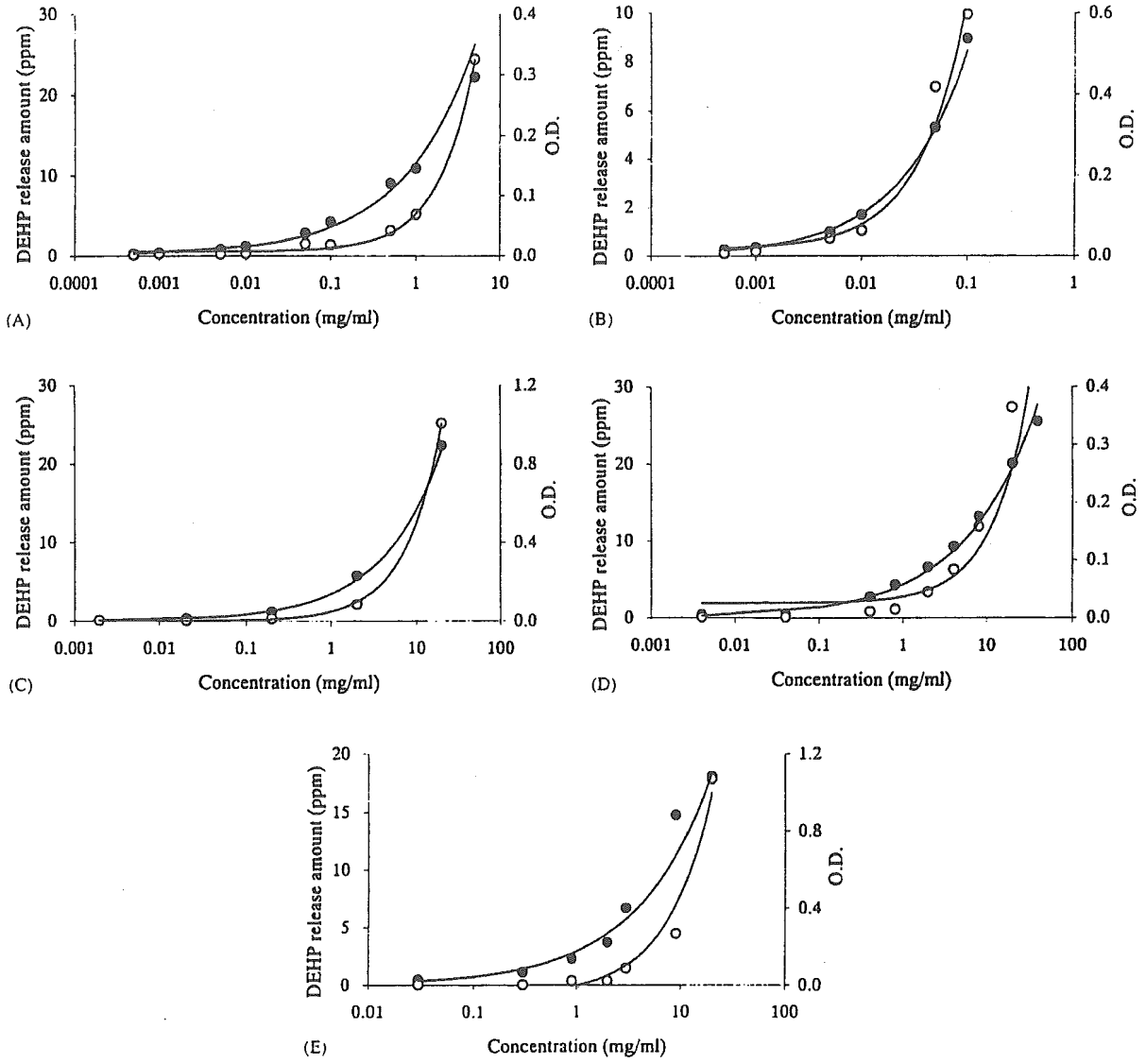

Fig. 2. Relationship between DEHP release potency (●) and methyl yellow solubility (○) of various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS. Absorbance of Sandimmun® and Tween® 80 was measured after five times dilution with distilled water.

and Gynecology, School of Medicine, Tokai University (Kanagawa, Japan). Based on the properties of drugs and additives contained in each pharmaceutical, these injections were divided into five groups, as follows: lipophilic injections (group 1), pH-dependent pharmaceuticals for solubilization (group 2), low solubility pharmaceuticals (group 3), pharmaceuticals suspected to induce DEHP migration (group 4), and hydrophilic injections as negative control (group 5), as shown in Table 1.

The release potency of DEHP from the PVC tubing was estimated by using 53 injections adjusted to the concentration used for medical treatment (Table 1). As shown in Table 3, Sandimmun®, Diprivan®, Ropion®, and Florid®-F, assigned to group 1, released large amounts of DEHP, and significant release was also observed by Prograf®, Sohvita®, Kaytwo® N, and Horizon®. In the other injections assigned to group 1, Predonine® (10 mg/ml) showed relatively low release of DEHP, and no remarkable release was recognized by

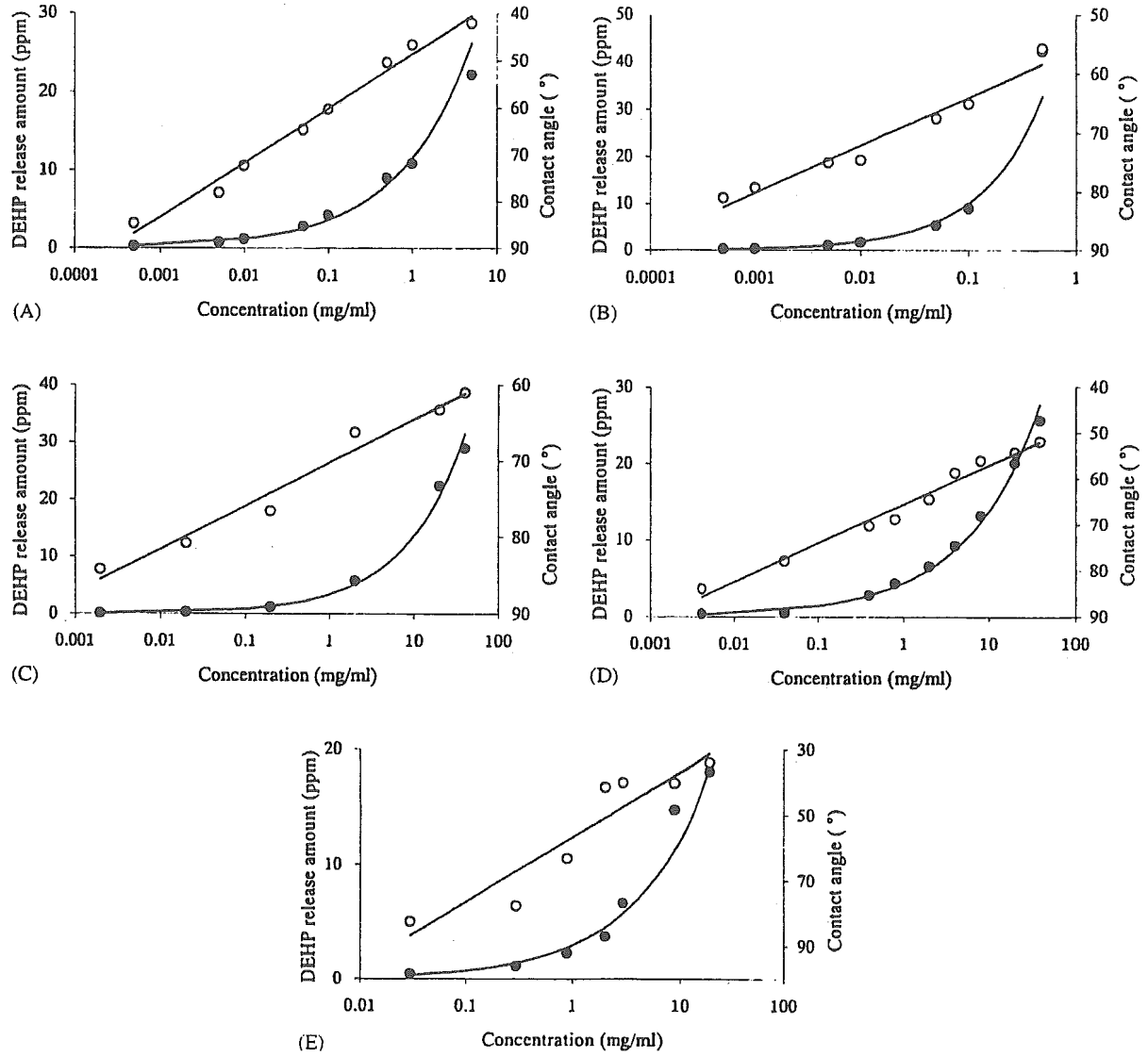

Fig. 3. Relationship between DEHP release potency (●) and static contact angle to PVC sheet (○) of various concentration of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS.

Humulin® R, Prostamon®, or Predonine® (1 mg/ml). On the other hand, no significant DEHP migration was observed by most of the other injections assigned to groups 2 through 5, and the concentration range of DEHP released into each injection was approximately 100–400 ppb. Exceptionally, Aleviatin® containing propylene glycol and ethanol (group 2) and Buminate® and Neuart®, which are human serum preparations (group 4), released relatively high amounts of DEHP, and Elaspol® (group 2) released a relatively low amount of DEHP.

The amount of methyl yellow, which exhibited the highest response regarding the increase of absorbance described above, dissolved in each pharmaceutical is listed in Table 3 as the absorbance at 450 nm. In this solubility test using lipophilic pigment, Sandimmun®, Buminate®, Florid®-F, Aleviatin®, Horizon®, Kaytwo® N, Diprivan®, and Ropion®, all of which showed potent DEHP release, showed high absorbance (over 0.8). However, absorbance of Prograf®, Neuart®, Sohvita®, and Elaspol® were lower than approximately 0.05. On the other hand, the

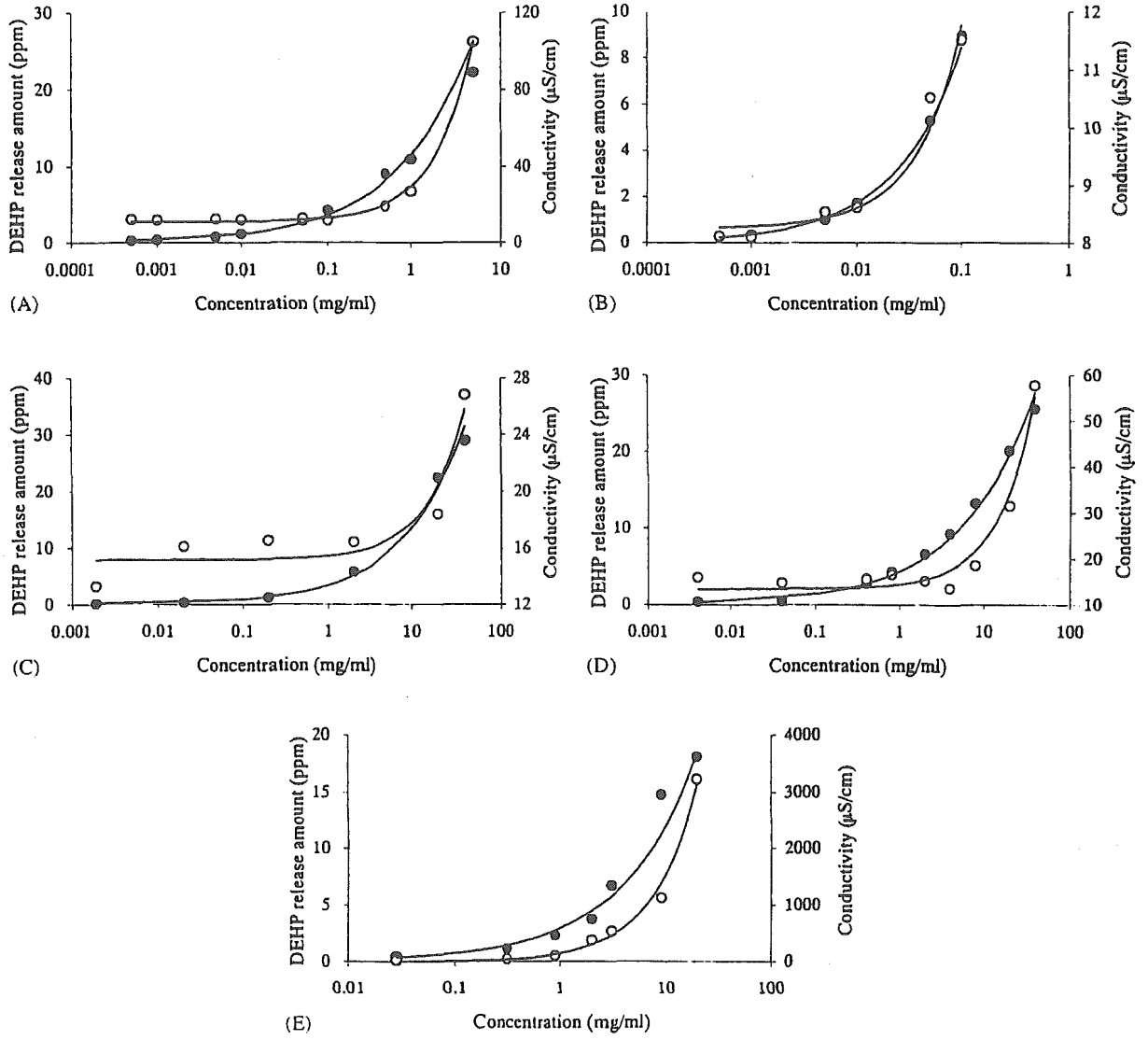

Fig. 4. Relationship between DEHP release potency (●) and electrical conductivity (○) of various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS.

values of other injections that demonstrated low potency of DEHP release were lower than 0.026. Exceptionally, absorbance of Optiray® and of Pantol® was approximately 0.1.

Static contact angle values of 53 pharmaceuticals to PVC sheet are listed in Table 3. All pharmaceuticals that did not exhibit remarkable release of DEHP from medical grade PVC tubing showed relatively large contact angles ranging from approximately 70°–90°. On the other hand, among the injections showing high potency of DEHP release, Florid®-F, Horizon®, Sandimmun®, and Aleviatin® exhibited low contact angles of 36.68° ± 2.81°, 48.74° ± 2.66°, 52.73° ± 0.93°, and 58.30° ± 2.53°, respectively. However, static contact angle of Predonine® (10 mg/ml), Diprivan®, Prograf®, Sohvita®, Ropion®, Buminate®, Kaytwo® N, Elaspol®, and Neuart®, all of which also released DEHP from PVC sheet, were relatively high, with values ranging from 72.83° to 88.61°.

The relationship between the released amount of DEHP and the value of the physicochemical properties

Table 3
DEHP release capacity and physicochemical properties of pharmaceutical injections used in this study

Product name	DEHP amount migrated into injections		Contact angle to PVC sheet		Solubility of methyl yellow[a]	
	ppb	S.D.	°	S.D.	O.D. at 450 nm	S.D.
Group 1						
Sandimmun®	27363.9	384.8	52.73	0.925	0.989	0.000
Prograf®	4091.9	31.9	78.11	1.418	0.041	0.001
Diprivan®	19451.2	852.5	78.17	0.961	5.983[b]	0.103
Ropion®	17838.5	821.6	81.31	1.778	19.500[b]	0.007
Sohvita®	1157.1	5.1	81.32	1.362	0.008	0.001
Kaytwo® N	8457.5	62.9	82.20	1.102	4.105[c]	0.007
Humulin® R	281.6	6.0	76.11	2.338	0.003	0.001
Prostarmon®-F	185.8	17.3	88.41	0.451	0.001	0.000
Florid®-F	30098.3	423.3	38.68	2.810	1.366	0.028
Horizon®	2008.8	257.6	48.74	2.656	2.596	0.150
Predonine® 10 mg/ml	915.6	182.3	72.83	2.122	0.022	0.001
Predonine® 1 mg/ml	407.1	2.4	87.46	0.445	0.002	0.000
Group 2						
Gaster®	166.0	0.9	87.83	0.445	0.003	0.001
Droleptan® 2.5 mg/ml	171.0	0.6	77.74	0.880	0.008	0.001
Droleptan® 50 μg/ml	167.4	24.6	89.55	0.521	0.002	0.001
Elaspol®	885.7	10.6	86.59	1.871	0.002	0.000
Aleviatin®	5009.0	288.1	58.30	2.534	1.872	0.015
Methotrexate®	372.8	6.8	88.64	0.926	0.001	0.001
Serenace®	50.6	2.5	77.59	1.881	0.005	0.000
Bosmin®	290.3	24.6	86.63	0.819	0.006	0.000
Group 3						
Partan M	462.7	4.2	88.52	0.898	0.007	0.000
Musculax®	192.7	1.5	87.60	2.737	0.001	0.001
Carbenin®	237.0	1.2	87.14	1.205	0.001	0.001
Minomycin®	150.0	8.9	88.65	0.900	0.012	0.001
Perdipine®	211.6	24.0	87.28	1.961	0.002	0.001
Bisolvon®	174.9	23.7	85.38	0.629	0.017	0.000
Modacin®	301.0	0.5	88.86	0.870	0.002	0.001
Diflucan®	210.5	1.2	88.08	0.610	0.002	0.001
Doyle®	296.7	2.6	86.16	1.814	0.002	0.001
Adona®	246.1	3.0	88.00	2.189	0.001	0.001
Group 4						
Atonin®-O	423.1	0.8	87.48	1.170	0.002	0.001
Atarax®-P	430.8	144.4	88.53	1.242	0.002	0.001
Zantac®	197.9	29.5	88.85	0.468	0.002	0.001
Kenketsu Venoglobulin®-IH	243.9	14.3	83.98	1.888	0.018	0.001
Pantol®	412.1	18.2	69.78	1.093	0.087	0.000
Buminate®	10080.8	84.1	81.68	1.915	1.130	0.057
Neuart®	2008.2	21.8	88.61	0.930	0.003	0.001
Millisrol®	267.6	8.9	87.74	0.630	0.002	0.000
Metilon®	302.8	3.8	86.80	1.745	0.001	0.001
Erythrocin®	92.2	0.7	81.49	3.162	0.003	0.000
Dalacin® S	274.9	4.0	84.56	1.232	0.002	0.001
Group 5						
Tienam®	205.1	1.6	88.64	0.909	0.002	0.000
Glucose®	284.6	4.8	87.38	1.333	0.002	0.001
Fesin®	244.5	5.5	87.97	1.859	0.026	0.011

Table 3 (*Continued*)

Product name	DEHP amount migrated into injections		Contact angle to PVC sheet		Solubility of methyl yellow[a]	
	ppb	S.D.	°	S.D.	O.D. at 450 nm	S.D.
Actit®	262.8	5.0	86.88	2.117	0.002	0.001
Atropine sulfate	200.7	5.1	87.99	1.065	0.001	0.001
Viccillin® for injection	262.3	6.8	88.85	0.886	0.003	0.000
Neophyllin®	301.1	4.0	89.77	0.466	0.001	0.005
Fosmisin®-S	289.6	6.7	88.39	0.462	0.001	0.000
Calcicol®	179.4	4.3	88.20	1.259	0.001	0.001
Cefamezin® α	215.1	0.9	87.93	1.171	0.003	0.001
PN-Twin® No.2	328.5	5.0	88.37	0.941	0.001	0.000
Succin®	228.6	2.1	89.20	0.226	0.002	0.001
Optiray®	404.0	79.5	85.49	0.761	0.162	0.002
Proternol®-L	326.3	8.6	87.75	1.425	0.002	0.001

[a] Values after substracting blank value.
[b] Measured after 50 times dilution.
[c] Measured after five times dilution.

is shown in Figs. 5 and 6. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day), and the times required for intravenous injection and instillation through transfusion set was assumed to be 5 min and 1 h, respectively. Although it is known that the released amount of DEHP from PVC tubing is influenced by drip rate (Hanawa et al., 2000; Hanawa et al., 2003), this factor was not considered in this risk assessment. When body weights of adult and neonate patients were assumed to be 50 and 3 kg, respectively, the absolute amounts of DEHP corresponding to the lower limit (40 μg/kg/day) of TDI value restricted by JMHLW represented 2000 and 120 μg per day, respectively. As shown in Fig. 5, a good proportional correlation was recognized between the DEHP release potency and methyl yellow solubility of each pharmaceutical. The response was found to be linear with correlation coefficient exceeding 0.707 for the pharmaceuticals administered by instillation and

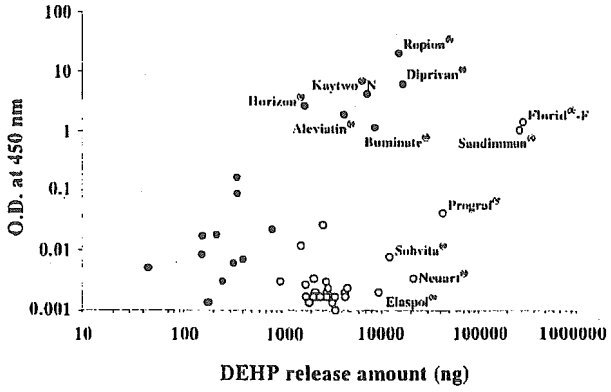

Fig. 5. Relationship between the released amount of DEHP and methyl yellow solubility of the medical use concentration of 53 pharmaceuticals. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day), and the times required for intravenous injection (●) and instillation (○) through transfusion set were assumed to be 5 min and 1 h, respectively.

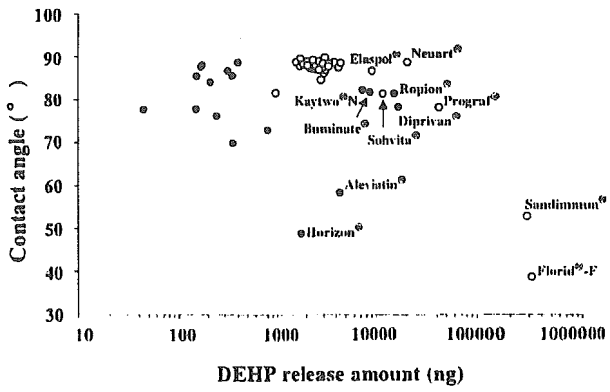

Fig. 6. Relationship between the released amount of DEHP and static contact angle of the medical use concentration of 53 pharmaceuticals. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day), and the times required for intravenous injection (●) and instillation (○) through transfusion set were assumed to be 5 min and 1 h, respectively.

0.819 for the pharmaceuticals by intravenous injection. Most of the pharmaceuticals administered by instillation did not cause DEHP exposure to patients over the lower limit of the TDI value. It was noted, however, that Sandimmun® and Florid®-F exhibited release of DEHP over the lower limit (120 μg) for neonates. When the threshold of DEHP exposure in medical treatment using transfusion set to neonate patients was set at 0.8 as absorbance of methyl yellow, only Sandimmun® and Florid®-F of all the pharmaceuticals administered by instillation showed high absorbance (i.e., over the threshold). Although Prograf®, Neuart®, Sohvita®, and Elaspol® could release relatively large amounts of DEHP, the exposure amounts to neonate patients were under the lower limit of TDI value and the absorbance of each pharmaceutical was lower than 0.8 in methyl yellow solubility test. On the other hand, none of the pharmaceuticals demonstrating significant release potency of DEHP from PVC tubing (Table 3) when administered to the patients by intravenous injection through transfusion set, including Diprivan®, Ropion®, Buminate®, Kaytwo® N, Aleviatin®, and Horizon®, caused DEHP exposure over the lower limit of TDI value, largely because of the short time required for administration. It was demonstrated, however, that methyl yellow solubility test could reflect the real potency of DEHP release, by which Diprivan®, Ropion®, Buminate®, Kaytwo® N, Aleviatin®, and Horizon® showed high absorbance (more than 0.8). These results clearly indicate that the risk of DEHP exposure to the patients could be predicted by methyl yellow solubility test.

Similar risk assessment was performed with static contact angle to PVC sheet of pharmaceuticals as a marker, the results of which are shown in Fig. 6. The risk of DEHP release caused by Sandimmun® and Florid®-F could be predicted by creating a borderline at an angle of 60°. All other injections, with the exception of Horizon® and Aleviatin®, exhibited a large angle more than the set value. It was suggested that the pairing of propylene glycol and ethanol, contained only in Horizon® and Aleviatin® as additives, may be responsible for DEHP release and low value of static contact angle, and that the angle was not influenced by the concentrations of soy bean oil, glycerin, and lecithin contained in Kaytwo® N, Ropion®, and Diprivan®. The concentration of HCO-60 must be very significant regarding DEHP release and low contact angle, because although Prograf® contains the same or similar surfactant as Florid®-F and Sandimmun®, the medical use concentration of Prograf® is relatively low compared to those of Sandimmun® and Florid®-F; hence, Prograf® shows a high contact angle on this test. From these results, it was suggested that static contact angle to PVC sheet of pharmaceuticals could be a useful marker to predict the risk of DEHP exposure to neonate patients. It seems, however, that in contrast with the results of the methyl yellow solubility test, the contact angle to PVC sheet of pharmaceuticals does not always reflect the real potency of DEHP release, based on the findings that Kaytwo® N, Ropion®, Buminate®, and Diprivan® showed relatively high contact angles despite their high potency of DEHP release (Table 3).

4. Conclusions

In the present study, the DEHP release behavior of pharmaceutical injections was compared with the potency of physicochemical properties of the injections in order to develop a simple method for predicting the level of DEHP migrating from PVC medical devices into the injections. It was shown that although some pharmaceuticals had high release potency of DEHP from PVC products, most of the pharmaceuticals tested did not cause significant DEHP exposure to patients in the form applied for medical use. However, neonate patients may be exposed to DEHP over the lower limit of TDI value when Sandimmun® and Florid®-F are administered by instillation through transfusion set. The risk could be predicted by methyl yellow solubility test, the results of which were closely related to DEHP release potency of pharmaceuticals. Some pharmaceuticals possess their own color characteristic, and the measurement of absorbance of methyl yellow may be inhibited by a color having a λ_{max} similar to that of methyl yellow. In this case, however, it appears that Sudan III and 1,4-diamino-anthraquinone, which have different λ_{max}, can be used instead of methyl yellow as marker pigments. Thus, the solubility test of lipophilic pigments is very simple and rapid in comparison with the typical and complicated elution tests of DEHP using GC-MS and LC-MS, and it may be applicable in the medical field, particularly in hospital, as one of the methods for the safety and risk assessment of DEHP exposure originating from the use of PVC products.

Acknowledgement

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High-throughput determination of mono- and di(2-ethylhexyl)phthalate migration from PVC tubing to drugs using liquid chromatography–tandem mass spectrometry

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Abstract

The risk assessment of di(2-ethylhexyl) phthalate (DEHP) that migrated from polyvinyl chloride (PVC) medical devices is an important issue for hospitalized patients. Many studies have been conducted to determine the level of DEHP migration. A recent report has indicated that DEHP in blood bags was hydrolyzed by esterase to mono(2-ethylhexyl) phthalate (MEHP). Therefore, a method for the simultaneous determination of DEHP and MEHP was developed. The migration of DEHP and MEHP from PVC tubing to drugs was examined. Although we detected MEHP in the drugs, we found no enzymatic activity involved in the migration process. Some reports have indicated that hydrolysis may have occurred during sterilization by autoclaving. However, we did not perform any heat treatment. It is speculated that the MEHP migrated directly from the PVC tubing. The simultaneous determination of DEHP and MEHP is required for risk assessment, as MEHP may be even more toxic than the parent compound.

Keywords: Liquid chromatography–tandem mass spectrometry; Di(2-ethylhexyl)phthalate; Mono(2-ethylhexyl)phthalate; Drugs

1. Introduction

Polyvinyl chloride (PVC) is one of the most widely used polymeric materials in medicine. Flexible PVC is used for the manufacture of blood and blood component storage bags, intravenous solution dispensing sets, blood tubing, and so on. As PVC per se is a rigid polymer, additives in the form of plasticizers are incorporated into it to increase its flexibility and low-temperature properties. The esters of phthalic acid, particularly di(2-ethylhexyl)phthalate (DEHP), are the most preferred plasticizers for medical grade PVC. However, because these additives are not bound to the base polymer by covalent bonds, their permanence is low. 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 general toxicity of DEHP has been evaluated [5,9–12], and a risk assessment study has suggested that it is relatively safe for humans. Recently, however, it has been considered that the level of DEHP exposure to humans, particularly high risk patients, must be monitored, based on the finding that DEHP exerts an adverse effect on young rodents. The US Food and Drug Administration's Center for Devices and Radiological Health and Health Canada have reported the risk assessment of DEHP that migrated from PVC medical devices in hospitalized patients [13,14].

It has been reported that DEHP is hydrolyzed enzymatically to mono(2-ethylhexyl)phthalate (MEHP) [15], and that MEHP may be even more toxic than the parent compound. In vitro studies have found that MEHP inhibits FSH-stimulated cAMP accumulation in cultured Sertoli cells [16–20], in addition to reducing 17β-estradiol production and aromatase mRNA expression [21,22]. These results suggest that MEHP

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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.

Therefore, a method for determining DEHP and MEHP with high sensitivity, precision, and selectivity is required. Most of the conventional simultaneous analyses of DEHP and MEHP involve liquid chromatography(LC)/ultraviolet(UV) detection [23,24], LC/mass spectrometry(MS) [2,7] and gas chromatography(GC)/MS [25–27]. However, those methods lack sensitivity, precision and selectivity. Inoue and co-workers [2,7] have reported the utility of the column-switching LC/MS method for the direct analysis of DEHP because of its high throughput and low contamination. In addition, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has high sensitivity and selectivity. Therefore, the column-switching LC–MS/MS method was developed.

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 tubing that is used for transfusion, infusion, and donation of blood. This was kindly supplied by two manufacturers and was not sterilized prior to use.

The drugs used for the DEHP and MEHP migration tests were Prograf® (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan), FLORID®-F (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan), and Lastet injection (Nippon Kayaku Co., Ltd., Tokyo, Japan). These were used after dilution with 5% glucose solution for injection (Otsuka Pharmaceuticals Co., Tokyo, Japan) to the desired concentration based on the package inserts.

2.2. Instrumentation

A Series 1100 liquid chromatograph from Agilent Technologies (USA) was coupled to an API 4000™ (Applied Biosystems Japan, Tokyo, Japan) equipped with a Turbo Ionspray™ 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 × 2.0 mm, 5 μm particle size) from Kanto Chemical was used for the separation. An Oasis® HLB extraction column (20 mm × 2.1 mm, 25 μm particle size) from Waters was used for the extraction and clean-up.

2.3. Standard solution and quantitative procedure

DEHP, DEHP-d_4, MEHP and MEHP-d_4 stock solutions were prepared in acetonitrile. They were mixed to make the desired ratio and serially diluted with 50% acetonitrile for the preparation of calibration curves.

2.4. Chromatographic and extraction conditions

The column switching system was used for sample injection. After 20 μl of the sample was injected with an

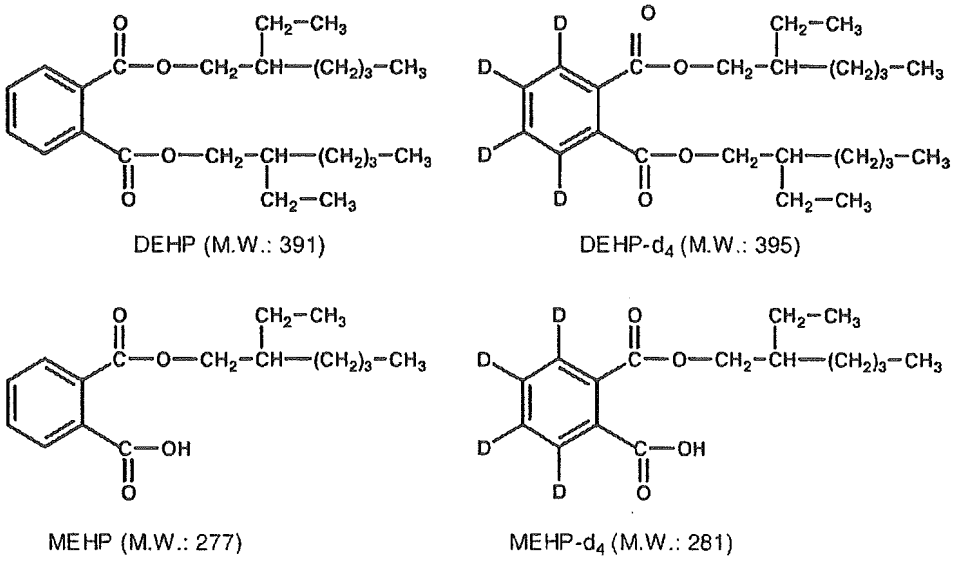

Fig. 1. Chemical structures of DEHP, MEHP and their surrogate compounds.

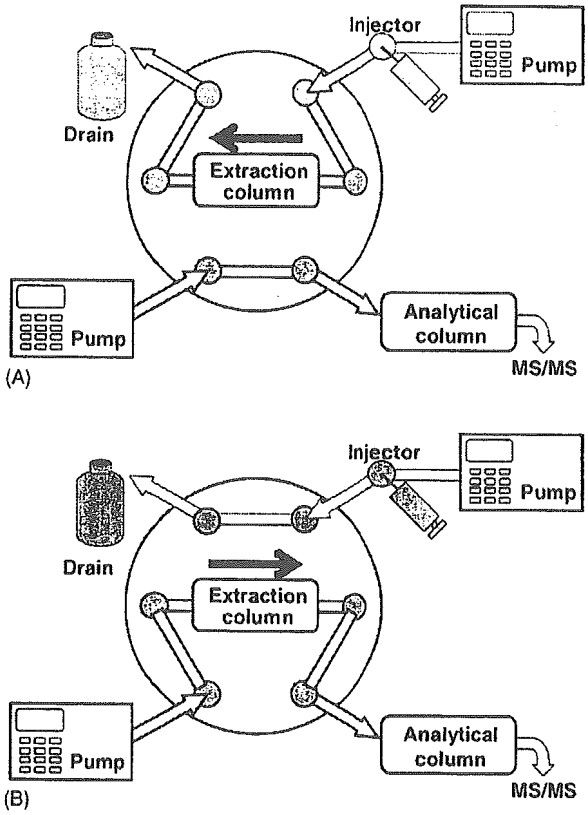

Fig. 2. Schematic representation of the column-switching LC–MS/MS system. (A) Configuration for sample loading and washing; (B) Configuration for sample elution.

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. While the eluate from the extraction column was directed to waste during the 3 min, the sample was extracted on the on-line extraction column. The matrices in the sample were eluted whereas DEHP and MEHP were retained on the extraction column. Then, the extraction process was performed after the on-line solid phase extraction was accomplished. After 3 min, the switching valve was changed to configuration B (Fig. 2). This configuration connected the extraction column to the analytical column and the MS detector in the flow path of the Agilent LC pump. The column oven was maintained at 40 °C for LC. The 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). The time program for the column-switching LC–MS/MS system is summarized in Table 1.

2.5. 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 are shown in Fig. 3. 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), and MEHP-d_4 (m/z 281 → 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).

Table 1
Time program for the proposed column switching-LC/MS/MS method

Time (min)	Solvent A (%)	Solvent B (%)	Configuration
0.0	100	0	Loading and washing
3.0	100	0	
3.1	0	100	Elution and separation
8.0	0	100	
8.1	100	0	Conditioning

Solvent A: water; solvent B: acetonitrile/water = 90/10 (v/v).

2.6. Migration test

The two kinds of PVC tubing were cut to 10 cm length and filled with the drugs (tube length, 8 cm). The tubing was subjected to extraction with shaking at room temperature for 1 h. The extracts were pipetted into another test tube, and put in vials containing 50 ng of DEHP-d_4 or MEHP-d_4. Then, the samples were appropriate dilute, consequently subjected to LC–MS/MS.

3. Results and discussion

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

Our previous report [7] which performed simultaneous determination of DEHP and MEHP in serum using column switching-LC/MS, required almost 30 min for analysis. In addition, other report [8] using LC–MS/MS method required 30 min to perform the DEHP and MEHP simultaneous analysis in serum. On the other hand, the column switching system combined with LC–MS/MS method performed the high-throughput and high-precision analysis that needs almost 10 min.

In this method, the limits of quantificaion (signal-to-noise ratio > 10) of DEHP and MEHP were 2.5 and 0.75 ng/ml with

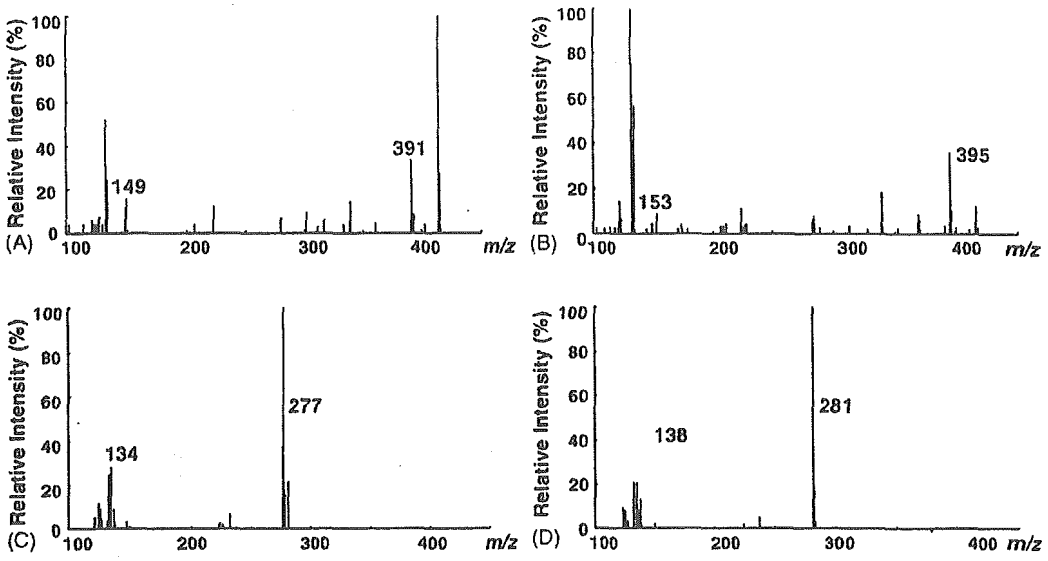

Fig. 3. Product ion spectra of DEHP, MEHP and their surrogate compounds. (A) DEHP (B) DEHP-d_4 (surrogate compound for DEHP). (C) MEHP (D) MEHP-d_4 (surrogate compound for MEHP).

Table 2
Validation data for determination of DEHP and MEHP migration from PVC tubing to drugs

	DEHP		MEHP	
	Quantitative range (ng/ml)	Correlation (r)	Quantitative range (ng/ml)	Correlation (r)
Glucose	2.5–100	0.999	0.75–100	0.999
Prograf®	2.5–50	0.999	0.5–50	0.999
FLORID®-F	2.5–50	0.999	0.25–50	0.996
Lastet inj.	5–50	0.999	0.5–50	0.999

the standard solutions, respectively. For DEHP measurement, the 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, the 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). DEHP and MEHP concentrations in the drugs were measured; however, as the the matrices of the drugs were different from each other, a known concentration of the standard solution was added to the drugs, and a calibration curve was obtained for each drug (Table 2)

We examined the recovery using 5% glucose solution. 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 (Table 3). The chromatograms obtained by the recovery test are shown in Fig. 4.

Table 3
Recoveries of DEHP and MEHP from glucose solution

50 ng/ml spiked	Recovery ± S.D. (%)
DEHP	99.2 ± 3.2
MEHP	109.0 ± 3.4

5% glucose solution, n = 6.

3.2. Determination of DEHP and MEHP migration from PVC tubing

The proposed method was applied to the determination of DEHP and MEHP migration from the PVC tubing (Table 4). The level of DEHP migration was almost the same for the two tubing, that is, it was speculated that the DEHP content

Table 4
Levels of DEHP and MEHP migration to various drugs from PVC tubing

Sample	DEHP concentration (mean ± S.D., μg/ml)	
	Company A	Company B
Glucose	0.12 ± 0.03	0.13 ± 0.06
Prograf®	4.60 ± 0.17	4.40 ± 0.10
FLORID®-F	53.99 ± 3.63	54.64 ± 2.90
Lastet inj.	27.04 ± 0.62	28.88 ± 1.53
	MEHP concentration (mean ± S.D., μg/ml)	
	Company A	Company B
Glucose	0.56 ± 0.05	0.20 ± 0.00
Prograf®	0.39 ± 0.04	0.12 ± 0.01
FLORID®-F	ND*	ND*
Lastet inj.	ND*	ND*

n = 3. The samples were appropriate dilute, consequently subjected to LC–MS/MS.
* One thousandth dilution were performed.

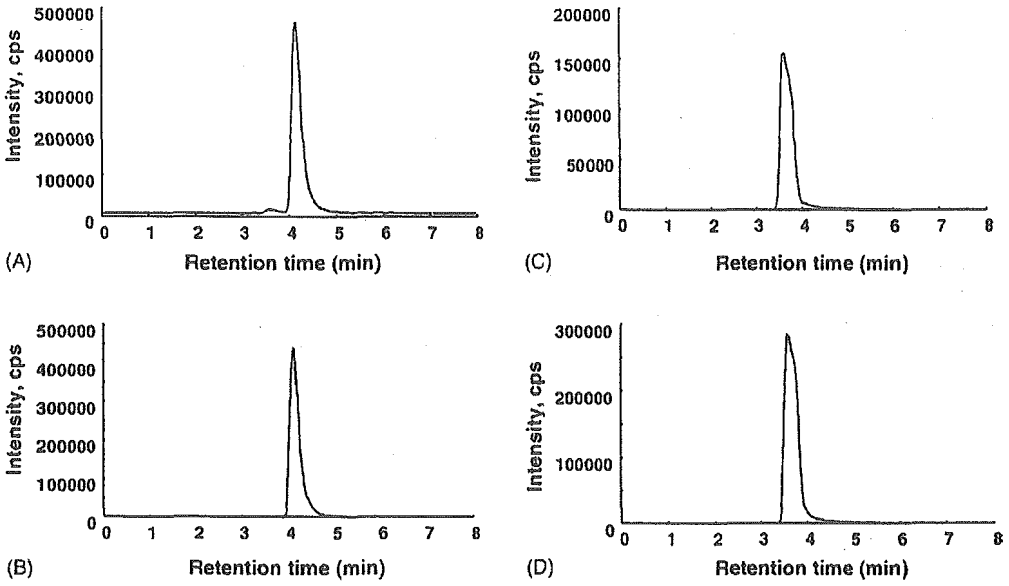

Fig. 4. MRM chromatograms of DEHP, MEHP and their surrogate compounds in glucose solution spiked with 50 ng of DEHP or MEHP. (A) DEHP (*m/z* 391 → 149); (B) DEHP-d_4 (*m/z* 395 → 153); (C) MEHP (*m/z* 277 → 134); (D) MEHP-d_4 (*m/z* 281 → 138).

was almost the same for the two tubings. On the other hand, MEHP was detected in 5% glucose solution and Prograf. The level of DEHP migration to the same drug was almost the same for the two tubings. By contrast, the level of MEHP migration differed by almost threefold between the two tubings even when the same drug was used.

The 5% glucose solution has been used for dilution of all drug, however, drug additives including surfactants and their concentration were different (Table 5). Owing to the other report [28], DEHP migration was dependent on the concentration of drug additives such as HCO-60. High concentration of drug additives such as HCO-60 as shown FLORID®-F and Lastet inj. in the Table 5 might be contributed to migrate the DEHP. In comparison, MEHP was more hydrophilic than DEHP (DEHP: log P = 7.19, MEHP: log P = 3.35 calculated by log P predictor from ChemSilico) so that MEHP have less migration than DEHP.

It was thought that 5% glucose solution had very little effect on DEHP migration. When we measured the levels of DEHP and MEHP migration with 5% glucose solution, the level of DEHP migration was found to be lower than that of MEHP migration. It has been reported that DEHP was hydrolyzed by such enzymes as lipases to MEHP in blood bags. However, that the drugs used in this study have enzymatic activity is not plausible. Some reports have indicated that hydrolysis may have occurred on sterilization by autoclaving [29,30]. However, we did not perform any heat treatment in this study. In addition, the level of MEHP migration was different between the two tubings. Moreover, we comfirmed that MEHP was also migrated from PVC sheet with just water. Taken together, we hypothesized that MEHP already existed in the PVC tubing and migrated directly from it.

To date, the mechanism underlying the migration of DEHP from PVC medical devices remains unknown. Further research of MEHP and DEHP migration from PVC medical devices is required.

Table 5
Additives in diluted drugs

Drugs	Additives	Concentration
Glucose	Nothing	-
Prograf®	Polyoxyethylated hydrogenated castor oil (HCO-60)	80 ppq
	Dehydrated ethanol	Unknown
FLORID®-F	Polyoxyethylated hydrogenated castor oil (HCO-60)	1000 ppm
Lastet inj.	Polyethylene glycol 400 (PEG-400)	240 ppm
	Polysorbate 80 (Tween 80)	32 ppm
	Ethanol	Unknown
	Citric acid	Unknown

Acknowledgements

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Reducing the migration of di-2-ethylhexyl phthalate from polyvinyl chloride medical devices

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Abstract

We attempted to determine the processing conditions for decreasing the migration of phthalate esters, particularly di-2-ethylhexyl phthalate (DEHP), from polyvinyl chloride (PVC) products using a drug solvent after dilution based on the package insert. PVC sheets and PVC tubing were subjected to optical irradiation (ultraviolet (UV), visible light irradiation) and heat treatment to determine whether they are deteriorated by these treatments. UV irradiation to one side of the PVC sheet decreased the levels of DEHP migration from the sheets by almost 50%, although the amount of DEHP content in PVC sheet was observed no significant change. On the other hand, the levels of DEHP migrating from the inner surface of PVC tubing UV-irradiated from the outer surface were not decreased compared with the control. Therefore, the surface structure was examined by conducting Fourier transform infrared spectroscopy (FT-IR), electron spectroscopy for chemical analysis (ESCA) and static angle of contact measurement. In FT-IR analysis, we found that the UV-irradiated PVC sheets were exhibited broadened absorption bands with time. In ESCA analysis, the chlorine content was decreased and the oxygen content was increased with time in UV-irradiated PVC sheets. Moreover, the other treated PVC sheets shows no significant change compared with the non-UV-irradiated PVC sheet. Therefore, the surface structure of the UV-irradiated PVC sheet was changed. As a result, the migration of DEHP from PVC products can be decreased with simple treatment, such as UV-irradiation. This could be a useful method to develop novel PVC products.

Keywords: Di-2-ethylhexyl phthalate (DEHP); Polyvinyl chloride (PVC); Medical device; UV irradiation; Surface structure

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1. Introduction

Phthalate esters are widely used as industrial plasticizers. In particular, di-2-ethylhexyl phthalate (DEHP) is used in the manufacture of polyvinyl chloride (PVC) products and other plastics to achieve the desired softness, flexibility and stability for specific applications. PVC is employed in the production of floor tiles, food wrapping film, industrial tubing, and medical devices (Huber et al., 1996), and is the most widely used polymer because of its availability. In general, PVC products used in medicine contain up to 40% by weight of this plasticizer (Ljunggren, 1984); however, it has been reported that some species of phthalate esters including DEHP exhibit reproductive and developmental toxicity (Arcadi et al., 1998; Gray et al., 1999). DEHP is not chemically bound to the PVC polymer and may leach when a medical device is heated or when the PVC comes in contact with surrounding media, such as blood, serum, plasma, drugs and intravenously administered fluids. The migration of DEHP from PVC medical devices into the surrounding media has been reported to result in toxicity (Tickner et al., 2001; Yakubovich and Vienken, 2000; Hill et al., 2001). Extraction occurs either by leaching or after an extracting material (blood, IV fluid) diffuses into the PVC matrix and dissolves the plasticizer, which is relatively lipophilic (Rock et al., 1986). In Japan, the Ministry of Health, Labour and Welfare has set the tolerable daily intake (TDI) of DEHP at 40–140 μg/kg/day (MHLW, 2000) and the use of DEHP as a plasticizer has been regulated so that it cannot be used in the manufacture of, for example, infant toys and plastic gloves for handling food. DEHP has been reported to leach from PVC medical devices containing fat-soluble drugs to be administered orally. Depending on the conditions at the time of use, a patient may be exposed to high levels of DEHP through medical treatment (USFDA, 2001; Health Canada, 2002).

Many studies have been reported on the release behavior of DEHP from PVC medical devices under various conditions (Hanawa et al., 2000; Jenke, 2001; Faouzi et al., 1995), because it is essential that the exposure be precisely determined in order to evaluate its significance as an integral part of the risk assessment of DEHP to human health. However, the quality change of PVC products during storage has been not estimated so far. It is possible that the content and release behavior of DEHP may be influenced by optical irradiation and temperature change during storage. Moreover, in this study, a DEHP migration test using PVC products treated with optical irradiation (visible and ultraviolet) and heating as external factors during storage was performed using a drug solvent for injection after dilution to the required concentration based on the package insert. In addition, the surface structure was examined by conducting Fourier transform infrared spectroscopy (FT-IR), electron spectroscopy for chemical analysis (ESCA) and static angle of contact measurement. When the PVC products were irradiated with UV, degradation occurred (Takeishi and Okawara, 1970). The tensile test was also performed as PVC products may deteriorate due to irradiation and heat treatment. The results of this study led to the development of processing conditions for decreased DEHP migration from PVC products, and provided novel information relevant to risk assessment and product development.

2. Materials and methods

2.1. Materials and chemicals

The test materials were a medical PVC sheet (1 cm × 3 cm, thickness: 0.4 mm) used in the manufacture of blood bags, and PVC tubing (length 10 cm, i.d. 2.13 mm) used for the transfusion, infusion, and donation of blood.

The drug solvent used for the DEHP migration tests was Sandimmun® (250 mg cyclosporine per ampoule (5 ml), Novartis Pharma Co., Tokyo, Japan). It is used for injection after dilution with 50 mg/ml glucose to the required concentration (0.5 mg/ml as cyclosporine concentration) based on the package insert.

Phthalate esters, di-2-ethylhexyl phthalate and DEHP-d_4, were purchased from Kanto Chemical Co. (Tokyo, Japan). Hexane, anhydrous sodium sulfate, a sodium salt of DEHP analytical grade, analytical grade diethyl ether, and HPLC grade distilled water were used in the experiments.

2.2. Pretreatment of PVC sheet and tubing

2.2.1. Control

A PVC sheet maintained in the shade and at room temperature was used as a negative control.

2.2.2. Heat treatment

The PVC sheets were kept at temperatures of 4, 37 and 60 °C for 1 week, 2 weeks, 1 month, 2 months and 3 months. The positive control was a PVC sheet kept at 100 °C for 25 days.

2.2.3. Optical irradiation

The embossed side is the outer surface of a blood bag. Some PVC sheets were irradiated with visible light using fluorescent lamp placed at a distance of 75 cm. On the other hand, the other PVC sheets were irradiated UV-ray using UV germicidal lamp placed at distance of 60 cm (UV intensity: 52.5 $\mu W/cm^2$) in clean-bench. These PVC sheets were irradiated for 1 week, 2 weeks, 1 month, 2 months or 3 months. After irradiation, the samples were stored in the shade. The positive control for visible light irradiation was a PVC sheet exposed to sunlight for approximately 1 year. The positive control for UV irradiation was a PVC sheet irradiated with a 254-nm UV lamp at a distance of 3 cm for 25 days. PVC tubing cut to a length of 10 cm was irradiated with a 254-nm UV lamp at a distance of 3 cm for 14 days.

2.3. GC–MS

A Hewlett-Packard HP 6890 Series GC system equipped with an auto-injector (Agilent Technologies, Palo Alto, CA) and a JMS700 spectrometer (JEOL, Tokyo, Japan) were used for gas chromatography-mass spectrometry (GC–MS). Chromatographic separations were performed with a BPX-5 fused silica capillary column (25 m × 0.22 mm i.d., film thickness: 0.25 μm, SGE Japan, Kanagawa, Japan).

A sample (2 μl) was injected in the pulsed splitless mode. The injector temperature was 260 °C. Helium was used as the carrier gas at a flow rate of 1 ml/min. The column temperature was programmed from 120 to 300 °C (held for 2 min) at a rate of 10 °C/min. The electron impact (EI)-mass spectrum was recorded at 70 eV for qualitative analysis, and ions of *m/z* 149.024 (DEHP) and 153.049 (DEHP-d_4) were selected as quantitative ions in selective ion monitoring (SIM) analysis (resolution = 5000) using the lock and check method of calibrating standard ions (*m/z* 168.989 of PFK). Quantitative analysis of each sample was repeated five times for calibration curves and twice for the other samples. The preparation of calibration curves and the calculation of quantitative data were performed using computer software TOCO, version 2.0 (total optimization of chemical operations), applying the function of mutual information (FUMI) theory (Hayashi and Matsuda, 1994; Hayashi et al., 1996, 2002; Haishima et al., 2001).

2.4. Migration test

The migration of DEHP from PVC sheets was examined in 5 ml of Sandimmun® prepared according to the instructions on the package insert. PVC sheets, which were irradiated or heat-treated were kept in test tubes and extraction was carried out by shaking at room temperature for 1 h. A 0.1 ml aliquot of the extract was pipetted into another test tube, and 2 ml of distilled water and 5 ml of diethyl ether containing 50 ng/ml DEHP-d_4 were added. The mixture was then subjected to extraction with shaking for 10 min. After centrifugation at 3000 rpm for 10 min, the organic phase was collected and dehydrated with anhydrous sodium sulfate, and subjected to GC–MS analysis.

PVC tubing cut to 10 cm length was used in the DEHP migration test, and filled with Sandimmun® (tube length, 8 cm; capacity, 0.285 cm^3; and surface area, 5.35 cm^2). The tubing was subjected to extraction with shaking at room temperature for 1 h. The extract was transferred into another test tube and treated in the same manner as that for PVC sheets.

2.5. Determination of DEHP compounds in PVC sheet by GC–MS

A PVC sheet sample (0.02 g) was dissolved in 20 ml of THF by soaking overnight at room temperature. A 0.1 ml aliquot of the solution was pipetted out and diluted with 2.0 ml of diethyl ether. A 0.1 ml aliquot was obtained, mixed with 50 ng/ml DEHP-d_4 (1 ml) and diethyl ether (8.9 ml), and then analyzed by GC–MS.

2.6. Analysis of surface structure

2.6.1. Infrared spectrometry

A JIR-SPX 200 (JEOL, Tokyo, Japan) was used for FT-IR spectroscopy coupled with attenuated total reflection (ATR) analysis. To analyze the PVC sheets, we used a germanium crystal, and the incidence angle was set at 45°.

2.6.2. Electron spectroscopy for chemical analysis

ESCA measurements were performed using an ESCA-3200 (Shimadzu, Kyoto, Japan). Only the inner side of the blood bag was measured for the heat treatment group and the visible light irradiation group, whereas both sides of the blood bag were measured for the UV irradiation group.

2.6.3. Static angle of contact

A solution of Sandimmun®, prepared according to the instructions on the package insert, was added dropwise to PVC sheets. After 120 s, the width and height of the droplet were measured with a G-1-1000 (ERMA, Tokyo, Japan). The static angle of contact with Sandimmun® was computed as follows:

$$L^2 = \left(\frac{w}{2}\right)^2 + (L - h)^2$$

$$\sin \delta = \left(\frac{w/2}{L}\right)$$

L is radius of droplet (mm); w is width of droplet (mm); h is height of droplet (mm); and (δ) static angle of contact.

Only the inner side of the blood bag was measured for the heat treatment group and the visible light irradiation group, whereas both sides of the blood bag were measured for the UV irradiation group.

2.7. Tensile test

A PVC sheet (0.7 cm × 3 cm, center width: 0.4 cm, thickness: 0.04 cm) was used as the sample (Fig. 1). Measurements were performed using an Autograph AG-20 kNG (Shimadzu, Kyoto, Japan) at a speed of 40 mm/min.

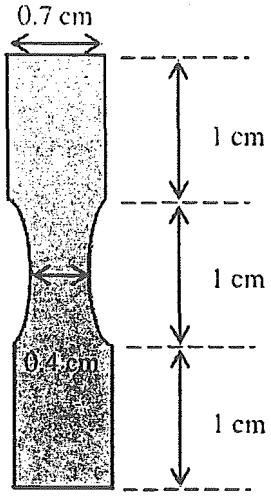

Fig. 1. The PVC sheet used in the tensile test A PVC sheet: 0.7 × 3 cm, center width: 0.4 cm, thickness: 0.04 cm.

3. Results and discussion

3.1. Determination of DEHP released from PVC products by GC–MS

First, the background was analyzed in order to examine the accuracy of the GC–MS method. When 50 ng/ml DEHP-d_4 with diethyl ether solution was used as the internal standard, 0.93 ± 0.31 ng/ml DEHP ($n = 5$) was detected in the internal standard. The DEHP background peaks in the internal standard solution were trace levels (S_0). In addition, the precision (SD) was expressed as SD_0. The limit of detection (LOD) and the limit of quantification (LOQ) of DEHP were calculated using S_0 and SD_0; LOD ($S_0 + 3 \times SD_0$) and LOQ ($S_0 + 10 \times SD_0$) were 1.9 and 4.0 ng/ml, respectively. A calibration curve was obtained for the peak ratio of DEHP to DEHP-d_4 versus the DEHP concentration level. The response was found to be linear in the validated range with a correlation coefficient (r) exceeding 0.999. Furthermore, the 95% confidence interval calculated by TOCO was sufficiently narrow to determine the amount of DEHP released from the PVC products. We found that this GC–MS method could be used for DEHP analysis with high accuracy.

The levels of DEHP that migrated from the PVC sheets were then determined, and the time course is shown in Fig. 2. Heat treatment and optical irradiation were each performed for 1 week, 2 weeks, 1 month, 2 months, and 3 months. At 2 months, the levels of DEHP migrating into Sandimmun® were slightly decreased by heat treatment and visible light irradiation, however no remarkable change was observed between the treatments, or between those treatments and their respective positive controls. The level of DEHP migration from the heat-treated PVC sheets has decreased the temperature-dependent. The most possible factor for the temperature-dependent, the sublimation/vaporization was occurred by heat treatment in

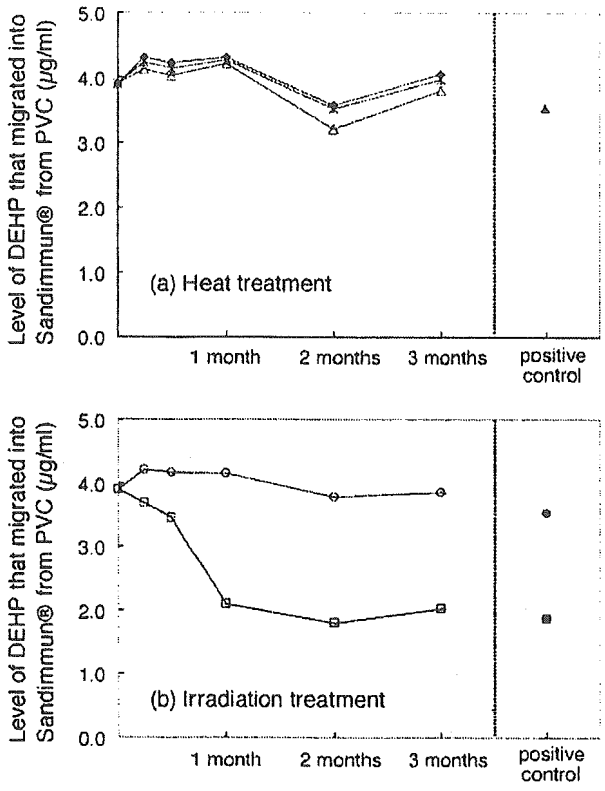

Fig. 2. Level of DEHP migrating into Sandimmun® injection from PVC sheet samples. (a) Heat treatment: PVC sheets were kept at 4 °C (◆); 37 °C (*) and 60 °C (△). Heated positive control: PVC sheets were kept at 100 °C for 25 days (▲). (b) Irradiation treatment: PVC sheets were irradiated with visible light (○) and UV (□). The visible irradiated positive control (●) was irradiated with sunlight for approximately 1 year. The UV-irradiated positive control (■) was irradiated with a UV lamp at 254 nm (3 cm, 25 days). The quantitative data were performed using computer software TOCO, Version 2.0 (Total Optimization of Chemical Operations), applying the function of mutual information (FUMI) theory.

PVC sheet. On the other hand, the significant change on migration was observed in UV-irradiated PVC sheets. The levels of DEHP migrating from the PVC sheets showed a time-dependent decrease in the UV irradiation group. At 1 month after UV irradiation, the level of DEHP migrating from the PVC sheet was reduced to approximately half that of the negative control. No significant change was observed thereafter, even if irradiation was continued. In addition, the level of DEHP migrating from the PVC sheet after UV irradiation over 3 months was not different from that of the positive control. We hypothesized that the reduction by half of the DEHP level has caused by UV-irradiated sides. We thought the UV-irradiated side (outer surface) of PVC sheet induces suppression in DEHP migration, and inner surface of PVC sheet dose not influence in migration. In order to confirm this hypothesis, we examined the PVC tubing that was able to distinguish outer and inner surface. The outer surface of the PVC tubing was subjected to strong UV irradiation. Subsequently, the level of DEHP migrating from the inner surface of the PVC tubing was determined. As a result, it was found that the level of DEHP released from the inner surface of the PVC tubing was almost the same as that of the negative control PVC tubing. It was concluded that the inner surface of the PVC tubing was not influenced by UV irradiation from the outside, since there was no change in the levels of DEHP released when compared with the control.

3.2. DEHP content examination

The DEHP content in the PVC sheets subjected to heat treatment or optical irradiation was determined. No significant difference in the DEHP content was found between the heat treatment groups and the visible light irradiation group. The positive controls of the two groups had almost the same DEHP content. On the other hand, the DEHP content in the UV-irradiated PVC sheets decreased slightly with time (Table 1). The most possible factor for the time-dependent, the sublimation/vaporization was occurred by UV-irradiated PVC sheet.

The rate of decrease in the DEHP content of the UV-irradiated PVC sheet was not equivalent to that of the level of migration. Therefore, the level of the suppression of DEHP migration was more remarkable than that of decreasing-content of DEHP.

3.3. Surface analysis

3.3.1. Surface analysis by FT-IR

FT-IR with ATR spectra was obtained from PVC sheets subjected to optical irradiation or heat treatment. Fig. 3a shows a characteristic absorption band at 635 cm^{-1}, due to C–Cl stretching vibration from PVC. We also observed absorption due to C–H from the aromatic compound and the carbonyl group from DEHP at 742 and 1720 cm^{-1}, respectively. Furthermore, an absorption band due to the alkane C–H bond from PVC and DEHP was found at nearly 1250 cm^{-1}.

Table 1
DEHP content in PVC sheet samples (w/w, %)

	4 °C	37 °C	60 °C	Visible light	UV light
1 week	31.2 ± 0.09	31.9 ± 0.61	33.2 ± 0.35	34.1 ± 1.65	36.2 ± 2.14
2 weeks	32.6 ± 0.44	33.3 ± 0.25	31.7 ± 0.03	34.8 ± 1.36	34.7 ± 3.32
1 month	32.9 ± 0.39	34.2 ± 0.45	35.0 ± 1.11	34.1 ± 0.85	33.7 ± 5.11
2 months	33.2 ± 0.12	33.9 ± 0.25	33.3 ± 0.43	32.8 ± 0.18	29.4 ± 0.63
3 months	33.8 ± 0.04	32.9 ± 0.26	30.9 ± 0.34	29.5 ± 4.05	27.1 ± 0.37

Negative control samples: 36.0 ± 2.60%; positive control samples subjected to heat treatment: 32.4 ± 0.45%; positive control samples irradiated with visible light: 32.6 ± 0.70%; positive control samples irradiated with UV: 30.8 ± 0.53%.

The FT-IR spectra of the heated-treated and visible light-irradiated PVC sheets were almost the same as that of the negative control. The spectrum was rectified using software because the ATR spectrum depended on the wavelength to calculate the areas of the characteristic absorption bands for DEHP or PVC. When the area ratios for the heat-treated or visible light-irradiated PVC sheets were compared with those of the negative control, no clear change was seen. On the other hand, as shown in Fig. 3b–f, the UV-irradiated PVC sheets were found to exhibit broadened absorption bands with time. These results led us to hypothesize that UV irradiation caused a change in the surface structure. The FT-IR spectrum of the non-UV-irradiated side was the same as that of the negative control, indicating that there was no change in the surface structure.

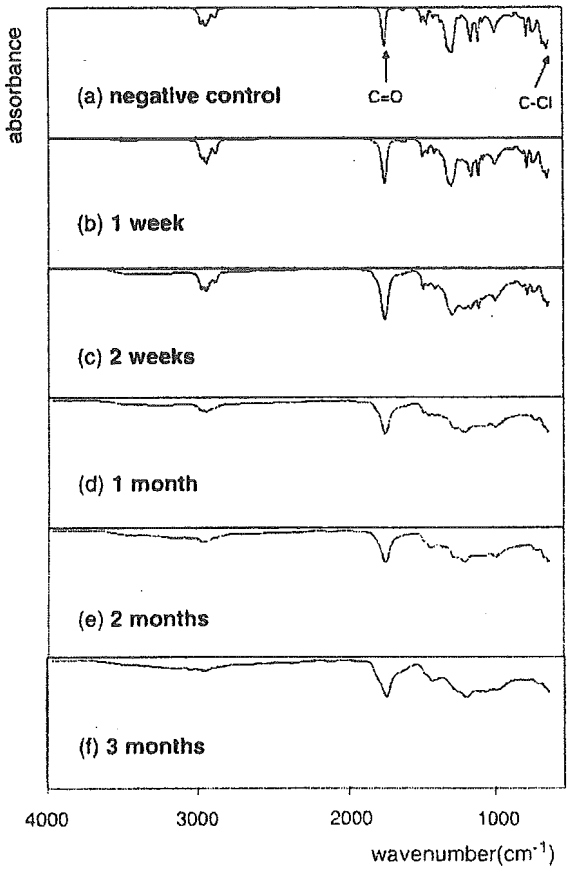

Fig. 3. FT-IR spectra of UV-irradiated and negative control PVC sheets FT-IR spectra of PVC sheets: negative control (a); and those irradiated with UV light for 1 week (b); 2 weeks (c); 1 month (d); 2 months (e) and 3 months (f).

3.3.2. *Surface analysis by ESCA*

Surface analysis of the PVC sheets was carried out and carbon, oxygen, chlorine and silicon were found on the sheet surface. As shown in Fig. 4a, the surface structure of the PVC sheets was not influenced by heat treatment or visible light irradiation because the composition ratio was maintained. On the other hand, in the UV-irradiated PVC sheets (Fig. 4b), the chlorine content was decreased and the oxygen content was increased with time. For the inner surface of the UV-irradiated PVC sheets, the composition ratio was hardly changed compared to the negative control in the period of 1 week to 1 month. However, after 2 months, the composition ratio was not changed at all compared with the negative control.

3.3.3. *Surface analysis by static angle of contact measurement*

In order to evaluate the affinity of the PVC sheets and the actual concentration of the Sandimmun® injection, we measured the static angle of contact. The static angle of contact was 37.1 ± 0.84 and 53.4 ± 0.93° for the outer and inner surfaces of the non-treated PVC