Measurement of formaldehyde concentrations in a
subatmospheric steam-formaldehyde autoclave

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SUMMARY A method has been developed for measuring formaldehyde concentrations in a sub-
atmospheric steam-formaldehyde autoclave. Data obtained using this method indicate that the
concentration of formaldehyde in the chamber atmosphere is not homogeneous and that it decreases
rapidly with time. The penetration of formaldehyde vapour into narrow tubes has also been
investigated and was shown to be dependent on the length-to-bore ratio of the tubes. The form-
aldehyde concentration within the tubes could be increased by using a lower vacuum in the air
removal stage at the beginning of the cycle.

Subatmospheric steam-formaldehyde (SASF) auto-
claves have over the past few years begun to be used
in increasing numbers in the United Kingdom and
in the Scandinavian countries. Assessment of the
efficiency of different commercial autoclaves cur-
rently in production has been hampered by the
paucity of detailed information on the conditions
required to ensure sterilisation. Given the present
state of knowledge, the SASF process should be
regarded as a chemical disinfection process which,
under optimal conditions, may be capable of
sterilising. The efficiency of a chemical disinfection
process depends on a number of factors such as the
type of organism to be destroyed, the temperature,
and the nature and concentration of the disinfectant.
One particular aspect of the SASF process that has
received little attention has been the fate of the
formaldehyde injected into the autoclave chamber
during a cycle.

When assessing the effect of changes in the con-
centration of disinfectant, it is necessary to determine
the concentration of disinfectant in its active form
which comes into direct contact with the organism.
A number of physical barriers may prevent access of
the disinfectant to the organism, for example,
packaging, organic or inorganic material surround-
ing the organism (blood, protein, salt crystals, soil),
air pockets, etc. Some substances may react chemi-
cally with the disinfectant, thus reducing the concen-
tration of active disinfectant available to destroy the
organism. Formaldehyde is known to polymerise,
and it seems unlikely that the polymers, particularly
insoluble ones, will be the active biocidal molecules.

One of the prime requirements in SASF autoclaves
is that the formalin solution used should be efficiently
vaporised. This is particularly important if the vapour
has to penetrate through packaging or into narrow
tubes such as catheters. Yet few reports indicate that
any attempt has been made to correlate the amount
of formalin injected into the autoclave with the
concentration of formaldehyde subsequently appearing
in the vapour or gaseous state within the
chamber. One is left with the impression that it is
simply taken for granted that all the formalin is
vapourised and remains so. Even if it is assumed that
all the formalin does in fact vaporise initially, there
are a number of factors that may alter this situation
inside the chamber of the autoclave. Condensation
of the vapour on cool surfaces would be the most
obvious factor. Another is the dissolution of gaseous
formaldehyde in water which is present as a result
of steam condensing in the early parts of the cycle
(when air is being removed and the load is still cool).
Weymes et al. (1975) have measured the relative
concentration of formaldehyde during SASF cycles
and found that a rapid decrease occurred initially.
The drop in formaldehyde concentration during the
sterilisation period was approximately exponential.

This paper describes a new method developed for
measuring formaldehyde concentrations in the
vapour or gaseous phase during SASF cycles. It also
presents data obtained using this method.

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Material and methods

APPARATUS
The autoclave used was a Miniclave 80 (Chas F. Thackray Ltd, Leeds) similar to the one described by Gibson (1977). The chamber volume is 0.022 m³. The model used incorporated a number of special modifications to permit more flexibility in the type of cycle chosen. For greater accuracy of measurement for experimental purposes, injection of formalin was made manually from a syringe directly into the steam generator block.

ASSAY OF FORMALDEHYDE IN CHAMBER EFFLUENT
A bleed in the chamber extraction valve allows condensate to be withdrawn continuously from the chamber. The effluent was collected via a coil condenser into a flask containing anhydrous sodium sulphite (2.4 g per ml of formalin injected into the autoclave). The flask was standing in ice. As the effluent was collected, it dissolved the sodium sulphite, allowing it to react with any formaldehyde present. After completion of effluent collection, the reaction mixture was titrated with standard (1 N) hydrochloric acid using thymolphthalein indicator (1 ml of 1 N hydrochloric acid is equivalent to 0.03003 g formaldehyde).

MEASUREMENT OF FORMALDEHYDE INSIDE AUTOCLAVE CHAMBER
To measure the concentration of formaldehyde present as a gas or vapour in the chamber atmosphere, a method of sampling was devised. Basically, the method consists of sealing glass ampoules placed inside the chamber and analysing their formaldehyde content by a standard colorimetric assay. The seals were achieved by using heat-shrinkable plastic sleeving, 6.4 mm in diameter (RS Components Ltd). When this type of plastic tubing is heated above 120°C it shrinks to about half its diameter and forms a tight grip around glass. The way in which the ampoules were sealed is shown in Figure 1. The plastic softens at the temperature at which the autoclave operates but the seals have been found to be gas-tight as long as the pressure inside the ampoules is equal to or lower than the surrounding pressure. For this reason, once the ampoules had been sealed, the pressure inside the chamber was not allowed to fall below that at which sampling had taken place. This meant aborting cycles before the vacuum normally in the elution and drying stages of SASF cycles.

To assay the formaldehyde in the ampoules, distilled water was injected into the sealed ampoules through the plastic sleeving. The resulting aqueous samples were assayed by a modification of the chromotropic acid method developed by Altshuller et al. (1961). An aliquot of the sample was slowly added to 9.0 ml of chromotropic acid solution (0.1% w/v in sulphuric acid) in a glass-stoppered test tube. Distilled water was slowly added to make up the aqueous aliquot to 1.0 ml. The solution was then shaken and allowed to cool before its absorbance at 580 nm was measured in a Cecil CE 292 spectrophotometer. The corresponding formaldehyde concentration was determined from a calibration graph prepared with standard formaldehyde solutions. Sampling was first done with commercial injection ampoules whose nominal volume when sealed was 2.0 ml, 3.5 ml, or 7.0 ml. As the glass and geometry of each type of ampoule differed, it was decided subsequently to use glass tubing (4.6-4.8 mm bore) to make calibrated ampoules whose nominal volume when sealed was either 1.2 ml or 2.2 ml. The ampoules were placed approximately 7 cm from the chamber door at a height 15 cm above the bottom of the chamber. Unless otherwise stated, 2.0 ml formalin (38% w/v) was injected per cycle. A steam only cycle was run between each formalin cycle to remove residual formaldehyde from the chamber. The ampoules were sealed at either of two times: (a) at time T (that is, as soon as the selected sterilising temperature had been reached, about 1-2 minutes after injection of the formalin), or (b) at time T + 5 minutes.

Results

ASSAY OF FORMALDEHYDE IN CHAMBER EFFLUENT
The average results for a number of cycles are shown in Table 1. It is apparent that with the rapid increase in the amount of formaldehyde in the effluent, the

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Fig. 1 Method for sealing ampoules: a, calibrated glass ampoule made from glass tubing; b, plastic sleeving shrunk onto ampoule; c, Nichrome wire heating coil; d, glass rod (T-shaped to prevent it falling into the ampoule). When the metal coil is heated, the plastic sleeving shrinks tightly around the glass rod, thus sealing the ampoule.
Measurement of formaldehyde concentrations in a subatmospheric steam-formaldehyde autoclave

Table 1  Amount of formaldehyde in chamber effluent

<table>
<thead>
<tr>
<th>Time after formalin (2.0 ml) injected (min)</th>
<th>Percentage HCHO recovered in effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>43</td>
</tr>
<tr>
<td>T + 1</td>
<td>49</td>
</tr>
<tr>
<td>T + 5</td>
<td>71</td>
</tr>
<tr>
<td>T + 9</td>
<td>83</td>
</tr>
</tbody>
</table>

T = time taken (1-2 min) for chamber to reach selected sterilisation temperature (73 ± 2°C) after formalin injection.

formaldehyde in the vapour or gaseous phase in the chamber must soon be considerably less than that injected into the autoclave. The decrease in the amount of formaldehyde inside the autoclave is approximately exponential (Fig. 2).

**MEASUREMENT OF FORMALDEHYDE INSIDE AUTOCLAVE CHAMBER**

Initial results obtained with commercial injection ampoules and with glass-tubing ampoules are shown in Figs 3 and 4 respectively. A striking feature of the results in Fig. 3 is the manner in which the average formaldehyde concentration decreases as the volume of ampoule used increases. The same feature is apparent in Figure 4. Another feature present in both Figs 3 and 4 is the wider scatter of individual results with smaller ampoules than with larger ones. The scatter is particularly noticeable in the results shown in Fig. 4 which were obtained with the ampoules made from glass tubing whose glass was thicker (0.7 mm) than that of the injection ampoules (0.4 mm). Since it was thought that these features might be due to condensation inside the ampoules, further trials were carried out using prewarmed ampoules.

**Fig. 3** Concentration of formaldehyde at time T. Sampling with commercial injection ampoules not prewarmed: a, 7.0 ml ampoules, average = 9.3 μg/ml; b, 3.5 ml ampoules, average = 17.5 μg/ml; c, 2.0 ml ampoules, average = 25.5 μg/ml.

**Fig. 4** Concentration of formaldehyde at time T. Sampling with glass tubing ampoules not prewarmed: a, 2.2 ml ampoules, average = 23.5 μg/ml; b, 1.2 ml ampoules, average = 35.1 μg/ml.

The results in Fig. 5 (a, b) show that when ampoules were prewarmed by subjecting them to a steam-only cycle immediately before the experimental cycle, both the average formaldehyde concentration and the scatter of individual results were considerably

![Fig. 2](http://jcp.bmj.com/)

**Fig. 2** Exponential decrease of formaldehyde in autoclave during sterilisation stage.
Fig. 5 Concentration of formaldehyde at time T. 
a + b, formalin injected = 2·0 ml; c + d, formalin injected = 4·0 ml; 
a, 1·2 ml ampoules, average = 7·5 \( \mu \)g/ml; 
b, 2·2 ml ampoules, average = 6·5 \( \mu \)g/ml; 
c, 1·2 ml ampoules, average = 14·9 \( \mu \)g/ml; 
d, 2·2 ml ampoules, average = 13·0 \( \mu \)g/ml.

Fig. 6 Concentration of formaldehyde at time T + 5 min. Formalin injected = 4·0 ml. 
a, 1·2 ml ampoules, average = 2·0 \( \mu \)g/ml; 
b, 2·2 ml ampoules, average = 3·1 \( \mu \)g/ml.

From the amounts of formaldehyde recovered in the effluent at the times of sampling (Table 1), it can be calculated that when 2·0 ml of formalin is injected into the autoclave, the theoretical concentration of formaldehyde in the chamber atmosphere should be about 20 \( \mu \)g/ml and 10 \( \mu \)g/ml at times T and T + 5 minutes respectively. This assumes complete vaporisation and homogeneous distribution of the formalin remaining in the chamber. The measured concentrations (Figs 5 and 7) appear to indicate that this is not the case.

At this stage a number of problems, particularly air leaks into the chamber, necessitated our returning the autoclave to the manufacturers for overhaul. At the same time a number of modifications were made to the autoclave. The procedures and experiments outlined above were then repeated, and the results are shown in Figures 7-9. The time taken to reach the selected sterilisation temperature (73 ± 2°C) after formalin injection was much shorter (20-40 seconds) after the autoclave had been overhauled. It was decided, therefore, to fix the times for sealing ampoules at 1 and 5 minutes after injection of the formalin (referred to hereafter as time F).

The most striking feature in all these results is that the average concentrations of formaldehyde in the chamber atmosphere are more than double those obtained previously with similar volumes of formalin. The amounts of formaldehyde in the effluent were again determined and the theoretical concentrations in the chamber calculated. The comparison between the theoretical and measured concentrations is shown.

Reduced. Subsequent measurements were all made with prewarmed ampoules. Sampling was also carried out with 4·0 ml formalin being injected into the autoclave, and the results are shown in Fig. 5 (c, d). The pattern of results is similar to that in Fig. 5 (a, b) but the average formaldehyde concentration is approximately doubled. The concentration of formaldehyde in the chamber at time T + 5 minutes is shown in Fig. 6, and it can be seen that the average concentration has been considerably reduced. It is also noteworthy that, whereas in Fig. 5 the average formaldehyde concentration was slightly greater in 1·2 ml ampoules than in 2·2 ml ampoules, the opposite occurs in Figure 6. It may be that initial diffusion of formaldehyde into the longer (2·2 ml) ampoules is slower than into the shorter (1·2 ml) ampoules, but then diffusion of formaldehyde out of the longer ampoules would also be slower.
Measurement of formaldehyde concentrations in a subatmospheric steam-formaldehyde autoclave

![Graphs showing formaldehyde concentration over time](image_url)

Fig. 7 Concentration of formaldehyde at time $F + 1$ min.
- a, 2-2 ml ampoules, average = 15.0 µg/ml;
- b, 1-2 ml ampoules, average = 17.5 µg/ml.

in Table 2. Once again the measured concentrations are much lower than would be expected with complete vaporisation and homogeneous distribution of the formalin still remaining in the chamber. As before, 2-2 ml ampoules gave slightly lower average concentrations per ml than 1-2 ml ampoules at the beginning of the sterilising stage (time $F + 1$ min) but slightly higher average concentrations per ml later on (time $F + 5$ min).

Whereas most sets of results in Figs 5 and 6 give normal distributions about the mean, those in Figs 7 and 8 show skewed distributions, especially when larger volumes of formalin were injected into the autoclave. The deviation from a normal distribution is less apparent at time $F + 5$ minutes (Fig. 9) although this may be due to the smaller scatter of results obtained in graphs with lower average formaldehyde concentrations.

The next experiment was designed to test the penetration of formaldehyde into the distal end of longer ampoules. Mention should be made that again a number of minor modifications to the autoclave resulted in slightly higher average formaldehyde concentrations, and this should be borne in mind when comparing these with previous results.

Lengths of glass tubing (bore 3.5 mm) were connected to 1-2 ml ampoules with the same heat-shrinkable plastic sleeving previously described. A small glass rod (1 cm long) was placed within the plastic sleeving to allow the 1-2 ml ampoule to be

<table>
<thead>
<tr>
<th>Formalin injected (ml)</th>
<th>Time of sampling (min)</th>
<th>Percentage of HCHO recovered in effluent</th>
<th>Approximate theoretical concentration of HCHO left in chamber (µg/ml)</th>
<th>Average measured concentration of HCHO in chamber atmosphere (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>F + 1</td>
<td>24</td>
<td>26</td>
<td>17.5</td>
</tr>
<tr>
<td>2.0</td>
<td>F + 3</td>
<td>65</td>
<td>12</td>
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<tr>
<td>4.0</td>
<td>F + 1</td>
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<td>49</td>
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<tr>
<td>4.0</td>
<td>F + 5</td>
<td>71</td>
<td>20</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>1-2 ml amps</td>
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<td></td>
<td>2-2 ml amps</td>
<td></td>
<td></td>
<td>6.0</td>
</tr>
</tbody>
</table>

Fig. 8 Concentration of formaldehyde at time $F + 1$ min. Formalin injected = 4-0 ml.
- a, 2-2 ml ampoules, average = 27.6 µg/ml;
- b, 1-2 ml ampoules, average = 31.5 µg/ml.
sealed as before. Two different lengths of tubing were used: 105 mm and 210 mm giving length-to-bore ratios of 30:1 and 60:1 respectively. Two sets of cycles were run: in the first set, the pressure during the steam pulses to remove air from the chamber ranged from \(0.34 - 0.18\) bar \((0.34 \times 10^5 - 0.18 \times 10^5 \text{ Pa})\) while in the second set the pulses were deeper, the pressure ranging from \(0.34\) to \(0.10\) bar \((0.34 \times 10^5 - 0.10 \times 10^5 \text{ Pa})\).

The results are shown in Figures 10 and 11. With the larger length-to-bore ratio, there is a sharp decrease in the average formaldehyde concentration in the 1-2 ml ampoule (Fig. 10). However, when the deeper pulsing sequence is used, the average concentration of formaldehyde that enters the 1-2 ml ampoules is markedly increased (Fig. 11a). Measurement of the concentration of formaldehyde remaining in the 1-2 ml ampoules at time \(F + 5\) minutes again shows a reduced value (Fig. 11b) from that at time \(F + 1\) minutes (Fig. 11a).

Experiments with tubing of larger bore (4.5 mm) gave results (not shown) that were similar to those with 3.5 mm bore tubing of corresponding length-to-bore ratio.

Experiments were also conducted in which 1-2 ml ampoules placed 6 cm and 15 cm respectively above...
the bottom of the chamber were sealed simultaneously. The results are shown in Fig. 12, and there is a clear difference between the two positions, the average concentration 6 cm above the bottom of the chamber being significantly higher (one-third) than the average concentration 15 cm above the bottom of the chamber.

![Graph](image)

**Fig. 12** Concentration of formaldehyde in 1-2 ml ampoules at different heights within chamber at time F + 1 min.

a, ampoules 15 cm above chamber bottom, average = 25.8 µg/ml;  
b, ampoules 6 cm above chamber bottom, average = 34.9 µg/ml.

**Discussion**

Kelsey (1967), reviewing gaseous antimicrobial agents, pointed out some of the problems associated with this type of sterilant. Particular attention was directed towards the difficulties encountered in ensuring that all the correct conditions were obtained to achieve reliable sterilisation with ethylene oxide gas. Since then much fundamental research has been carried out on ethylene oxide sterilisation, and Ernst (1975), reviewing some of this research, stated that 'in many applications ethylene oxide provides greater assurance of sterilisation than steam'. Although this statement is open to debate, there is little doubt that significant progress has been made in understanding some of the fundamental factors that govern the efficiency of the ethylene oxide process. Unfortunately, the same cannot be said of the subatmospheric steam-formaldehyde process. Although offering early promise as a relatively simple, effective, and economical process for treating contaminated non-autoclavable items, doubts have recently been raised as to its reliability as a sterilising process (Aycliffe et al., 1977; White, 1977). Like ethylene oxide sterilisation, it is undergoing an uncertain phase of development as closer scrutiny indicates the complexity of controlling a system in which there exists water, formaldehyde, and air (as well as methanol) in a dynamic equilibrium of liquid, vapour, and gas. Most of the published literature deals with the ability of a variety of SASF cycles to inactivate microbial organisms placed within test pieces designed to simulate some of the instruments and objects to be sterilised in practice. Most, though by no means all, of this work fails to probe in a fundamental way the factors that determine the optimal conditions under which the process will operate reliably. A particular case in point is the relative lack of information regarding the fate and activity of formaldehyde inside the autoclave chamber during a cycle. It is in an attempt to try to improve this situation that this research has been carried out.

We have shown that the concentration of formaldehyde present in the vapour or gaseous phase in the chamber cannot be assumed to be equivalent to the amount of formaldehyde present in the formalin injected into the autoclave. With a rapid reduction in formaldehyde concentration, the 'sterilising period' used in many SASF autoclaves may in fact be superfluous. Weymes et al. (1975) argue that it can be eliminated and that reliable penetration and sterilisation is achieved with six deep pulses—down to a pressure of 30 mm Hg (4 × 10⁵ Pa). Alternatively, it could be argued that greater effort should be made to determine exactly why this decrease in formaldehyde concentration occurs with a view to preventing or minimising it.

We have shown that deeper pulsing in the initial stages of the cycle does in fact increase the penetration of formaldehyde in long, narrow tubing. When the concentration of formaldehyde was measured 5 minutes after formalin injection, it was found that the drop in concentration (about 41%; see Fig. 11) was much smaller than that experienced in 1-2 ml and 2-2 ml ampoules without extension tubing (about 60-70%; see Figs 7-9). In other words, it is more difficult to get formaldehyde into the distal end of long tubing but, once it gets in, it remains there longer. When much longer tubing was used (length-to-bore ratio of 120:1 or more), no formaldehyde could be detected in the distal end, even when larger volumes of formalin were injected into the autoclave. Unfortunately, the autoclave used in this work did not enable us to reduce the pressure still further during the stage of air removal.

A striking increase in formaldehyde concentration in the chamber atmosphere was noted after the overhauling and modifications to the autoclave. It is
probable that reducing air leaks into the chamber was largely responsible for this major change in formaldehyde concentration. However, some of the modifications may also have been partly responsible (modifications included replacement of valves, re-lagging the steam generator and changing thermostat switches on it, re-positioning a steam-pressure control unit).

The phenomenon of the skewed distribution of results obtained, especially at higher formaldehyde concentrations (as seen in Figs. 7, 8, 10, 12, and, to a lesser extent, 5c and 5d), is possibly an indication of non-homogeneous distribution of the vapour. Larger formaldehyde vapour concentrations, possibly in droplet form, might account for some of the high results obtained with a number of samples.

The difference in the average formaldehyde concentrations obtained at different heights within the chamber indicates that layering of gases and vapours may be taking place. The autoclave used has only a small chamber, and it would be interesting to determine to what extent layering takes place within a larger chamber. Handlos (1977) measured formaldehyde residuals in various materials exposed to SASF cycles in different types of autoclaves. In one autoclave, polyester fibres placed near the chamber door retained between 30 and 72 µg cm⁻² of formaldehyde whereas those placed in the centre retained only 23-28 µg cm⁻².

Ernst and Doyle (1968) and Ernst et al. (1970) have reported that, in ethylene oxide sterilisers, condensation of vapour, temperature distribution gradients, air pockets, and the design of the steriliser are all factors that can contribute to non-homogeneous chamber atmosphere. Although SASF cycles have by and large eliminated the problems of residual polymerised formaldehyde formerly associated with 'formaldehyde cabinets', polymerisation may still play a part in reducing the concentration of formaldehyde in the gaseous phase. Carruthers and Norrish (1936) noted that polymerisation observed in experiments with formaldehyde required the presence of a cold surface where the polymer could be formed. Thus one is faced with the practical problem of eliminating cold surfaces (on which polymerisation can take place) but of doing so without producing substantial quantities of condensed moisture which could impede penetration of formaldehyde or in which formaldehyde might dissolve. In addition, Nordgren (1939), Kelsey (1967), and Ernst (1975) have all stressed the importance of correct humidity when sterilising bacterial spores. Alder (1968) reported the use of dry superheated steam in SASF cycles. All these factors indicate that more attention should be paid to the quality of steam supplied in SASF cycles.

The method we have developed to measure the formaldehyde concentration may not give an absolute measurement of the formaldehyde concentration in the chamber atmosphere. However, it has shown that simply withdrawing samples from the chamber, as was done by Wymes et al. (1975), does not provide realistic information about the amount of formaldehyde that finds its way within objects, in particular those with narrow lumens. Further, we have shown how the amount of formaldehyde within objects (glass ampoules in these experiments) may be affected by the condensation of moisture, which in turn will depend on the nature and temperature of these objects. The method has also proved its value in revealing that modifications to the autoclave can result in a change, in this case favourable, in the formaldehyde concentration in the chamber atmosphere.

Experience accumulated so far in practice seems to indicate that at a minimum temperature (65-73°C), within a defined range of relative humidity (possibly 70-90%) and at a minimum formaldehyde concentration (as yet undetermined) in the gaseous or vapour phase, it is possible to inactivate all microorganisms including bacterial spores. The limits within which these parameters can be altered and the possible ways in which they can best be achieved still remain to be determined.

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