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EFFECT OF STERILIZATION ON MECHANICAL AND BLOOD PROPERTIES OF MEDICAL GRADE POLYVINYL CHLORIDE

MARYAM POOSTCHI AND HAMED BAGHERI*

Faculty of Interdisciplinary Sciences and Technologies,
Tarbiat Modares university,Tehran,Iran

**Corresponding author: hbagheri@modares.ac.ir*

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ABSTRACT: The use of phthalates as a plasticizer in plasticized polyvinyl chloride (PVC) always poses the threat of migration of phthalates into the environment through medical equipment. Phthalates can be used with natural-based plasticizers, such as Epoxidized soybean oil (ESBO) known as phthalate's scavenger and PVC stabilizers. PVC formulations were characterized by different combinations of di (2-ethylhexyl) phthalate (DEHP) 30-40% with 5% ESBO. PVC flexibility increased significantly in the presence of ESBO, without a change in strength (tensile test). The decrease of the Tg temperature by adding ESBO in Differential Scanning Calorimetry indicated that ESBO preserved DEHP in the polymer. Also, it was shown that the sterilization process with Ethylene Oxide, similar to ESBO, decreased the Tg of polymer. DEHP migration was evaluated at a maximum level to the environment using the Gas Chromatography test. Samples containing ESBO showed less hemolysis.

ABSTRAK: Penggunaan phthalates sebagai plasticizer dalam plastik polyvinyl chloride (PVC) selalu menimbulkan ancaman penghijrahan phthalates ke alam sekitar melalui peralatan perubatan. Phthalates boleh digunakan dengan plasticizer berasaskan semula jadi, seperti minyak kacang soya Epoxidized (ESBO) yang dikenali sebagai pemulung phthalate dan penstabil PVC. Formulasi PVC dicirikan oleh kombinasi yang berbeza di (2-ethylhexyl) phthalate (DEHP) 30-40% dengan 5% ESBO. Fleksibiliti PVC meningkat dengan ketara di hadapan ESBO, tanpa perubahan kekuatan (ujian tegangan). Penurunan suhu Tg dengan menambahkan ESBO dalam Calorimetri Pengimbasan Berbeza menunjukkan bahawa ESBO mengekalkan DEHP dalam polimer. Juga, ditunjukkan bahawa proses pensterilan dengan Etilena Oksida, serupa dengan ESBO, menurunkan Tg polimer. Penghijrahan DEHP dinilai pada tahap maksimum ke lingkungan menggunakan uji Kromatografi Gas. Sampel yang mengandungi ESBO menunjukkan kurang hemolisis.

KEYWORDS: *Di (2-ethylhexyl) phthalate, polyvinyl chloride, plasticizer, Blood bag, Epoxidized soybean oil*

1. INTRODUCTION

In the years before 1970, glass bottles were used as containers for human blood preservation and were reused after cleaning and sterilization [1]. Now containers are special plastic bags with appropriate properties that meet today's needs regarding the maximum stability of the product and cell survival [2]. Today, all blood containers are sterilized and disposable. Blood bags used for blood preservation are made of polyvinyl chloride (PVC) [1–3].

PVC has a wider range of use in the construction and auto industries, as well as in the medical industry due to its characteristics, i.e. inert properties, transparency, easy sterilization,

compatibility with chemical compositions, and low cost [3–6]. Semi-hard and soft PVC comprises numerous parts of plastic materials used in the medical industry. Many sterilized disposable containers and tools, such as catheters, preservation bags for injecting fluids, medical tubes, preservation bags for blood and plasma, and dialysis equipment, are made of PVC. Materials used in these tools, especially blood preservation bags and medical tubes, cannot be replaced with other polymers [7–10].

PVC is considered a hard polymer with high Tg that is required to be plasticized for medical equipment, such as medical tubes and bags [11]. Various plasticizers, such as phthalates, are utilized in PVC. Phthalates are compounds formed as a result of phthalic acid esterification, which adds about 30-40% weight to PVC and plays a major role in its flexibility [9, 12]. Di-(2-ethylhexyl)-phthalate (DEHP) is the most widely used plasticizer. Most concerns are about the migration of DEHP to the environment due to the non-covalent bond between DEHP (phthalates) molecules and vinyl chlorides [5, 6, 10, 13–16].

Epoxidized soybean oil (ESBO) is an organic compound produced by soybean oil epoxidation [17]. The compound is used as a lubricant and stabilizer in PVC [5, 18–20]. Food products preserved in glass containers are sealed with PVC films or linings. ESBO is an additive used during PVC manufacture. ESBO can resist the release of HCl molecules due to its bonding with chlorine atoms in PVC [6, 15, 20, 21].

The United States Food and Drug Administration announced that the DEHP study on lab animals did not prove the safe use of DEHP for human beings. Thus, DEHP is used only for medical instruments with no continual contact with fluids in a patient's body. Furthermore, it is strongly advised not to utilize PVC–DEHP containing medical instruments for vulnerable patients, such as infants and pregnant women. Medical instrument manufacturing companies are also advised to remove DEHP from their products [22].

Sterilization is a major process in manufacturing medical tools. Most soft PVC products should be sterilized before use. Regarding the significance of the sterilization process in producing medical PVC tools, this study investigated the sterilization process effect on the migration of the plasticizer in the PVC bulk. The migration of the plasticizer probably increases due to special conditions in the sterilization process related to pressure and temperature.

The present study mainly discussed how to modify PVC blood preservation bags and find suitable polymer compositions with higher safety, flexibility, and blood compatibility, as well as lower migration rate. The study also evaluated the effect of various factors, including sterilization with ethylene oxide gas and addition of secondary plasticizers, such as ESBO, on the migration rate of plasticizers into the blood. We investigated certain properties of blood preservation bags and their stability and flexibility, including the release rate of plasticizers into the blood and blood compatibility (as clot formation for each PVC film with different percentages of plasticizers).

2. MATERIALS AND METHODS

2.1 Materials:

The materials were used PVC S6058 with the K-value 60 manufactured by Iranian Petrochemical Industries Co., di-2 (ethylhexyl) phthalate manufactured by Azar Shimi Co. (Iran), 2,6-di-tert-butyl-4-methylphenol with Vulkanox BHT manufactured by LanXess (Germany), ESBO manufactured by MBT (South Africa), Ca-Zn stearate manufactured by Poorya Exir Co. (Iran), chloroform with CAS No. 3-66-67 and the molecular mass of 119.38g/mol manufactured by Merck (Germany), Ethanol of 98% purity manufactured by

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Jahan Khorram Co. (Iran), distilled water and physiologic serum manufactured by Sepidaj Pharmaceutical Co. (Iran), and sheep blood obtained from Darvash Co. (Iran).

2.2 Sample preparation:

The plasticizer percentage of each sample (30-40%) was determined based on previous studies [16, 23, 24]. The three samples contained 30%, 35%, and 40% DEHP. The test was also repeated with ESBO, which was added as much as 5% to the samples four, five, and six as a secondary plasticizer (at the same DEHP content). The number of samples along with their plasticizer contents is listed in Table 2.1. The samples zero and seven were used as evidence samples; none of the two samples contained DEHP, although the sample seven contained 5% ESBO.

Table 2.1: Percentage of each plasticizer was used in samples.

Sample No.	Primary plasticizer (DEHP) %	Secondary plasticizer (ESBO) %
0	0	0
1	30	0
2	35	0
3	40	0
4	30	5
5	35	5
6	40	5
7	0	5

After determining the plasticizer percentage in each sample, its component was measured in 100 units (phr), as shown in Table 2.2. The weight of each sample was kept at 60g to fit the capacity of an internal mixer device. The fraction of each component, including PVC, a stabilizer, an antioxidant, a plasticizer, and ESBO, was calculated based on 100 units(phr), as shown in Table 2.2.

Table2.2: Components of a sample weighing 60g

Sample No.	Composition	Sample No.	Composition
1	PVC+30%DEHP (Non-sterilized)	7	PVC+30%DEHP (sterilized)
2	PVC+35%DEHP (Non-sterilized)	8	PVC+35%DEHP (sterilized)
3	PVC+40%DEHP (Non-sterilized)	9	PVC+40%DEHP (sterilized)
4	PVC+30%DEHP+5%ESBO (Non-sterilized)	10	PVC+30%DEHP+5%ESBO (sterilized)
5	PVC+35%DEHP+5%ESBO (Non-sterilized)	11	PVC+35%DEHP+5%ESBO (sterilized)
6	PVC+40%DEHP+5%ESBO (Non-sterilized)	12	PVC+40%DEHP+5%ESBO (sterilized)

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Each sample was mixed with a W50 Brabender mixer (Germany) to obtain a homogeneous mixture. The mixing device was set at 160°C and 80 rpm, and the samples were mixed for two min. The sample without the plasticizer was selected as a control sample of zero migration. However, the sample was removed from the study because of its poor properties.

Sample films with 5mm thickness were prepared. Hot pressing was conducted with a device manufactured by Sanjesh Baspar Novin Co. (Iran), while cold pressing was performed with a device manufactured by Toyo Seiki (Japan).

Each sample was fixed in two dumbbell-shaped casts where their dimensions were worked out according to ISO 6621 standards—length 15cm, width 2cm, and thickness 3mm. The samples then underwent hot pressing at 165°C for four min under 60Nm torque, followed by cold pressing (room temperature) for three min under 60Nm torque.

Half of the dumbbell samples were sterilized to compare the characteristics before and after sterilization. Each sample was put into a sterilized tank (Jiangyin Huaqing Machinery, China) of 90% ethylene oxide gas at 50°C and 50KP for eight h.

Table 2.3 shows the composition of each prepared sample, including figures attributed to it, which were utilized later.

Table 2.3: Sample nomenclature and abbreviations

	PVC		DEHP		ESBO		Ca-Zn St		BHT	
Sample No.	gr	phr	gr	phr	Gr	phr	Gr	Phr	gr	phr
0	58.53	100	0	0	0	0	01.17	2	0.29	0.5
1	40.96	100	18.02	44	0	0	0.82	2	0.25	0.5
2	38.10	100	20.95	55	0	0	0.76	2	0.19	0.5
3	34.99	100	24.14	69	0	0	0.70	2	0.17	0.5
4	37.85	100	18.17	48	03.03	8	0.76	2	0.19	0.5
5	34.78	100	21.22	61	03.13	9	0.70	2	0.17	0.5
6	31.83	100	24.19	76	03.18	10	0.64	2	0.16	0.5
7	55.81	100	0	0	05.00	5	02.00	2	0.50	0.5

2.3 Mechanical testing:

Both sterilized and non-sterilized samples underwent examination separately, based on ISO-527 standards, using TCS2000 Universal Testing (Gotech Testing Machines, Taiwan). The study samples had an average thickness of 2.4mm and width of 10mm.

2.4 Differential scanning calorimetry (DSC):

Differential Scanning Calorimetry (DSC) measurements were carried out on a DSC-1 unit manufactured by Mettler Toledo, Switzerland. About 4-5mg of the polymer sample was sealed in an aluminum pan. The polymer sample was first heated to 130 °C at a rate of 10°C/min under nitrogen atmosphere for three min to erase the thermal history. Then, it was cooled to -70°C at

a rate of 10°C/min for five min, and subsequently, heated to 130°C at a rate of 10°C/min. Both the cooling and heating traces were recorded.

2.5 Gas chromatography analysis (GC):

2.5.1 Sample extraction

All glassware was washed in 70% alcohol solutions and then put into the laboratory oven at 70°C for 15 min. The samples were cut in certain pieces using sterilized scissors and then put into a watch glass. The samples were weighed individually, and sterilized forceps were used to place the samples in an Erlenmeyer flask immediately after it was filled with as much as 50cc chloroform solution. The samples were tapped, and the flask was placed in the oven at 25°C for 24 h. Then, the flask was removed from the oven, and the pieces were taken out of the chloroform solution and put back in a watch glass. The pieces were again put into the oven at 70°C for 30 min to be dried. The solutions were preserved in other tapped dishes to be analyzed by gas chromatography.

2.5.2 Preparation of standard solution

Dodecane, with the chemical formula C12H26, was utilized to prepare standard solutions. The solutions were prepared at 100, 200, and 500ppm concentrations. The 500ppm solution was more like the samples than the other two solutions.

Chromatography was performed on the CP3800 gas chromatograph manufactured by Varian, USA. The injector temperature was set at 280°C with split-less mode, and the detector temperature was 300°C. The column was CP-Sil 5 CB made from silica with an inner diameter of 0.25mm and a length of 25m. Nitrogen-bearing gas at 40psi pressure and 1µl injection volume was applied to a flame Ionization detector. The test was conducted with the following temperature condition passing through the liquid column:

The initial temperature of 90°C was held for three min and increased at a heating rate of 15°C/min up to 280°C, which was held for five min. The total heating time was 20.67 min.

2.6 Hemolysis test:

The samples were cut into 1cm × 1cm films with sterilized scissors and a knife under sterilized conditions. The cut films were added to 100 million red globules lacking serum. Then, they were delivered to a container with 1 mL biological buffer using physiologic buffer and put in a SHIN SAENG Finetech rotary oven, (South Korea) at 37°C for one h at 100rpm. After an hour, the samples were centrifuged at 10,000rpm for five min in a Sigma3-30K centrifuge (ATR, USA). The supernatant was tested using the WPA Biowave II spectrophotometer (Biochrom, UK) at a 540nm wavelength to determine the released hemoglobin.

3. RESULTS AND DISCUSSION

3.1 Tensile test

Increasing the plasticizer to PVC led to increased flexibility (Figs. 1a, b) by decreasing the tensile stress and increasing the maximum stain at break. Considerable flexibility was observed in the samples 5 and 6 to which ESBO was added, confirming the ESBO plasticizer effect on PVC.

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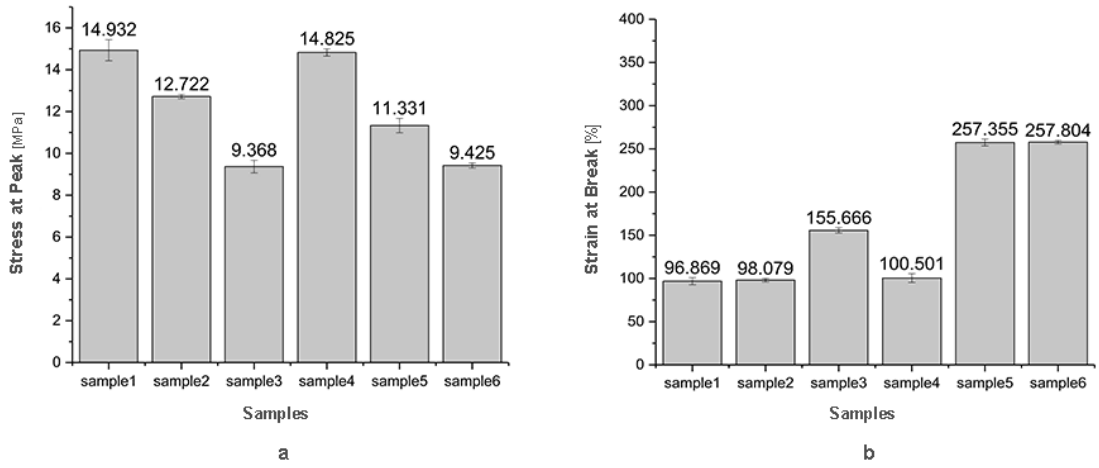

Figure 3.1 mechanical properties of stretch PVC films evaluated: Yield stress (a) and strain at breakpoint (b) before sterilization process.

The stress-strain diagram demonstrated that ultimate stress decreased with increasing the plasticizer in the samples 1 to 6. Furthermore, by adding ESBO to the samples, ultimate stress decreased continually with increasing the plasticizer.

Both samples 2 and 4 with the same plasticizer content had different strains at break so that the maximum strain was 2% more in the sample 4 compared to the sample 2. The strain rate difference was insignificant in these samples due to the low plasticizer content. However, increasing the plasticizer content up to 40% led to a meaningful difference in the samples 3 and 5. In other words, replacing 5% DEHP with 5% ESBO increased the polymer strain from 156% to 257%. Accordingly, ESBO had higher plasticizing effect than DEHP and its plasticizing characteristics in PVC. When a plasticizer (such as DEHP) is added, its molecules will be placed in various sites between polymer chains. This helps PVC become more flexible and leads to a decrease in Tg.

The effect of plasticizers on Tg is attributed to the increase in the free volume that enhances molecular mobility. Although plasticizers reduce chain entanglement density, the length scale of the chain contributing to Tg (~50 C-C bond) is smaller than the entanglement Mw (~200 C-C). It means that the chain entanglement density effect should not be considerable in typical plasticizer contents (~10-20%); unless one uses much higher plasticizer contents or co-plasticizers such as ESBO.

All the samples represented similar behavior both before and after sterilization. The mechanical behavior of the samples after sterilization is shown in Figure 3.2.

The sterilization process did not have considerable effects on maximum strain at the break of the samples. The sample strains considerably increased after sterilization, indicating that sterilization tended to increase its flexibility. Moreover, samples with 5% ESBO better preserved their mechanical properties.

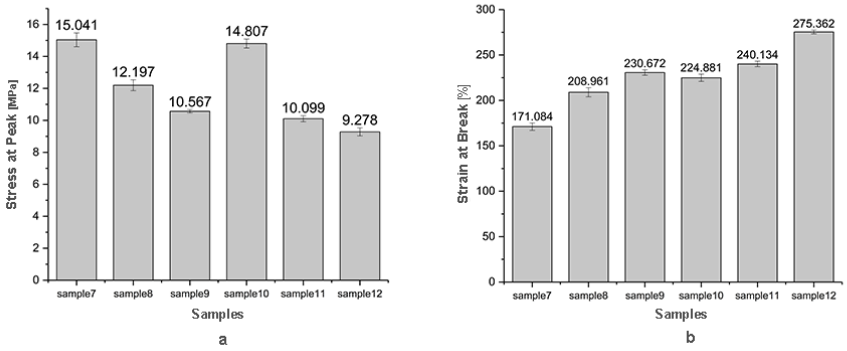

Figure 3.2 mechanical properties of stretch PVC films evaluated; Yield stress (a) and strain at breakpoint (b) after sterilization process.

The results of mechanical testing after sterilization confirmed that the strain increased at the break of the polymer with the ESBO plasticizer. The other plasticizers did not show such a maximum strain level at break. However, DEHP showed maximum strain at break after sterilization.

These results can be attributed to the fact that DEHP molecules diffuse to polymer chains because of more heating during the sterilization process and lead to increasing the distance of polymer chains and thus increasing maximum strain at break. Increasing DEHP provides more void spaces between chains, leading to improving chain sliding against each other and increasing polymer flexibility.

When ESBO was added to the polymer, it caused higher increased molecular distances between PVC chains by the combined effect of ESBO and DEHP molecules. DEHP was emplaced between PVC chains and, finally, increased polymer flexibility. Actually ESBO has a synergistic effect when used with DEHP and increases the polymer flexibility.

Sarath Josh et al. (2012) studied the temperature effect on the DEHP migration and release in blood preservation bags and confirmed the increased DEHP penetration index with increasing the temperature [25, 26]. In other words, DEHP molecules were stabilized between PVC chains with increasing the temperature. During the sterilization process, the temperature increased to 60°C when ethylene oxide gas was penetrated to the polymer bulk. Therefore, during the sterilization process, DEHP molecules were better mixed with PVC molecules with increasing the temperature up to 60°C. The polymer was annealed, and its mechanical properties were improved.

3.2 DEHP release from PVC

3.2.1 DSC analysis

Table 3.3 and Figure 3.1 show the second heating DSC curves of the four samples. As shown in Table 3.3, the Tg temperature decreased with increasing ESBO (Table 3.1). Accordingly, the Tg temperature decreased 4°C in the sample 5 compared to the sample 2 and more than 13°C in the sample 11 compared to the sample 8.

Decreasing the Tg temperature with increasing ESBO indicates that ESBO, used as a stabilizer for DEHP, can be simultaneously used as a secondary plasticizer. Furthermore, the

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existence of ESBO in the PVC polymer bulk not only stabilizes DEHP into the PVC structure but also reduces the Tg temperature in the samples.

Table 3.1 Tg comparison in the samples 2, 5, 8 and 11

Sample No.	T_{start} (°C)	T_{final} (°C)	T_g (°C)
2	-23.10	-4.73	-13.38
5	-37.50	-27.43	-17.09
8	-24.45	-5.13	-14.22
11	-31.27	-23.63	-27.29

The results showed that the Tg temperature of the polymer decreased after sterilization because of more molecular diffusion of the plasticizer into the polymer bulk.

The above findings are confirmed by the tensile test results, where the samples exhibited a higher maximum strain after sterilization. As shown in the mechanical test results, maximum strain at break increased with an increase in the plasticizer content. The DSC test results indicated that maximum strain at break increased by decreasing Tg and increasing the free volume in the polymer chains.

In other words, the DSC test results confirmed the tensile test results. A decrease in Tg indicated an increase in the plasticizer effect on PVC because of the synergistic effect of ESBO and the sterilization process (Fig. 3.3).

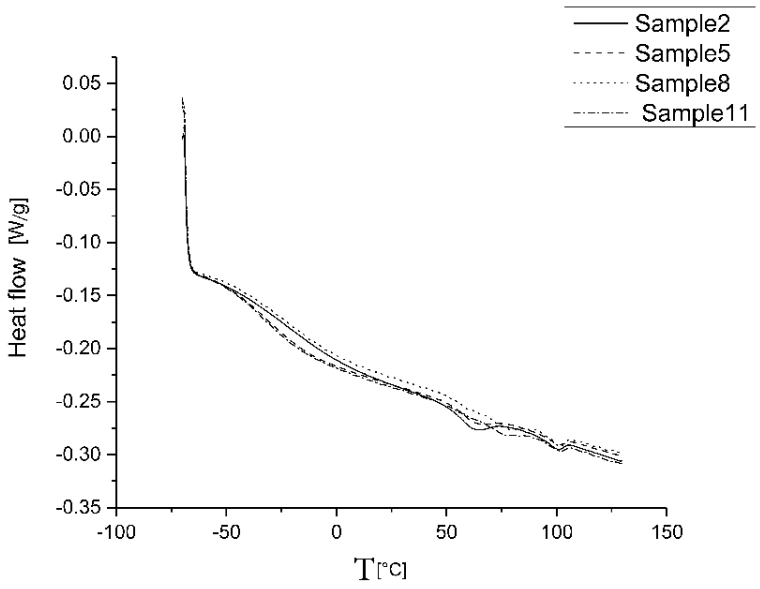

Figure 3.3: DSC curve of the samples 2, 5, 8 and 11

3.2.2 Gas chromatography test analysis

The DEHP concentration in the samples 8 and 11 was calculated to be 1333.4948 ppm and 1362.5368 ppm, respectively, with a difference of 30 ppm that can be neglected (Fig. 3.4)

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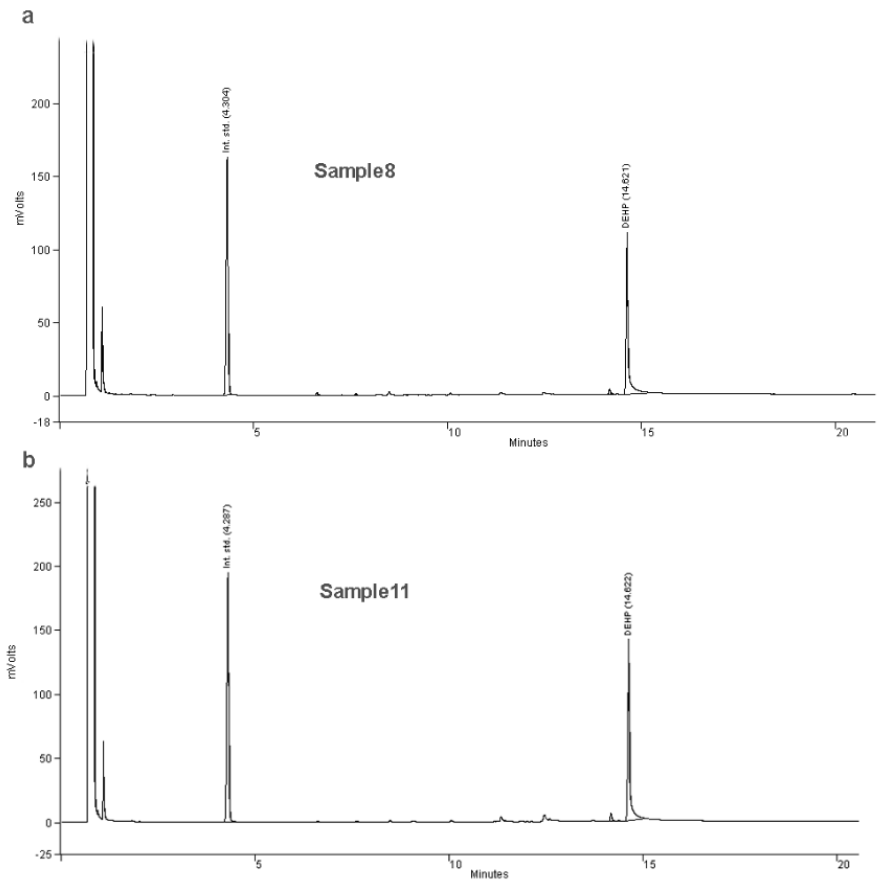

Figure 3.4 Chromatograms of samples 8 (a) and 11 (b).

Given the results in Figure 3.4 showing the release rate of DEHP into the extraction solution, it can be concluded that ESBO has no role in the migration of DEHP from the polymer bulk.

3.3 Hemolysis analysis

Samples with hemolysis more than 2% are considered slightly hemolytic, according to analysis standards of medical tools. According to the results, the samples had no considerable hemolysis effect; maximum hemolysis was in the sample 7 at 3.3%, containing 30% DEHP (Fig. 3.5). However, it appears that adding ESBO to PVC decreased the PVC hemolysis effect. Thus, the sample 10 with 0.22% hemolysis percentage and the samples 11 and 12 with 0.88% hemolysis percentage were known as non-hemolytic samples, while the samples 7 and 8 were slightly hemolytic with 3.3% and 2% hemolysis percentages, respectively. Therefore, the results indicated that the samples were non-hemolytic.

Haishima et al. demonstrated that PVC plasticizers, including a 6-carbon ring such as DEHP, had an effective role in suppressing blood cell hemolysis [27]. Moreover, Miller et al. reported that adding small amounts of DEHP to red blood cells reduced their hemolysis to 20%, which were preserved at 4°C for 35 days [28, 29]. The present study confirms these results.

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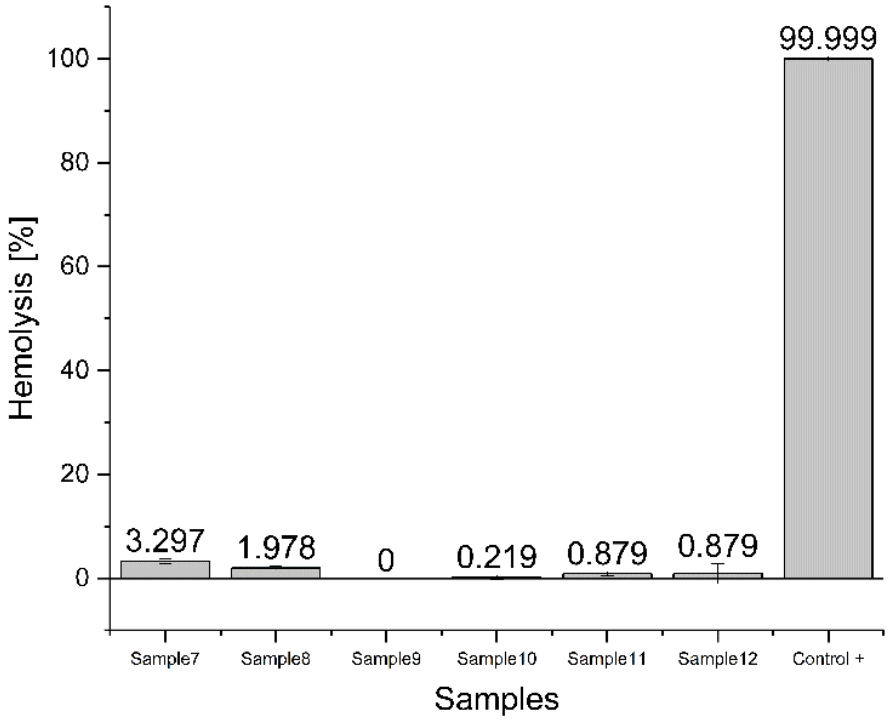

Figure 3.5 Hemolysis of the samples 7 – 12

4. CONCLUSION

For more than 50 years, DEHP has been used as a plasticizer in PVC in blood preservation bags and also in most medical tools. Ethylene oxide sterilization is an essential process in manufacturing medical tools.

The results of tensile tests and heat analysis on the samples demonstrated that 5% ESBO led to DEHP stabilization within the polymer structure and improved its mechanical properties with decreasing temperature (Tg) and increasing polymer flexibility. Moreover, sterilization using ethylene oxide gas improved mechanical properties of the samples.

The results of the gas chromatography test on solutions extracted from the sterilized samples, both with and without ESBO, indicated that ESBO had no effect on the migration of DEHP molecules into the non-polar environment.

According to the hemolysis test results, DEHP release into the blood was extremely low, as DEHP is a non-polar molecule whereas blood is a polar one. Moreover, all samples containing 5% ESBO were non-hemolytic.

Finally, it can be summarized that sterilization has no significant effect on the migration of the plasticizer from the polymer bulk; however, its annealing effects help improve the mechanical properties of PVC medical tools.

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