 5-h solution had turned slightly yellow. The 21-h sample was still clear, but much yellower in color. The 45-h solution had turned a brownish color with a small amount of precipitate present. The 141-h sample was dark brown in appearance with a large amount of a tarlike precipitate present. These solutions, plus an unheated control, were quantitatively diluted to a level of 2% and analyzed by the GC and titration methods. Results are summarized in Table I.

TABLE I

COMPARATIVE ANALYSIS OF INTACT AND DEGRADED GLUTARALDEHYDE SOLUTIONS USING THREE METHODS

Solution	*Glutaraldehyde found (mg/ml)*		
	GC	*I_2 titration*	*Hydroxylamine titration*
Initial	20.7	20.8	21.4
5 h at 98°	19.2	20.1	21.2
21 h at 98°	17.7	19.4	20.0
45 h at 98°	15.0	17.3	18.6
141 h at 98°	9.1	12.6	14.7

It can be seen that the GC procedure gives lower values for the decomposed samples, suggesting enhanced specificity. The hydroxylamine titration consistently gives the highest values, suggesting that it is less sensitive to molecular changes and produces a titer as long as aldehyde groups are still left intact. To verify this, a sample of the tarlike precipitate was isolated and dissolved in tetrahydrofuran (THF) at a concentration of 20 mg/ml and analyzed by the two methods.

GC analysis indicated that no free glutaraldehyde was present whereas the hydroxylamine titration gave a result of 6 mg/ml. This indicates that the decomposition products probably contain reactive aldehyde groups which render the titration methods non-specific for glutaraldehyde. Such an assumption supports work done by Richards and Knowles[3], who concluded that impurities found in commercial glutaraldehyde solutions were primarily α,β-unsaturated aldehydes, formed by aldol condensation reactions (Fig. 4). The decomposition product was also analyzed with other GC columns and conditions, both silylated and unsilylated. No peaks could be found, however, again indicating the presence of a high-molecular-weight, nonvolatile material such as the condensation product shown.

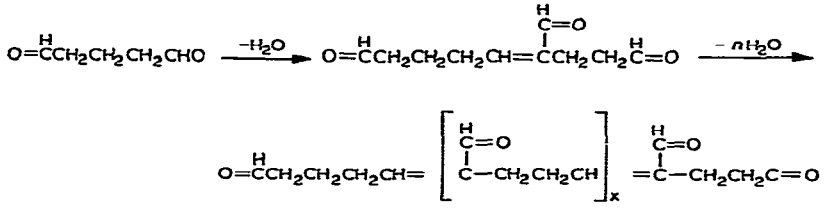

Fig. 4. Aldol condensation reaction of glutaraldehyde.

Nuclear magnetic resonance (NMR) analysis of the tarlike substance also supported the condensation product hypothesis. A sample of the decomposition product was dissolved in deuterated chloroform and scanned on a Varian EM-360 (60-MHz) spectrometer. A multiplet at 400 Hz indicates unsaturated protons. A triplet and multiplet at 570 and 600 Hz, respectively, suggest two types of aldehyde protons with different coupling constants corresponding to α,β-unsaturated and α,β-saturated carbonyl groups. Also present are very broad multiplets in the 100-Hz region, which are evidence for polymeric methylene protons.

IR analysis of a chloroform solution of the material gave a strong peak at 1720 cm^{-1}, which is indicative of aldehydes, and also a strong peak at 1680 cm^{-1}, which suggests the presence of α,β-unsaturated carbonyl groups.

For UV analysis, a solution of the decomposition product was made up in THF at a level of 18.6 μg/ml. It was scanned from 350 to 210 nm. Results showed a strong peak at 230 nm, corresponding to an $E^{1\%}_{1\,cm}$ of about 160. Peaks in this region are usually associated with impurities in commercial solutions of glutaraldehyde[8] and purity determinations have been based on the aqueous $A_{235/280}$ ratio. However, the peak due to glutaraldehyde at 280 nm has an extremely low $E^{1\%}_{1\,cm}$ of less than one. Therefore, even if the ratio of peaks at 235 and 280 nm in a commercial sample is equal to one, the amount of impurity actually present is still very small.

Since glutaric acid has been a suspected decomposition product of glutaraldehyde, the degraded sample was subjected to GC analysis. Solutions of standard glutaric acid and the decomposed sample were acidified to pH 1, extracted with methyl isobutyl ketone, concentrated and then reacted with diazomethane to form the methyl ester. Good peak shape was obtained for glutaric acid dimethyl ester on a Carbowax column at 140°, but no corresponding peak was found in the decomposed solution.

Table II indicates the precision of the GC method. Five 2-ml aliquots of a commercial 2% solution were analyzed on three separate days. Results show the good precision of the method.

TABLE II

RESULTS OF PRECISION STUDY SHOWING POTENCY OF A COMMERCIAL GLUTARALDEHYDE SOLUTION (2%) RUN ON THREE SEPARATE DAYS

Glutaraldehyde (mg/ml)		
Day 1	*Day 2*	*Day 3*
20.7	20.4	20.4
20.6	20.3	20.5
20.5	20.2	20.1
20.6	20.5	20.3
20.7	20.3	20.0
Average = 20.4		
S.D. = 0.21		
C.V. = 1.02%		

A GC method for the analysis of glutaraldehyde has been presented. It offers a simple and rapid alternative method to existing methods when interferences make these methods impractical. It has been shown to be accurate, precise and specific.

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