International Journal of Pharmaceutics 430 (2012) 86–88

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Di-(2-ethylhexyl)-phthalate migration from irradiated poly(vinyl chloride) blood bags for graft-vs-host disease prevention

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ARTICLE INFO

Article history:
Received 23 December 2011
Accepted 23 March 2012
Available online 3 April 2012

Keywords:
Di-(2-ethylhexyl)-phthalate
Differential scanning calorimetry
Graft-vs-host disease
Poly(vinyl chloride)
Glass transition temperature

ABSTRACT

Irradiation with 20–25 kGy is a process commonly used for sterilizing poly(vinyl chloride) (PVC) medical devices. Moreover, whole blood and blood components undergo additional irradiation with 25–50 Gy to inhibit the proliferative capacity of lymphocytes and reduce the risk of transfusion-associated graft-vs-host disease (GVHD). Di-(2-ethylhexyl)-phthalate (DEHP) plasticized PVC is extensively used for the production of flexible medical devices including blood bags, but since DEHP is not covalently bound to PVC, it tends to migrate and leach out of the medical device, with harmful consequences for the patients.

In this study, the effects of different doses of gamma irradiation on DEHP migration from PVC blood bags was investigated using differential scanning calorimetry (DSC) analysis.

Our findings indicate that irradiation with 25–100 Gy reduces the ability of DEHP to migrate from the blood bags, and in the case of a primary container a correlation between the doses of gamma ray irradiation was also observed. In particular, a decrease in DEHP leachability was obtained by increasing the dose of gamma ray irradiation.

1. Introduction

Irradiation with gamma-rays is considered to be a safe and effective method for sterilizing commercially available polyvinyl chloride (PVC) medical devices (United States Pharmacopeia Convention, 1995; International Organization for Standardization, 1995). Moreover, blood and blood components are subjected to an additional, effective irradiation process to inhibit the proliferative capacity of lymphocytes and reduce the risk of transfusion-associated graft-vs-host disease (GVHD) (Butson et al., 2000; Fearon et al., 2005; Góes et al., 2004).

In this regard, in the (industrial) PVC sterilization process, recommended gamma irradiation dose levels should range from 20 to 25 kGy (United States Pharmacopeia Convention, 1995; International Organization for Standardization, 1995) and in the case of blood irradiation from 25 to 50 Gy (Luban and DePalma, 1996; Pelszynski et al., 1994).

Phthalates are a group of chemicals, widely used primarily as plasticizers to impart flexibility otherwise to rigid PVC in various industrial and consumer products, including personal care products, medical devices and pharmaceuticals. However, they are not covalently bound to the polymer and leak out with time and use into the environment, thus becoming ubiquitous environmental contaminants. DEHP is the most widely used plasticizer in PVC medical devices, such as blood bags (Guo and Kannan, 2011; Koch et al., 2011; Latini et al., 2010; Latini, 2005; Romero-Franco et al., 2011; Rudel et al., 2011; Wittassek et al., 2011).

Phthalates have been identified as active endocrine disruptors and as developmental and reproductive toxicants in laboratory animals.

In humans, phthalate exposure has been associated with various adverse health outcomes. Thus, the potential consequences of human exposure to phthalates have raised concern in the general population (Frederiksen et al., 2011; Jurewicz and Hanke, 2011; Koch and Calafat, 2009).

In particular, neonates in the neonatal intensive care unit (NICU) environment are a population at particularly increased risk due to their small body size, physical condition and multiple

Abbreviations: DEHP, di-(2-ethylhexyl)-phthalate; DSC, differential scanning calorimetry; GVHD, graft-vs-host disease; NICU, neonatal intensive care unit; PVC, poly(vinyl chloride); Tg, glass transition temperature.

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http://dx.doi.org/10.1016/j.ijpharm.2012.03.039

Fig. 1. Picture of the blood bag studied.

medical-device-related DEHP exposure, each increasing exposure levels (Center for Devices and Radiological Health, 2001; Latini et al., 2010).

Recently, it has been shown that irradiation of PVC sheets with 0–50 kGy gamma-rays produced a decreased DEHP migration in proportion to the dose of gamma-ray irradiation, and increased (mono(2-ethylhexyl)phthalate (MEHP) and phthalic acid (PA)) migrations (Ito et al., 2006, 2008, 2009).

The aim of this study was to evaluate by differential scanning calorimetry (DSC) analyses DEHP migration from PVC blood bags, after exposure to an additional irradiation at doses ranging from 25 to 100 Gy commonly used to prevent GVHD.

2. Materials and methods

2.1. Chemicals and materials

The test material was PVC blood bags composed of a primary container (sac) and a tube (Fig. 1), which after the common industrial irradiation process for sterilizing were subjected to additional γ-ray irradiation (Linac 6MV, doses of 25, 50, 100 Gy). Samples that did not undergo gamma ray irradiation were used as control.

2.2. Differential scanning calorimetry (DSC)

The DSC analyses were performed using a Mettler DSC-822 (Mettler Toledo, Milan, Italy) under 80 mL/min nitrogen flow. The amount of samples employed was approximately 10 mg. All samples were subjected to a double cooling–heating cycle at 10 °C/min between −90 °C and 130 °C. The glass transition temperatures (Tg) were taken at the inflection point of sample devitrification.

The analyses were carried out on both the sacs and the tubes.

All measurements were carried out in triplicate and average data were used for statistical analysis.

2.3. Migration test

The migration of DEHP from PVC blood bag was examined in cyclohexane; 1 g of finely cut pieces from the PVC sacs or tubes was kept in test tubes (15 mL) and extraction was carried out with 10 mL of cyclohexane by shaking at room temperature for 24 h.

2.4. Data analysis

Results of DSC were tested for statistical significance by calculating standard deviation at 95% confidence intervals using IgorPro software (Wavemetrics, Lake Oswego, OR, USA).

Table 1
Tg values of irradiated and non-irradiated blood bags.

Sample	Tg before extraction (°C)	Tg after extraction (°C)
Irradiated tube 100 Gy	−32.0 ± 1.3	16.4 ± 0.8
Irradiated tube 50 Gy	−32.5 ± 1.5	16.4 ± 1.2
Irradiated tube 25 Gy	−30.2 ± 0.8	17.4 ± 1.1
Non-irradiated tube	−32.1 ± 1.1	19.6 ± 0.9
Irradiated sac 100 Gy	−24.8 ± 1.5	15.1 ± 0.8
Irradiated sac 50 Gy	−25.1 ± 0.9	18.9 ± 1.1
Irradiated sac 25 Gy	−23.7 ± 1.1	20.6 ± 1.5
Non-irradiated sac	−24.0 ± 1.4	23.5 ± 1.3

3. Results and discussion

DSC analyses were employed to determine the glass transition temperature (Tg) of the investigated samples. Tg is a temperature characteristic for each virgin polymer and is essential for our study since it is known to decrease with the addition of plasticizer to a virgin polymer. In order to determine the effect of gamma-ray irradiation on DEHP leaching from blood bags, a comparison between the glass transition temperature of the samples before and after extraction with cyclohexane was performed. An increase in the Tg values after extraction was expected as a result of the release of plasticizer from the devices.

There are several accepted ways to assign Tg to the transition obtained by DSC (Kerc and Stcic, 1995). In this study, Tg was taken at the inflection point of sample devitrification.

Given the importance of the plasticizing effect of cyclohexane on the samples, all samples used in the study were subjected to a double cooling–heating cycle at 10 °C/min between −90 °C and 130 °C in order to remove any residual solvents. The Tg from these two scans was reproducible, thus indicating the absence of residual cyclohexane in the samples.

The Tg values of sacs and tubes with and without irradiation are reported in Table 1.

Taking into account the Tg values of the samples before extraction, no significant differences were observed between the Tg values of the irradiated and non-irradiated samples. These results highlight that no significant release of plasticizers occured due to the irradiation process (Figs. 2 and 3).

DSC analyses performed on blood bags after extraction showed that gamma ray irradiation influences the leachability of DEHP. In particular, data relative to the sac showed that higher doses of gamma ray irradiation correlate with lower Tg values, and as a consequence, with a minor release of DEHP, as shown in Fig. 2.

In the case of the tube, a minor influence of gamma ray irradiation on DEPH release from the samples was observed (Fig. 3). Tubes not subjected to irradiation showed a greater tendency to release DEHP, as is highlighted by the highest Tg value (Table 1). However, the dose of gamma rays does not seem to affect DEHP leaching from

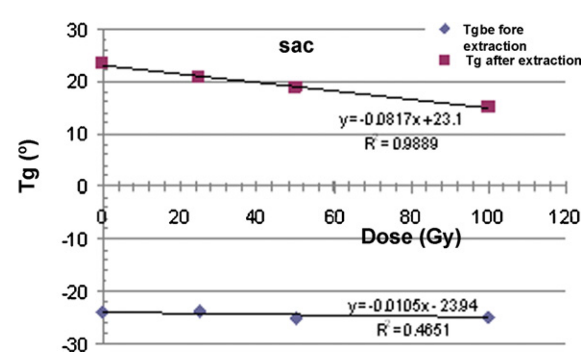

Fig. 2. Fitting of data from Table 1 relative to the sac.

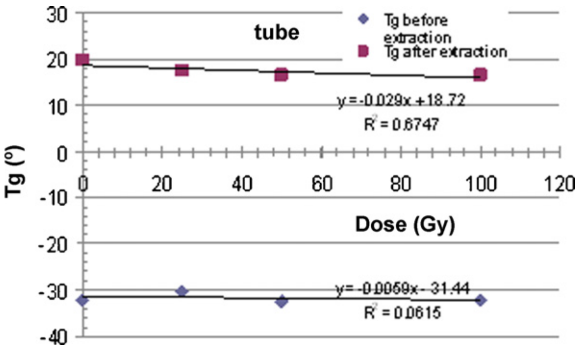

Fig. 3. Fitting of data from Table 1 relative to the tube.

PVC tubing. In fact, no significant difference between the Tg values of irradiated samples with different doses was observed (Table 1).

4. Conclusion

The risk assessment of DEHP leaching from PVC medical devices is an important issue for patients. In particular, newborns in the neonatal intensive care unit (NICU) environment are a population at markedly increased risk. As a consequence, these plasticizers should be promptly replaced with alternative and better-quality materials (Latini et al., 2010).

Our findings indicate that additional irradiation with gamma rays used to prevent GVHD further reduces the ability of DEHP to migrate from the investigated blood bags.

Moreover, in the case of a primary container a correlation between the dose of gamma ray irradiation and the release of DEHP was observed. In particular, a decrease in DEHP leachability was obtained by increasing the dose of gamma ray irradiation. These findings would appear to confirm those previously reported (Ito et al., 2006, 2008, 2009), although the radiation doses used in the study were much higher (by about a 10^3 factor) than those tested in our study and recommended to prevent GVHD.

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