

Effect of sterilization process on the formation of mono(2-ethylhexyl)phthalate from di(2-ethylhexyl)phthalate

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Abstract

The risk assessment of di(2-ethylhexyl)phthalate (DEHP) migrating from polyvinyl chloride (PVC) medical devices is an important issue. Many studies have been conducted to determine the level of DEHP migration. A recent report has indicated that DEHP in blood bags is hydrolyzed by esterase into mono(2-ethylhexyl)phthalate (MEHP). However, MEHP is thought to be even more toxic than the parent compound. Therefore, a method for the simultaneous determination of DEHP and MEHP was developed. The limits of quantification (LOQs) of DEHP and MEHP were 2.5 and 0.75 ng/ml, respectively. In this study, the effect of sterilization process on the levels of DEHP and MEHP migration was investigated. The level of migration of DEHP from gamma(γ)-ray sterilized PVC sheet was low compared with that of the unsterilized control. By contrast, the level of MEHP migration from the γ -ray sterilized PVC sheet was high compared with that of the unsterilized control. In addition, a high content of MEHP was found in the γ -ray sterilized PVC sheet.

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1. Introduction

Polyvinyl chloride (PVC) is one of the most widely used polymer materials in the medical field. Flexible PVC is used in the manufacture of blood storage bags, blood tubing, and so on. Plasticizers are incorporated into PVC medical devices to increase their flexibility. The esters of phthalic acid, particularly di(2-ethylhexyl)phthalate (DEHP), are the most preferred plasticizers for medical grade PVC. However, the migration of DEHP from PVC medical devices has been reported [1–5]. DEHP in PVC products easily migrates into foods, drugs and body fluids [6–8]. The toxicity of DEHP has been evaluated [5,9–12], and a risk assessment study has suggested that it is relatively safe for humans.

However, it has been reported that DEHP is hydrolyzed enzymatically into mono(2-ethylhexyl)phthalate (MEHP) [13–15]. In vitro studies have indicated that MEHP inhibits FSH-stimulated cAMP accumulation in cultured Sertoli cells [16–20],

in addition to reducing 17 β -estradiol production and aromatase mRNA expression [21,22]. These results suggest that MEHP is an active metabolite of DEHP, and that any toxic effects of orally ingested DEHP are more likely to be due to the properties of the corresponding monoester rather than the intact DEHP, and that MEHP may be even more toxic than the parent compound. Medical devices are sterilized because they directly contact or are inserted into the human body. It has been reported that DEHP is hydrolyzed by such enzymes as lipases into MEHP in blood bags [13–15]. Some reports have indicated that the hydrolysis may have occurred during sterilization by autoclaving [23,24]. For medical devices, such sterilization processes as autoclaving, gamma(γ)-ray irradiation, and exposure of ethylene oxide gas (EOG), are usually performed.

Therefore, we investigated the effect of the sterilization process on the levels of migration of DEHP and MEHP from PVC medical devices. The PVC sheets that were subjected to various sterilization processes were extracted with purified water, 5% glucose solution or polyoxyethylated hydrogenated castor oil 60 (HCO-60). Moreover, the contents of DEHP and MEHP in the PVC sheets were examined. We have developed the column-

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switching (CS) liquid chromatography–tandem mass spectrometry (LC–MS/MS) as the method for determining DEHP and MEHP with high sensitivity and selectivity.

2. Experimental (materials and methods)

2.1. Chemicals and materials

Environmental analytical grade DEHP and DEHP- d_4 were purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). MEHP and MEHP- d_4 were purchased from Hayashi Pure Chemical Industries (Osaka, Japan). The structures of DEHP, MEHP and their surrogate standards are shown in Fig. 1. Phthalic acid esters, analytical grade acetonitrile and acetone were used in the experiments. The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

The test material was PVC sheet that was subjected to γ -ray irradiation (^{60}Co : 24.2 kGy), autoclaving ($115^\circ\text{C} \times 40$ min) or EOG ($50^\circ\text{C} \times 8$ h) as the sterilization process. In addition, commercially available PVC tubing on which γ -ray sterilization (20–25 kGy) was performed was used. None of the sterilization processes were performed on the control sample that was kindly supplied by the manufacturer.

The extraction solvents were 5% glucose solution for injection (Otsuka Pharmaceuticals Co., Tokyo, Japan), polyoxyethylated hydrogenated castor oil 60 (HCO-60) (Wako Pure Chemical Industries Ltd., Osaka, Japan) and purified water.

2.2. Instrumentation

A Series 1100 liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000TM (Applied Biosystems Japan, Tokyo, Japan) equipped with a Turbo IonsprayTM ionization source. Mass spectrometry data were processed with Analyst 1.3.2 software. A Shimadzu (Kyoto, Japan) LC-10 AS pump was used for providing flow through the extraction column

to load and wash the sample and to equilibrate the extraction column. A Mightysil[®] RP-18 GP column (5 mm \times 2.0 mm, 5 μm particle size) from Kanto Chemical was used for the separation. An Oasis[®] HLB extraction column (20 mm \times 2.1 mm, 25 μm particle size) from Waters was used for the extraction and clean-up.

2.3. Chromatographic and extraction conditions

The column-switching system was used for sample injection [25]. After 10 μl of the sample was injected with an auto-sampler, it was loaded onto the extraction column by flowing pure water at the rate of 1 ml/min using the LC-10 AS pump for 3 min. The matrices in the sample were eluted whereas DEHP and MEHP were retained on the extraction column. After the 3 min period, the switching valve was changed to configuration B (Fig. 2). The column oven was maintained at 40°C . Separation was carried out with a mobile phase of acetonitrile/water (90/10, v/v) at a flow rate of 0.2 ml/min. The eluate from the analytical column was directed to the electrospray MS. After elution for 8 min, the switching valve was returned to the original position (configuration A in Fig. 2).

2.4. MS/MS conditions

The working parameters for turbo ionspray ionization MS/MS were as follows: declustering potentials, 81 V (DEHP and DEHP- d_4) and -60 V (MEHP and MEHP- d_4); curtain gas flow rates, 20 psi (DEHP and DEHP- d_4) and 30 psi (MEHP and MEHP- d_4); nebulizer gas (N_2) pressure, 30 psi; and turbo ionspray gas (N_2) pressure, 0 psi. The ion source temperature was maintained at 650°C and the turbo ionspray voltages for DEHP (DEHP- d_4) and MEHP (MEHP- d_4) were 5500 and -4500 V, respectively. DEHP and DEHP- d_4 were detected in the positive mode, whereas MEHP and MEHP- d_4 were detected in the negative mode. The product ion mass spectra of DEHP, DEHP- d_4 , MEHP and MEHP- d_4 obtained by the LC–MS/MS system

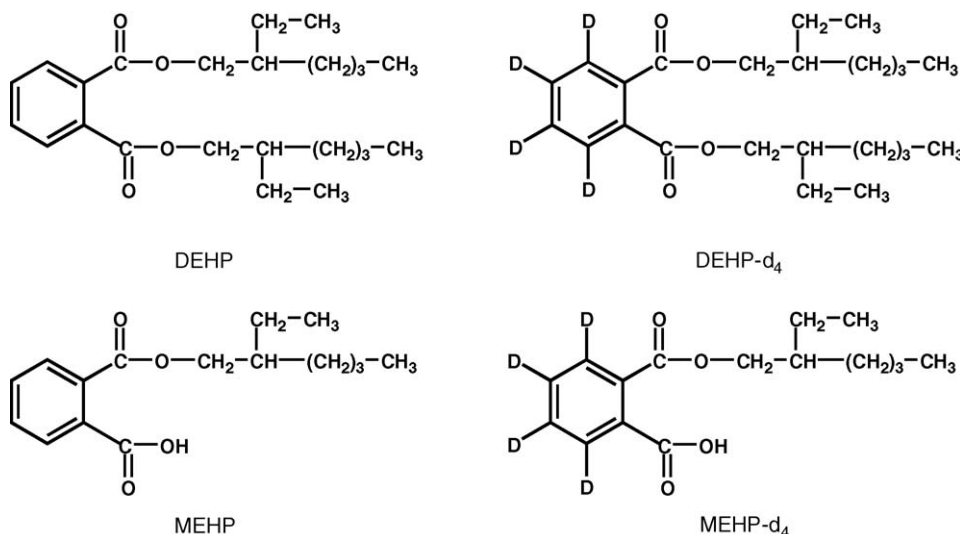


Fig. 1. Chemical structures of DEHP, MEHP and their internal standards.

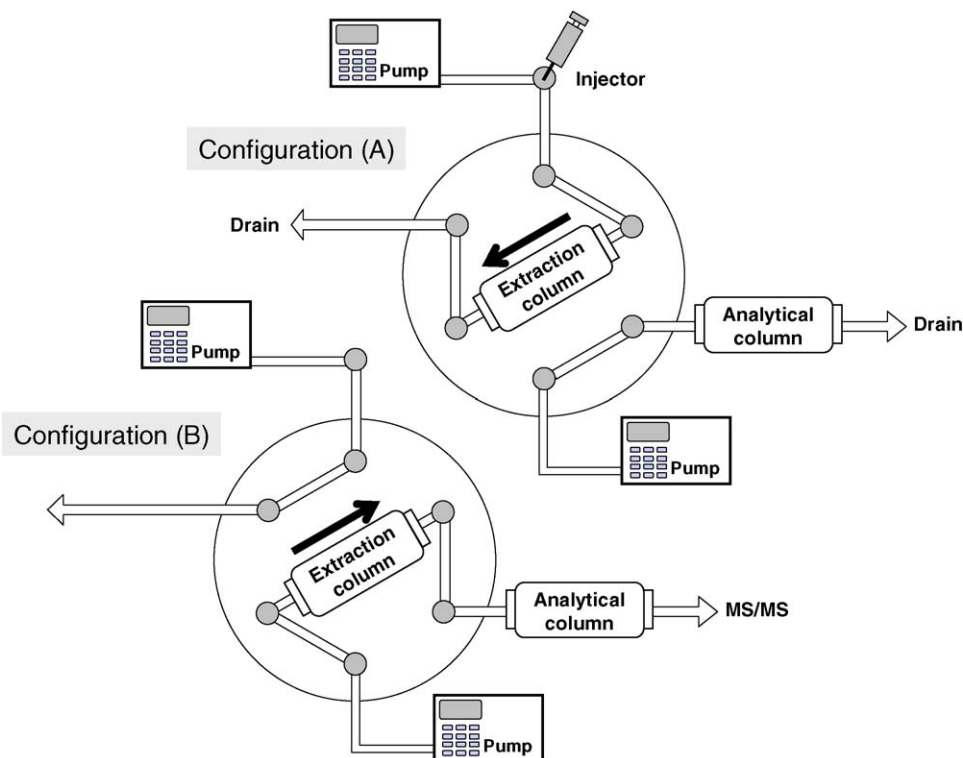


Fig. 2. Schematic representation of the column-switching LC-MS/MS system. (A) Configuration for sample loading and washing; (B) configuration for sample elution.

are shown in Fig. 3. The combinations of precursor ion and product ions were as follows: DEHP (precursor ion \rightarrow product ion, m/z 391 \rightarrow 149), DEHP- d_4 (m/z 395 \rightarrow 153), MEHP (m/z 277 \rightarrow 134), and MEHP- d_4 (m/z 281 \rightarrow 138). The collision gas (N_2) pressures were set at 2 units (DEHP and DEHP- d_4) and 1 unit (MEHP and MEHP- d_4).

2.5. Migration test

The migration of DEHP and MEHP from the PVC sheet (1 cm \times 3 cm) was examined in 5 ml of each solvent. HCO-60 is a surfactant that is used in the formulation of such drugs as Prograf[®] and is involved in the migration of DEHP. In addition,

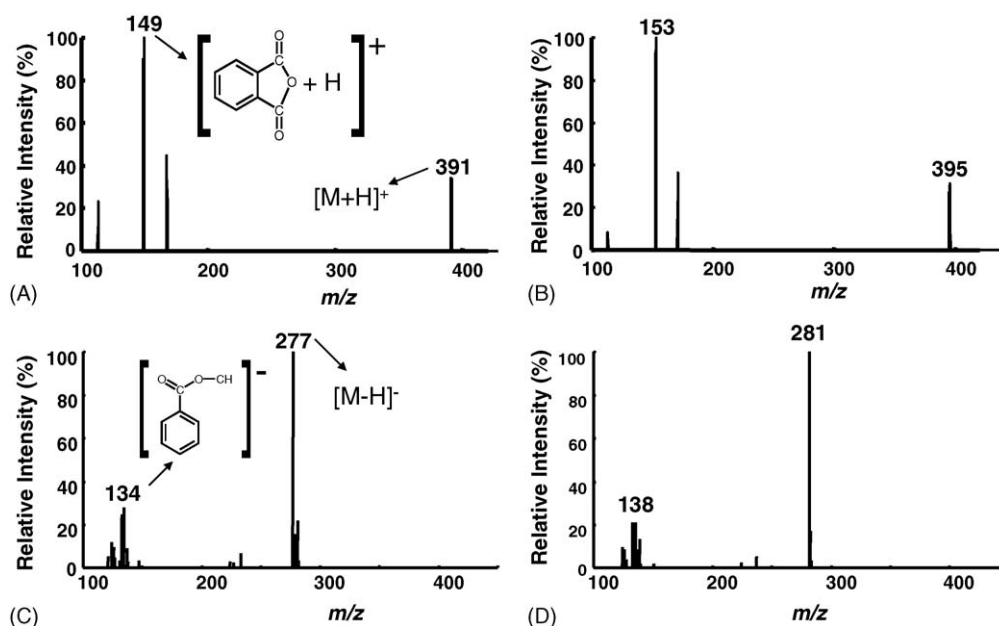


Fig. 3. Product ion mass spectra of DEHP, MEHP and their internal standards. (A) DEHP; (B) DEHP- d_4 (internal standard for DEHP); (C) MEHP; (D) MEHP- d_4 (internal standard for MEHP).

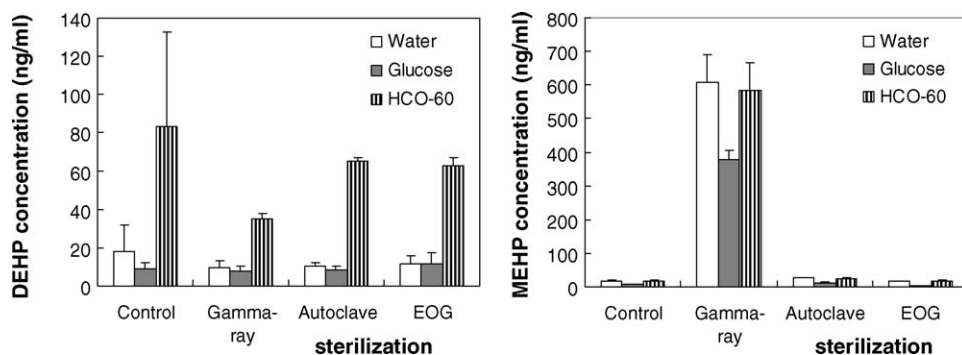


Fig. 4. Levels of DEHP and MEHP migration into various solutions from PVC sheet samples. Each plotted column is the mean the levels of DEHP or MEHP with triplicate analysis ($n=3$). The error bar represents the standard deviation (S.D.).

it has reported that the level of DEHP migration was dependent on the concentration of HCO-60 [25]. We prepared 0.02 mg/ml HCO-60 for the migration test [26]. The samples were kept in test tubes and extraction was carried out with shaking at 37 °C for 1 h. A 1 ml aliquot of the extract was pipetted into another test tube, and DEHP- d_4 and MEHP- d_4 were added. Then, the sample solution was appropriate diluted prior to LC–MS/MS analysis.

The PVC tubing was cut to 10 cm length and filled with the solvents (tube length, 8 cm). The tubing was subjected to extraction with shaking at 37 °C for 1 h. The extracts were pipetted into another test tube containing DEHP- d_4 and MEHP- d_4 . Then, all the samples were appropriate diluted prior to LC–MS/MS analysis.

2.6. Contents of DEHP and MEHP in PVC

A PVC sample (5 mg) was completely dissolved in 5 ml of THF. The solution was appropriate diluted with acetonitrile. Then, the internal standard was added prior to the analysis.

3. Results and discussion

3.1. Analysis of DEHP and MEHP by on-line SPE–LC–MS/MS

In the proposed method, the limits of quantification (LOQs) (signal-to-noise ratio >10) of DEHP and MEHP were 2.5 and 0.75 ng/ml with the standard solutions, respectively. For DEHP measurement, a calibration curve was obtained by plotting the peak-area ratio (DEHP/DEHP- d_4) versus DEHP concentration, and was linear over the range of 2.5–500 ng/ml ($r=0.998$). For MEHP measurement, a calibration curve was obtained by plotting the peak-area ratio (MEHP/MEHP- d_4) versus MEHP concentration, and was linear over the range of 0.75–500 ng/ml ($r=0.997$). We also examined the recovery using 5% glucose solution. For the glucose solution that was spiked with 50 ng/ml DEHP and MEHP, the average recoveries of DEHP and MEHP were 99.2% (R.S.D. = 3.2%, $n=6$) and 109.0% (R.S.D. = 3.4%, $n=6$), respectively.

3.2. Determination of DEHP and MEHP migration from PVC sheet and tubing

The developed method was applied to the determination of DEHP and MEHP migration from the PVC sheets that were subjected to various sterilization processes (Fig. 4). The migration of DEHP and MEHP from all the PVC sheets was observed. The level of DEHP migration had the following order: HCO-60 > water \geq 5% glucose solution, similar to the report of Hanawa et al. [25]. Furthermore, when the PVC sheets were extracted with purified water and HCO-60, the levels of DEHP migration from all the PVC sheets that were subjected to the sterilization processes, particularly γ -ray sterilization, were low compared with the unsterilized control. On the other hand, the levels of MEHP migration from the unsterilized control, autoclaved and EOG sterilized PVC sheets were not different, whereas the γ -ray sterilized PVC sheet released a large amount of MEHP.

Then, the levels of DEHP and MEHP migration from the commercially available PVC tubing sterilized by γ -ray were compared with those of the unsterilized one (Fig. 5). As expected, DEHP more easily migrated in HCO-60 than in water or glucose solution. In HCO-60, the level of DEHP migration from the γ -ray sterilized PVC tubing was low compared with that of the unsterilized one. Moreover, the unsterilized PVC tubing released little MEHP whereas the γ -ray sterilized PVC tubing released approximately 30–40 times more MEHP compared with the unsterilized one. In addition, MEHP was also released from the γ -ray sterilized PVC tubing that was extracted with water or glucose solution.

3.3. Contents of DEHP and MEHP in PVC sheet and tubing

The developed method was also applied to determine the contents of DEHP and MEHP in the PVC sheets (Table 1) and the PVC tubing (Table 2). We thought that the DEHP contents were almost the same in the various sterilized sheets because the DEHP contents were 26.8–27.8% (w/w) in the sterilized PVC sheets. By contrast, MEHP was detected in only the γ -ray sterilized PVC sheet. Therefore, PVC material might inherently contain MEHP, and the MEHP migrated directly into the solvent.

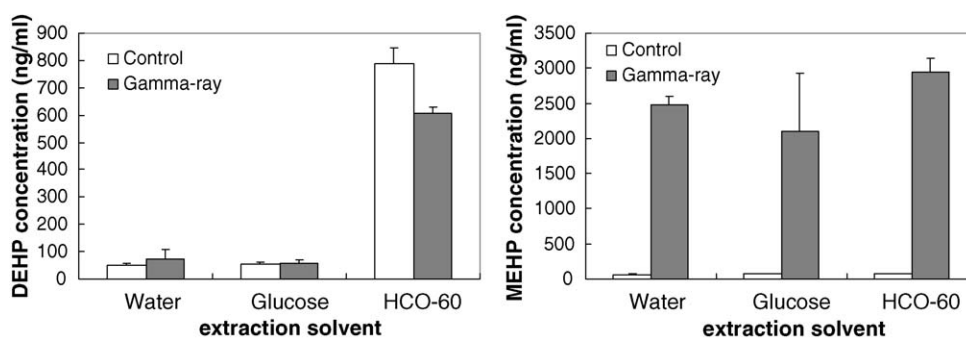


Fig. 5. Level of DEHP and MEHP migration into various solutions from commercially available PVC tubing. Each plotted column is the mean the levels of DEHP or MEHP with triplicate analysis ($n=3$). The error bar represents the standard deviation (S.D.).

Table 1
Contents of DEHP and MEHP in PVC sheet samples treated with various sterilization

	Control	Gamma-ray	Autoclave	EOG
DEHP (% w/w)	32.1 ± 5.7	27.8 ± 0.8	26.8 ± 1.6	26.8 ± 0.8
MEHP (mg/g)	<0.25	0.38 ± 0.05	<0.25	<0.25

Mean \pm S.D., $n=3$.

The commercially available PVC tubing which was sterilized by γ -ray was confirmed to contain MEHP, although the level was below the LOQ. A high dilution ratio was required because of the difference in level between DEHP and MEHP. Therefore, MEHP could not be determined in some of the sterilized PVC sheets.

In this study, the following phenomena were observed: (1) the level of DEHP migration from the γ -ray sterilized PVC sheet was low compared with that of the unsterilized sheet. Surface processing, an example of which is plasma treatment, is known to suppress DEHP migration [27,28]. We speculated that a similar surface processing occurred with γ -ray irradiation; (2) the level of MEHP migration from the γ -ray sterilized PVC sheet was significantly high compared with that of the unsterilized control. In addition, MEHP was released from the γ -ray sterilized PVC sheet regardless of the solvent used. We hypothesized that MEHP was inherently contained in the PVC sheet, and then directly migrated from it. To confirm this hypothesis, we determine the DEHP and MEHP contents in the PVC sheet; (3) MEHP was detected in the γ -ray sterilized PVC sheet. Although MEHP was also found to migrate from the PVC sheets sterilized by EOG or autoclaving, MEHP was not detected in them. We speculated that the MEHP contents in the other sterilized sheets were very low compared with that in the γ -ray sterilized PVC sheet. In addition, the most plausible reason for not detecting MEHP in

the PVC sheets was the high dilution ratio of the samples; (4) although MEHP was also detected in PVC tubing, its amount could not be determined.

Taking these into consideration, we surmised that MEHP was inherently present in PVC and migrated directly from it, although MEHP was thought to be hydrolyzed by enzymes as lipases or by autoclave sterilization until now. In addition, we speculated that the sterilization by γ -ray was sufficient to decompose DEHP into MEHP in PVC materials. We conducted a risk assessment of MEHP migration. The level of MEHP migration was calculated as follows: we found that when γ -ray sterilized PVC sheet (1 cm \times 3 cm) was extracted with 5 ml of solvent, approximately 600 ng/ml MEHP migrated from it. Therefore, the amount of MEHP migration was 3.0 μ g (=600 ng/ml \times 5 ml). As the superficial area involved in the migration was 6 cm², the level of MEHP migration per unit area was 0.5 μ g/cm². The superficial area of the commercially available infusion set was 101.3 cm² at the maximum. Therefore, the amount of MEHP migration from the infusion set was 50.65 μ g (=101.3 cm² \times 0.5 μ g/cm²). When this infusion set was used for 2 days in a patient with 50 kg body weight, the patient was exposed to 0.51 μ g/kg/day of MEHP. In 2001, the Center for Devices and Radiological Health of the U.S. Food and Drug Administration reported DEHP assessment as ‘‘Safety assessment of DEHP released from PVC medical devices.’’ In Annex C of the report, ‘‘Aggregate safety assessment for coexposure to DEHP and MEHP,’’ the relative potency of MEHP/DEHP was calculated to be 10. Therefore, an MEHP exposure of 0.51 μ g/kg/day meant a DEHP exposure of 5.1 μ g/kg/day. In addition, in this report, the tolerable intake (TI) of DEHP was 40 μ g/kg/day by oral administration and 600 μ g/kg/day by parenteral route. Although the level of MEHP that migrated from the γ -ray sterilized PVC did not exceed the TI, we must investigate the effect of formation of MEHP from DEHP by γ -ray sterilization for high risk patients such as infant and pregnant women.

Table 2
Contents of DEHP and MEHP in commercially available PVC tubing

	Control	Gamma-ray
DEHP (% w/w)	44.7 ± 2.9	53.9 ± 2.5
MEHP (mg/g)	<0.25	<0.25 (0.23)

Mean \pm S.D., $n=3$.

Acknowledgements

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ilized by γ -ray, autoclaving or EOG were kindly supplied by the Terumo Corporation (Tokyo, Japan) and JMS Corporation (Hiroshima, Japan).

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