Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 297 (2005) 30–37
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Release behavior of diethylhexyl phthalate from the polyvinyl-chloride tubing used for intravenous administration and the plasticized PVC membrane

Hanawa Takehisa [a,*], Endoh Naoko [a], Suzuki Masahiko [a], Terada Katsuhide [b], Ohkuma Moriyuki [c], Sakuta Keizoh [c], Tanaka Mutsuko [d], Kawano Kenji [d], Nakajima Shin'ichiro [a], Oguchi Toshio [a]

[a] *Department of Pharmacy, University Hospital, Faculty of Medicine, University of Yamanashi, 1110 Shimokato, Tamaho, Yamanashi 409-3898, Japan*
[b] *Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan*
[c] *Drug Formulation Department, Central Research Laboratories, Kaken Pharmaceutical Co. Ltd., 301 Gensuke, Fujieda, Shizuoka 426-8646, Japan*
[d] *Faculty of Pharmaceutical Sciences, Niigata College of Pharmacy, 5-13-2 Kamishinei, Niigata 950-2081, Japan*

Received 5 November 2004; received in revised form 28 January 2005; accepted 19 February 2005
Available online 20 April 2005

Abstract

The release behavior of diethylhexyl phthalate (DEHP) from polyvinyl-chloride (PVC) tubing, which composes materials in an intravenous administration set, was investigated using polysorbate 80 (Tween 80) aqueous solutions. When Tween 80 solution was circulated in PVC tubing, the amount of DEHP released increased with increasing circulation velocity and temperature. In order to clarify the effect of temperature on the release behavior of DEHP, PVC films containing varying amounts of DEHP were mounted on a cylindrical shaft and rotated at 5 and 40 °C. The cumulative amount of DEHP released increased with an increase in temperature, the diffusion coefficients [$D \times 10^{-10}$ cm^2 min^{-1}] at 5 and 40 °C were 9.1 and 156.0, respectively. The glass transition temperature (T_g) of PVC films decreased with an increase in DEHP in the PVC film, as measured by differential scanning calorimeter (DSC) and release of DEHP occurred at temperatures above T_g. These results indicate that the release of DEHP from PVC tubing is closely associated with the state of the PVC and is related to diffusion of DEHP throughout the PVC.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Diethylhexyl phthalate; PVC tubing; Tween 80; Release behavior; Glass transition temperature; Diffusion

1. Introduction

In clinical use, polyvinyl-chloride (PVC) tubing is the most widely used material for enteral tube

* Corresponding author. Tel.: +81 55 2739810; fax: +81 55 2736672.
E-mail address: t-hanawa@yamanashi.ac.jp (H. Takehisa).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.02.015

feeding or intravenous administration of medication. To provide pliability and strength, diethylhexyl phthalate (DEHP) is added to the PVC as a plasticizer. Previously, we reported that isosorbide dinitrate and/or cinnamic acid adsorb into the tubing of an intravenous administration set (IAS) (Nakajima et al., 1985; Nakazawa et al., 1988). Water-insoluble drugs, such as miconazole, cyclosporin, and tacrolims, also adsorb into IAS concurrent with the release of DEHP (Kawano et al., 1992, 1994; Suzuki et al., 2000). Many studies have reported the general toxicity of DEHP. Zacharewski et al. (1998) reported that DEHP has no uterotrophic activity despite its weak estrogenic activity. Animal studies have revealed that diets containing DEHP result in damage to the testicles and reproductive organs (Poon et al., 1997; Lamb et al., 1987). Although the effect of DEHP on the human body in not well understood, DEHP intake from intravenous treatment or enteral tubing should be avoided. The Ministry of Health, Labor, and Welfare of Japan (JMHLW) classified DEHP as a chemical agent suspected of causing endocrine disruption (1998) and restricted oral tolerable daily intake (TDI) to 0.14 mg/(kg day) (2000). In addition, results from AdvaMed and other important studies prompted the Center for Devices and Radiological Health (CDRH) and the U.S. Food and Drug Administration (FDA) to propose a parenteral tolerable intake (TI) of DEHP of 0.6 mg/(kg day) (CDRH, 2001; AdvaMed, 2001). In recent days, PVC tubing containing tris(2-ethylhexyl) trimillitate as the alternative plasticizer of DEHP has been developed. However, because of its high cost, it has not been widely disseminated yet.

Previous studies have investigated the release of DEHP from PVC tubing, promoted by surfactants such as polysorbate 80 (Tween 80) and polyethylene castor oil (HCO60), which are used as solubilizers for water-insoluble drugs (Muramatsu et al., 2000; Hanawa et al., 2000, 2003). The results demonstrated that the concentration of DEHP released increased with an increase in Tween 80 concentration. As aqueous solution containing Tween 80 or HCO60 was dripped through PVC tubing, the concentration of released DEHP decreased with an increase in drip rate. A comparison of the release behavior of DEHP in a solution containing surfactant alone to a solution used for commercial injections indicated that DEHP release was similar in both cases. Thus, the concentration of dissolved DEHP can be estimated by measuring the concentration of the surfactant and drip rate in practical use. In addition, Kawano et al. (1992) demonstrated that DEHP content in the PVC influenced DEHP release; DEHP release was not observed at levels below 20 wt.%. However, few reports exist concerning the relation between release behavior of DEHP and the environmental conditions such as temperature and DEHP content in the PVC.

To investigate the mechanism of DEHP release from PVC in detail, we firstly examined the effect of surrounding conditions such as temperature and circulating velocity on the release of DEHP from PVC tubing. Successively, we prepared PVC membranes containing various amounts of DEHP, and examined the influence of DEHP content and temperature on the release of DEHP.

2. Experimental

2.1. Materials

Polysorbate 80 (Tween 80) was purchased from Sigma (St. Louis, MO). Diethylhexyl phthalate (DEHP) and di-*n*-pentyl phthalate were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Distilled water for injection was JP grade. The polyvinyl-chloride (PVC) tubing used for the experiments was from a Terufusion® intravenous administration set (IAS, Terumo, TS-A256PK027, Tokyo, Japan).

2.2. Measurement of DEHP

DEHP concentration in the sample solution was determined by high-performance liquid chromatography (HPLC). HPLC conditions were: column, Shodex® C18M-4D (4.6 mm i.d. × 150 mm length, Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, 60:100:25 acetonitrile:methanol:distilled water; elution rate, 1.5 ml/min; internal standard, di-*n*-pentyl phthalate; detector, SPD-10AVP; column oven, CTO-10AVP; calculator, C-R8A (225 nm, Shimadzu Co., Kyoto, Japan). The retention times of the internal standard and DEHP under these chromatographic conditions were approximately 2.8 and 7.9 min, respectively. A calibration curve was constructed by plotting the ratio of peak area of DEHP to that of the internal standard against the concentration of DEHP. Data were fitted to

a least squares linear regression, which yielded the linearity of the standard curve as $r > 0.995$. Limits of detection (LOD) and limits of quantitation (LOQ) were 0.02 and 0.22 μg/ml, respectively.

2.3. Methodology evaluation of DEHP release from PVC tubing

A Tween 80 solution (2.0 mg/ml) was prepared by dissolving the Tween 80 in distilled water. The concentration of Tween 80 applied in this study, i.e. 2.0 mg/ml, is much higher that that of critical micelle concentration in distilled water observed in our previous study (Hanawa et al., 2000). However, in actual use, it may become the concentration near 2.0 mg/ml depending on medical supplies. One meter of PVC tubing (i.d. 0.21 cm, content of DEHP 2.2 g) from the IAS was attached to a peristaltic pump, and the Tween 80 solution was circulated through the PVC tubing at constant velocity (66, 375, or 1125 ml/h) and temperature (5, 20 or 40 °C). Sample solutions were collected at suitable intervals and the amount DEHP released was measured.

2.4. Preparation of PVC film

After 20 ml of tetrahydrofuran (THF) was added to 1.2–1.8 g of bulk PVC to dissolve the PVC completely, 0.2–0.8 ml DEHP was added to the PVC solution. This solution was transferred to a Petri dish and allowed to stand at room temperature over a period of time > 2 weeks (Kakiuchi et al., 2001). The solution was then reduced to dryness at 50 °C for 24 h to yield PVC films with a DEHP content of 10–90%.

2.5. Measurement of DEHP released from PVC film

PVC film [30 mm × 60 mm × 0.1 mm; $L \times H \times D$] was mounted on a cylindrical shaft and rotated at 300 rpm in a known volume of Tween 80 solution at constant temperature (5 or 40 °C). The concentration of DEHP in the Tween 80 solution was measured intermittently.

2.6. Thermal analysis

The thermal behavior of PVC membranes were measured by differential scanning calorimetry (DSC) using a Mettler DSC822 Stare System (Greifesee, Switzerland) at a heating rate of 5 °C/min in the range −60 to 115 °C under the stream of N_2 gas (10 mL/min). Approximately 100 mg of the PVC membranes were sealed in the sample pan for the liquid sample (40 μl). Glass transition temperatures (T_g) of PVC films were determined from the midpoint of a small endothermic rise of the pre- and post-transition baselines according to the method described by JIS K7121 (1987).

3. Results and discussion

3.1. Release behavior of DEHP from PVC tubing

Fig. 1 depicts the release profile of DEHP from PVC tubing at varying surrounding temperatures and circulation velocities of Tween 80 solution. In all cases, the cumulative amount of DEHP released increased linearly, suggesting that the release of DEHP from PVC tubing follows zero-order release kinetics. As

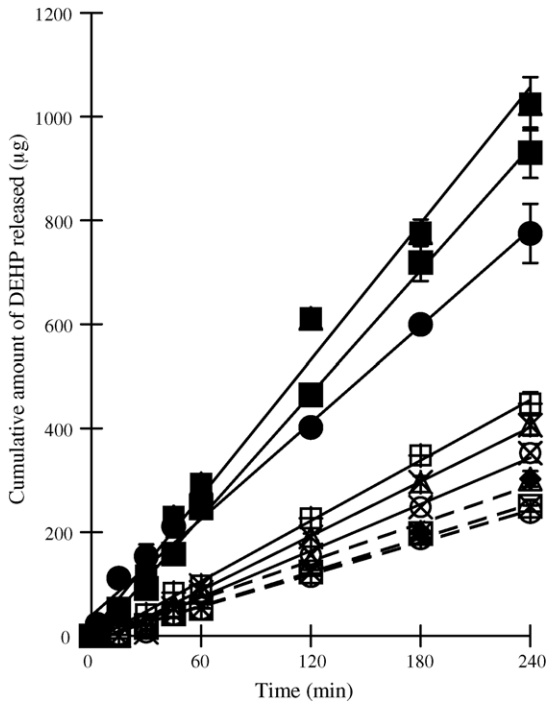

Fig. 1. Release profiles of DEHP from PVC tubing in Tween 80 solution at varied circulation velocities and temperatures. Circulated at: 66 ml/h and (+) 5 °C; (◯) 20 °C; (●) 40 °C, 375 ml/h and (×) 5 °C; (△) 20 °C; (▲) 40 °C, 1125 ml/h and (♦) 5 °C; (□) 20 °C; (■) 40 °C. Data are expressed as mean ± S.D. ($n = 3$).

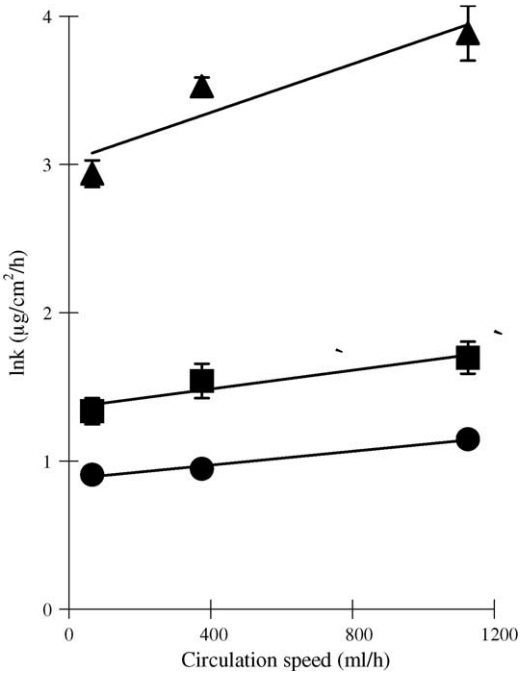

Fig. 2. Apparent release rate constant of DEHP released from PVC tubing at various temperatures. At temperatures of: (●) 5 °C; (■) 20 °C; (▲) 40 °C. Data are expressed as mean ± S.D. ($n = 3$).

can be seen in Fig. 2, the apparent release rate constants increased with an increase in circulation velocity and temperature. The circulation velocity seems to be a contributor to cause the release of DEHP. The increase in apparent release rate constant with circulation velocity appeared to result from changes in the thickness of a diffusion layer on the surface of the PVC tubing. That is, the thickness of this diffusion layer became thinner as circulation velocity increased. Amarantos et al. (1987) and Papadokostaki and Petropoulos (1998) investigated the kinetics of the release of 4-aminobenzene from cellulose acetate matrices and showed that the thickness of the stagnant boundary solution layers reduced with an increase in stirring rate. Also in this study, the increase of the apparent release rate constant appears to be related to the increase of diffusion coefficient with circulation velocity.

Though the possibility of the influence of the mechanical stress applied to the PVC tubing by using of peristaltic was regarded as another factor to the increase of diffusion coefficient with circulation velocity, the details were not able to be clarified in this study.

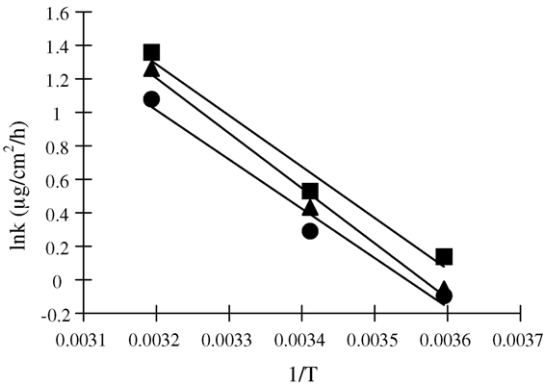

Fig. 3. Effect of temperature on apparent release rate constant of DEHP released from PVC tubing at various circulating velocities. Circulated at: (●) 66 ml/h; (▲) 375 ml/h; (■) 1125 ml/h.

In this study, the temperature showed a strong effect on the apparent release rate constant. To clarify the temperature dependency of DEHP release behavior, the relation between the apparent release rate constants and the reciprocal of absolute temperature were plotted (Fig. 3) and showed good linearity ($r^2 = 0.976$–0.989).

The cumulative amount of DEHP released at 40 °C after 240 min was 1025 μg (Fig. 1), which is negligible compared to that of the TI (0.6 mg/(kg day)) proposed by CDRH and FDA. Although a transfusion is not allowed to circulate through the PVC tubing in actual medical treatment, in case a PVC tubing is placed in vein to do the continuous administration for a long time, a large amount of DEHP may flow into the inside of the body.

3.2. Release behavior of DEHP from PVC film

Numerous studies have been reported on the release behavior of DEHP from PVC materials. However, an investigation about the relationship between the DEHP release behavior from PVC film into solutions and the thermal properties of PVC film has not been reported. In this study, the release of DEHP from PVC film containing various amounts of DEHP into a Tween 80 solution circulating at different rates was investigated. Fig. 4 shows the release behavior of DEHP from PVC film at 5 or 40 °C. The cumulative amount of DEHP released increased with an increase in the time and temperature in the same manner of the PVC tubing. The release and diffusion of DEHP was based on the

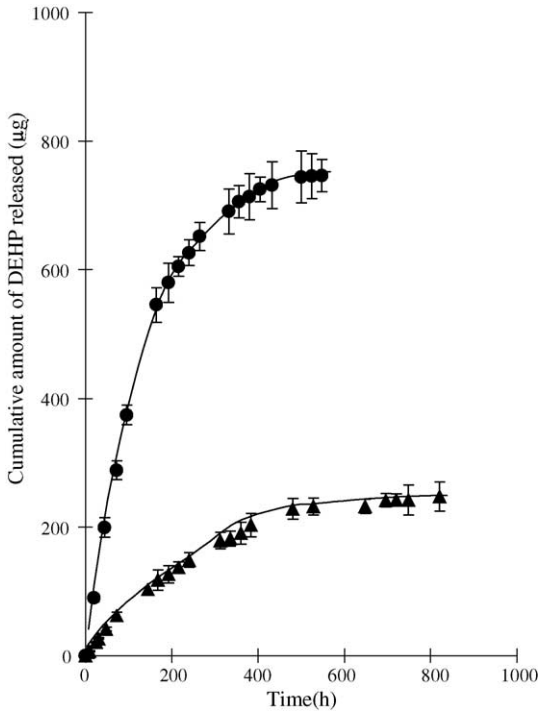

Fig. 4. Release behavior of DEHP from PVC film at different temperatures at temperatures of: (▲) 5 °C; (●) 40 °C. Data are expressed as mean ± S.D. ($n = 3$).

assumptions that (i) PVC films are perfectly planar and DEHP primarily diffuses through the surface of the film (i.e., release of DEHP through the edge of the film is negligible), and (ii) the volume of leaching solvent is very large compared to the amount of released DEHP. Using these assumptions, we applied Fick's law described by the well known relation:

$$\frac{M_t}{M_\infty} = 2\sqrt{\frac{Dt}{\pi l^2}} \quad (1)$$

where M_t represents the total amount of DEHP released at time t and M_∞ is the amount originally present; D is the diffusion coefficient; and l denotes the thickness of the film.

Fig. 5 illustrates the relation between M_t/M_∞ and $t^{1/2}$ at 5 and 40 °C. The cumulative amount of DEHP released increased with an increase in temperature. Furthermore, the functional relation of M_t/M_∞ and $t^{1/2}$ demonstrated good linearity at the initial portion ($r^2 = 0.997$ at 5 °C, 0.996 at 40 °C). This suggests that DEHP from PVC films into an "infinite" bath (i.e., 2 mg/ml of Tween 80 solution) can be described by Fick's law. The diffusion coefficients calculated from the modulus of the straight line are listed in Table 1. Temperature dependency is related to intermolecular forces induced by orientation and/or an induction effect between DEHP and PVC, despite a lack of covalent bonding between DEHP and PVC. Therefore, at high temperatures, these intermolecular forces are weakened by thermal vibration, allowing the release of DEHP from the PVC matrix.

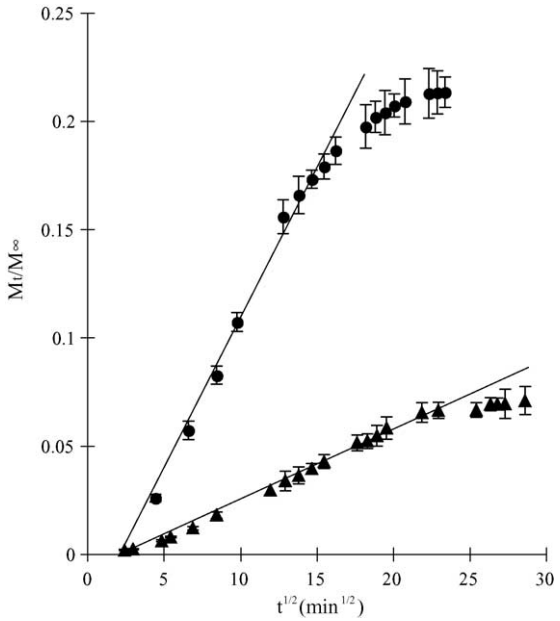

Fig. 5. Plots of M_t/M_∞ against $t^{1/2}$ for the surrounding temperature. At temperatures of: (▲) 5 °C; (●) 40 °C. Data are expressed as mean ± S.D. ($n = 3$).

3.3. Relation between release of DEHP and PVC film characteristics

The variation in diffusion coefficients at different temperatures appears to be related to a rearrangement

Table 1
Diffusion coefficients of DEHP through the PVC film at various temperatures

Temperature (°C)	Membrane thickness (μm)	$D \times 10^{-10}$ (cm^2 min^{-1})
5.0	10.0	9.1
40.0	10.0	156.0

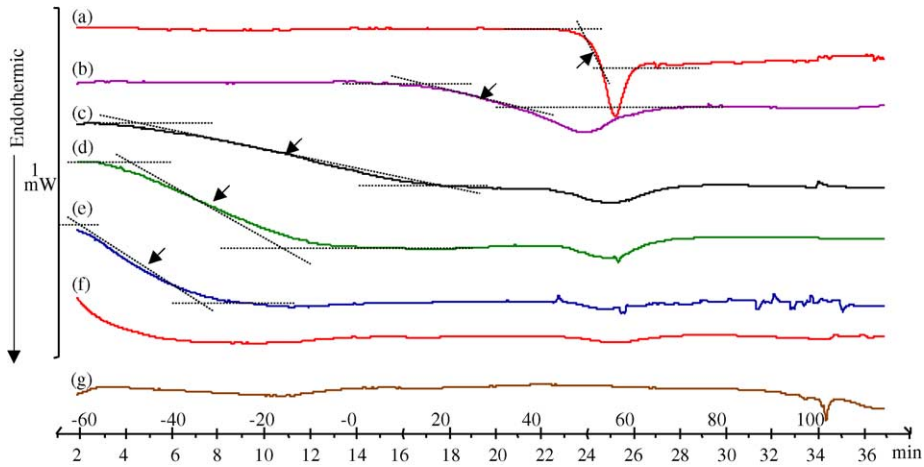

Fig. 6. DSC curves of PVC films containing various amounts of DEHP. DEHP amounts (wt.%): (a) 9.76; (b) 18.4; (c) 30.4; (d) 43.6; (e) 53.1; (f) 63.7; (g) 90.0%.

of the polymer structure. Glass transition temperature (T_g), as measured by DSC, is an important indicator of the microstructural property of a polymer (Kim et al., 2003; Tarvainen et al., 2002). Fig. 6 illustrates the DSC thermograms of PVC films containing various amounts of DEHP. PVC films containing 9.76 and 18.4% DEHP yielded thermograms exhibiting well-defined glass transitions. However, for films with a DEHP content greater than 63.7%, the thermal changes corresponding to glass transition were blurred, preventing determination of T_g. The T_g value decreased with an increase in DEHP concentration (Fig. 7(a)), suggesting that the polymeric structure of PVC requires more thermal energy and higher concentrations of DEHP to loosen its intermolecular bonds. To examine the release of DEHP from PVC film at temperatures just below and above the T_g, PVC films containing 15.2% DEHP (with a T_g of approximately 32.4 °C estimated by extrapolation using Fig. 7(a)) were investigated at various temperatures (Fig. 7(b)). The

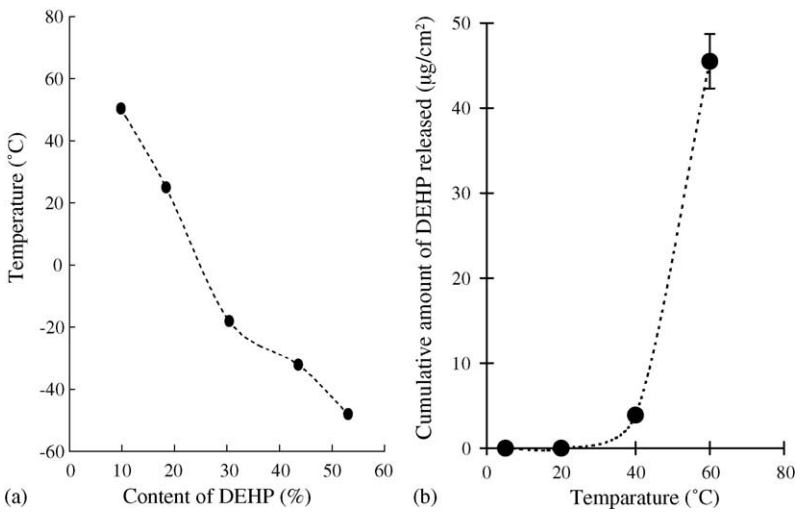

Fig. 7. Effect of DEHP amount contained in PVC on T_g value and release of DEHP. (a) Effect of the amount of DEHP contained in PVC films on their T_gs. (b) Release of DEHP from PVC film containing 15.2% DEHP. Data are expressed as mean ± S.D. ($n = 3$).

release of DEHP was observed at temperatures above 40 °C, and the amount of DEHP released significantly increased at 60 °C. Papaspyrides (1991) prepared PVC sheets containing various amounts of dioctylphthalate (DOP) and investigated the release behavior of DOP from PVC sheets. Results revealed that an increase in DOP content and elevation of immersion temperature facilitated DOP release. Papaspyrides (1991) demonstrated that diffusion of low molecular weight substances into and out of a polymer occurs mainly in the amorphous phase; the mechanism changes for the polymer in a glassy state below the glass transition region or in the rubbery state above the glass transition region. Papaspydides also reported that the increase in DEHP release from PVC film above the T_g was related to the change in the characteristics of the PVC film, i.e., the increase in DEHP content in a PVC film reduces T_g; at temperatures above the T_g, DEHP molecules in the rubbery state are apt to diffuse throughout the PVC film.

4. Conclusions

The amount of DEHP released from PVC tubing used for IAS increased with circulation velocity and temperature. The increase in apparent release rate constant with circulation velocity was related to the thickness of a diffusion layer on the surface of the PVC tubing, which became thinner as circulation speed increased. In this study, the temperature showed a strong effect on the apparent release rate constant. The diffusion coefficients increased with an increase in temperature. That is, the change in the state of PVC at elevated temperatures affects DEHP release, as indicated by the important role play by the T_g of the PVC film in the diffusion of DEHP throughput the film. These results indicate: (a) DEHP on the surface of the PVC film diffuses through the PVC film/Tween 80 solution interface into the liquid, (b) DEHP diffuses into the Tween 80 solution, and (c) the release of DEHP from the surface of the PVC film leads to diffusion of DEHP from within the PVC film to the film surface. The repetition of steps (a)–(c) results in the release of DEHP over a prolonged period of time. These results are similar to those obtained by Papaspyrides (1991) and with previous studies from this laboratory (Tanaka et al., 2002; Hanawa et al., 2003).

The result obtained in this research will be considered to be significant when the preservation temperature of the medical supplies for injection containing surfactant as solubilizer should be set.

Such the circulation of a transfusion carried out in this study is not allowed in actual medical treatment. However, in case a PVC tubing is placed in vein to do the continuous administration for a long time, a large amount of DEHP may flow into the inside of the body. There is no change in DEHP being a foreign substance for the human body, and inflow inside of the body should be avoided.

Although the PVC tubing containing tris(2-ethylhexyl) trimellitate as an alternative plasticizer to DEHP has been developed recently, its dissemination is not enough. Haishima et al. (2004) demonstrated that the release of DEHP was obviously suppressed by covalently coating the inner surface of the PVC tubing with heparin. In our future study, the solubilizers which do not cause the release of DEHP, and the coating substances which can suppress the release of DEHP from the surface of PVC tubing should be investigated simultaneously.

References

AdvaMed, 2001. 21-day repeat dose male reproductive tract study of di(2-ethylhexyl) phthalate (DEHP) administered either intravenously or orally to rats starting at neonatal age 3–5 days, with satellite recovery group through 90 days of age. Study number 11947.

Amarantos, G.S., Papadokostaki, K., Petropoulos, H.J., 1987. Finite bath and boundary layer effects on solute release kinetics from solid matrices. J. Membr. Sci. 34, 207–223.

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. Table 4-1, Rockville, MD, USA.

Haishima, Y., Matsuda, R., Hayashi, Y., Hasegawa, C., Yagami, T., Tsuchiya, T., 2004. Risk assessment of di(2-ethylhexyl)phthalate released from PBC blood circuits during hemodialysis and pump-oxygen therapy. Int. J. Pharmaceut. 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 polyvinylchloride tubing for intravenous administration. Int. J. Pharmaceut. 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 ad-

ministration based on HCO60. Int. J. Pharmaceut. 267, 141–149.

Japanese Standards Association, 1987. Japanese Industrial Standard, Testing Methods for Transition Temperatures of Plastics, JIS K7121, Tokyo, Japan.

Kakiuchi, M., Aoki, Y., Watanabe, H., Osaki, K., 2001. Viscoelastic properties of poly (vinylchloride) gels: effect of plasticizer type. Nihon Reoroji Gakkaishi 29, 53–59.

Kawano, K., Nakazawa, K., Terada, K., Nakajima, S., 1992. The leaching of diethylhexyl phthalate from the administration set into intravenous cyclosporine solutions. Jpn. J. Hsp. Pharm. 18, 454–457.

Kawano, K., Nakazawa, K., Takamatsu, S., Nakajima, S., 1994. Sorption of miconazole from the solution to the intravenous administration set. Yakuzaigaku 54, 275–279.

Kim, H.J., Kim, S.H., Lee, H.C., Nah, J.-W., Hahn, A., 2003. DEHP migration behavior from excessively plasticized PVC sheets. Bull. Korean Chem. Soc. 24, 345–349.

Lamb IV, 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.

Muramatsu, E., Hanawa, T., Suzuki, M., Tanaka, M., Kawano, K., Nakajima, S., 2000. Investigation of the effect of the coexistence of surfactant on the release behavior of diethylhexyl phthalate from the polyvinyl chloride tubing. Jpn. J. Hosp. Pharm. 25, 471–477.

Nakajima, S., Kawano, K., Nakazawa, K., 1985. Loss of isosorbide dinitrate from solutions in administration set. Yakuzaigaku 45, 285–290.

Nakazawa, K., Kawano, K., Ito, M., Amemiya, H., Nakajima, S., 1988. The effect of pH in the solutions on the absorption of cinnamic acid into the administration set. Yakugaku Zasshi 108, 1110–1113.

Papadokostaki, G.K., Petropoulos, H.J., 1998. Kinetics of release of a model disperse dye from supersaturated cellulose acetate matrices. J. Controlled Rel. 54, 251–264.

Papaspyrides, D.C., 1991. Prior sample history vs. plasticizer migration phenomena in flexible poly(vinyl chloride) sheets. J. Vinyl Tech. 13, 74–79.

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.

Suzuki, M., Takamatsu, S., Muramatsu, E., Nakajima, S., Tanaka, M., Kawano, K., 2000. Loss of tacrolimus solution content and leaching of di-2-ethylhexyl phthalate in practice injection of precision continuous drip infusion. Jpn. J. Hosp. Pharm. 26, 7–12.

Tanaka, M., Kawano, K., Hanawa, T., Suzuki, M., Nakajima, S., 2002. Leaching mechanisms of di-(2-ethylhexyl)phthalate from polyvinyl-chloride tube during the administration of enteral nutrition. J. Pharm. Sci. Technol. Jpn. 62, 146–152.

Tarvainen, M., Sutinen, R., Peltone, S., Tiihonen, P., Paronen, P., 2002. Starch acetate – a novel film-forming polymer for pharmaceutical coatings. J. Pharm. Sci. 91, 282–289.

Zacharewski, T.R., Meek, M.D., Clemons, J.H., Wu, Z.F., Fielden, M.R., Matthews, J.B., 1998. Examination of the in vitro and in vivo estrogenic activities of eight commercial phthalate esters. Toxicol. Sci. 46, 282–293.