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Studying DEHP migration in plasticized PVC used for blood bags by coupling Raman confocal microscopy to UV spectroscopy

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ABSTRACT

Plasticized PVC is widely used to make medical devices such as tubing, perfusion bags and blood bags. By using confocal Raman microscopy on a PVC sheet plasticized with around 40% of di-(2-ethylhexyl)phthalate (DEHP), we propose a simple and sensitive approach to studying and understanding the diffusion of plasticizers from polymers into the surrounding media. Moreover, we sought to correlate our findings to standard measurements conducted by UV spectroscopy. This study showed differences in the concentration gradient observed due to the diffusion of the plasticizer inside a PVC sheet. We can thus follow the critical DEHP ratios that can impact the diffusion process. Water and ethanol were chosen as storage media: in ethanol, the lowest concentration of DEHP was observed at the surface resulting in the formation of a less plasticized layer near the interface; unlike ethanol, PVC sheets stored in water showed a greater concentration of DEHP on the film surface as an exudation of DEHP onto the surface.

1. Introduction

The migration of small molecules of plasticizers is a well-known problem. It has become a health concern in the case of plasticized PVC. This is related to the possible toxic effects of the released plasticizers on consumer and/or patient health [1]. Due to its flexibility, toughness, optical clarity and weldability, as well as its very low cost, plasticized PVC is widely used in the medical field for making blood bags and flexible tubing for infusors. The most commonly used primary plasticizers have low molecular weights and are esters derived from phthalic acid or organic acids such as trimetillates, citrates or adipates [2]. For several decades, the di-(2-ethylhexyl)phthalate(DEHP), has been the most commonly used plasticizer in medical devices [3]. DEHP is still the most extensively used plasticizer for blood bag applications due to its release that enables a better preservation of red blood cells during storage. For other medical devices, alternative plasticizers are now added to PVC, as DEHP is suspected to be an endocrine disruptor [1]. It may particularly affect patients exposed to high levels of contamination due to long term chronic infusion procedures or multiple infusion procedures, for example, neonates in medical care units or dialyzed patients.

Many studies have been made on plasticizers and in particular on DEHP migration, considering different contact media and conditions [4–11]. Diffusion is favored by lipophilic media. Most studies relate to the medical field [12–18] and to food packaging [19–22].

DEHP migration in blood was studied at different temperatures (storage at 4 °C or at ambient temperature), in different blood products, (whole blood, red cell concentrate, platelet concentrate, plasma) and for storage times between 2 days and several weeks [23–25].The levels of DEHP in blood products can be found in an EPA report [26]. For example, after one week of storage at 4 °C, 100 to 275 mg/L of DEHP was detected in plasma and after 3 days at ambient temperature, around 200 to 300 mg/L of DEHP was detected in the platelets with plasma. These very different conditions of storage are due to the diversity of blood products and to their different expiry dates: after being collected in a first blood bag, the whole blood is separated into red cell concentrate, plasma and platelet concentrate. Concentrates are a suspension of red blood cells or platelets in a mixture of plasma and anticoagulant nutrient solution. These solutions contain, for example, citrate as an anticoagulant, dextrose as a nutrient and adenine or mannitol as red blood preservatives. Due to rapid platelet degradation at 4 °C, this separation process must occur on the day of blood collection or after one night at 22 °C. For the production of red cells exclusively, whole collected blood can however be stored at 4 °C for 48–72 h prior to separation. The red cell concentrate can then be stored for 42 days between 2 and 8 °C, but the platelet concentrate can only be preserved for 5 days at between 20 and 24 °C. In the case of plasma, it can be frozen and used one year later.

For DEHP migration studies, different analytical techniques have been used [27].The main results obtained were the amounts of DEHP leached over time. Migration was quantified either by following the mass change of the sample, or by quantifying the amount of DEHP either

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in polymer or in the surrounding medium. For this quantification, analytical techniques such as UV spectroscopy, chromatography or radiometric methods (use of labeled plasticizers) [5] were used. To obtain DEHP concentration profiles in the material, Messadi et al. stacked seventeen 0.2 mm thick films in storage solution and analyzed the DEHP content of each film by chromatography [28,8,9].

In this study we propose a simpler approach to the characterization of the distribution of the plasticizer inside the PVC during the diffusion process. We also obtain greater accuracy by conducting a mapping of the plasticizer localization in the PVC film *in situ* over time, using a Raman spectrometer coupled to a confocal microscope. The advantage of this approach is that no sample preparation is needed: no stack films are used, the sample does not need to be cut before analysis and the analyzed volume (1 μm^3) is much smaller than that of the stack films. This method enables chemical imaging giving the location of the different components of the sample. The use of a confocal microscope makes it possible not only to explore the sample in XY directions, but also in depth (Z), with spatial resolutions in the 0.5–1 μm range.

Results of Raman analyses were compared with those of a more conventional analysis that consists in quantifying the amount of plasticizer in the whole film and in solution by UV spectroscopy. As blood products are aqueous electrolytes with biological entities and organic molecules that increase their lipophilic nature and change their extractive power [29], we selected two simple storage media having different polarities for this study: one is water, the other ethanol, a medium much more favorable to the extraction of the plasticizer. As a model for the plasticized PVC sample, we used a commercial extruded sheet of PVC containing around 40% DEHP.

2. Materials and methods

2.1. Sample

The studied sample was a commercial sheet used to produce blood bags. It mainly consists of PVC and DEHP (Fig. 1). Sheets were approximately 0.33 mm thick.

2.2. Sample storage

The samples were stored at 25 °C in ethanol 96%, or in WFI water from several hours to several weeks. A disk of 2.5 cm in diameter was cut and stored in a hermetic glass vial filled with the storage liquid.

2.3. UV analysis

The amount of DEHP in the film and the solution was quantified by using the absorbance of the band at 275 nm which is characteristic of DEHP. For the film, the DEHP content was calculated by directly

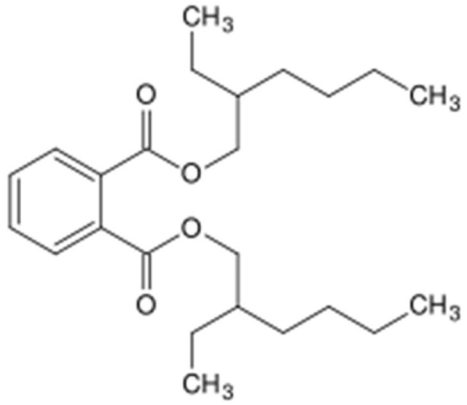

Fig. 1. DEHP formula.

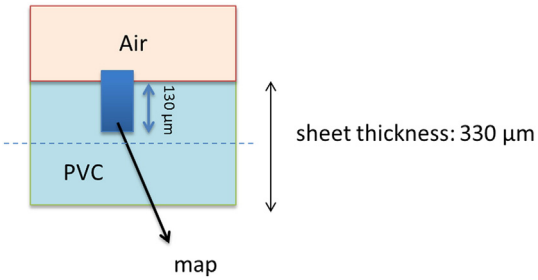

Fig. 2. Schematic representation of the PVC sheet area analyzed with Raman confocal microscopy.

analyzing the film with air as the reference substance and by comparing its absorbance to the absorbance of the film containing a ratio r = 0.4 of DEHP ($r = \frac{m_{DEHP}}{m_{DEHP}+m_{PVC}}$). The UV spectrophotometer was a double-beam Jasco (Easton, USA) V-550. The reference sample for DEHP analysis in the surrounding liquid was water or ethanol.

2.4. Raman analysis

A LabRam HR evolution Raman spectrophotometer (Horiba Jobin-Yvon, Villeneuve d'Ascq, France), equipped with a 10 mW, 632.8 nm He–Ne laser and a MPLFLN 100X (0.90 NA, Olympus), long focal distance microscope objective lens, was used. The confocal pinhole was set to 200 μm. The collected light was filtered through an edge filter and dispersed with a 2 cm^{-1} spectral resolution using a grating of 600 grooves/mm over a wave number range of 400–3200 cm^{-1}. Spectral acquisition preprocessing and processing were performed using LabSpec 6.1.9 software (Horiba Scientific, Villeneuve d'Ascq, France). For each spectrum, a 5 s exposure time was used with 2 accumulations. Confocal maps were obtained in a point-by-point mode perpendicular to the SC surface with a 1 μm step-size. Firstly, the distribution of DEHP in the whole sheet depth was studied by producing a spectrum with every 10 μmin as sample depth (objective displacement).

The diffusion process was studied as follows: at each time point, the sample was removed from the storage liquid and analyzed by creating a 100 * 20 μm^2 map (Fig. 2). A Raman spectrum was produced every 2.5 μm. Two sets of samples were analyzed for t = 0, t = 1 week and t = 2 weeks in order to check the reproducibility of the diffusion maps. It should be noted that the depths given here correspond to the

Table 1
Correspondences between objective displacement and depth in the film.

Objective displacement (μm)	Corrected depth (μm)
5	9
10	19
15	27
20	35
25	43
30	51
35	59
40	67
45	75
50	83
55	91
60	99
65	107
70	115
75	122
80	131
85	138
90	146
95	154
100	162