

Reduction of formaldehyde concentrations in the air and cadaveric tissues by ammonium carbonate

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Abstract

The reduction of formaldehyde by ammonium carbonate was examined in cadavers and *in vitro*. Formaldehyde concentrations in the air (10 cm above human cadavers) and in various cadaveric tissues were measured with or without perfusion of ammonium carbonate solution into formaldehyde-fixed cadavers. Air samples were monitored using Kitagawa gas detector tubes. For measurement of formaldehyde in tissues, muscles and organs were cut into small pieces and tissue fluids were separated out by centrifugation. These specimen fluids were diluted, supplemented with 3-methyl-2-benzothiazolinone hydrazone hydrochloride and quantified by spectrophotometry. In five cadavers without ammonium carbonate treatment, the formaldehyde concentrations in the air above the thorax and in various tissue fluids were 1.2–3.0 p.p.m. and 0.15–0.53%, respectively. Arterial reperfusion of saturated ammonium carbonate solution (1.0, 1.5 or 2.0 L) into five formaldehyde-fixed cadavers successfully reduced the formaldehyde levels, both in the air (0.5–1.0 p.p.m.) and in various tissue fluids (0.012–0.36%). *In vitro* experiments demonstrated that formaldehyde concentrations decreased, first rapidly and then gradually, with the addition of ammonium carbonate solution into fluids containing formaldehyde. It was confirmed that formaldehyde reacted with the ammonium carbonate and was thereby changed into harmless hexamethylenetetramine. The application of ammonium carbonate solution via intravascular perfusion and, if necessary, by infusion into the thoracic and peritoneal cavities, injection into muscles and spraying on denuded tissues can be anticipated to reduce formaldehyde to satisfactorily low levels in cadaveric tissues and, consequently, in the air, which may provide safe and odorless dissecting rooms.

Key words: ammonium carbonate, cadaver, formaldehyde, hexamethylenetetramine, spectrophotometry.

Introduction

The dissection of cadavers is a basic and essential practice for studying human anatomy. For the preparation of human cadavers, formaldehyde has been most widely used for many years. Formaldehyde is a good and potent fixative available at a reasonable price. However, this reagent has recently attracted attention as a health hazard for students and instructors, including the fact that it frequently causes various physical symptoms, such as burning eyes, lacrimation, irritation of the airways and dermatitis (Pabst, 1987; A kbar-Khanzadeh *et al.*, 1994; Kim *et al.*, 1999; Mizuki & Tsuda, 2001; Tanaka *et al.*, 2003). In most medical and dental universities in Japan, formaldehyde-based solutions are infused via an artery (usually the femoral artery) for the initial fixation of cadavers. After a certain period of initial fixation, cadavers are then preserved in another fluid, which usually contains little or no formaldehyde, to reduce the formaldehyde content in cadavers during storage. In spite of immersion in a formaldehyde-free fluid, formaldehyde levels in the air in anatomy dissection rooms are high (Skisak, 1983; A kbar-Khanzadeh *et al.*, 1994; Kim *et al.*, 1999; Mizuki & Tsuda, 2001) and far exceed the upper limit of acceptable exposure levels (0.5 p.p.m.; Japan Society for Occupational Health, 1988). Frequent air ventilation of dissecting rooms (25 room air changes per hour) does not necessarily solve this problem (Tanaka *et al.*, 2003). Dissection tables with local exhaust ventilation can markedly reduce airborne formaldehyde (Coleman, 1995; Martin *et al.*, 1995), but are very expensive and may hasten drying of the tissues (Keil *et al.*, 2001). However, fixation of cadavers without formaldehyde may result in poor tissue preservation and possible survival of contagious microorganisms. Therefore, it is preferable to fix and thoroughly disinfect cadavers using formaldehyde for a sufficient period of time, followed by removal of the formaldehyde. However, the conventional methods used to achieve this are unsatisfactory and better methods are needed.

Formaldehyde contains an aldehyde group that is known to be chemically active. Formaldehyde reacts with various substances, in particular with ammonia, and thereby produces harmless hexamethylenetetramine (also called hexamine or methenamine; Nagakubo *et al.*, 1961; van der Eerden & van Nie, 1981). van der Eerden and van Nie (1981) successfully decreased free formaldehyde by perfusion of 2% ammonium. However, this method has not been widely accepted, perhaps because ammonia is difficult to handle owing to its toxicity and strong, unpleasant odor. Instead of ammonia, we used ammonium carbonate, which also reacts with formaldehyde and probably forms hexamethylenetetramine, as ammonia does. In the present study, an attempt was made to decrease formaldehyde using ammonium carbonate. Formaldehyde levels in the air and cadaveric tissues (more precisely, the fluids separated from cadaveric tissues) were compared between formaldehyde-fixed cadavers with or without ammonium carbonate reperfusion. Detoxification of formaldehyde by ammonium carbonate was also studied *in vitro*.

Materials and methods

Ten cadavers had been infused with 5 L of so-called 10% formalin (this solution actually contains approximately 3.7% formaldehyde in water and the net concentration of formaldehyde is used in the present paper) via the femoral artery on one side. Then, the cadavers were preserved in a fluid containing 39.6% ethanol and 6.6% methanol. One group of five cadavers (cases 1–5) was not subjected to further treatment until dissection. The other group of five cadavers (cases 6–10) was reperfused with 1.0, 1.5 or 2 L saturated ammonium carbonate solution by gravity mediated flow into the same artery used for the infusion of formaldehyde solution. No bubble formation was observed during or after perfusion, whereas bubbling occurred *in vitro* upon mixing of 3.7% formaldehyde solution with 10% ammonium carbonate solution.

The present study was conducted within the parameters of the written permissions we received from the donors and their surviving relatives and did not include any specific issues that required approval from the ethics committees of our institutions. The present investigation conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000).

Measurement of formaldehyde in the air

Known volumes of air samples (500 mL) at a position 10 cm above the cadavers were passed through Kitagawa gas detector tubes (171 SC; As One, Tokyo, Japan) at 24°C (otherwise, the measured values were corrected to be equivalent at this temperature) and formaldehyde concentrations were determined on the basis of the color changes of the detector tubes.

Measurement of formaldehyde in cadaveric tissues

Cadaveric tissues (1–2 g) were cut into small pieces (less than several mm in all dimensions) and were packed in a centrifuge tube for filtration (Ultrafree-CL; pore size 0.65 µm; Millipore, Bedford, MA, USA) and the liquid component in the tissues was separated out by centrifugation at 360 g for 10 min. Some muscle specimens yielded lipids or little fluid after centrifugation. In such cases, specimens were further centrifuged at 1450 g for 5 min or specimens were replaced by another sample of the same muscle. In a preliminary experiment, specimen fluids of 2.9–22.3% tissue wet weight were obtained. The yield of fluids varied, possibly depending on the types of tissues, packing conditions and filter permeabilities, and did not necessarily reflect the water content of the specimens. This fluid was diluted, in most cases, over a range of one in 1000 to one in 10 000 and was assayed using a formaldehyde detection kit (LR-FOR; Kyoritsu Chemical-Check Laboratory, Tokyo, Japan), following the manufacturer's instructions, with the aid of a spectrophotometer (UVmini 124; Shimadzu, Kyoto, Japan) equipped with water analysis program software (Shimadzu) specially programmed for the kit mentioned above. In this system, formaldehyde reacted with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) to produce a blue substance. The extent of coloration, which correlates with the amount of formaldehyde, was quantified by spectrophotometry. This highly sensitive method was easy to perform and required only a short measuring time and the details have been reported previously by Chan *et al.* (2001). Formaldehyde concentrations of sample fluids were calculated on the basis of the absorption of 625 nm light using the following equation (Kyoritsu Chemical-Check Laboratory, Tokyo, Japan):

$$\text{Formaldehyde concentration (mg/L)} = 0.5775 \times \text{absorbance at 625 nm} - 0.0289$$

For measurement, 25 mL of appropriately diluted specimen fluid was supplemented with reagent R-1 from the kit (each dose is packed separately by the manufacturer), stirred and allowed to stand with occasional agitation. Ten minutes later, two drops of solution R-2 (in the kit) were added, stirred and allowed to stand for 3 min. This mixture was then poured into a glass cell for spectrophotometry and the absorbance was measured 5 min after the addition and stirring in of R-2. Attention was paid that all specimen fluids were kept at $25 \pm 1^\circ\text{C}$ during the measurement. Specimen fluids centrifuged at 360 g for 5 min and 1450 g for 15 min also gave quite similar results to samples centrifuged at 360 g for 10 min, all at room temperature.

To evaluate the accuracy of measurements, a standard formaldehyde solution in methanol (1 mg/mL) was purchased (Kanto Chemical, Tokyo, Japan). This solution was diluted one in 2000 to a final concentration of 0.5 mg/L. This standard sample was assayed 10 times by the method described above. The measured values (mean \pm SD) were 0.515 ± 0.025 mg/L. The manufacturer claims that the kit can measure formaldehyde concentrations ranging from 0.05 to 0.8 mg/L. However, in our hands, absorbance over approximately 1.0 (solutions over 0.6 mg/L formaldehyde) tended to produce lower values than they should have. In addition, serial dilution of sample fluids may enhance errors. For dilution of fluids, we used an electronic balance that can measure in the order of 1 mg to minimize errors, because a balance is more accurate than pipettes.

Formaldehyde detoxification in vitro

Formaldehyde solutions containing 6 millimol formaldehyde (3.7%, 4.860 g each) were mixed with 0, 2, 4, 6, 8, 10, 12, 14 or 16 g aqueous 10% ammonium carbonate solution and the total weight of each specimen was adjusted to 48.600 g as precisely as possible by adding pure water. These fluids were appropriately diluted and formaldehyde concentrations were determined by spectrophotometry. The mixtures of formaldehyde and ammonium carbonate solutions were also assayed without volume adjustment by the addition of water.

Detection of hexamethylenetetramine in vitro

A mixture of formaldehyde and ammonium carbonate solutions was analyzed using a mass spectrometer (QSTAR; Applied Biosystems, Foster City, CA, USA) to examine whether hexamethylenetetramine was formed.

Results

Formaldehyde concentrations in the air and cadaveric tissues without ammonium carbonate reperfusion

Formaldehyde concentrations in the air and cadaveric tissues during dissection are presented in Table 1. Formaldehyde levels in the air 10 cm above the head (the calvarium was removed), thorax and abdomen of open cadavers ranged from 1.2 to 3.0 p.p.m. These values are comparable with the data of breathing zone formaldehyde concentrations at Okayama University (1.8–3.8 p.p.m.; A. Ohtsuka, pers. comm.). Formaldehyde levels in tissue fluids ranged from 0.15 to 0.53% (Table 1).

Formaldehyde concentrations after infusion of 1.0, 1.5 or 2.0 L saturated ammonium carbonate solution

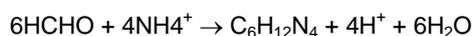
Formaldehyde levels in the air and tissues in cases 6–10 were reduced to various degrees compared with levels determined for cadavers without reperfusion (cases 1–5; Table 1). After infusion of 2.0 L saturated ammonium carbonate solution, most portions of cadavers showed significantly low levels of formaldehyde. It was noticed that the odor of formaldehyde was modest and the muscles were softer. Neither deterioration nor other problems were recognized in cadavers treated with ammonium carbonate during the entire period of the dissection.

Formaldehyde detoxification in vitro

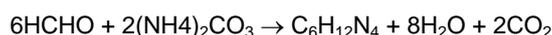
As the volume of ammonium carbonate added was increased *in vitro*, formaldehyde concentrations decreased, at first rapidly and then gradually (Fig. 1a,b). The mixture of formaldehyde and ammonium carbonate solutions was found to contain hexamethylenetetramine by mass spectrometry (Fig. 2). After drying, the mixture left a colorless or whitish substance.

Discussion

It was clearly demonstrated in the present study that formaldehyde levels can be reduced by ammonium carbonate. Formaldehyde reacts with ammonia and ammonium ions, producing hexamethylenetetramine, as follows (Nagano & Higashino, 1961):



Based on this chemical reaction, formaldehyde was presumably changed into hexamethylenetetramine by ammonium carbonate as follows:

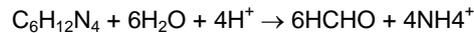


The formation, *in vitro*, of bubbles, probably carbon dioxide, and the results of mass spectrometry confirmed our speculation.

The idea to eliminate free formaldehyde by ammonia was tried by van der Eerden and van Nie (1981). They perfused 10 formaldehyde-fixed cadavers with a fluid containing 2% ammonia and a preservative. Although the volume infused was not described, the authors stated that the smell of formaldehyde and irritation of the skin and mucous membranes of people handling the cadavers were absent after ammonia perfusion. Furthermore, ammonia perfusion resulted in softer tissue consistency in the cadavers, which is consistent with the findings of the present study, and excellent mobility of the neck, shoulder and limbs. van der Eerden and van Nie (1981) speculated that formaldehyde reacted with ammonia to form hexamethylenetetramine. However, their method of fixation and perfusion has not become widely accepted, perhaps because of the difficulty in handling ammonia. In the present study, ammonium carbonate was used instead of ammonia. Ammonium carbonate solution is non-toxic and easy to handle. In addition, hexamethylenetetramine is a harmless reagent used for therapeutic purposes (Mandell & Sande, 1985; Japan Pharmaceutical Information Center, 2002).

Concerning formaldehyde concentrations, high levels of formaldehyde in the air were detected above cadavers without ammonium carbonate infusion in the present study, in agreement with findings of other studies (Kim *et al.*, 1999; Mizuki & Tsuda, 2001), and clearly there is a need to reduce formaldehyde levels. Infusion of ammonium carbonate solution resulted in reduced levels of formaldehyde in cadaveric tissues. After the infusion of 1.0 L ammonium carbonate solution (case 6), the right gastrocnemius muscle showed significantly lower formaldehyde concentrations, probably owing to profuse perfusion of ammonium carbonate solution in this tissue from the right femoral artery. However, considerable levels of formaldehyde remained in other tissues, probably for the following reasons: (i) ammonium carbonate solution was distributed inhomogeneously in the cadaver; and (ii) formaldehyde concentrations decrease gradually after the infusion of a certain amount of ammonium carbonate. Neutralization of the total amount of formaldehyde infused (approximately 185 g; 3.7% × 5 L) theoretically needs approximately 70 g ammonia, which roughly equals 233 g commercially available ammonium

carbonate of good quality (bottles labeled with an ammonia content of more than 30%). One litre of saturated ammonium carbonate contains nearly this quantity of ammonium carbonate, because ammonium carbonate dissolves to a concentration of over 20% at room temperature. However, as shown in Fig. 1a,b, formaldehyde did not disappear, even after an amount of ammonium carbonate greater than the theoretically needed quantity was added. This finding may be partly explained by the decomposition of hexamethylenetetramine as follows (Mandell & Sande, 1985):



After the infusion of 1.5 or 2.0 L ammonium carbonate solution, formaldehyde concentrations of various tissues were markedly decreased. Supplementary application of ammonium carbonate solution by infusion into the thoracic and peritoneal cavities, injection into muscles and spraying on denuded soft tissues would further reduce formaldehyde levels in cadaveric tissues and, consequently, in the air.

In our hands, the reperfusion of prefixed cadavers requires some labor and causes difficulties that vary depending on the condition of the cadavers. It is preferable to leave a plastic T tube *in situ* after the first perfusion with formaldehyde-based fluids and reuse it for secondary perfusion with ammonium carbonate. The period between the initial perfusion for fixation and reperfusion for neutralization can be variable. However, reperfusion into cadavers should be performed at a time sufficiently in advance of the use of the cadavers in class, because the perfused fluid takes some time to diffuse. Copper instruments should be avoided because ammonium carbonate erodes copper products (H. Kodera, unpubl. data).

After inactivation of formaldehyde, a method to suppress bacterial and fungal proliferation is necessary. Preservatives (van der Eerden & van Nie, 1981) or storage in phenoxyethanol (Frölich *et al.*, 1984) have been used to prevent microbial overgrowth and tissue maceration. This problem still needs to be solved.

Recently, a glutaraldehyde-based fixative and its neutralizer have become available commercially (Anatomical Embalming Fluid and Formaldehyde Neutralizer, Trisco, Baltimore, MD, USA). The mechanism of neutralization is not explained by the supplier and this solution is relatively expensive. In addition, reperfusion for neutralization is supposed to be conducted 24 or 48 h after the first perfusion. In contrast, our method allows the fixation of cadavers using conventional methods at a reasonable cost and neutralization can be performed at any time after fixation. In conclusion, the reduction of formaldehyde by ammonium carbonate seems very useful, easy to perform and relatively inexpensive. This method may lead to safe and odorless anatomy dissection rooms without special ventilation systems.

Acknowledgments

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Figure 1. Changes in formaldehyde concentrations after the addition of ammonium carbonate *in vitro* with the total volume of each specimen (a) adjusted to be the same by the addition of deionized water or (b) not adjusted before dilution. With the addition of 10% ammonium carbonate, formaldehyde levels decrease ($n = 3$ each; mean \pm SD), at first steeply and then gradually, with or without volume adjustment before dilution.

Figure 2. Results of mass spectrometry. A high peak of hexamethylenetetramine (formula weight 140.1 plus $H^+ = 141.1$) is obvious. The small peak on the right (arrow) indicates hexamethylenetetramine containing one ^{13}C atom. Ordinate, counts of detected particles; Abscissa, mass-to-charge ratio.

Table 1 Methods of preparation of cadavers and formaldehyde concentrations in the air and fluids of various tissues

	<ts10>Case									
	1	2	3	4	5	6	7	8	9	10
Bodyweight (kg)	42	47	54	45	60	61	57	50	44	48
'10%' formalin (L)	5	5	5	5	5	5	5	5	5	5
Ammonium carbonate (L)	0	0	0	0	0	1.0	1.5	2.0	2.0	2.0
Formaldehyde concentration										
Air above the head (p.p.m.)	2.1	–	–	–	–	1.5	–	–	–	–
Air above the thorax (p.p.m.)	3.0	1.8	2.5	1.2	2.1	0.7–0.8	0.5	1.0	0.5	0.5
Air above the abdomen (p.p.m.)	–	1.8	2.3	2.0	2.2	–	0.6	0.3	0.2	1.0
Lung (%)	–	0.26	0.32	0.27	0.23	–	0.025	0.060	0.049	0.061
Liver (%)	0.52	0.15	0.21	0.22	0.28	0.22	0.013	0.032	0.012	0.058
Kidney (%)	0.53	0.25	0.25	0.24	0.26	0.32	0.014	0.016	0.014	0.076
Left brachioradialis muscle (%)	–	0.29	0.39	0.29	0.30	0.36	0.25	0.063	0.041	0.040
Right brachioradialis muscle (%)	0.43	0.25	0.34	0.29	0.32	–	0.28	0.210	0.087	0.055
Left gastrocnemius muscle (%)	0.43	0.21	0.35	0.31	0.38	0.37	0.31	0.016	0.017	0.022
Right gastrocnemius muscle (%)	–	0.25	0.29	0.28	0.41	0.047	0.31	0.012	0.012	0.017

Atmospheric formaldehyde concentrations were lower in cases 1–5 than for cases 6–10. The formaldehyde level of the right gastrocnemius muscle in case 6 is significantly decreased. After infusion of a larger amount of ammonium carbonate solution, most cadaveric tissues showed very low levels of formaldehyde.

–, not measured.

Fig. 1a

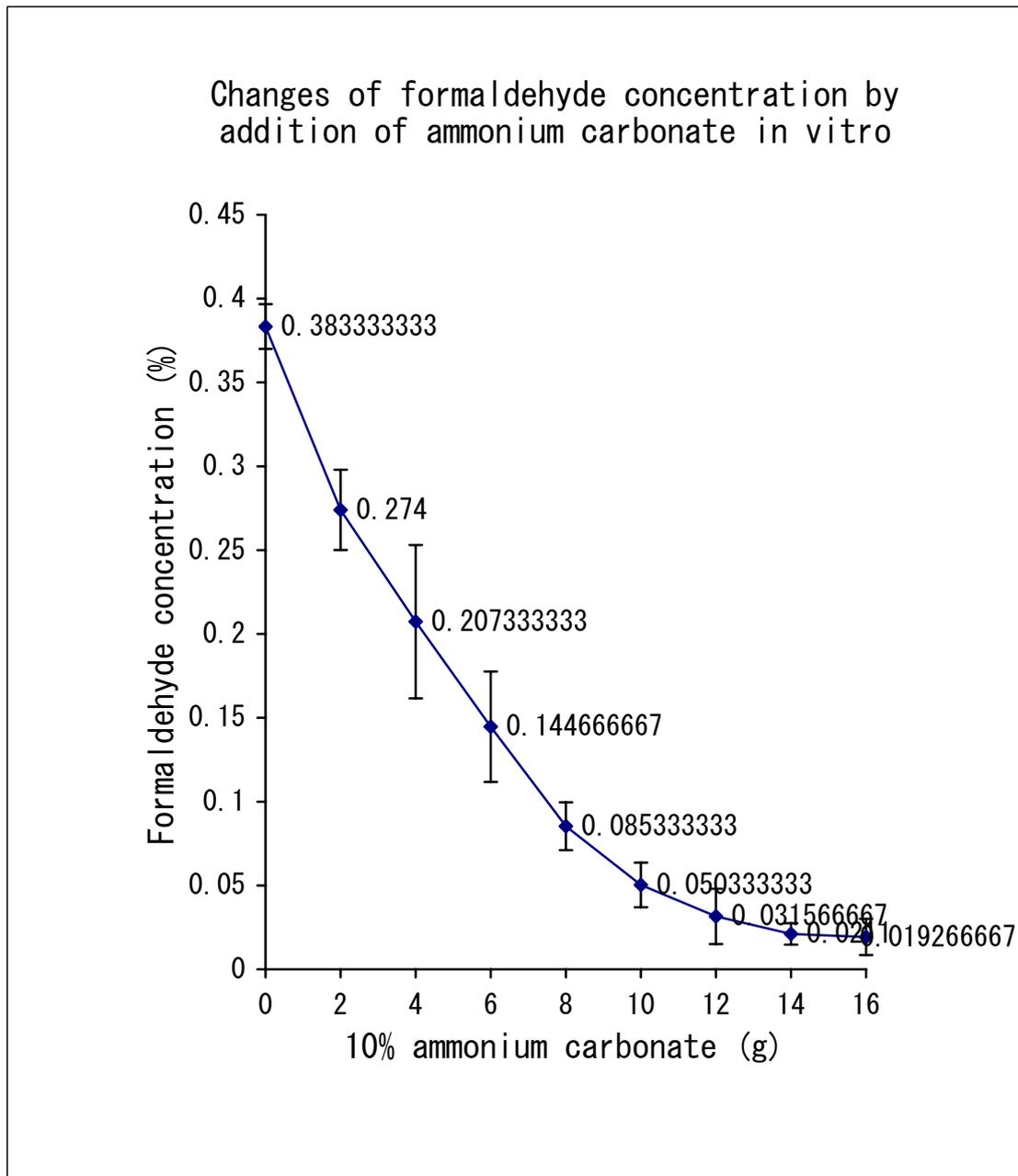
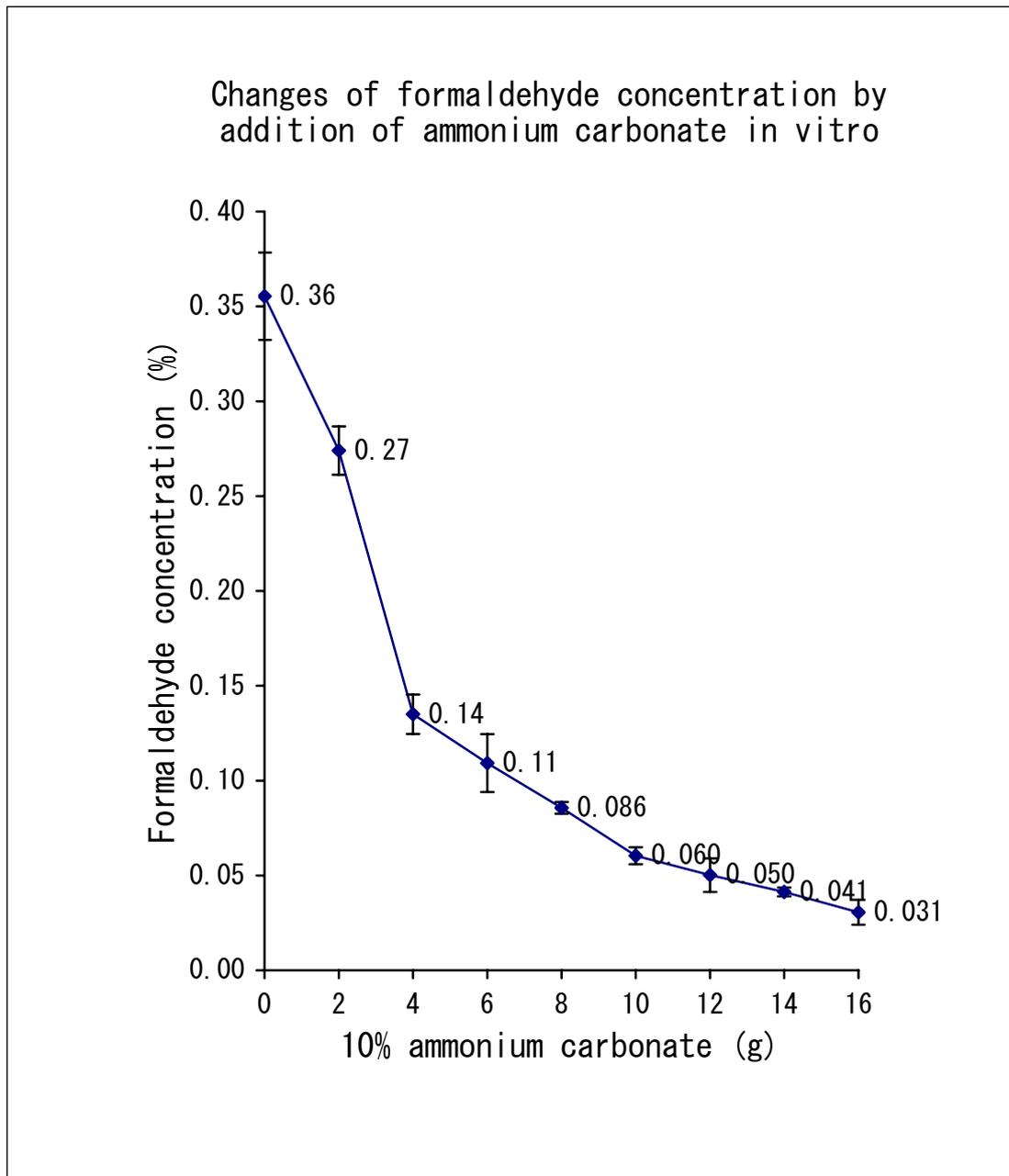


Fig. 1b



+TOF MS: 2.600 to 3.900 min from 1003-2.wiff
a=3.56952589635713310e-004, t0=-2.67128773942422410e+001, subtracted (0.500 to 1....

Max. 787.9 counts.

