Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

International Journal of Pharmaceutics 298 (2005) 126–142

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Development of a simple method for predicting the levels of di(2-ethylhexyl) phthalate migrated from PVC medical devices into pharmaceutical solutions

Yuji Haishima[a,*], Fumie Seshimo[b], Tae Higuchi[b], Haruko Yamazaki[b], Chie Hasegawa[a], Shun-ichiro Izumi[c], Tsunehisa Makino[c], Keisuke Nakahashi[d], Rie Ito[b], Koichi Inoue[b], Yoshihiro Yoshimura[b], Koichi Saito[b], Takeshi Yagami[a], Toshie Tsuchiya[a], Hiroyuki Nakazawa[b]

[a] *Division of Medical Devices, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*
[b] *Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan*
[c] *Department of Obsterics and Gynecology, School of Medicine, Tokai University, Bohseidai, Isehara-city, Kanagawa 25-1193, Japan*
[d] *Terumo Corporation, 2-44-1 Hatagaya, Shibuya-ku, Tokyo 151-0072, Japan*

Received 12 February 2005; received in revised form 21 March 2005; accepted 10 April 2005
Available online 23 May 2005

Abstract

This study deals with the development of a simple method for predicting the elution levels of di-2-ethylhexyl phthalate (DEHP) from medical devices made of polyvinyl chloride (PVC) by using the physicochemical properties of pharmaceutical injections as a marker. GC-MS analysis showed that the release of DEHP from medical grade PVC product was concentration-dependently increased by extraction with two kinds of lipophilic injections (Sandimmun® and Prograf®) and three kinds of surfactants (HCO-60, Tween® 80, and SDS). The solubility of lipophilic pigments such as Sudan III, methyl yellow, and 1,4-diamino-anthraquinone against these solutions were also increased in a concentration-dependent manner, in which methyl yellow showed the highest response regarding the increase of optical density (O.D.). Further, electrical conductivity and static contact angle to the PVC sheet of the solutions were also increased or decreased in the same manner. As a result of the comparative study, significant correlation was found between DEHP release levels and these three physicochemical properties, particularly methyl yellow solubility, of the solutions tested. To evaluate the relationship in detail, DEHP release levels from PVC tubing and methyl yellow solubility of 53 injections used in gynecologic and obstetric fields were determined. None of the hydrophilic medicines showed any significant release of DEHP, and all showed low solubility of methyl yellow. On the other hand, the lipophilic medicines releasing a large amount of DEHP showed high solubility of methyl yellow (greater than O.D. 0.8). These

---

[*] Corresponding author. Tel.: +81 3 3700 4842; fax: +81 3 3707 6950.
E-mail address: haishima@nihs.go.jp (Y. Haishima).

doi:10.1016/j.ijpharm.2005.04.009

results indicate that a significant proportional relationship exists between DEHP release potency and methyl yellow solubility of pharmaceutical solutions, and the risk of DEHP exposure to the patients administered pharmaceuticals through transfusion set could be easily predicted by the solubility test without complicated elution tests of DEHP using GC-MS or LC-MS.

Keywords: DEHP; PVC; Medical device; Prediction; Risk assessment

---

1. Introduction

Phthalate esters, and DEHP in particular, have been extensively used as plasticizers due to the increased flexibility of PVC a plastic polymer used in a wide array of products including medical devices such as tubings, intravenous bags, blood containers, and catheters. DEHP is easily eluted from PVC products into not only foods but also pharmaceuticals and body fluids that come in contact with the plastic, and the migrated DEHP is directly and/or indirectly introduced into the human body (Allwod, 1986; Loff et al., 2000; Tickner et al., 2001). Some phthalates including DEHP are considered to be a toxic compound exhibiting effects similar to those of endocrine disruptors in rodents; they have antiandrogenic effects in male rats during the development of the male reproductive system and the production of normal sperm (Poon et al., 1997; Lamb et al., 1987; Tyl et al., 1988), and decrease the 17β-estradiol level in blood in female rats (Davis et al., 1994). General toxicity of DEHP has been well evaluated, and so far the result of risk assessment to human health indicates that this compound is relatively safe to humans. However, because the reproductive and developmental toxicity of DEHP to the human body is not well understood, it has recently been suggested that precautions be taken to limit the exposure of humans, particularly that of high risk patient groups such as male neonates, male fetuses, and peripubertal males, to DEHP. The concern is that DEHP's potency might have adverse effects on humans similar to those demonstrated on young rodents.

Taking the above into consideration, several agencies and official organizations in the world individually evaluated the safety of DEHP released from PVC products (Center for Devices and Radiological Health, 2001; Health Canada, 2002), and the Japanese Ministry of Health, Labor and Welfare (JMHLW) restricted the oral tolerable daily intake (TDI) value to 40–140 μg/kg/day.

It is very important that the exposure amount be exactly determined to conduct a risk assessment of the effect of DEHP on human health. Although some studies on the elution of DEHP from PVC medical devices have been performed as one of the JMHLW projects (Haishima et al., 2004; Inoue et al., 2003a,b; Takatori et al., 2004), it is not easy to identify the release behavior of DEHP from the variety of PVC products used in Japan by elution test under conditions that are the same as or similar to those of medical use. In addition, analytical methods having high sensitivity, precision, selectivity of quantitative ions, and low background, such as tandem LC-MS, high resolution GC-MS, and column-switching LC-MS methods, are required to determine DEHP for clinical assessment. Thus, regardless whether an investigation is in vivo or in vitro, the release test of DEHP is at present time-consuming and labor-intensive.

Jenke (2001) reported that the chemical compatibility assessment considers two distinct yet complementary mechanisms by which a device and its contacted solution can interact. These mechanisms include the migration of a chemical component out of the device and into the contacted solution (leaching) and the sorption of contained solution components by the device (binding). Alternatively, the product/device interaction can be modeled based on a rigorous scientific assessment of the physicochemical processes. Such models are based on the linear correlation of polymer/solution interaction constants with solvent/water partition coefficients (Nasim et al., 1972; Pitt et al., 1988; Hayward et al., 1990; Kenley and Jenke, 1990; Jenke, 1991; Jenke et al., 1991; Atkinson and Duffull, 1991; Roberts et al., 1991; Jenke et al., 1992). In addition, it is known that extraction occurs either by leaching or after an extracting material such as blood and pharmaceutical solutions diffuses into the PVC matrix and dissolves the plasticizer, which is relatively lipophilic. In consideration of these issues, we suspected that the release behavior of DEHP from PVC medical devices may be predicted from the physicochemical properties of pharmaceutical injections applied to the devices, without a complicated elution test.

In the present study, to develop a simple method for predicting the release level of DEHP from PVC medical devices, we examined the relationship between the release potency of DEHP from PVC product and physicochemical properties such as the solubility of lipophilic pigments, electrical conductivity, and the static contact angle to PVC sheet, using two kinds of lipophilic injections and three kinds of surfactants as test solutions. Further, to evaluate the relationship in detail, DEHP release levels from PVC tubing and the physicochemical properties of 53 injections used in gynecologic and obstetric fields were determined.

2. Materials and methods

2.1. Chemicals and utensils

Medical grade PVC sheet for blood container and PVC tubing for transfusion set were provided by Terumo Co. (Tokyo, Japan).

Sandimmun® (50 mg/ml cyclosporine) and Prograf® (5 mg/ml tacrolimus) were provided by Novartis Pharma K.K. (Tokyo, Japan) and Fujisawa Pharmaceutical Co., Ltd. (Tokyo, Japan). The other 51 injections listed in Table 1 were purchased from commercial companies. Polyoxyethene hydrogenated castor oil 60 (HCO-60) provided by Nikko Chemicals Co. (Tokyo, Japan), polysorbate 80 (Tween® 80, ICN Biomedicals Inc., Ohio, USA), and sodium lauryl sulfate (SDS, Sigma Aldrich Japan, Tokyo, Japan) were used as surfactants. In these materials, Sandimmun®, Prograf®, HCO-60, Tween® 80, and SDS were used as pretest solutions for evaluating the relationship between release potency of DEHP and physicochemical properties of pharmaceuticals.

Methyl yellow (Wako Pure Chemical Industries, Ltd., Osaka, Japan), Sudan III (Sigma Aldrich Japan, Tokyo, Japan), and 1,4-diamino-anthraquinone (Tokyo Kasei Co., Tokyo, Japan) were used as lipophilic pigments. DEHP and DEHP-d_4 were purchased from Kanto Chemical Co. (Tokyo, Japan). Hexane, anhydrous sodium sulfate, sodium chloride of phthalate esters of analytical grade, diethyl ether of dioxin of analytical grade, and distilled water of HPLC grade were used in this study.

All utensils were made of glass, metal, or teflon, and were heated at 250 °C for more than 16 h before use.

2.2. Classification of pharmaceuticals

As shown in Table 1, based on the properties of principal drugs and additives contained in each pharmaceutical, 53 injections used in this study were divided into five groups. Expression rule on solubility of the drugs has been established in general notices in the Japanese Pharmacopoeia IX edition regarding the relationship between descriptive term and the degree of dissolution. Pharmaceuticals such as Sandimmun® and Prograf® containing principal drugs that are expressed as practically insoluble or insoluble to water in the instruction manuals were assigned to group 1 as lipophilic injections. Most of pharmaceuticals in this group were contained various additives such as surfactants, oils, glycerin, ethanol, benzyl alcohol, and so on. The principal drugs of pharmaceuticals classified into group 2 are also insoluble or very slightly soluble to water, but these drugs can be dissolved in acidic or basic solutions. Gaster®, Droleptan®, Elaspol®, Aleviatin®, Methotrexate® Parenteral, Serenace®, and Bosmin® were assigned to this group, and pH of each pharmaceutical is expressed in the instruction manuals as 4.7–5.7, 2.5–4.5, 7.5–8.5, approximately 12, 7.0–9.0, 3.5–4.2, and 2.3–5.0, respectively. Pharmaceuticals consisted of drugs that are slightly soluble or sparingly soluble to water were classified into group 3. Solubility of principal drugs contained in the pharmaceuticals assigned to groups 4 and 5 was expressed as very soluble, freely soluble, or soluble to water in each instruction manual. Pharmaceuticals of group 5 are hydrophilic injections as negative control regarding DEHP migration. Although pharmaceuticals assigned to group 4 are also hydrophilic injections, these pharmaceuticals were suspected to induce DEHP migration, because some of them are human serum products or containing chlorobutanol, phenol, and benzyl alcohol as additives.

2.3. Solubility test of lipophilic pigments

One millilitre of each surfactant solution and pharmaceutical injection was added to each lipophilic pigment (5 mg) followed by sonication for 10 min at room temperature and centrifugation at 3000 rpm for 10 min. The supernatant was passed through a membrane filter (pore size 0.2 μm) and the filtrate (100 μl) was

Table 1
List of phamaceutical injections used in this study

Product name	Principal drug	Concentration for medical use	Additives	Medication	Color
Group 1[a]					
Sandimmun®	Cyclosporin	500 μg/mL	Polyoxyethene castor oil, ethanol	Instillation	Clear
Prograf® injection 5 mg	Tacrolimus hydrate	10 μg/mL	Absolute ethanol, HCO-60	Instillation	Clear
1% Diprivan® injection	Propofol	10 mg/mL	Soybean oil, concentrated glycerin, pure egg-yolk lecithin, edetate sodium pH adjuster	Intravenous injection	White emulsion
Ropion®	Flurbiprofen axetil	10 mg/mL	Pure soybean oil, pure egg-yolk lecithin, concentrated glycerin	Intravenous injection	White emulsion
Sohvita®	Vitamins including fat-soluble vitamin	Whole amount of Sobita was mixed with PN-Twin No.2 (2.2 L)	Sodium citrate, pH adjuster, sodium pyrosulfite, sodium thioglycollate, HCO-60, benzyl alcohol, polysorbate 80	Instillation	Yellow (clear)
Kaytwo® N	Menatetrenone	5 mg/mL	Aminoethylsulfonic acid, sesame oil, pure soybean lecithin, D-sorbitol, concentrated glycerin, pH adjuster	Intravenous injection	Buff yellow (translucence)
Humulin® R	Insulin human	40 units/mL	Concentrated glycerin, *m*-cresol, pH adjuster	Intravenous injection	Clear
Prostarmon®-F	Dinoprost	2 mg/mL		Instillation	Clear
Florid®-F	Miconazole	1 mg/mL	HCO-60	Instillation	Clear
Horizon®	Diazepam	5 mg/mL	Propylene glycol, ethanol, benzyl alcohol, sodium benzoate, benzoic acid	Intravenous injection	Buff yellow (clear)
Predonine®	Prednisolone sodium succinate	① 10 mg/mL, ② 1 mg/mL	Dried sodium carbonate, sodium hydrogenphosphate, sodium dihydrogenphosphate crystal	① Intravenous injection, ② instillation	Clear
Group 2[a]					
Gaster®	Famotidine	20 mg/mL	L-Aspartic acid, D-mannitol	Instillation	Clear
Droleptan®	Droperidol	① 2.5 mg/mL, ② 50 μg/mL	*p*-Oxymethyl benzoate, *p*-oxypropyl benzoate pH adjuster (acidic)	① Intravenous injection, ② instillation	Clear
Elaspol®	Sivelestat sodium hydrate	1 mg/mL	D-Mannitol, pH adjuster	Intravenous injection	Clear
Aleviatin®	Phenytoin	50 mg/mL	Sodium hydroxide, propylene glycol, ethanol	Intravenous injection	Clear
Methotrexate® parenteral	Methotrexate	0.2 mg/mL	Sodium chloride, sodium hydroxide	Instillation	Clear
Serenace®	Haloperidol	5 mg/mL	Glucose, lactic acid, sodium hydroxide	Instillation	Clear
Bosmin® injection	Epinephrine	0.25 mg/mL	Chlorobutanol, sodium hydrogen sulfite, hydrochloric acid, sodium chloride, pH adjuster	Intravenous injection	Clear
Group 3[a]					
Partan M injection	Methylergometrine maleate	0.2 mg/mL		Intravenous injection	Clear
Musculax® intravenous	Vecuronium bromide	2 mg/mL	D-Mannitol	Intravenous injection	Clear
Carbenin® for intravenous drip infusion	Panipenem Betamipron	5 mg/mL	pH Adjuster	Instillation	Achroma yellow (clear)

Table 1 (*Continued*)

Product name	Principal drug	Concentration for medical use	Additives	Medication	Color
Minomycin® intravenous for drip use	Minocycline Hydrochloride	1 mg/mL		Instillation	Clear
Perdipine®	Nicardipine Hydrochloride	0.1 mg/mL	D-Sorbitol, pH adjuster	Instillation	Clear
Bisolvon® injection	Bromhexine Hydrochloride	2 mg/mL	Glucose	Intravenous injection	Clear
Modacin® injection	Ceftazidime	10 mg/mL	Sodium carbonate	Instillation	Clear
Diflucan® intravenous solution	Fluconazole	1 mg/mL		Instillation	Clear
Doyle® for injection	Aspoxicillin	50 mg/mL	Sodium chloride	Instillation	Clear
Adona® (AC-17) injection	Carbazochrome sodium sulfonate	0.05 mg/mL	Sodium hydrogensulfite, D-sorbitol, propylene glycol	Instillation	Clear
Group 4[a]					
Atonin®-O	Oxytocin	0.01 units/mL	Chlorobutanol	Instillation	Clear
Atarax®-P Parenteral solution	Hydroxyzine Hydrochloride	0.05 mg/mL	Benzyl alcohol, pH adjuster	Instillation	Clear
Zantac® injection	Ranitidine hydrochloride	0.1 mg/mL	pH adjuster, phenol	Instillation	Achroma yellow (clear)
Kenketsu venoglobulin®-IH YOSHITOMI	Human immunoglobulin G	50 mg/mL	D-Sorbitol, pH adjuster	Intravenous injection	Clear
Pantol® injection	Panthenol	250 mg/mL	Benzyl alcohol	Intravenous injection	Clear
Buminate® 25%	Human serum albumin	250 mg/mL	Sodium *N*-acetyl tryptophan, sodium caprylate, sodium hydrogen carbonate	Intravenous injection	Clear
Neuart®	Human antithrombin III	25 units/mL	Sodium chloride, sodium citrate, D-mannitol	Instillation	Achroma yellow (barely opacity)
Millisrol® injection	Nitroglycerin	0.5 mg/mL	D-Mannitol, pH adjuster	Instillation	Clear
Metilon®	Sulpyrine	2.5 mg/mL	Benzyl alcohol	Instillation	Clear
Erythrocin®	Erythromycin Lactobionate	2.5 mg/mL	Benzyl alcohol	Instillation	Clear
Dalacin® S injection	Clindamycin phosphate	3 mg/mL	Benzyl alcohol	Instillation	Clear
Group 5[a]					
Tienam® for intravenous drip infusion	Imipenem Cilastatin sodium	5 mg/mL	Sodium hydrogencarbonate	Instillation	Achroma yellow (clear)
Glucose® injection	5% glucose			Instillation	Clear
Fesin®	Ferric oxide, saccharated	0.4 mg/mL		Instillation	Clear
Actit® injection	Maltose, sodium chloride, potassium chloride, magnesium chloride, potassium dihydrogen phosphate, sodium acetate			Instillation	Clear
Atropine sulfate injection	Atropine sulfate	0.5 mg/mL		Intravenous injection	Clear
Viccillin® for injection	Ampicillin sodium	10 mg/mL		Instillation	Clear
Neophyllin®	Aminophyline	0.5 mg/mL	Ethylenediamine	Instillation	Clear
Fosmisin®-S Bag 2g for intravenous drip infusion	Fosfomycin sodium	20 mg/mL	Glucose solution	Instillation	Clear
Calcicol®	Calcium gluconate	85 mg/mL		Instillation	Clear
Cefamezin® α	Cefazolun sodium hydrate	10 mg/mL		Instillation	Clear
PN-Twin® No.2	Amino acids, electrolytes		Sodium hydrogen sulfite	Instillation	Clear
Succin®	Suxamethonium chloride	2 mg/mL		Instillation	Clear
Optiray®	Ioversol	320 mg/ml as iodine		Intravenous injection	Clear
Proternol®-L injection	*l*-Isoprenaline hydrochloride	1 μg/mL	Sodium hydrogen sulfite L-cysteine hydrochloride	Instillation	Clear

[a] A detailed information on this classification was described in the part of Section 2.

transferred to a 96-well plate, and absorbance of the sample was measured by μQuant (BIO-TEK Instruments, Inc., Vermont, USA) at 450 nm for methyl yellow, 530 nm for Sudan III, and 590 nm for 1,4-diaminoanthrazuinone.

2.4. Measurement of static contact angle and electrical conductivity

Ten microlitre of each surfactant solution and pharmaceutical injection was dropped on PVC sheets. After 120 s, the width and height of the drops were measured with a G-1-1000 instrument (ERMA, Tokyo, Japan). The static contact angle was computed by the following formulas

$$r^2 = (w/2)^2 + (r-h)^2, \qquad \sin\delta = (w/2)/r$$

where, r is the radius of drop (mm), w the width of drop (mm), h the height of drop (mm), δ the static angle of contact.

Electrical conductivity of each test solution was measured by COS conductivity analyzer (CEH-12, Horiba, Tokyo, Tokyo).

2.5. Elution test of DEHP and determination of DEHP content

PVC sheet (1 cm × 3 cm, thickness: 0.4 mm) was put in a screw-capped glass tube, and 5 ml of pretest solutions (Sandimmun®, Prograf®, HCO-60, Tween® 80, and SDS) were added to the respective tubes. After shaking for 2 h at room temperature, an aliquot (0.1 ml) of the solution was taken into another glass tube, and distilled water (2 ml), sodium chloride (10 mg), and 5 ml of diethyl ether containing 50 ng/ml DEHP-d_4 were added to the tube. After shaking for 30 min followed by centrifugation at 3000 rpm for 10 min at room temperature, the organic phase was collected and dehydrated with anhydrous sodium sulfate followed by GC-MS analysis described below.

Pharmaceutical injections including Sandimmun® and Prograf® adjusted to the concentration used for medical treatment were enclosed in PVC tubing (inner diameter, 2.13 mm) cut to 10 cm length. The length and volume of the enclosed injection were 8 cm and 0.285 ml, respectively, and the surface area in contact with the enclosed injection was 5.35 cm^2. After shaking the tube for 1 h at room temperature, the enclosed test solution was transferred to a screw-capped glass tube, and the sample for GC-MS analysis was prepared by the same method as that described above.

To determine DEHP content, PVC sheet and tubing (20 mg) were dissolved in 20 ml of THF by soaking overnight at room temperature. An aliquot (0.1 ml) of the solution was diluted 10,000 times with diethyl ether containing 50 ng/ml DEHP-d_4, and then analyzed by GC-MS. DEHP contents of the PVC sheet and tubing used in this study were 36.2 and 32.9% (w/w), respectively.

2.6. GC-MS analysis

A JMS700 instrument (JEOL, Tokyo, Japan) equipped with a Hewlett-Packard HP6890 series GC system and an auto-injector (Agilent Technologies, Palo Alto, CA) were used for GC-MS analysis (resolution = 5000). Chromatographic separation was performed with BPX-5 fused silica capillary column (25 m × 0.22 mm I.D., film thickness: 0.25 μm, SGE, Melbourne, Australia).

The sample (2 μl) was injected in the pulsed splitless mode. The injector temperature was 260 °C. Flow rate of helium carrier gas was 1 ml/min. Column temperature was programmed as initial temperature to 120 °C for 2 min then increasing to 300 °C at 10 °C/min. Electron impact (EI)-mass spectrum was recorded at 70 eV, and the ions of m/z 149.024 for DEHP and 153.049 for DEHP-d_4 were selected as the quantitative ions in the selective ion mode (SIM) analysis 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 lines and three times for the other samples. Preparation of calibration curves and calculation of quantitative data were performed by the computer software TOCO (Total Optimization of Chemical Operations), Version 2.0, practicing the function of mutual information (FUMI) theory (Hayashi and Matsuda, 1994; Hayashi et al., 1996, 2002; Haishima et al., 2001, 2004).

3. Results and discussion

3.1. Precision of quantitative GC-MS analysis and release profile of DEHP from PVC sheet

Background analyses of DEHP originating from each reagent and GC-MS instrument showed that

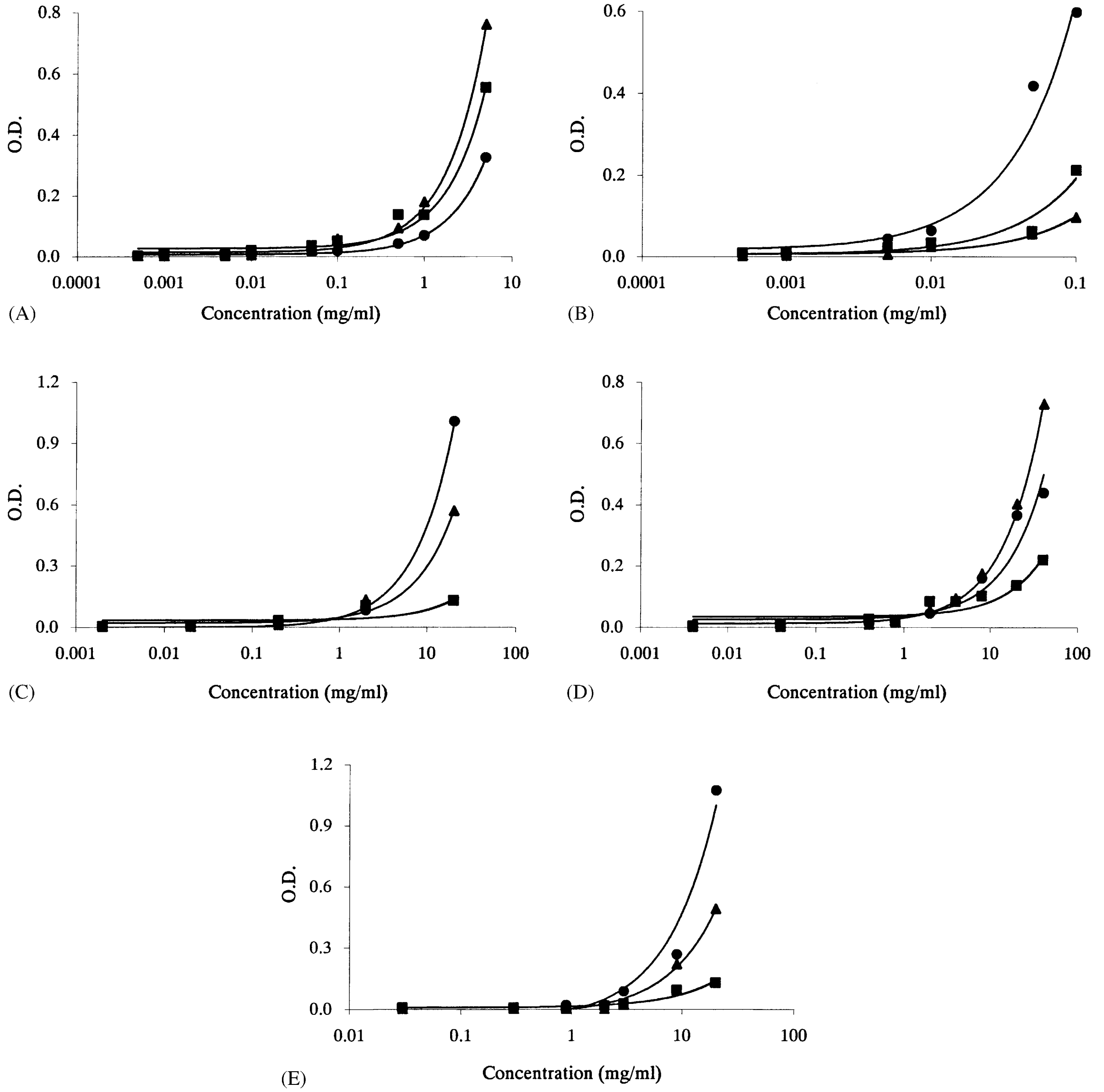

Fig. 1. Lipophilic pigment solubility against various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS. Methyl yellow(●), Sudan III (■), and 1,4-diamino-anthraquinone (▲) were used as the pigments. Absorbance of methyl yellow dissolved in Sandimmun® and Tween® 80 was measured after five times dilution with distilled water.

0.93 ± 0.31 ng/ml DEHP ($n = 5$) was detected as background contamination when 50 ng of the internal standard (DEHP-d_4) was used in the quantitative analyses. On the basis of the background value, the experimental LOD and LOQ were calculated as 1.85 and 4.01 ppb, respectively. A calibration curve was obtained for the peak ratio of DEHP to DEHP-d_4 versus DEHP concentration level. The response was found to be linear in the validated range (5–200 ppb) with correlation coefficient (r) exceeding 0.999. Further, the 95% confidence interval calculated by TOCO was sufficiently narrow, indicating that the present GC-MS method could be used for DEHP analysis with high accuracy.

Table 2
DEHP release capacity and physicochemical properties of lipophilic injections and surfactants

Solution (mg/ml)	Release amount of DEHP		Lipophilic pigments' solubility[a]						Electrical conductivity		Contact angle to PVC sheet	
			Methyl yellow[b]		Sudan III		1,4-diamino anthraquinone					
	ppm	S.D.	O.D. at 450 nm	S.D.	O.D. at 530 nm	S.D.	O.D. at 590 nm	S.D.	μS/cm	S.D.	°	S.D.
Sandimmun®												
0.0005	0.22	0.003	0.001	0.002	0.001	0.001	0.001	0.001	12.13	0.56	84.69	1.35
0.001	0.35	0.01	0.003	0.003	0.009	0.001	0.001	0.001	11.93	0.82	nt	nt
0.005	0.77	0.01	0.003	0.001	0.006	0.002	0.003	0.001	12.55	0.46	78.17	1.77
0.01	1.16	0.01	0.004	0.001	0.020	0.001	0.005	0.001	12.02	0.61	72.36	0.21
0.05	2.84	0.01	0.019	0.001	0.036	0.000	0.019	0.001	12.46	0.31	64.72	0.55
0.1	4.22	0.03	0.018	0.001	0.051	0.001	0.059	0.001	11.66	0.55	60.39	0.97
0.5	9.01	0.05	0.042	0.001	0.137	0.001	0.094	0.001	18.91	0.36	50.47	1.48
1	10.90	0.15	0.069	0.001	0.136	0.001	0.180	0.004	26.90	0.78	46.65	1.98
5	22.19	0.26	0.325	0.001	0.555	0.002	0.762	0.005	104.80	1.32	42.05	1.62
Prograf®												
0.0005	0.25	0.01	0.006	0.001	0.009	0.001	0.002	0.002	8.11	0.26	81.07	0.26
0.001	0.34	0.02	0.010	0.001	0.009	0.005	0.004	0.001	8.09	0.32	79.38	1.01
0.005	0.99	0.01	0.043	0.001	0.022	0.002	0.006	0.001	8.53	0.15	75.06	0.66
0.01	1.71	0.003	0.063	0.001	0.033	0.005	0.025	0.001	8.61	0.22	74.66	1.52
0.05	5.31	0.05	0.418	0.005	0.062	0.002	0.057	0.001	10.52	0.45	67.54	0.88
0.1	8.95	0.04	0.597	0.004	0.211	0.005	0.097	0.001	11.51	0.38	65.07	0.87
0.5	42.26	1.64	nt	nt	nt	nt	nt	nt	nt	nt	55.67	0.83
HCO-60												
0.002	0.09	0.01	0.003	0.003	0.005	0.001	0.001	0.001	13.27	0.52	84.22	1.92
0.02	0.28	0.01	0.003	0.003	0.010	0.001	0.001	0.001	16.07	0.66	80.79	1.39
0.2	1.15	0.01	0.011	0.001	0.033	0.001	0.012	0.001	16.51	0.43	76.54	2.48
2	5.72	0.04	0.083	0.001	0.106	0.002	0.135	0.001	16.39	0.59	66.23	0.34
20	22.32	0.25	1.006	0.005	0.130	0.013	0.571	0.007	18.36	0.64	63.31	5.18
40	28.90	0.22	nt	nt	nt	nt	nt	nt	26.80	0.80	61.02	0.70
Tween® 80												
0.004	0.38	0.01	0.001	0.001	0.005	0.001	0.002	0.002	15.93	0.38	84.01	1.28
0.04	0.49	0.01	0.001	0.001	0.009	0.001	0.002	0.003	14.82	0.29	77.91	0.40
0.4	2.77	0.02	0.011	0.001	0.027	0.001	0.010	0.003	15.60	0.41	70.28	0.87
0.8	4.30	0.03	0.015	0.002	0.018	0.001	0.017	0.001	16.49	0.35	68.78	1.23
2	6.58	0.03	0.045	0.001	0.083	0.002	0.055	0.001	15.20	0.47	64.43	6.80
4	9.26	0.15	0.083	0.001	0.083	0.004	0.094	0.003	13.49	0.33	58.70	1.03
8	13.17	0.17	0.159	0.002	0.101	0.001	0.175	0.003	18.50	0.50	56.05	0.33
20	20.07	0.32	0.365	0.007	0.136	0.001	0.403	0.002	31.40	0.82	54.21	0.53
40	25.56	0.20	0.438	0.004	0.219	0.002	0.728	0.004	57.70	0.91	51.89	0.61
SDS												
0.03	0.44	0.005	0.001	0.001	0.009	0.001	0.001	0.001	20.90	0.59	82.48	1.29
0.3	1.10	0.02	0.002	0.001	0.006	0.002	0.001	0.001	41.90	0.72	77.65	0.57
0.9	2.25	0.01	0.021	0.019	0.007	0.001	0.001	0.001	102.20	1.33	63.15	0.93
2	3.70	0.01	0.022	0.001	0.018	0.001	0.002	0.001	373.00	1.56	41.51	0.63
3	6.67	0.03	0.088	0.001	0.027	0.001	0.024	0.001	533.00	1.96	40.03	1.21
9	14.75	0.09	0.268	0.003	0.094	0.001	0.220	0.003	1120.00	2.42	40.23	0.64
20	18.05	0.18	1.071	0.014	0.129	0.003	0.491	0.004	3220.00	2.68	33.94	3.09

nt, not tested.

[a] Values after substracting blank value.

[b] O.D. of Sandimmun and Tween 80 was measured after five times dilution with distilled water.

Release test of DEHP from medical grade PVC sheet was performed using GC-MS analysis. Two kinds of pharmaceuticals and three kinds of surfactants were used as the test solutions for DEHP extraction. Qualitative analysis of DEHP was performed by scan mode EI-MS (Haishima et al., 2004), and the release profile of DEHP from the sheet is shown in Table 2. Sandimmun® and Prograf®, typical lipophilic injections containing polyoxyethene castor oil or HCO-60, and ethanol as additives, were found to release DEHP from the sheet concentration-dependently. Significant release of DEHP was observed at concentrations higher than 0.05 mg/ml, and the released amounts reached 22.19 ± 0.26 ppm by Sandimmun® (5 mg/ml) and 42.26 ± 1.64 ppm by Prograf® (0.5 mg/ml). Three kinds of surfactant, including HCO-60, Tween® 80, and SDS, were also found to release DEHP from the PVC sheet in a concentration-dependent manner. In particular, the release was significantly increased more than the concentration of approximately 1 mg/ml that is critical micelle concentration (CMC) of each surfactant, and the released amounts reached 28.90 ± 0.22, 25.56 ± 0.20, and 18.05 ± 0.18 ppm by the extraction with 40 mg/ml of HCO-60, Tween® 80, and 20 mg/ml of SDS, respectively (Table 2).

3.2. Determination of physicochemical property of test solution

Three kinds of physicochemical properties of Sandimmun®, Prograf®, HCO-60, Tween® 80, and SDS were measured to determine whether the properties could be used as markers to predict the level of DEHP released by these solutions from medical grade PVC sheet as described above. As shown in Fig. 1 and Table 2, the absorbance of each lipophilic pigment, including methyl yellow, Sudan III, and 1,4-diaminoanthraquinone, which have different absorption maximums, dissolved in each solution was increased in proportion to the rise of the solution concentration. Of the three kinds of lipophilic pigment, methyl yellow exhibited the highest response regarding the increase of absorbance, and the response of Sudan III was the lowest.

In order to evaluate the affinity of the test solutions against PVC sheet, static contact angle to the surface of PVC sheet was measured. As shown in Table 2, the angle of each solution was decreased in a concentration-dependent manner, indicating that the affinity was increased according to the rise of solution concentration. The electrical conductivity of each test solution was also measured as a marker predicting DEHP release level. As shown in Table 2, electrical conductivity of all the solutions except Prograf® was increased in a concentration-dependent manner. In particular, the value of SDS, an ionic surfactant, was remarkably increased according to the increase of concentration. On the other hand, no significant change was observed in the electrical conductivity of Prograf®.

As shown in Figs. 2–4, the profiles of these physicochemical properties appear to significantly relate to the release behaviors of DEHP from medical grade PVC sheet by the extraction with the solutions. However, some pharmaceuticals may exhibit very low electrical conductivity, similar to that of Prograf® (Fig. 4 and Table 2), and the value is greatly influenced by the amounts of electrolytes present in solution rather than by the lipotropy of the solution, which is not the case for other two physicochemical properties. Taking the above findings into consideration, electrical conductivity may be not useful as a marker to predict the level of DEHP released from PVC medical devices. On the other hand, no such disadvantage was recognized in the lipophilic pigment solubility test, in which good correlation to the release behavior of DEHP was observed (Fig. 2), indicating that the DEHP release level from PVC medical devices could be predicted by the test. Although static contact angle value appears to change linearly according to the concentration of the test solution, the value suggests that this property may also be useful as a marker (Fig. 3).

3.3. Detailed evaluation of the relationship between release potency of DEHP and physicochemical properties of pharmaceuticals

A detailed investigation was performed to evaluate the relationship between release behavior of DEHP from medical grade PVC tubing used as a transfusion set and the physicochemical properties, namely lipophilic pigment solubility and static contact angle, of pharmaceuticals. For this investigation, 53 pharmaceutical injections including Sandimmun® and Prograf® as positive control were scientifically selected from 180 injections used in the department of Obstetrics

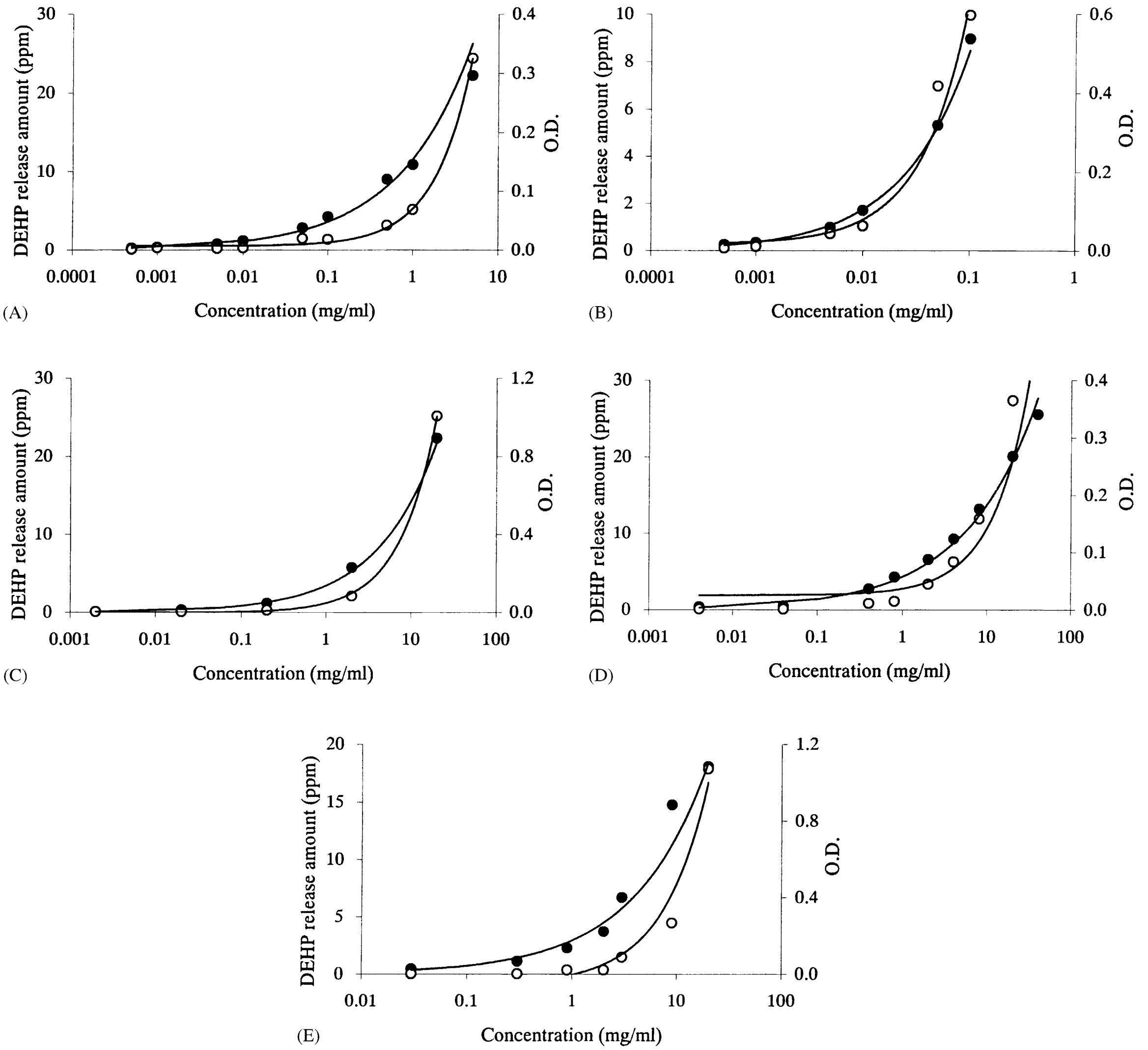

Fig. 2. Relationship between DEHP release potency (●) and methyl yellow solubility (○) of various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS. Absorbance of Sandimmun® and Tween® 80 was measured after five times dilution with distilled water.

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

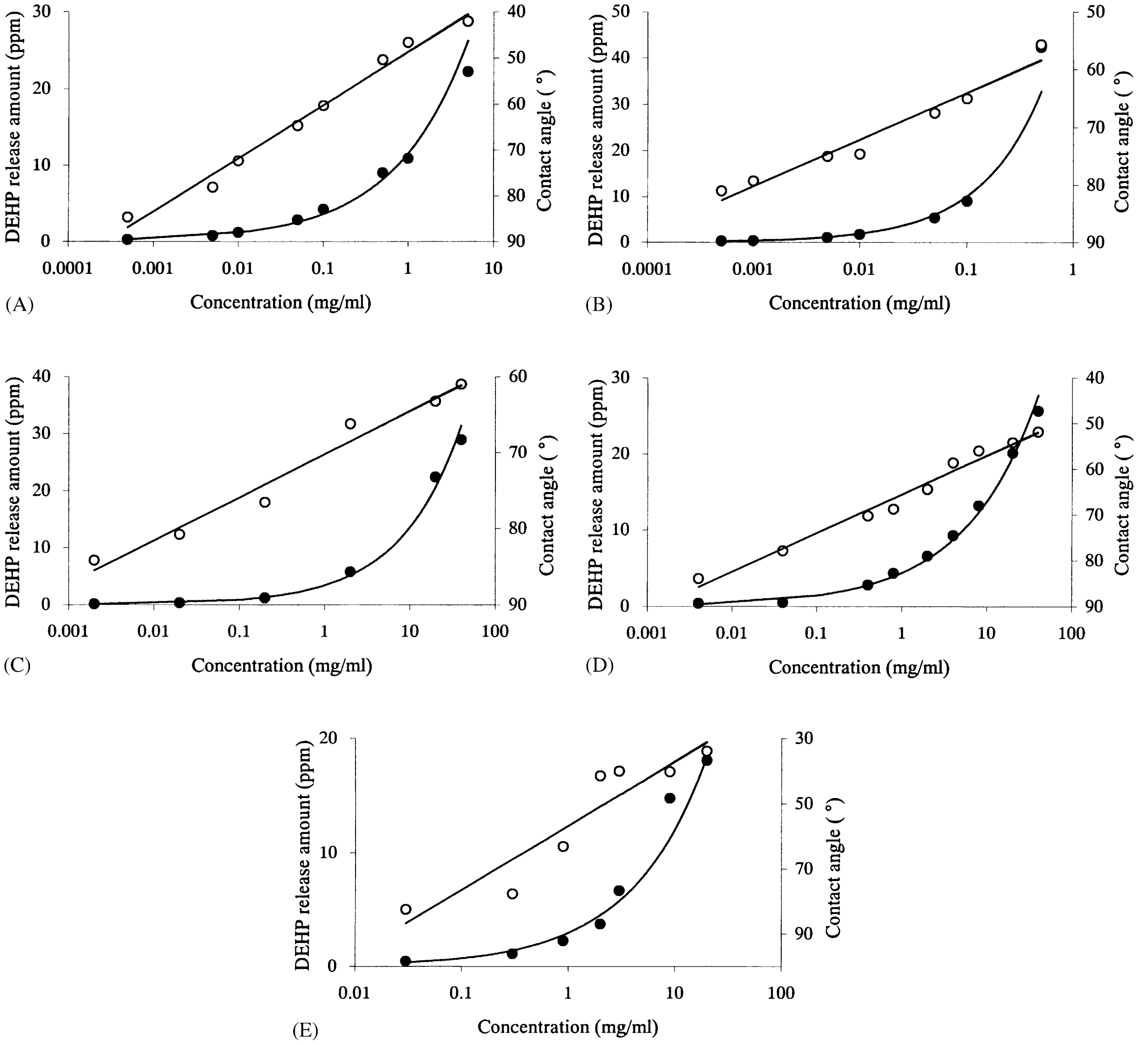

Fig. 3. Relationship between DEHP release potency (●) and static contact angle to PVC sheet (○) of various concentration of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS.

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

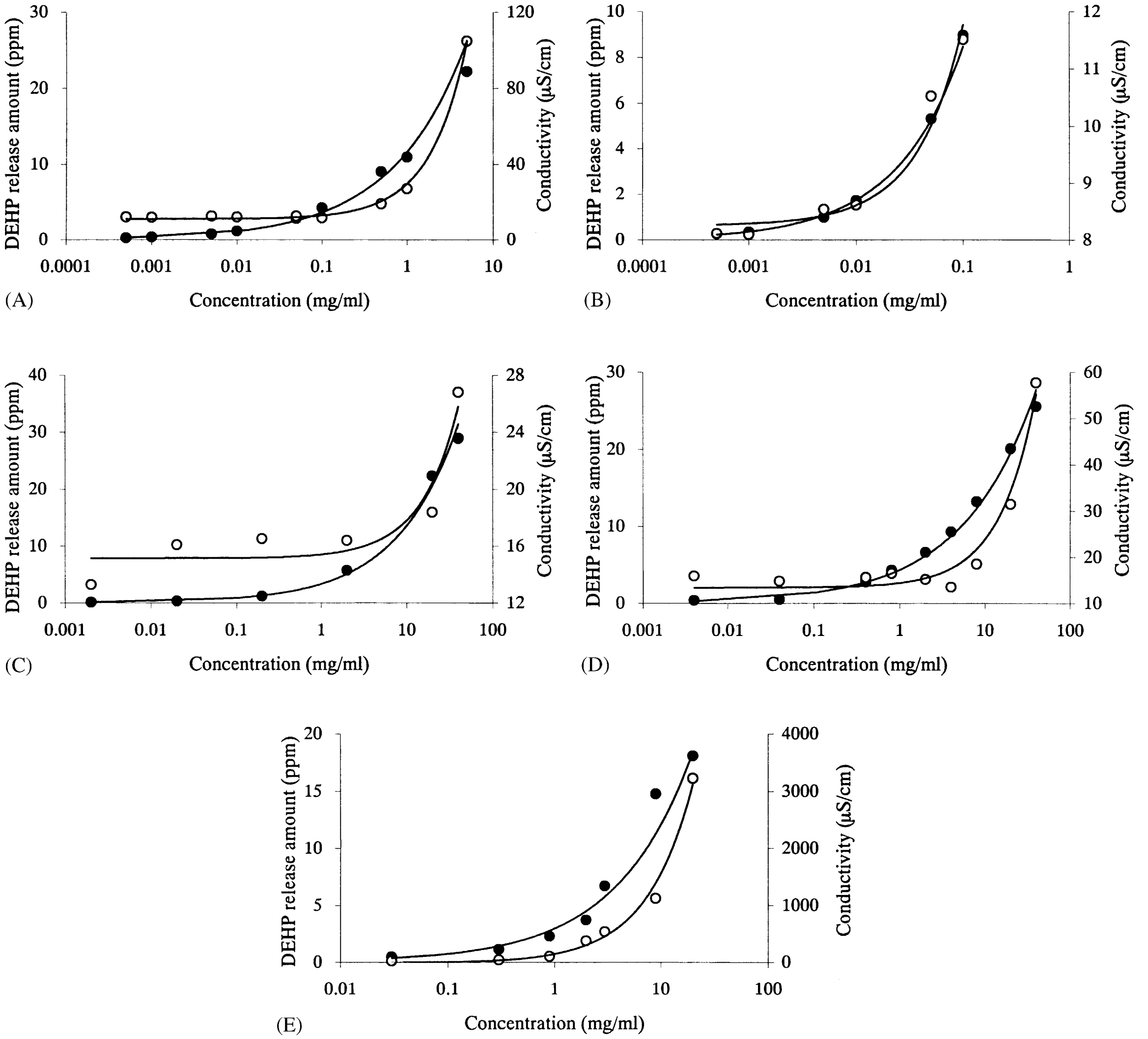

Fig. 4. Relationship between DEHP release potency (●) and electrical conductivity (○) of various concentrations of (A) Sandimmun®, (B) Prograf®, (C) HCO-60, (D) Tween® 80, and (E) SDS.

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

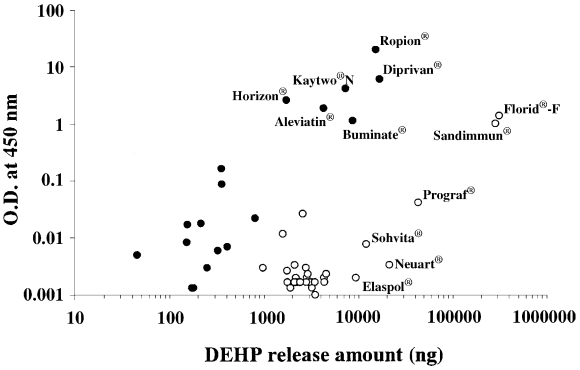

Fig. 5. Relationship between the released amount of DEHP and methyl yellow solubility of the medical use concentration of 53 pharmaceuticals. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day),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

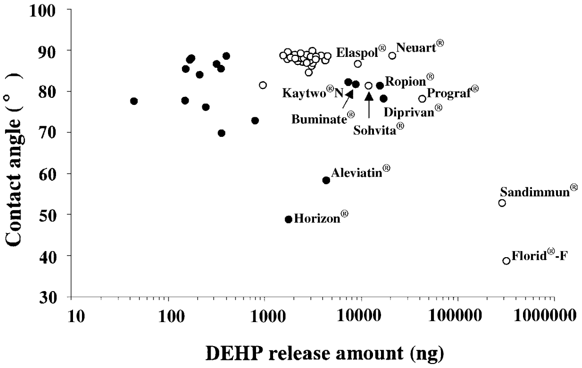

Fig. 6. Relationship between the released amount of DEHP and static contact angle of the medical use concentration of 53 pharmaceuticals. The released amount of DEHP was calculated as the absolute value when 3 m of PVC tubing (inner diameter, 2.13 mm) is used for medical treatment (one time per day), 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

This work was supported by grant H14-Iyaku-005 and H15-Risk-017 from the Ministry of Health, Labor, and Welfare of Japan. We greatly appreciate cooperation of pharmaceutical companies that have given us Sandimmun® and Prograf® injections.

References

Allwod, M.C., 1986. The release of phthalate ester plasticizer from intravenous administration sets into fat emulsion. Int. J. Pharm. 29, 233–236.

Atkinson, H., Duffull, S.B., 1991. Prediction of drug loss from PVC infusion bags. J. Pharm. Pharmacol. 43, 374–376.

Center for Devices and Radiological Health, U.S. Food and Drug Administration, 2001. Safety assessment of di(2-ethylhexyl) phthalate (DEHP) released from PVC medical devices. Web site at http://www.fda.gov/cdrh/nespg.html.

Davis, B.J., Maronpot, R.R., Heindel, J.J., 1994. Di-(2-ethylhexyl)phthalate suppressed estradiol and ovulation in cycling rats. Toxicol. Appl. Pharmacol. 128, 216–223.

Haishima, Y., Hayashi, Y., Yagami, T., Nakamura, A., 2001. Elution of bisphenol-A from hemodialyzers consisting of polycarbonate and polysulfone resins. J. Biomed. Mater. Res. Part B: Appl. Biomater. 58, 209–215.

Haishima, Y., Matsuda, R., Hayashi, Y., Hasegawa, C., Yagami, T., Tsuchiya, T., 2004. Risk assessment of di(2-ethylhexyl)phthalate released from PVC blood circuits during hemodialysis and pump-oxygenation therapy. Int. J. Pharm. 274, 119–129.

Hanawa, T., Muramatsu, E., Asakawa, K., Suzuki, M., Tanaka, M., Kawano, K., Seki, T., Juni, K., Nakajima, S., 2000. Investigation of the release behavior of diethylhexyl phthalate from the polyvinyl-chloride tubing for intravenous administration. Int. J. Pharm. 210, 109–115.

Hanawa, T., Endoh, N., Kazuno, F., Suzuki, M., Kobayashi, D., Tanaka, M., Kawano, K., Morimoto, Y., Nakajima, S., Oguchi, T., 2003. Investigation of the release behavior of diethylhexyl phthalate from polyvinyl chloride tubing for intravenous administration based on HCO60. Int. J. Pharm. 267, 141–149.

Hayashi, Y., Matsuda, R., 1994. Deductive prediction of measurement precision from signal and noise in liquid chromatography. Anal. Chem. 66, 2874–2881.

Hayashi, Y., Matsuda, R., Poe, R.B., 1996. Probabilistic approach to confidence intervals of linear calibration. Analyst 121, 591–599.

Hayashi, Y., Matsuda, R., Haishima, Y., Yagami, T., Nakamura, A., 2002. Validation of HPLC and GC-MS systems for bisphenol-A leached from hemodialyzers on the basis of FUMI theory. J. Pharm. Biomed. Anal. 28, 421–429.

Hayward, D.S., Kenley, R.A., Jenke, D.R., 1990. Interactions between polymer containers and parenteral solutions: the correlation of equilibrium constants for polymer–water partitioning with octanol–water partition coefficients. Int. J. Pharm. 59, 245–253.

Health Canada, 2002. Expert Advisory Panel on DEHP in Medical Devices. Web site at http://www.hc-sc.gc.ca/hpb-dgps/therapeut/hemleng/whatsnew.html.

Inoue, K., Kawaguchi, M., Okada, F., Yoshimura, Y., Nakazawa, H., 2003a. Column-switching high-performance liquid chromatography electrospray mass spectrometry coupled with on-line of extraction for the determination of mono- and di-(2-ethylhexyl) phthalate in blood samples. Anal. Bioanal. Chem. 375, 527–533.

Inoue, K., Higuchi, T., Okada, F., Iguchi, H., Yoshimura, Y., Sato, A., Nakazawa, H., 2003b. The validation of column-switching LC/MS as a high-throughput approach for direct analysis of di(2-ethylhexyl)phthalate released from PVC medical devices in intravenous solution. J. Pharm. Biomed. Anal. 31, 1145–1152.

Jenke, D.R., 1991. Effect of solution phase composition on the interaction between aqueous model solutes and polymeric container materials. Pharm. Res. 8, 782–786.

Jenke, D.R., 2001. Evaluation of model solvent systems for assessing the accumulation of container or extractables in drug formulations. Int. J. Pharm. 224, 51–60.

Jenke, D.R., Kenley, R.A., Hayward, D.S., 1991. Interactions between polymeric containers and their contained solution: modeling of polymer–water solute partitioning via coupled solvent–water partition coefficients. J. Appl. Polym. Sci. 43, 1475–1482.

Jenke, D.R., Chess, E.K., Zietlow, D.C., Rabinow, B.E., 1992. Model for estimating the accumulation of solutes leaching from polymeric containers into parenteral solutions. Int. J. Pharm. 78, 115–122.

Kenley, R.A., Jenke, D.R., 1990. Determination of solute–polymer interaction properties and their application to parenteral product container compatibility evaluations. Pharm. Res. 7, 911–918.

Lamb, J.C., Chapin, R.E., Teague, J., Lawton, A.D., Reel, J.R., 1987. Reproductive effects of four phthalic acid esters in the mouse. Toxicol. Appl. Pharmacol. 88, 255–269.

Loff, S., Kabs, F., Witt, K., Sartoris, J., Mandl, B., Niessen, K.H., Waag, K.L., 2000. Polyvinylchloride infusion lines expose infants to large amounts of toxic plasticizers. J. Pediatr. Surg. 35, 1775–1781.

Nasim, K., Meyer, M.C., Autin, J., 1972. Permeation of aromatic organic compounds from aqueous solutions through polyethylene. J. Pharm. Sci. 61, 1775–1780.

Pitt, G.C., Bao, Y.T., Andrady, A.L., Samuel, P.N.K., 1988. The correlation of polymer–water and actanol–water partition coefficients: estimation of drug solubility in polymers. Int. J. Pharm. 45, 1–11.

Poon, R., Lecavalier, P., Mueller, R., Valli, V.E., Procter, B.G., Chu, I., 1997. Subchronic oral toxicity of di-*n*-octyl phthalate and di(2-ethylhexyl)phthalate in the rat. Food Chem. Toxicol. 35, 225–239.

Roberts, M.S., Kowaluk, E.A., Polack, A.E., 1991. Prediction of solute sorption by polyvinyl chloride plastic infusion bags. J. Pharm. Sci. 80, 449–455.

Takatori, S., Kitagawa, Y., Kitagawa, M., Nakazawa, H., Hori, S., 2004. Determination of di(2-ethylhexyl)phthalate in human serum using liquid chromatography-tandem mass spectrometry. J. Chromatogr. B 804, 397–401.

Tickner, J.A., Schettler, T., Guidotti, T., McCally, M., Rossi, M., 2001. Health risks posed by use of di-2-ethylhexyl phthalate (DEHP) in PVC medical devices: a critical review. Am. J. Ind. Med. 39, 100–111.

Tyl, R.W., Price, C.J., Marr, M.C., Kimmel, C.A., 1988. Developmental toxicity evaluation of dietary di(2-ethylhexyl)phthalate in Fischer 344 rats and CD-1 mice. Fundam. Appl. Toxicol. 10, 395–412.