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A determination method of pristine multiwall carbon nanotubes in rat lungs after intratracheal instillation exposure by combustive oxidation–nondispersive infrared analysis

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ABSTRACT

This paper describes a method for determination of multiwall carbon nanotubes (MWCNTs) in rat lungs after intratracheal instillation exposure. The MWCNTs were quantitatively decomposed to CO_2 by combustive oxidation and were then determined by non-dispersive infrared analysis. Samples were pretreated by acid digestion, muffle ashing and *in situ* preheating to remove interferences due to coexisting biological carbon from the lung tissue sample, while preserving the MWCNTs as in its their original form. The preservation was confirmed by transmission electron microscopic observation of the pretreated samples of exposed lung tissues and by the fact that the recoveries of MWCNTs spiked to the lung tissues were close to 100%. The detection limit for MWCNTs obtained by the proposed method was 0.30 μg and the repeatability as expressed by the relative standard deviation was 5.6% ($n = 4$). The method was sufficiently sensitive and precise to apply to real samples of rat lung to investigate the *in vivo* persistence of intratracheally instilled MWCNTs. To our knowledge, this is the first report of this type of sample pretreatment and direct determination of pristine MWCNTs without modification or tagging. Conventional indirect methods use tagging with other compounds or metal impurities in the CNTs for detection, and the detachment of these tags can increase uncertainties in the determination of the CNTs. The tags can also change how the CNTs persist *in vivo*, which can lead to an incorrect understanding of the persistence of pristine CNTs *in vivo*.

1. Introduction

Carbon nanotubes (CNTs) [1,2] are a promising material in industrial [3] and biomedical [4,5] fields because of their unique physicochemical properties, such as high electrical and thermal conductivity, and rigidity. The number of commercial applications of CNTs is increasing rapidly, and it is necessary to evaluate the risk of CNTs to human health [6,7]. Especially, the evaluation of pristine CNTs should be investigated, because the CNTs are starting materials to produce many carbon nanomaterials by molecular modification and therefore the risk is a criterion for those of all of the modified CNTs.

The histopathological responses of animals experimentally exposed to pristine CNTs have been used to evaluate the toxicity of CNTs. Toxicity has been evaluated using bio-markers and microscopic observations of the occurrence of tumors in organs [8–11]. By contrast, there is little quantitative data on the persistence of pristine CNTs *in vivo* after exposure, because of a lack of validated methods for the determination of CNTs *in vivo*. This data would be indispensable for a risk evaluation of CNTs.

Since most CNTs have few intrinsic physicochemical properties that are useful for detection, most reported methods for *in vivo* CNTs have involved detection of marker substances immobilized on pristine CNTs. Tagging of radioisotopes to CNTs has been used to obtain *in vivo* images of CNTs [12–15]. However, radioisotope procedures are expensive, as they require specialized laboratories, instruments and trained operators. It is also not easy to apply radioisotope procedures to the quantification of CNTs in the organs of test animals,

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because it is difficult to evaluate whether the tag molecules retain on the CNTs at long-terms after the exposure. Tagging with fluorescent molecules has been applied to *in vitro* samples [16], but application to *in vivo* samples is difficult because self-adsorption of the fluorescent molecules and coexisting substances can influence the results. It should be noted that the *in vivo* persistence and toxicity of the tagged CNTs are essentially different to those of the pristine CNTs, because their physicochemical and biological properties are altered by the tag [17,18]. Consequently, tagging can increase uncertainties in the determination of CNTs and lead to misinterpretation of the persistence of pristine CNTs *in vivo*. Indirect determination of pristine CNTs has been performed by determining the content of metal impurities, such as cobalt, originally contained as catalysts in the CNTs [19,20]. However, it is difficult to control the amounts of the impurities and to eliminate long-term leaching from the CNTs. For an aggregated single-walled carbon nanohorn (SWNH), gadolinium oxide was embedded into aggregated SWNHs and the content of Gd in mice organs was used to estimate the residual amounts of SWNHs [21]. However, this method encounters the same difficulties as discussed above for the metal catalyst method.

Only a few analytical methods for direct determination of pristine CNTs *in vivo* have been proposed. Intrinsic Raman scattering [17] and near-infrared fluorescence [22,23] could be used for *in vivo* imaging to directly determine single-walled CNTs (SWCNTs). However, these methods are limited to specific SWCNTs, such as those with below 2 nm or semiconducting SWCNTs. These methods are unsuitable for other CNTs, such as larger and/or metallic SWCNTs and multiwall CNTs (MWCNTs). ^{13}C-labeling of SWCNTs and isotope ratio mass spectrometry analysis of ^{13}C has also been investigated for determining the residual amount of CNTs [24]. However, synthesis of ^{13}C-labeled CNTs is expensive and complicated, and it is difficult to confirm that all the introduced ^{13}C atoms are located in the CNTs. Most of these reported methods are not suitable for determination of pristine CNTs in the organs of test animals. Appropriate methods should be developed that are simple and inexpensive for ever-growing users of CNTs in various industrial fields.

In this study, we established a method for the determination of pristine MWCNTs in rat lung tissue. This method combined combustive oxidation of the MWCNTs to CO_2, and subsequent non-dispersive infrared (NDIR) analysis. Interferences due to coexisting carbon mainly originating from the lung tissue were removed by sample pretreatment combining acid digestion, muffle ashing and *in situ* preheating. This pretreatment selectively oxidized the carbon and not the MWCNTs based on their differences in decomposition characteristics. Consequently, determination of MWCNTs in rat lung was achieved by an easy and inexpensive procedure. The proposed method was validated through recovery and repeatability tests. A pilot study on the *in vivo* persistence of MWCNTs was then conducted with real lung samples after intratracheal instillation exposure.

2. Experimental

2.1. Reagents

MWCNTs were obtained from Nikkiso Co., Ltd. (Tokyo, Japan). Their specific surface area measured by a BET method was 77.0 m^2/g. The combustion rate of the MWCNTs measured by thermogravimetry under atmospheric condition (ramp rate; 10 °C/min, maximum temperature; 1000 °C) was $99.6 \pm 1.9\%$ (w/w, $n = 4$). Nitric acid and hydrochloric acid were of ultra-pure grade (Kanto Kagaku, Tokyo, Japan). Tetramethylammonium hydroxide was of analytical grade (Wako Pure Chemical Industries, Tokyo, Japan). Trition-X-100 (polyoxyethylene-*p*-isooctylphenol, Alfa Aesar, Ward Hill, MA, USA), sodium hypochlorite, sodium dodecylsulfate, and glucose were of commercially available grade (Wako Pure Chemical Industries). Ultra-pure water from a Milli-Q system (Mill-Q TOC, Millipore Corp., Bedford, MA, USA) was used in all experiments.

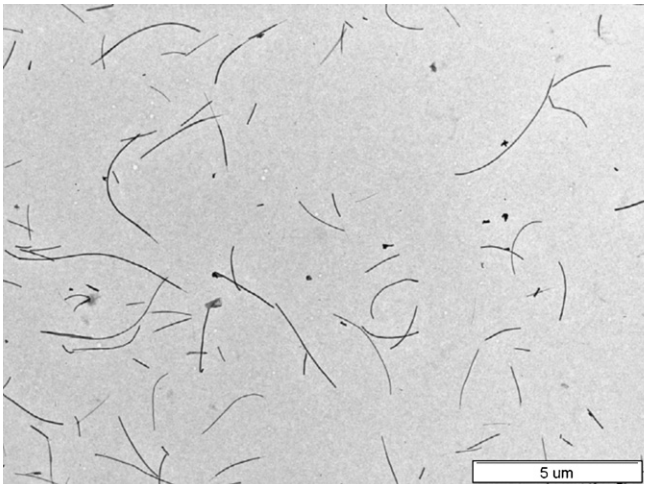

Fig. 1. TEM photographs of MWCNTs in a dispersion solution.

2.2. Preparation of dispersion solutions of the MWCNTs

MWCNTs dispersed in solution with Triton-X were used for the instillation exposure of MWCNTs to rats. Preparation of the MWCNTs dispersions will be detailed elsewhere. Briefly, the MWCNTs were ground with ethanol. The ethanol suspension of the MWCNTs was mixed with fructose at 120 °C, and a solidified dispersion of the MWCNTs was obtained after drying and cooling. The solidified dispersion was ground using a planetary ball mill for 20 min. After soaking the ground product in water and heating at 80 °C for 24 h, the suspension was filtered to remove fructose from the MWCNTs. The MWCNTs were then ultrasonically dispersed in an aqueous solution of Triton-X. The dispersed MWCNTs were separated according to their length by centrifugation. The fraction recovered between 3000 × *g* and 20,000 × *g* was used for the instillation tests. Transmission electron microscopy (TEM) (Fig. 1) showed that the MWCNTs in this fraction were mostly isolated from each other. Their median diameter (48 nm) and mean length (1 μm) were obtained by measuring the MWCNTs ($n = 1060$) on the TEM photographs. Their minimum and maximum lengths were 0.07 and 8.91 μm, respectively, and the length distribution is shown in Table 1.

2.3. Intratracheal instillation of MWCNTs into rat lungs and homogenization of the exposed lungs

Eight-week-old male Wistar rats were obtained from CLEA Japan, Inc. (Tokyo, Japan). The rats were fed a standard diet, and allowed to acclimatize to their environment for one week before experimentation. The animals were housed in cages with two rats per cage, with standardized conditions as follows: lights on at 7 am, lights off at 7 pm, 20–40 lx during the light phase, temperature 22 °C, humidity 70%. At 9 weeks of age the rats were exposed to 200 μg (0.66 mg/kg body weight) or 1 mg (3.3 mg/kg body weigh) of MWCNTs dispersed in 0.4 mL of distilled water with 0.05% Triton X. Intratracheal instillation of the control rats was with aqueous solutions of 0.05% Triton-X. Rats from the 200 μg-exposure and control groups were dissected at 3 days, 1 week and 1 month after instillation, while rats from the 1 mg-exposure group were dissected at

Table 1
Length distribution of MWCNTs based on measurements from TEM photographs of a dispersion solution.

MWCNT length, L (μm)	Frequency (%)
$L < 0.10$	0.1
$0.10 \le L < 0.14$	0
$0.14 \le L < 0.20$	5.1
$0.20 \le L < 0.27$	6.8
$0.27 \le L < 0.38$	11.1
$0.38 \le L < 0.54$	7.5
$0.54 \le L < 0.75$	12.5
$0.75 \le L < 1.05$	10.9
$1.05 \le L < 1.48$	15.8
$1.48 \le L < 2.07$	14.2
$2.07 \le L < 2.89$	8.2
$2.89 \le L < 4.05$	5.3
$4.05 \le L < 5.67$	1.9
$5.67 \le L < 7.94$	0.4
$7.94 \le L < 11.11$	0.2
$11.1 \le L$	0

3 days and 1 week. The details of the intratracheal instillation have been reported previously [25].

Before dissection, the exposed rats were anesthetized with an intraperitoneal injection of pentobarbital. At autopsy, blood was taken from the abdominal aorta. The lungs were then perfused with physiological saline, dissected and weighed. The left lung of each rat was used for this study and the right one was used for gene analysis in another study, which will be described elsewhere. The pretreated left lungs were stored at −80 °C until homogenization. The lung samples were cut into small pieces with scissors and then homogenized with three times their weight of saline in an ice bath with an electric homogenizer (Polytron RT3100; Kinematica AG, Switzerland) at 10,000 rpm. The samples were stored at −20 °C until analysis.

All procedures and animal handling followed the guidelines in the Japanese Guide for the Care and Use of Laboratory Animals as approved by the Animal Care and Use Committee, University of Occupational and Environmental Health, Japan or by the Institutional Animal Care and Use Committee, National Institute of Advanced Industrial Science and Technology.

2.4. Pretreatment of homogenized lung tissue for determination of MWCNTs

2.4.1. Acid digestion

A homogenized tissue sample (0.1 g) was thawed and redispersed by sonication (model 1510, B. Braun, Germany). The redispersed sample (approximately 0.4 g) was transferred to a 10 mL glass beaker and weighed. Three milliliters of 60% (v/v) nitric acid was added to the sample, and the sample was heated at 120 °C for 8 h on a hot plate (HF-61, Yamato Kagaku Co. Ltd., Japan). When the volume of nitric acid decreased by half, more nitric acid was added to maintain the initial volume. After heating, the sample solution was allowed to cool to room temperature. Five milliliters of water was added, which caused aggregation of the MWCNTs in the solution. The aggregated MWCNTs were collected on a quartz membrane filter (99.99% trapping efficiency, 0.3 μm dioctylphthalate, QR-100, Advantec, Japan), which had been preheated at 900 °C for 15 min in a muffle furnace (FP42, Yamato Kagaku Co. Ltd., Japan). The aggregated CNTs collected on the filter were washed five times with 5 mL of water each time.

2.4.2. Muffle-ashing

The CNTs on the filter were heated at 400 °C for 15 min in a muffle furnace to remove residual biological carbon after acid digestion.

2.4.3. In situ preheating in a combustive oxidation chamber

After the muffle-ashing, the filter with residue was transferred to a ceramic boat, and then inserted into the chamber of for combustive oxidation. The chamber temperature was held at 900 °C for 50 s to remove all of the residual carbon except for the MWCNTs.

2.5. Combustive oxidation–NDIR measurement

The MWCNTs were analyzed using a combustive oxidation chamber (SSM-5000A, Shimadzu, Japan) and a NDIR detector (TOC-V CPH, Shimadzu, Japan). The carrier gas was oxygen (purity >99.9%). The catalyst for the combustive oxidation was a mixture of platinum and cobalt oxide. The quartz filter after the pretreatment was transferred to a ceramic boat. Carbon dioxide generated by the combustive oxidation after the *in situ* preheating was used for quantification of the MWCNTs. The quantification of MWCNTs was performed with a glucose calibration curve.

2.6. TEM observation

To confirm the decomposition of biological substances and integrity of the MWCNTs, the lung tissues containing MWCNTs after the pretreatment were observed by TEM. The pretreated MWCNTs on a filter were dispersed in ethanol, and placed on a carbon mesh supported with a copper grid for the TEM observations. TEM observation was performed on an EM922 (Carl Zeiss SMT, Germany), with an acceleration voltage of 200 kV.

3. Results and discussion

3.1. Determination of MWCNTs by combustive oxidation–NDIR

To detect the MWCNTs by NDIR, they needed to be decomposed to CO_2. The MWCNTs decomposed to CO_2 under an oxygen condition when the temperature of the combustive oxidation chamber was increased to 900 °C (Fig. 2(b)). The temperature and oxygen conditions enabled the decomposition of the MWCNTs, because the MWCNTs decomposed completely at the temperature even under the atmosphere condition, which was confirmed by the thermogravimetric analysis of the MWCNTs (Fig. 3). To confirm if the decomposition conversion to CO_2 was quantitative or not, the conversion efficiency was compared with that for glucose, which can be easily decomposed to CO_2 and is typically used for calibration of the determination of carbon in a solid sample. The temporal profile of glucose is shown in Fig. 2(a). The relative sensitivity, calculated from the peak area, for MWCNTs was 98% of that for glucose in the range from 0 to 500 μg of carbon. The carbon purity of the MWCNTs defined as the combustion rate obtained by thermogravimetry was $99.6 \pm 1.9\%$ ($n = 4$). The agreement with these values suggests that the MWCNTs were completely decomposed to CO_2 at 900 °C with oxygen as the carrier gas. The coefficient constant of 0.999 indicated the linearity of the calibration curve for the MWCNTs was also satisfactory for the determination. The determination of MWCNTs alone was easily conducted by combustive oxidation–NDIR. However, the situation altered in the presence of rat lung tissue. Fig. 2(d) shows a temporal profile for a homogenized lung tissue sample spiked with MWCNTs. A very large peak from biological carbon in the lung tissue completely overlapped with the MWCNTs peak. For the MWCNTs dispersed in solution with Triton-X, the surfactant peak also partly overlapped with that of the MWCNTs (Fig. 2(c)).

3.2. Removal of coexisting biological carbon from lung tissue

To separate MWCNTs from coexisting carbon in the homogenized lung samples, digestion and/or solubilization of biological carbon were examined using acid, alkali, oxidant or surfactant

Table 2
Residual amount of biological carbon from homogenized lung tissues after pretreatment procedures.

Digestion		Muffle-ashing	*In situ* preheating	Residual amount of carbon (%)[a]
Reagents	Temperature and time			
Water	60 °C, 2 h			61
5% SDS	60 °C, 2 h			17
5% SHC	60 °C, 2 h			29
25% TMAH	60 °C, 2 h			34
60% HNO_3	60 °C, 2 h			15
60% HNO_3	86 °C, 7 h			0.53
60% HNO_3	99 °C, 8 h			0.19
60% HNO_3	120 °C, 8 h			0.12
60% HNO_3	120 °C, 8 h	400 °C, 15 min		0.026
60% HNO_3	120 °C, 8 h	400 °C, 15 min	900 °C, 50 s	Not detected (<0.0038[b])

The amounts of lung tissues (wet weight) and the initial amounts of the carbon were 0.1 g and 8 mg, respectively.

[a] The amounts were calculated by the following equation: the residual amount (μg) of carbon/the initial amounts of carbon (μg) × 100.

[b] The amount was estimated using the detection limit of the proposed determination method.

reagents. The resulting solution was filtered with a quartz membrane filter. The carbon remaining on the filter was determined by combustive oxidation–NDIR. To avoid decomposition of the MWCNTs, the reaction temperature was low and the reaction time was short. The mass fraction of residual carbon remaining after reaction at 60 °C for 2 h was determined against the initial amount (Table 2). The reduction of the amount of residual carbon with the different reagents was in the following order (greatest reduction to smallest reduction): 60% nitric acid < 5% sodium dodecylsulfate (SDS) < 5% sodium hypochlorite (SHC) < 25% tetramethylammonium hydroxide (TMAH) < water. HNO_3 was the most effective for removal of the biological carbon, and was selected as the digestion reagent for subsequent experiments. After digestion, the mass fraction of residual carbon reduced to 15% of the initial amount. The amount of residual carbon was in the order of 1 mg for every 0.1 g of lung tissue (wet weight), which was the amount of tissue typically used for determination of MWCNTs. The residual amount is unsatisfactory for determination of trace MWCNTs at 1 μg levels, which is necessary for investigating clearance after exposure to low amounts (10 or 100 μg per 0.1 g tissue (wet weight)) of MWCNTs. To improve the removal of the biological carbon from the tissue, the digestion temperature and time were investigated. The results are shown in Table 2. Increasing the temperature and time improved the removal rate of carbon. When the temperature and time were increased to 120 °C and 8 h, respectively, the mass fraction of residual carbon decreased to 0.12% of the initial amount. The absolute amount of the carbon in this case was 9.6 μg for 0.1 g of lung tissue (wet weight), which still created serious interferences for the determination of MWCNTs at 1 μg levels (Fig. 4(a)). To remove more residual carbon, an ashing procedure at 400 °C for 15 min in a muffle furnace was added after the acid-digestion. The ashing process reduced the residue to 0.026% of the initial amount, but a small peak corresponding to 2.0 μg still overlapped that of MWCNTs (Fig. 4(b)). Pretreatment with only muffle-ashing was examined for comparison, but it did not remove as much coexisting carbon as the procedure with digestion. The residual carbon was approximately 3% of the initial amount after muffle-ashing alone.

For combination pretreatment with muffle-ashing and acid-digestion, we suspected that the overlapping peak was due to

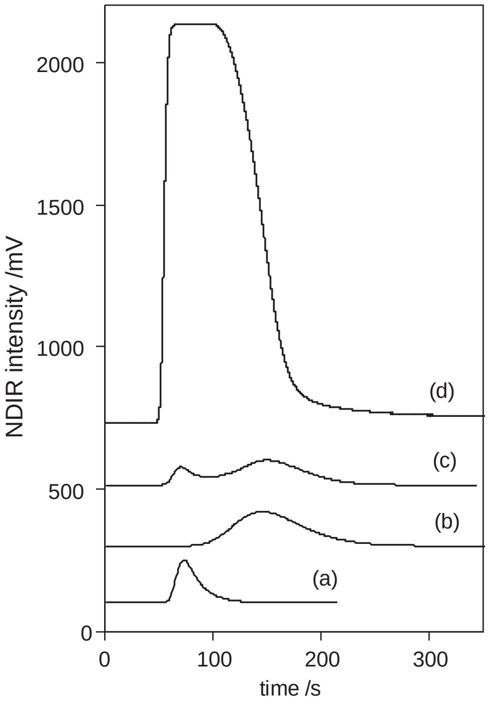

Fig. 2. Temporal profiles of combustive oxidation–NDIR measurement for (a) glucose, (b) MWCNTs, (c) MWCNTs dispersed with Triton-X, and (d) a homogenized sample of lung tissue from a control rat spiked with MWCNTs. The amounts of carbon for glucose (a) and MWCNTs (b) were 11 μg and 33 μg, respectively. The amounts of MWCNT and Triton-X in (c) were both 18 μg. The amounts of MWCNTs, Triton-X and lung tissue (wet weight) in (d) were 20 μg, 20 μg and 0.10 g, respectively. The NDIR spectra for (b)–(d) are elevated off the baseline for better visual observation.

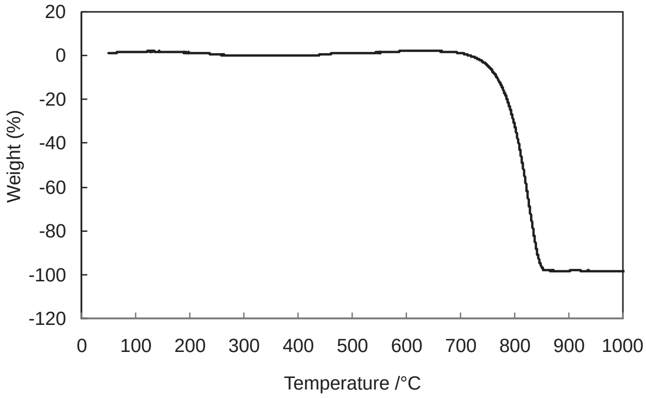

Fig. 3. A thermogravimetric analysis of MWCNTs under an atmosphere condition. The ramp rate and maximum temperature were 10 °C/min and 1000 °C, respectively.

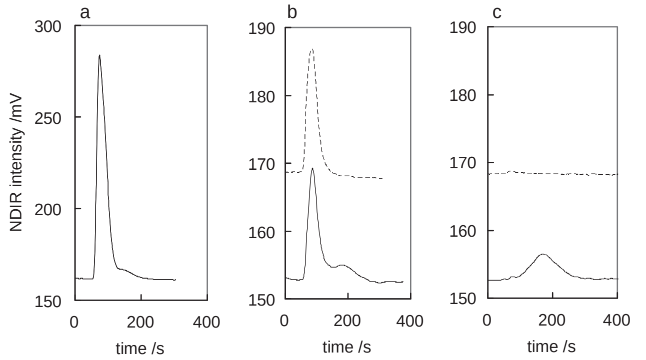

Fig. 4. Temporal profiles of combustive oxidation–NDIR measurement for homogenized samples of lung tissue spiked with MWCNTs after a sequential pretreatment: (a) acid-digestion, (b) muffle-ashing and (c) *in situ* preheating. The amounts of MWCNTs, Triton X and lung tissue (wet weight) were 1.0 μg, 1.0 μg and 0.10 g, respectively. The dashed lines show the profiles for the blank samples (only quartz filters). The NDIR spectra for the case of blank samples are elevated off the baseline for better visual observation.

contamination during the pretreatment, because it was observed in blank samples that did not contain any lung tissue or MWCNTs. A typical profile for a blank test is also shown in Fig. 4(b). The potential for airborne contaminants in the laboratory to adsorb on the quartz filter and produce this peak was investigated by storing the filter after muffle-ashing in a box purged with argon gas. However, this failed to remove the small peak. This suggested that the contamination occurred in the short time taken to transfer the filter from the muffle furnace to the argon filled box or the sample boat in the combustive oxidation chamber. An attempt was made to remove this contamination by preheating the filter in the combustive oxidation chamber and by minimizing its exposure to the atmosphere. After preheating the sample boat holding the filter at 900 °C in the chamber for a short time, the sample boat was removed from the chamber and then inserted again for the MWCNTs measurement. Increasing the preheating time reduced only the small carbon contamination peak. Finally, the preheating time was increased to 50 s, which resulted in almost complete elimination of the small carbon contamination peak in both the lung tissue + MWCNTs and blank samples (Fig. 4(c)). When the preheating time was further extended, the decomposition of MWCNTs started and then the MWCNTs was partly lost before the measurement. The optimized three-step sequential pretreatment of acid-digestion, muffle-ashing and *in situ* preheating allowed combustive oxidation–NDIR detection of MWCNTs in lung tissue without interference from co-existing biological carbon.

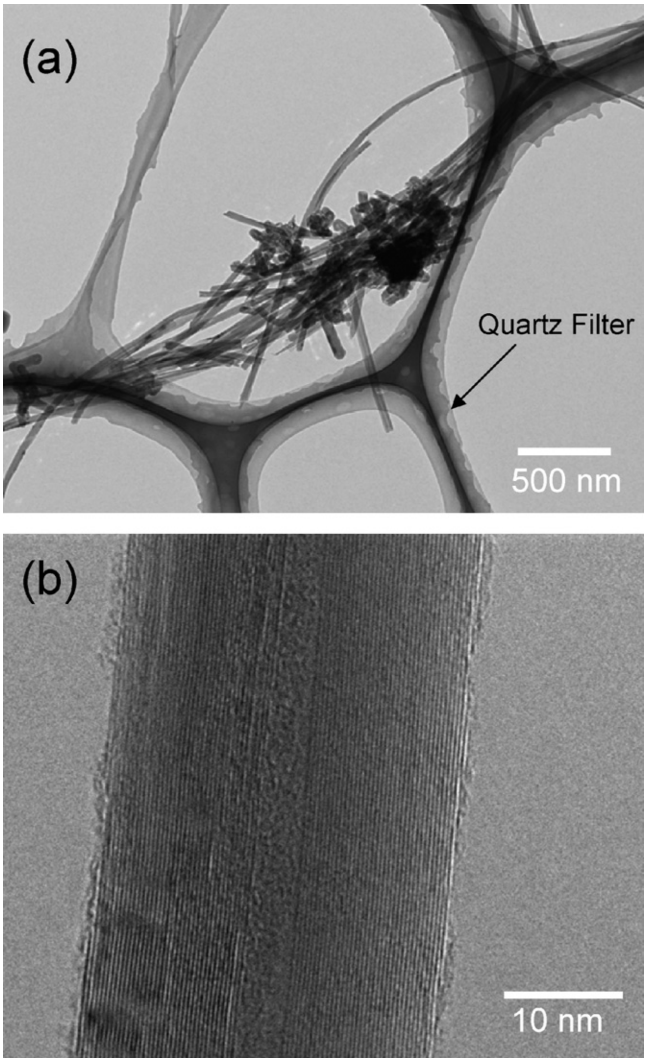

Fig. 5. (a) Low-resolution and (b) high-resolution transmission electron microscope photographs of the residual matter after pretreatment of the homogenized lung tissue from a rat 1 week after intratracheal instillation with MWCNTs (1 mg).

3.3. Validation of the method for determination of MWCNTs in lung tissue

To validate the proposed method, recovery tests were performed by spiking homogenized lung tissue (1 g, wet weight) from the control rats with MWCNTs (1 or 10 μg) dispersed in solution. The residual MWCNTs after pretreatment were determined using glucose calibration curves. The recoveries of MWCNTs after spiking with 1 and 10 μg of MWCNTs were 96.3 ± 0.6% ($n = 3$) and 98.6 ± 2.3% ($n = 5$), respectively. These results indicate that MWCNTs in lung tissue are not lost during sample pretreatment. To confirm this, the lung tissue residue after intratracheal exposure to MWCNTs and sample pretreatment was observed by TEM (Fig. 5). The low- and high-resolution micrographs showed rodlike and multilayer structures, respectively, which suggests that most of the residual matter was MWCNTs with the original structure intact. Additional indirect evidence that the residual matter was mostly MWCNTs was obtained from the temporal profile of the combustive oxidation–NDIR detection for the residual matter (Fig. 6). This profile was almost identical to that for the MWCNTs reagent used in this study (Fig. 2(b)). These results show that with the proposed method, complete decomposition of the coexisting carbon occurs without decomposition of the MWCNTs. Therefore, the combination of sample pretreatment and combustive oxidation–NDIR is satisfactory for determination of MWCNTs in lung tissue samples.

The detection limit (DL), which was defined as the amount that gave a signal-to-noise ratio of three, was 0.30 μg of MWCNTs. The signal and background levels were obtained from analyses of homogenized lung tissue from a control rat that was spiked with solutions containing 1 and 0 μg of MWCNT, respectively. The background noise was calculated as half of the difference between the maximum and minimum intensities obtained from four replicate analyses. The DL enables determination of trace MWCNTs in rat lung, which in turn allows long-term observation of MWCNTs' clearance of the low-dosage exposure test. The repeatability of the analytical method was evaluated by repetitive analyses of a lung sample after intratracheal instillation of 1 mg MWCNTs. The relative standard deviation (RSD) was 5.6% ($n = 4$) when the amount of MWCNTs measured was 42 ± 2 μg in a lung sample. Hence, the proposed method enabled precise determination of MWCNTs in lung tissue.

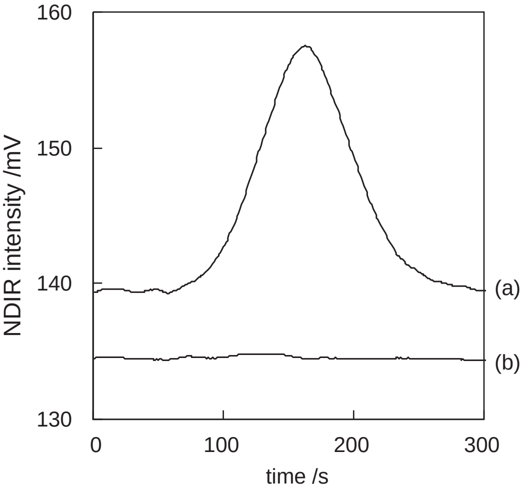

Fig. 6. Temporal profiles of combustive oxidation–NDIR analysis of lung tissues from rats intratracheal instilled with 200 μg of MWCNTs (a) and control rats (b). The amount of lung tissue (wet weight) analyzed in (a) and (b) was 0.055 g and 0.10 g, respectively. The measured amount of MWCNTs for (a) was 4.5 μg. The NDIR spectrum for (a) is elevated off the baseline for better visual observation.

The present analytical method was also applicable to the determination of MWCNTs in the dispersion solution containing Triton-X, which was easily decomposed along with biological carbon by the sample pretreatment.

3.4. *Application to the lung tissues of rats intratracheally instilled with MWCNTs*

The proposed method was applied in a pilot study to investigate the clearance of MWCNTs from rat lung after intratracheal instillation. The instillation was performed with MWCNTs (200 μg) dispersed in solution with Triton-X. Residual MWCNTs in the left lung of each rat were determined by the proposed method. Even the trace amounts on microgram levels of MWCNTs gave a distinct signal in NDIR (Fig. 6). By contrast, no signal was observed for the control rats (Fig. 6). The residual concentrations of MWCNTs in the left lung gradually decreased from 3 days to 1 month after instillation (Fig. 7). Since the deviations obtained for four rats were relatively larger than the determination precision, we estimate that the main reason is the individual difference among the rats. The distribution of MWCNTs in rat lungs after the instillation was heterogeneous: e.g., focal accumulation of agglomerated MWCNTs in the alveoli [26,27]. However, the homogeneity of the MWCNTs in the analytical samples for determinations of the MWCNTs was guarantied because of homogenizing whole of the left lung of a rat. Actually, the determination precision for a homogenized sample was less than 10% as described at Section 3.3. We are conducting further experiments to investigate clearance of MWCNTs over longer periods and after exposure by inhalation. The details from these experiments on the clearance kinetics of MWCNTs from rat lungs will be presented elsewhere.

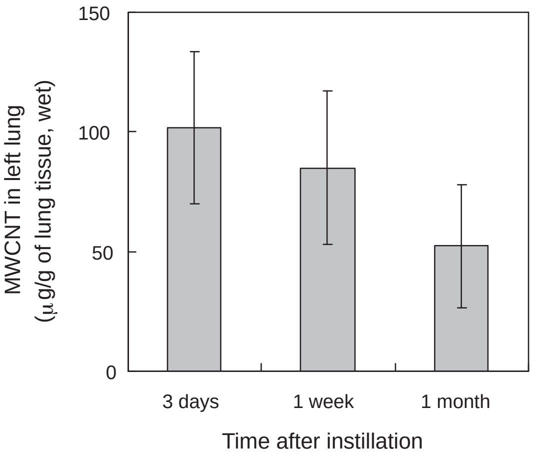

Fig. 7. Time-course of the clearance of MWCNTs from rat lung tissue after intratracheal instillation with 200 μg of MWCNTs. The gray bars and error bars represent the average concentrations and the standard deviations, respectively, obtained from three or four rats.

4. Conclusions

Residual MWCNTs in rat lungs after intratracheal instillation exposure were determined by combustive oxidation and NDIR. Samples were pretreated to remove coexisting biological carbon from lung tissue. The developed method is simple and can be conducted with conventional instruments. Consequently, it will be of use to many researchers to investigate the biological behavior of MWCNTs.

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