

# Iodometry: A straightforward “tank side-test” to determine formaldehyde concentrations in anatomical tanks

Michael Kohr<sup>a</sup>, Carla L. Janko<sup>a</sup>, Anne-Marie Schmidt<sup>a</sup>, Armin A. Weber<sup>b</sup>, Markus R. Meyer<sup>b</sup>, Stefan Wagenpfeil<sup>c</sup>, Carola Meier<sup>a</sup>, Thomas Tschernig<sup>a,\*</sup> 

<sup>a</sup> Institute for Anatomy and Cell Biology, Germany

<sup>b</sup> Experimental & Clinical Toxicology and Pharmacology, Saarland University, Medical Faculty, Homburg, Saar 66424, Germany

<sup>c</sup> Institute for Medical Biometry, Epidemiology and Medical Informatics, Saarland University, Medical Campus, Homburg, Saar, Germany

## ARTICLE INFO

### Key words:

Formaldehyde concentration  
Tank  
Basin  
Embalment  
Measurement  
Titration  
Mass spectrometry

## ABSTRACT

In anatomical preservation, low formaldehyde concentrations are recommended because of the carcinogenic potential of formaldehyde. However, in big basins with a varying number of bodies inside it may be difficult to estimate the formaldehyde concentration. Here, a feasible titration method, i.e. iodometry, was described to measure formaldehyde concentrations in anatomical preservation tanks. This method was compared to head-space mass spectrometry-. Results of triplet measurements of the iodometry were coherent and fitted to nominal formaldehyde concentrations. The formaldehyde concentrations in the preservation tanks were found to be in the anticipated ranges between 2 % and 4 %. Therefore, an estimation of the formaldehyde concentration in preservation tanks is possible using the described quick and cost-effective method.

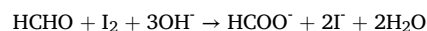
## 1. Introduction

Formaldehyde or methanal and its aqueous solution, formalin, are carcinogenic under certain conditions (Khoshakhlagh et al., 2023; Proitano et al., 2021; Vincent et al., 2024). Exposure to formaldehyde may occur by direct contact to formaldehyde-containing solutions and tissues or by inhalation. Recently, recommendations have been published to reduce the concentration of formaldehyde during preservation and storage of bodies in anatomical institutes (Kerner et al., 2023; Mohebimoushaei et al., 2025; Schnapper-Isl, Schmiedl, 2023; Waschke et al., 2019). The formaldehyde concentration in large tanks or basins, used for longer-term embalms of fixed bodies is often uncertain because of intermittent discharges and new entries of bodies. The bodies within the tanks absorb formaldehyde and the tank volume must be filled occasionally. To hold or to achieve sufficient concentrations it is necessary to monitor the formaldehyde concentration in the tanks. On the other hand, a higher concentration should be avoided, which also makes the intermittent measurement of the formaldehyde concentration necessary. Different methods are available. One is the colorimetric method using the Hantzsch reaction (Nash, 1953). Another one is also a titration method using sodium sulphite to react with formaldehyde in solution (Finkelde, 2020). To obtain semiquantitative information of the content

of formaldehyde in fluids test strips are available from different suppliers such as Machery-Nagel GmbH or Merck, Germany, and others. The most accurate method for this purpose might be the application of mass spectroscopy. The aim of the project was to establish and evaluate the use of iodometry in tank solutions of anatomical institutes. Therefore, the iodometry was established as described in textbook protocols and elsewhere (Cao et al., 2021). The method was evaluated using stock solutions and distinct dilutions which served as controls for the method. In a second step the formaldehyde concentrations of the conservation solution were determined in nine basins. These iodometric data were compared with the gold standard of mass spectroscopy.

## 2. Methods

All samples for analysis were taken from nearly full basins and from the surface. For this Technical Note the iodometry was chosen as a simple, rapid and cost-effective method to determine the formaldehyde concentration in preservation basins (Cao et al., 2021). This method is based on the following redox-reaction:



The amount of the consumed iodine is proportional to the

\* Correspondence to: Anatomy and Cell Biology, Toxicology, Saarland University, Campus Homburg, Kirrberger Straße, Homburg, Saar 66424, Germany.  
E-mail address: [Thomas.Tschernig@uks.eu](mailto:Thomas.Tschernig@uks.eu) (T. Tschernig).



**Fig. 1.** Pictures of the iodometry procedure. The brownish solution was decoloured with addition of natriumthiosulfate. Starch indicator solution might be added to better recognise the tipping point (see Methods).

formaldehyde concentration. With the titration of the iodine excess using thiosulfate, the amount of formaldehyde can be determined indirectly. Hereby, the brown iodine is reduced to colourless iodide (Fig. 1). To further facilitate the visualisation of the colour change, starch was used as an indicator (Bystrov, 1963; Yoshinaga et al., 2001). The procedure was performed under a hood and by room temperatures between 20 bis 25 ° Celsius. First, 10 g of the sample was weighed, and 1.5 ml sodium hydroxide solution (2 mol/L) was added. This solution was filled up to 250 ml with aqua dest. and 25 ml of this solution were transferred for further use. 15 ml sodium hydroxide solution (2 mol/L) and 50 ml iodine solution (0.05 mol/L) were added. After 15 min of incubation time 20 ml sulphuric acid (1.0 mol/L) were added leading to a dark brown colour. A burette (50 ml) was filled with natriumthiosulfate (0.1 mol/L). Under stirring the natrium-thiosulfate was added dropwise until a slight bleaching was visible. Now starch indicator solution (1 %) was added leading to a blue colour, and the titration was continued until a total decolourisation of the solution.

Standard solutions for calibration were mixed using Pearson's square („Mischungskreuz“). For example, 2.22 ml of a 36 % formaldehyde solution plus 37.78 ml aqua dest. yields a solution with a formaldehyde concentration of 2 %:

$$36\% - 2\% = 34 \text{ parts water}$$

$$2\% - 0\% = 2 \text{ parts stock solution}$$

$$\text{Mix ratio } 2: 34 \text{ or } 1: 17$$

$$\text{For } 40 \text{ ml final volume: } 1 + 17 = 18 \text{ parts}$$

$$1 \text{ part is } 40 \text{ ml} \div 18 = 2.22 \text{ ml}$$

Calculation of the formaldehyde concentration using the formula

$$n = C \times V$$

with

C = 0.05 mol/L (concentration of iodide solution),

V = used volume (L) (e.g. 10.5 ml = 0.0105 L),

n = amount of substance in (Mol)

Mass of formaldehyde

$$m = M \times n$$

with

m = mass in g

M = molar mass (Formaldehyde 30.03 g/mol)

n = calculated amount of mass (Mol)

Mass of formaldehyde in the solution (%) =  $m_{\text{sample}} / (m_{\text{formaldehyde}} \times 10) \times 100$

**Example:**

Expended volume of natriumthiosulfate

$$V = 25.6 \text{ ml}$$

Calculation

$$V_{\text{Probe}} = 50 \text{ ml} - 25.6 \text{ ml} = 24.4 \text{ ml} = 0.0244 \text{ L}$$

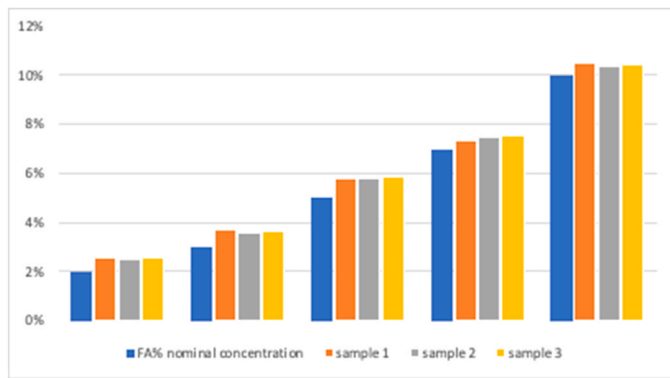
Amount of iodide

$$n = C \cdot V = 0.05 \text{ mol/L} \times 0.0244 \text{ L} = 0.00122 \text{ mol} = 1.22 \text{ mmol}$$

Amount of formaldehyde

$$m = M \cdot n = 30.03 \text{ g/mol} \times 0.00122 \text{ mol} = 0.0366366 \text{ g} = 36.6366 \text{ mg}$$

Sample (undiluted)



**Fig. 2.** Results of iodometrically measured formaldehyde concentrations in percent on the y-axis in samples of diluted stock solutions (2 %, 3 %, 5 %, 7 %, 10 %) as triplets on the x-axis.

$m(\text{sample}) = 1.015 \text{ g} = 1015 \text{ mg}$   
 Correction because of 1:10-dilution  
 $m_{\text{formaldehyde, corrected}} = 36.6366 \text{ mg} \times 10 = 366.366 \text{ mg}$   
 $\text{Mass of formaldehyde in the solution (\%)} = (366.366 \text{ mg}) / (1015 \text{ mg}) \times 100 = 36.095 \%$

The calculated mass of formaldehyde in the measured sample was 36.10 % which fits to the used stock solution (36.0 %).

### 3. Statistical analysis

For comparison of iodometry measurements with the gold standard of head-space mass spectrometry, we used the one-sample Wilcoxon signed-rank test for each series of measurements per basin. P-values are two-sided and subject to a significance level of 0.05.

### 4. Results

For determination of 36.0 % of nominal formaldehyde concentration the iodometry revealed results from 36.09 % to 36.12 %. The data of diluted solutions from 36 % stock (2, 3, 5, 5 and 7 %) were found to be slightly higher as measured using iodometry (Fig. 2).

Besides iodometry, head-space gas chromatography mass spectrometry (HS-GCMS) was applied to obtain a second set of results for comparison (Daoud Agha Dit Daoudy et al., 2018). The calibration was performed using a stock solution (36.0 % formaldehyde). An example of

iodometric measurements (triplets) from nine different basins was presented in in Fig. 3. The iodometric values were found to be in a close range. For each basin measurement series, there were no significant differences between head-space mass spectrometry and iodometry values with all the p-values > 0.08.

### 5. Discussion

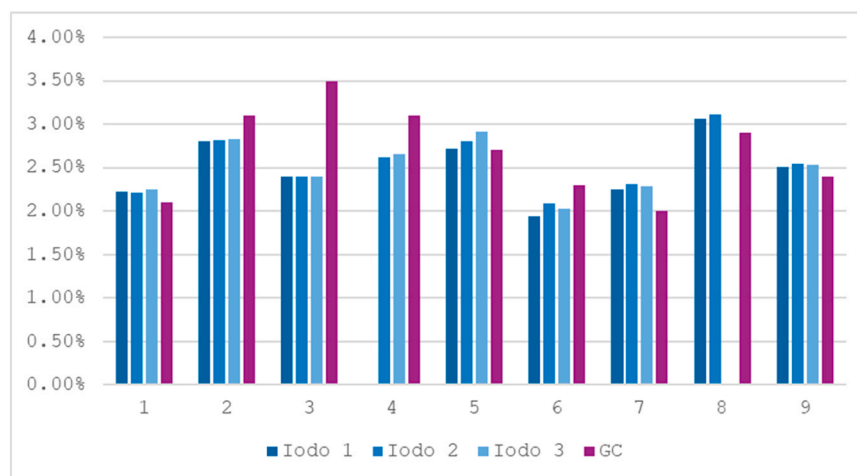
The stock solution and its dilutions were precisely measured using the iodometric method. In the basins the results showed nicely that the formaldehyde concentrations as determined by iodometry fitted to the measurements using HS-GCMS. No significant differences were observed. Nevertheless, so called matrix effects of distinct ingredients of the embalming solution and released (macro)molecules of the body donors may influence the results of HS-GCMS. In general, the HS-GCMS has a high sensitivity (characteristic fragment ion at  $m/z = 30.03$ ) and precision and might thus serve as the gold standard. However, HS-GCMS is requiring expensive equipment and highly specialised expertise. Iodometry, in turn, provides an easy-to-implement method, which is both rapid and cost-effective. A limitation of the presented study is that the samples were only taken from the surface of each basin and not from the middle section or from the ground. This was planned with the assumption of a uniform distribution of the formaldehyde concentration in each basin. This should be proved in experiments in the future. It has to be mentioned again that kits for semiquantitative determination of formaldehyde concentrations are available (among others Machery-Nagel GmbH or Merck, Germany). Its use might be a good addition for the surveillance of the formaldehyde concentration in the anatomical basins.

### 6. Conclusion

In conclusion, the old method of iodometric determination of formaldehyde is a straightforward and inexpensive “tank side-test” for routine control of the formaldehyde concentrations in preservation tanks in anatomical institutes.

### CRedit authorship contribution statement

**Carola Meier:** Writing – review & editing, Supervision. **Meyer Markus R:** Writing – review & editing, Methodology, Conceptualization. **Anne-Marie Schmidt:** Investigation. **Thomas Tschernig:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Stefan Wagenpfeil:** Data curation, Writing – original draft.



**Fig. 3.** Iodometrically measured formaldehyde concentrations in percent of samples from nine different basins containing storage solution and embalmed human bodies on the y-axis. All samples from nine basins on the x-axis were determined in doublets or triplets (blue columns). For comparison, the values, which were determined using HS-GCMS, were included (torquoise columns).

**Janko Carla L:** Investigation. **Michael Kohr:** Methodology. **Weber Armin A:** Investigation.

### Ethical Statement

Human bodies in this investigation came from the body donation programme of the Saarland University.

### Funding

The study received no funding.

### Declaration of Competing Interest

The authors declare that they have no competing interests.

### Acknowledgements

The authors are very grateful to all persons who donated their body to our Institute of Anatomy and Cell Biology. In addition, they thank Andreas Schmiedl (Institute of Anatomy, Medical School of Hannover) for excellent support with the manuscript and Alexander Grißmer (Institute of Anatomy, Saarland University) for excellent technical help.

### References

- Bystrov, S.P., 1963. Indicators for iodometry. *Aptech Delo* 12, 44–50.
- Cao, Y., Zhu, S., Zhang, L., Cui, Q., Wang, H., 2021. Qualitative and quantitative determination of trace aldehydes and ketones in food preservative propionic acid for quality improvement. *Anal. Methods* 13, 2989–2996.
- Daoud Agha Dit Daoudy, B., Al-Khayat, M.A., Karabet, F., Al-Mardini, M.A., 2018. A robust static headspace GC-FID method to detect and quantify formaldehyde impurity in pharmaceutical excipients. *J. Anal. Methods Chem.* 2018, 4526396.
- Finkelde, I., W.R., 2020. Comparing methods of determining formalin concentration in fluid preservatives. *Collect. Forum* 34, 32–52.
- Kerner, A.M., Biedermann, U., Bräuer, L., Caspers, S., Doll, S., Engelhardt, M., Filler, T.J., Ghebremedhin, E., Gundlach, S., Hayn-Leichsenring, G.U., Heermann, S., Hettwer-Steeger, I., Hiepe, L., Hirt, B., Hirtler, L., Hörmann, R., Kulisch, C., Lange, T., Leube, R., Meuser, A.H., Müller-Gerbl, M., Nassenstein, C., Neckel, P.H., Nimtschke, U., Paulsen, F., Prescher, A., Pretterklieber, M., Schliwa, S., Schmidt, K., Schmiedl, A., Schomerus, C., Schulze-Tanzil, G., Schumacher, U., Schumann, S., Spindler, V., Streicher, J., Tschernig, T., Unverzagt, A., Valentiner, U., Viebahn, C., Wedel, T., Weigner, J., Weninger, W.J., Westermann, J., Weyers, I., Waschke, J., Hammer, N., 2023. The chemicals between us-First results of the cluster analyses on anatomy embalming procedures in the German-speaking countries. *Anat. Sci. Educ.* 16, 814–829.
- Khoshakhlagh, A.H., Mohammadzadeh, M., Manafi, S.S., Yousefian, F., Gruszecka-Kosowska, A., 2023. Inhalational exposure to formaldehyde, carcinogenic, and non-carcinogenic risk assessment: A systematic review. *Environ. Pollut.* 331, 121854.
- Mohebmoushaei, S., Antipova, V., Biedermann, U., Brand-Saberi, B., Bräuer, L., Caspers, S., Doll, S., Engelhardt, M., Filler, T.J., Gericke, M., Gümişalan, Y., Gundlach, S., Hayn-Leichsenring, G.U., Heermann, S., Hiepe, L., Hirt, B., Hirtler, L., Kulisch, C., Konschake, M., Kürten, S., Lange, T., Lorenz, M., Nassenstein, C., Neckel, P.H., Nimtschke, U., Paulsen, F., Prescher, A., Pretterklieber, M.L., Schmiedl, A., Schumann, S., Spindler, V., Schulze-Tanzil, G., Tschernig, T., Unverzagt, A., Valentiner, U., Varga, I., Wedel, T., Weigner, J., Weninger, W.J., Westermann, J., Wimmer-Roell, M., Waschke, J., Hammer, N., 2025. Cluster analyses of contemporary embalming protocols in central European anatomy institutions: A collaborative effort to minimize chemical exposure. *Ann. Anat.* 260, 152403.
- Nash, T., 1953. The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Biochem. J.* 55, 416–421.
- Protano, C., Buomprisco, G., Cammalleri, V., Pocino, R.N., Marotta, D., Simonazzi, S., Cardoni, F., Petyx, M., Iavicoli, S., Vitali, M., 2021. The carcinogenic effects of formaldehyde occupational exposure: a systematic review. *Cancers (Basel)* 14.
- Schnapper-Isl, A., K.F., Schmiedl, A., 2023. Measuring low formaldehyde exposure values in the dissection hall after embalming human body donors with ethanol-based fixation methods. *Eur. J. Anat.* 27, 475–491.
- Vincent, M.J., Fitch, S., Bylsma, L., Thompson, C., Rogers, S., Britt, J., Wikoff, D., 2024. Assessment of associations between inhaled formaldehyde and lymphohematopoietic cancer through the integration of epidemiological and toxicological evidence with biological plausibility. *Toxicol. Sci.* 199, 172–193.
- Waschke, J., Bergmann, M., Bräuer, L., Brenner, E., Buchhorn, A., Deutsch, A., Dokter, M., Egu, D.T., Ergün, S., Fassnacht, U., Fietz, D., Gundlach, S., Heermann, S., Hirt, B., Kugelman, D., Müller-Gerbl, M., Neiss, W., Nimtschke, U., Plendl, J., Pretterklieber, M., Redies, C., Scaal, M., Schmidt, M.H.H., Schmiedl, A., Schnittler, H.J., Schomerus, C., Sebestény, T., Spittau, B., Steiniger, B., Tschernig, T., Unverzagt, A., Viebahn, C., Voigt, E., Weigner, J., Weyers, I., Winkelmann, A., Winkler, M., Paulsen, F., 2019. Recommendations of the working group of the Anatomische Gesellschaft on reduction of formaldehyde exposure in anatomical curricula and institutes. *Ann. Anat.* 221, 179–185.
- Yoshinaga, T., Shirakata, T., Dohtsu, H., Hiratsuka, H., Hasegawa, M., Kobayashi, M., Hoshi, T., 2001. Polyvinyl alcohol as a useful indicator on iodometry: volumetric and spectrophotometric studies on iodine-PVA and iodine-starch complexes. *Anal. Sci.* 17, 333–337.