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Note

Effect of gamma-ray irradiation on degradation of di(2-ethylhexyl)phthalate in polyvinyl chloride sheet

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ABSTRACT

The risk assessment of di(2-ethylhexyl)phthalate (DEHP) migration from polyvinyl chloride (PVC) medical devices is an important issue for patients. The aim of this study was to determine DEHP degradation and migration from PVC sheets. To this end, the method for the simultaneous determination of DEHP and its breakdown products (mono(2-ethylhexyl)phthalate (MEHP) and phthalic acid (PA)) was improved. Their migration levels from 0 to 50 kGy gamma-ray irradiated PVC sheets were determined. DEHP migration level decreased in proportion to the dose of gamma-ray irradiation, while MEHP and PA migration levels increased. The hardness and the elastic modulus of PVC sheets were examined, but no clear relationship between DEHP migration and these parameters was observed.

1. Introduction

Phthalate esters are widely used as industrial plasticizers. In particular, di(2-ethylhexyl)phthalate (DEHP) is used in the production of polyvinyl chloride (PVC) and other plastics to increase flexibility, softness, and stability for specific applications. PVC is one of the most widely used plastic polymers in such medical products as blood containers, blood tubing, and catheters. However, it has been reported that DEHP is easily released from PVC products into food, drugs, and body fluids [Earls et al., 2003; Inoue et al., 2003; Takatori et al., 2004; Ito et al., 2005]. DEHP is considered to exhibit reproductive and developmental toxicity [Lovekamp-Swan and Davis, 2003], carcinogenicity, and testicular toxicity [Tickner et al., 2001; Yakubovich and Vienken, 2000; Hill et al., 2001]. Some phthalates including DEHP are said to exhibit toxic effects, including antiandrogenic effects during reproductive system development and normal sperm production in male rat [Poon et al., 1997; Lamb et al., 1987; Tyl et al., 1988], and the decrease in blood 17β-estradiol level in female rat [Davis et al., 1994]. In addition, recent studies have shown that certain phthalate exposure levels in pregnant women are associated with the reproductive health of male infants [Latini et al., 2003; Swan et al., 2005; Marsee et al., 2006]. In Japan, The Ministry of Health, Labour and Welfare (2000) has set the tolerable daily intake (TDI) of DEHP at 40–140 $\mu g\,kg^{-1}\,day^{-1}$ and has regulated the use of DEHP as plasticizer in the manufacture of infant toys.

In our previous studies, we observed that not only DEHP but also mono(2-ethylhexyl)phthalate (MEHP) and phthalic acid (PA) migrated from PVC medical devices into simulated pharmaceuticals even without enzymatic hydrolysis [Ito et al., 2005, 2006, 2008]. DEHP migration was suppressed by the sterilization process, particularly gamma-ray sterilization [Ito et al., 2006]. In contrast, MEHP migration from gamma-ray sterilized PVC medical device was increased dramatically [Ito et al., 2006, 2008]. Since MEHP is thought to be even more toxic than DEHP, the formation of MEHP as a breakdown product of DEHP is a critical problem.

In this study, DEHP, MEHP, and PA migration levels were determined to confirm the effect of gamma-ray irradiation on the degradation of DEHP. Commercially available PVC medical devices are generally subjected to 20–25 kGy gamma-ray sterilization. Therefore, PVC sheets used in the manufacture of blood bags were irradiated with 1–50 kGy gamma rays. No sterilization process

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was performed on the control sample (non-irradiated gamma ray: 0 kGy). DEHP, MEHP, and PA migration levels were examined in relation to the dose of gamma rays. Moreover, the hardness and the elastic modulus of irradiated PVC sheet were examined because surface processing, an example of which is polyethylene glycol grafting, is known to suppress DEHP migration [Lakshmi and Jayakrishnan, 1998]. Then, the effect of gamma-ray irradiation on the PVC surface was studied to understand the relationship between DEHP, MEHP, and PA migration levels and the hardness and the elastic modulus of irradiated PVC sheets.

2. 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). PA and PA-d_4 were purchased from CDN Isotope Central Chemicals Co. Inc. (Tokyo, Japan). Phthalic acid esters, analytical grade acetonitrile, and acetone were used in the experiments. Analytical grade formic acid was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 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 subjected to gamma-ray irradiation (^{60}Co; 1, 5, 10, 25, 50 kGy). Commercial medical devices were irradiated with approximately 25 kGy gamma rays for sterilization. The control sample was not irradiated with gamma rays. The PVC sheets were 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 and LC–MS/MS conditions

A Series 1100 liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000^{TM} (Applied Biosystems Japan, Tokyo, Japan) equipped with a Turbo Ionspray^{TM} ionization source. Mass spectrometry data were processed with Analyst 1.3.2 software. An Inertsil-Ph3 column (50 mm × 2.1 mm, 5 μm particle size) from GL Sciences was used for separation.

After 5 μL of the sample was injected with an auto-sampler, it was loaded onto the analytical column by introducing the mobile phase at the flow rate of 0.2 ml min^{-1}. The auto-sampler was maintained at 4 °C to keep the sample cool. Acetonitrile (mobile phase A) and 0.05% formic acid in water (mobile phase B) were used. Separation was carried out with the following profile: mobile phase A/B was 15/85 (0–4 min) → 90/10 (4.01–15 min for elution) → 15/85 (15.01–25 min for equilibration) (v/v). The column oven was maintained at 40 °C for LC.

The working parameters for turbo ionspray ionization MS/MS were as follows: curtain gas, 20 psi (DEHP and DEHP-d_4 for the positive ion mode) and 20 psi (MEHP, PA, and their internal standards for the negative ion mode); nebulizer gas (N_2) pressure, 80 psi for the positive ion mode and 80 psi for the negative ion mode; and turbo ionspray gas (N_2) pressure, 60 psi for the positive ion mode and 60 psi for the negative ion mode. Ion source temperature was maintained at 650 °C and turbo ionspray voltages for the positive ion mode (DEHP, DEHP-d_4) and the negative ion mode (MEHP, PA, and their internal standards) were 5000 and −4500 V, respectively. Declustering potentials of DEHP, MEHP, and PA were 61 V, −60 V, and −35 V, respectively. DEHP and DEHP-d_4 were monitored in the positive ion mode, whereas MEHP, PA, and their internal standards were monitored in the negative ion mode. The combinations of precursor ion and product ions were as follows: DEHP (precursor ion → product ion, m/z 391 → 149), DEHP-d_4 (m/z 395 → 153), MEHP (m/z 277 → 134), MEHP-d_4 (m/z 281 → 138), PA (m/z 165 → 121), and PA-d_4 (m/z 169 → 125). The collision gas (N_2) pressure was set at 4 units for both positive and negative ion modes. These conditions were modified from those of our previous paper [Ito et al., 2008].

2.3. Effect of gamma-ray irradiation on migration test

The migration of DEHP, MEHP, and PA from PVC sheet (1 cm × 3 cm) into 5 ml of each extraction solvent was examined. 5% glucose solution, HCO-60, and purified water were used as extraction solvent. They also served as simulated pharmaceuticals. HCO-60 is a surfactant that is involved in the migration of DEHP into such drugs as Prograf®. The extent of DEHP migration was dependent on the concentration of HCO-60 [Hanawa et al., 2003]. In this study, 2 mg ml^{-1} HCO-60 was prepared for the migration test. The concentration of HCO-60 was set with reference to its content in commercial pharmaceuticals as surfactant. The samples were kept in test tubes and extraction was carried out by shaking at 37 °C for 1 h. An aliquot (1 ml) of the extract was pipetted into another test tube and DEHP-d_4, MEHP-d_4, and PA-d_4 were added. Then, the sample solution was appropriately diluted prior to LC–MS/MS analysis.

2.4. Instrumentation and conditions for indentation measurement

The hardness and the elastic modulus of non-irradiated (0 kGy) and irradiated PVC sheets (25 and 50 kGy) were measured by a depth-sensing nanoindentation technique with Nano indenter XP (MTS Systems Co., Oak Ridge, TN, USA). PVC sheets were indented with a Berkovich diamond tip to a maximum depth of 55 μm at room temperature (23 ± 1 °C). The indentation load–displacement behavior of gamma-ray irradiated PVC sheets was tested in the continuous stiffness measurement mode. As shown in Fig. 1, load–displacement curves were obtained to determine the

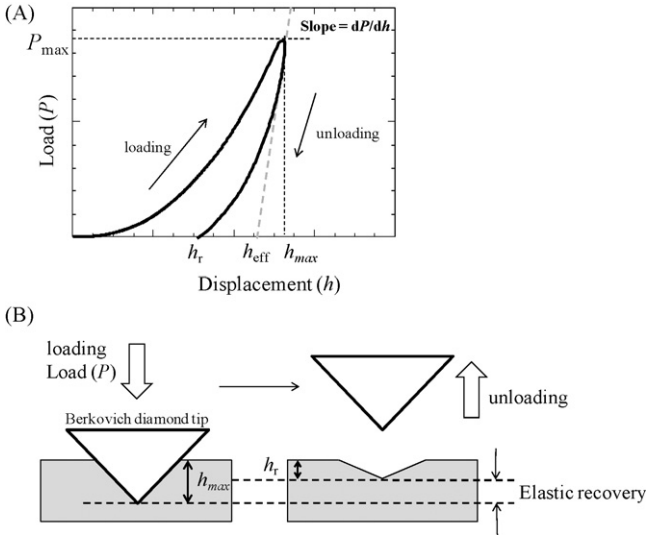

Fig. 1. Determination of hardness and the elastic modulus. (A) Typical load versus displacement curve. (B) Schematic diagram of indentation measurement.

Table 1
Figures of merit of LC–MS/MS method for determination of DEHP, MEHP, and PA.

	Range (ng ml^{-1})	Spiked conc. (ng ml^{-1})	Purified water		5% glucose		HCO-60	
	(r)		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DEHP	20–1000	100	97.5	6.2	97.2	4.4	98.5	1.8
	(0.998)	500	97.5	1.8	99.9	0.9	94.5	1.2
MEHP	2–1000	100	102	2.4	105	5.2	105	2.5
	(0.999)	500	98.2	3.1	101	1.8	104	3.9
PA	5–1000	100	97.0	7.8	90.4	7.0	107	3.4
	(0.999)	500	102	1.8	102	2.1	104	1.4

r: correlation coefficient; RSD: relative standard deviation ($n = 3$).

hardness (H) and the elastic modulus (E) of the sheets. Theoretical elastic modulus and theoretical hardness were calculated as follows:

$$E \approx \frac{\sqrt{\pi}}{2\beta}\frac{1}{\sqrt{kh_{eff}^2}}\frac{dP}{dh} \quad \text{and} \quad H = \frac{P_{max}}{kh_{eff}^2}$$

where β and k are constants. When the Berkovich diamond tip was used, $k = 24.56$ and $\beta = 1.034$.

Hardness and elastic modulus were calculated by multiplying modification coefficient (η) by the theoretical hardness and the theoretical elastic modulus. In addition, modification coefficient was confirmed with a load–displacement curve obtained from a calibration experiment using silica.

3. Results and discussion

3.1. Optimization of the LC–MS/MS method

In the scan mode, DEHP, MEHP, and PA were monitored at m/z 391, 277, and 165, which were assigned to $[M+H]^+$, $[M-H]^-$, and $[M-H]^-$, respectively. Moreover, in the product ion MS/MS measurement, selective reaction monitoring ions (SRMs) of DEHP, DEHP-d_4, MEHP, MEHP-d_4, PA, and PA-d_4 were set depending on their precursor ions. When the auto-sampler was maintained at room temperature, the peak shape was not good. However, when the auto-sampler was maintained at 4 °C to keep the sample cool, a reproducible peak area was obtained. In addition, the sample solution was acidified (1%) to improve separation. No interference from peaks of the other compounds present in the extraction solvents was noted.

3.2. Figures of merit of LC–MS/MS analysis for determination of DEHP, MEHP, and PA

In the proposed method, determination was achieved by stable isotope dilution analyses. The limits of detection (LODs) of DEHP, MEHP, and PA subjected to LC–MS/MS analysis were 5, 0.5, and 1 ng ml^{-1}, respectively, and the signal-to-noise (S/N) ratio was 3. In addition, the limits of quantification (LOQs) of DEHP, MEHP, and PA when S/N > 10 were 20, 2, and 5 ng ml^{-1}, respectively. The method showed good linearity and the correlation coefficients (r) were higher than 0.998 for all the analytes. The figures of merit of the present method are summarized in Table 1. Sensitivity and accuracy were sufficient for the determination of DEHP, MEHP, and PA migration levels from PVC sheets.

The recovery and precision of the method were assessed by replicate analyses ($n = 3$) of each solvent spiked at 100 and 500 ng ml^{-1} levels. Non-spiked and spiked samples were subjected to LC–MS/MS analysis. Recovery was calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were obtained by using calibration curves acquired from standard solutions containing the surrogate compounds. The recovery and precision were 97.0–102% (relative standard deviation—RSD: 1.8–7.8%), 90.4–105% (RSD: 0.9–7.0%), and 94.5–107% (RSD: 1.2–3.9%) for purified water, 5% glucose solution, and HCO-60, respectively (Table 1). Therefore, the method enables the precise determination of standards and may be applicable to the determination of DEHP, MEHP, and PA in pharmaceutical solutions containing HCO-60 as surfactant.

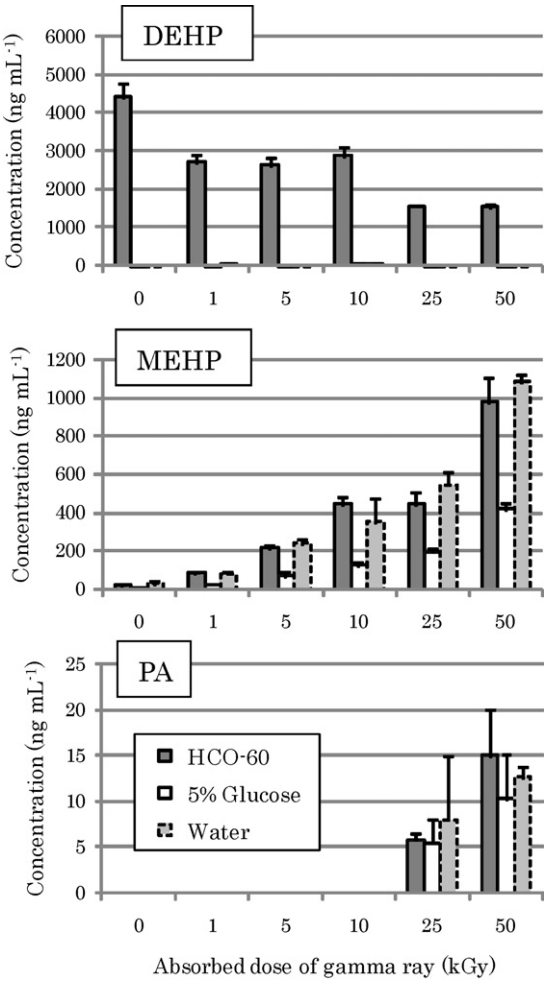

Fig. 2. Concentrations of DEHP, MEHP, and PA that migrated from gamma-ray irradiated PVC sheet. Extraction solvents HCO-60, 5% glucose solution, and water are represented by dark columns with a solid line, white columns with a solid line, and gray columns with a dotted line, respectively.

3.3. Effect of gamma-ray irradiation on DEHP degradation

DEHP, MEHP, and PA migration levels from gamma-ray irradiated PVC sheets are shown in Fig. 2. Similar to that reported in another paper [Ito et al., 2006], DEHP migration was marked when HCO-60 was used as the extraction solvent. DEHP, MEHP, and PA migration from PVC sheets was influenced by gamma-ray irradiation. The higher the dose of irradiated gamma rays, the lower DEHP migration level from the PVC sheets. In contrast, MEHP migration from the irradiated PVC sheets was dependent on the dose of gamma-ray irradiation. In the case of PVC sheets subjected to 1 and 5 kGy gamma-ray irradiation and non-irradiated (0 kGy), no PA was detected (below LOD). In PVC sheets exposed to 10 kGy gamma-ray irradiation, trace level of PA was detected (between LOD and LOQ). PA was clearly detected when the gamma-ray irradiation exceeded 25 kGy. The amount of PA that migrated from the 50 kGy gamma-ray irradiated PVC sheet was higher than that from the 25 kGy irradiated one. Therefore, PA migration from the irradiated PVC sheet was thought to be dependent on the gamma-ray irradiation dose applied to the PVC sheet.

It should be noted that the molar concentration of DEHP that was decreased upon gamma-ray irradiation was not equal to the total molar concentration of MEHP and PA that was increased upon gamma-ray irradiation; however, taking into consideration the fact that MEHP migration level was well correlated with the dose of gamma-ray irradiation, we can say that MEHP and PA were formed from the breakdown of DEHP by gamma-ray irradiation. Moreover, there is a possibility that other factors were involved in the migration of these compounds.

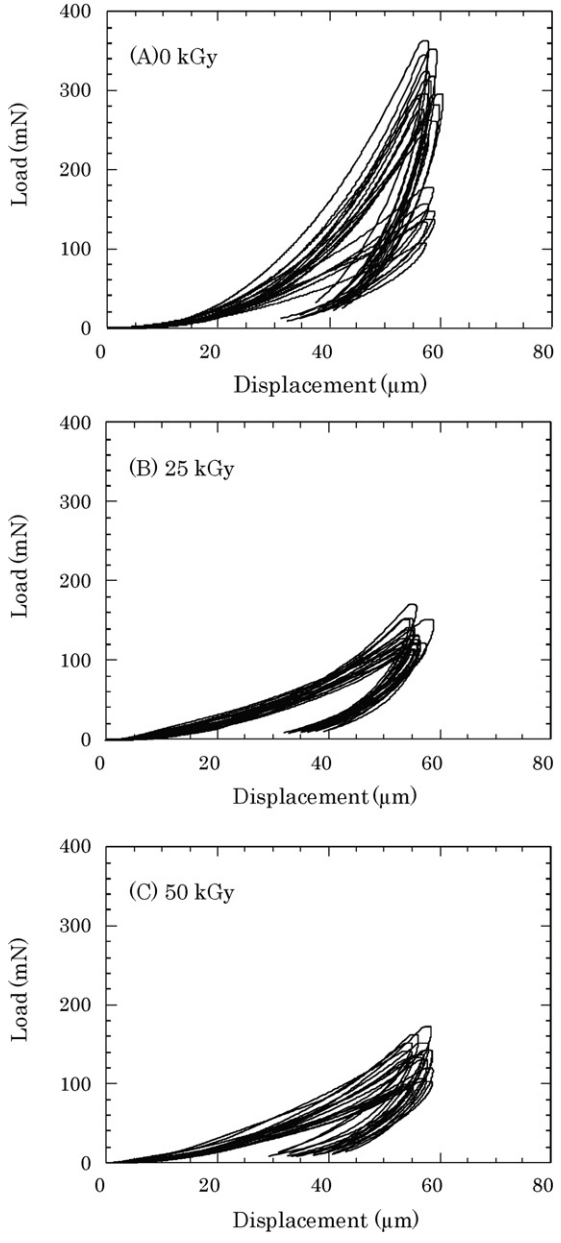

Fig. 3. Load–displacement curves for 0, 25, and 50 kGy gamma-ray irradiated PVC sheets. These load–displacement curves were obtained from (A) 0 kGy gamma-ray irradiated PVC sheet, (B) 25 kGy gamma-ray irradiated PVC sheet, and (C) 50 kGy gamma-ray irradiated PVC sheet.

3.4. Indentation measurement of gamma-ray irradiated PVC sheets

The load–displacement curves for non-irradiated (0 kGy) and irradiated (25 and 50 kGy gamma ray) PVC sheets are shown in Fig. 3. Although the load–displacement curves were scattered widely, replicate studies ($n = 20$) were conducted to determine the average behavior. It was thought that the contact state between the Berkovich diamond tip and the PVC sheet could not be kept in the same state because of the rough surface of the PVC sheet (Fig. 4). From the load–displacement curves, Young's modulus versus depth plots (Fig. 5A) and hardness versus depth plots (Fig. 5B) were obtained. As shown in Fig. 5, the correlation between hardness and depth or between elastic modulus and depth was observed. In particular, below 20 μm depth, a difference was observed between non-irradiated sample (0 kGy) and irradiated samples (25 and 50 kGy). However, it is thought that the data were influenced by the rough surface of the PVC sheet when monitoring was carried out at small depths. Similarly, when monitoring was carried out at large depths (≥40 μm), the data were influenced by the rough back surface as the PVC sheet itself was approximately 400 μm thick. The elastic modulus and the hardness at 1, 5, 10, and 20 μm depth from the surface are shown in Table 2. In calculating the elastic modulus, the Poisson ratio was empirically assumed to be 0.3. As a result, the elastic modulus of gamma-ray irradiated PVC sheet was higher than that of the non-irradiated sample. Surface PVC chains might be cross-linked or broken by gamma-ray irradiation [Mendizabal et al., 1996; Baccaro et al., 2003; Silva et al., 2008] and might be influenced by the migration of DEHP, MEHP, and PA.

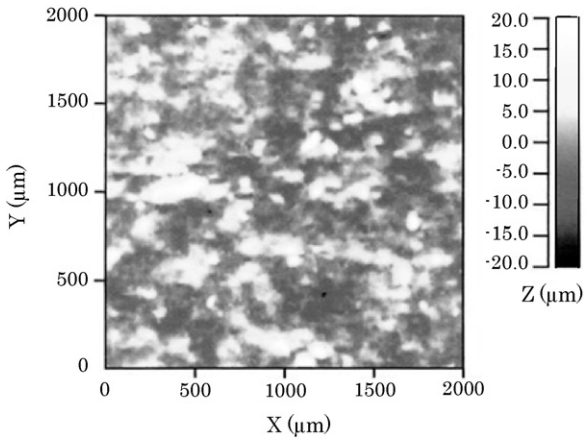

Fig. 4. 3D surface topography of non-irradiated PVC sheet. KLA-Tencor P-15 contact stylus profiler was used to measure the surface roughness of a sample PVC sheet.

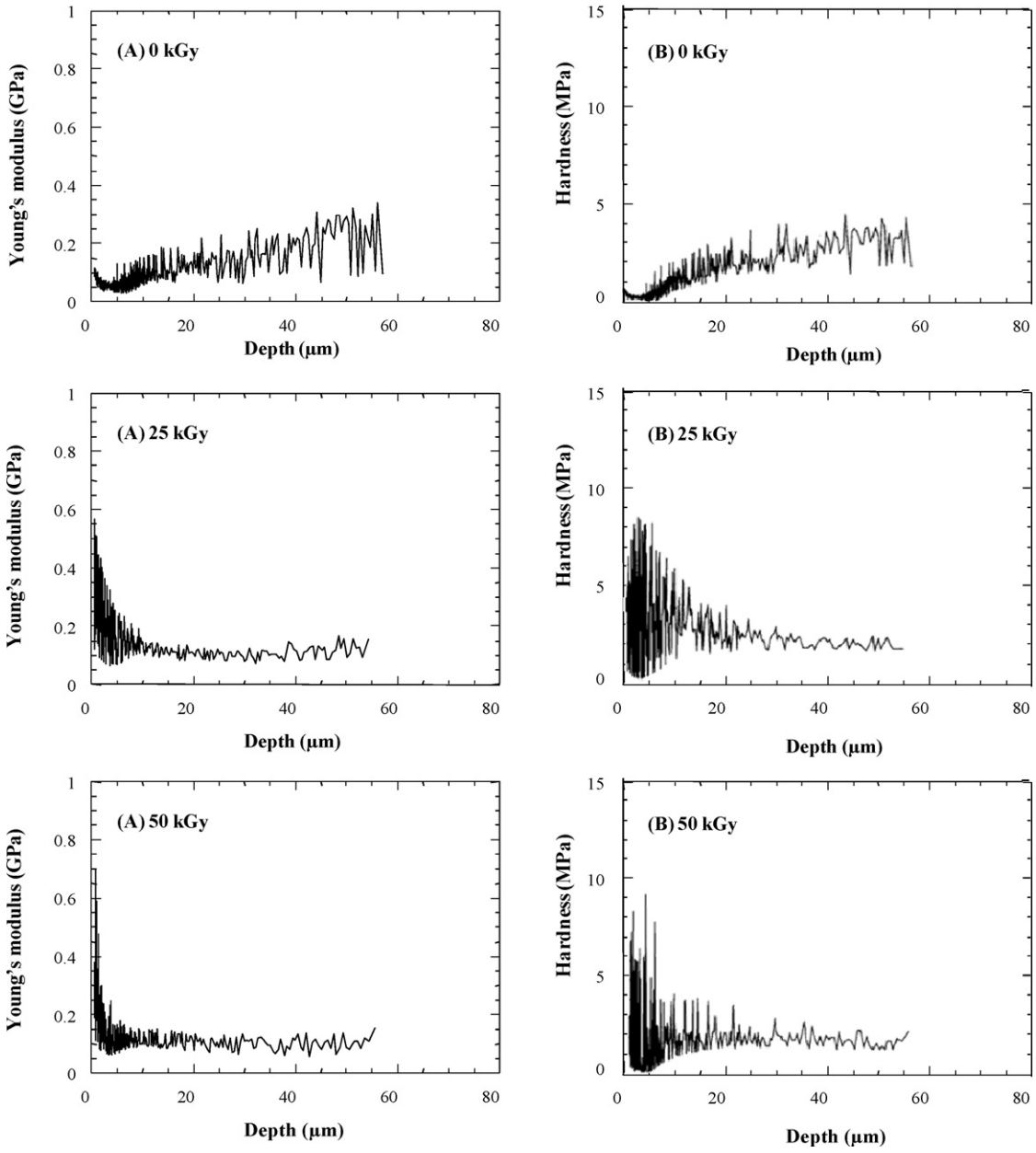

Fig. 5. Young's modulus versus depth (A) and hardness versus depth (B) diagrams. Top panels show data for non-irradiated PVC sheet (0 kGy). Middle panels show data for 25 kGy irradiated PVC sheet. Bottom panels show data for 50 kGy irradiated PVC sheet.

Table 2
Elastic modulus and hardness.

Depth (μm)	Control		25 kGy		50 kGy	
	Elastic modulus (MPa)	Hardness (MPa)	Elastic modulus (MPa)	Hardness (MPa)	Elastic modulus (MPa)	Hardness (MPa)
1	80 ± 17	0.42 ± 0.11	330 ± 120	2.9 ± 1.9	240 ± 140	2.0 ± 2.3
5	59 ± 22	0.37 ± 0.22	180 ± 50	4.3 ± 2.3	130 ± 37	2.3 ± 2.3
10	93 ± 35	1.1 ± 0.45	130 ± 21	3.6 ± 1.5	120 ± 19	2.2 ± 1.2
20	130 ± 32	2.0 ± 0.56	100 ± 14	2.5 ± 0.7	100 ± 19	2.1 ± 0.6

Poisson ratio of sample was assumed to be 0.3 when elastic modulus was calculated.

4. Conclusion

MEHP was formed from DEHP degradation depending on the dose of gamma-ray irradiation. Moreover, DEHP migration depended on its decomposition to MEHP or PA. The cross-linking or scission of surface PVC chains might be influenced by DEHP, MEHP, and PA migration from the PVC sheet. However, it is an undeniable fact that MEHP migration level increased with increasing dose of irradiated gamma rays. MEHP is thought to be more toxic than DEHP. Therefore, MEHP exposure should be taken into consideration in the assessment of DEHP exposure in high-risk patients. The gamma-ray sterilization process uses at least 20–25 kGy gamma rays as sterilization dose, and it is possible that even higher doses are used to sterilize medical devices. In the present paper,

apparent high correlations between hardness or elastic modulus of PVC surface and migration levels of DEHP, MEHP, or PA were not observed. However, any relationship between plasticizer migration and gamma-ray effect on the PVC surface is undeniable possibility. We should further study in greater detail the relationship between plasticizer migration and gamma-ray effect on the PVC surface.

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