Technical report: Effects of PUVA treatment on the optical properties of blood/tissue storage bags during extracorporeal photochemotherapy

Ali Umit Keskin *

Yeditepe University, Department of Biomedical Engineering, Kayisdagi, 34755 Istanbul, Turkey

Received 29 January 2007; accepted 7 August 2007

Abstract

Extracorporeal photochemotherapy (photopheresis, ECP) is a novel therapeutic method for patients who do not respond to immunosuppressive medications, and gaining interest in the treatment of Graft-vs-Host Disease. This paper is focused on the optical transmission properties of plastic bags which can be used in an independent (off-line) method of ECP, and reports the results of spectral measurements on various bags of different chemical compositions, with and without PUVA treatment. Regarding their higher and more uniform UVA transmission values, FEP based bags perform superior to the others. Considering its UVB absorption and UVA transmission properties, the EVA bag is a good choice, while Polyimide Kapton–FEP plastic film should not be considered for use in ECP. PUVA treatment of blood bags may affect their optical behaviour, and causes reduction of transmission of the material in UV range of the spectrum.

© 2007 Published by Elsevier Ltd.

Keywords: Graft-vs-Host Disease; Psoralen; Ultraviolet (UV) rays; Photopheresis; Extracorporeal chemotherapy; Plastic bag

1. Introduction

Extracorporeal photochemotherapy (photopheresis, ECP) is a novel therapeutic method for patients who do not respond to immunosuppressive medications [1–12]. ECP is a relatively new concept which generates much interest among researchers because it appears to be able to stimulate the immune system to treat a malignant disorder, yet causes selective immune suppression in autoimmune and alloimmune disorders [13].

In this procedure, blood is withdrawn from the subject, portions of the blood are separated and passed through an UVA field (ultraviolet rays with wavelength between 320 and 400 nm) in the presence of a dissolved UV sensitizer and DNA-intercalating agent 8-methoxypsoralen (8-MOP) C_{12}H_{8}O_{4}, all together known as PUVA process, and then returned to the subject. While an ECP method uses commercially dedicated equipment (Therakos, Exton, PA, USA), another form of ECP (so called, independent or off-line method) involves external PUVA treatment of leukocytes in a plastic bag [2,3,12] and it is the method of interest in the present study.
Although previously published papers on ECP applications mostly concentrate on the cellular objects, and rarely deal with problems due to medical materials (tubing, bags...) [14], it has been recently reported that some routine procedures (such as method of sterilization of storage containers) may affect cellular parameters [15]. This paper is focused on the optical properties of plastic container bags which are used in an independent (off-line) ECP procedure, and report experimental findings on these bags. The effect of independent ECP procedure on tubing is not considered here, since tubing is not exposed (or exposed partially) to UV radiation.

2. Materials and methods

The bags were obtained from several manufacturers and were of the following compositions: 1 – PVC with di-2-ethylhexylphthalate (DEHP), 2 – polyethylene vinyl acetate (EVA), 3 – fluorinated polyethylene propylene (FEP), 4 – sandwiched polyimide Kapton and FEP. In the first group, single blood bag systems made of PVC–DEHP film and with the blood capacity of 450 ml for collection storage and administration of whole blood, manufactured by Kansuk Laboratories (Istanbul, Turkey) (product code 6A217, steam sterilized, CE 0197 marked) were used. The CPDA-1 anticoagulant solution filled bags were emptied, washed with distilled water, gently dried using a paper towel and then 4 cm x 2 cm strips were cut from them using a scissor (providing two strips at a time). Optical transmittance analysis of the bags were performed using an UV–vis spectrophotometer (Beckman Coulter DU-800) in wavelength scanning mode (600 nm/min scan speed, 0.5 nm wavelength interval). The strips were placed orthogonal to the optical path of each open top glass cuvette (PN 75152), and cuvettes were placed in auto 6 cell unheated holder. Strips were inserted into the cuvettes having an optical path length of 1 cm by symmetrically bending each strip about its middle-long axis, and then leaning it firmly onto the real wall of the cell using a plastic pipette tip. This ensures leakage prevention through the cuvette during spectral measurements. All measurements including sample preparation and treatment procedures have been performed at lab temperature (22 ±1 °C), and without stretching the sample strips.

PVC sample strips in the first run were untreated; PVC–DEHP film strips in the second group were dipped into 50 cc of 8-methoxypsoralen (8-MOP)/distilled water solution containing 2.5 mg of 8-MOP (Gerot Pharmazeutika GmbH, Vien, Austria), and irradiated by UVA light for a duration of 20 min at 0.350 mW/cm² by putting them in a beaker standing on the lamp unit, altogether placed in a closed laboratory drawer. Intensity of UVA radiation was measured with a UV radiometer (UVA 365, Lutron Electronic Enterprise Co., Ltd.). At the end of UVA irradiation, the strips were washed with distilled water, dried using a paper towel, and inserted into the cuvettes. UV spectral studies were then performed for each group.

The same sample preparation and UV–vis measurement procedures were applied to all other bag specimens made of EVA (first sample: HPF 0679 Beldico SA/NV, Belgium, gamma sterilized, pyrogen free; second sample: Cryocyte 4R9955 Baxter-MiltenyiBiotec, Germany, gamma sterilized, non-pyrogenic bag, contains dry natural rubber and is rated for cryogenic blood preservation), Polyimide-Kapton-FEP (OriGen CryoBag, Origen Biomedical, Austin, TX), and FEP (PermaLife PL-70, Origen Biomedical, Austin, TX, gamma sterilized, non-pyrogenic).

Before scanning, spectral measurement device was calibrated using its internal procedure.

3. Results

Typical records simultaneously displaying UV transmission spectra of tested bag film specimens are given in Fig. 1 for PVC–DEHP, in Fig. 2 for EVA (Cryocyte, Baxter), in Fig. 3 for EVA (by

![Fig. 1. UV transmission spectra for PVC-DEHP (Kansuk Labs) bags (6A217). 1-Ununtreated sample (top), 2-Psoralen-UVA treatment for 20 min (below).](image-url)
Beldico), and in Fig. 4 FEP based films. UV–vis spectra of EVA bag samples of two different producers showed different transmittance patterns. (Baxter EVA bag is more UVA transparent than Beldico EVA bag). UV spectra for PVC–DEHP and EVA based bag samples display sharp decrease in light transmission in UVB (280–320 nm) range for bag samples of the first group which have not been treated with 8-MOP, and there is a gradual decrease of the same in the UV-A region, while the light transmission in visible range (not shown in Fig. 1) is almost constant function of wavelength. No significant minima or maxima are noted in all three ranges of the radiation spectrum. On the other hand, PUVA treated samples in all test groups somehow demonstrated reduction of UV transmission as a function of decreasing wavelength, and plasticized PVC material strongly blocked optical transmission at about 292 nm. Static electrification was observed on FEP samples, while EVA and PVC samples were easier to handle.

On the other hand, all PUVA treated samples somehow demonstrate reduction in UV transmission, which is more pronounced in PVC bag samples. Sandwiched Polyimide Kapton–FEP plastic film blocks UVA completely, as shown in Fig. 5.

4. Discussion and conclusion

An off-line (independent) procedure of ECP consists of the following steps [12]: (1) Buffy coat separation is performed using a cell separator (apheresis device). Here, one can obtain large quantity of mononuclear cells (purity about 90%) in a small volume (100–150 ml). The time required for the collection of buffy coat depends upon the equipment used (typically 2 h). (2) This buffy coat concentrate is adjusted to a constant volume for irradiation by
dilution in normal saline. Hematocrit of the final product is below 2%. (3) Dissolved UVA sensitizer (8-MOP) is added to the cell concentrate at a final concentration of 200 ng/ml. (4) It is transferred to an irradiation container (blood bag) to ensure an efficient irradiation with the UVA irradiator. After irradiation at 2 J/cm² for 20 min, the cells are re-infused into the patient. Independent ECP method does not use a closed device, works with manual operations. However, the procedure is less costly, easier to handle, and various companies are known to be trying to obtain commercial rights of its clinical implementation.

ECP success is highly dependent on effective PUVA procedure in which UVA emitting source has a significant contribution. This source is a fluorescent tube. (A typical example is Cosmopower S lamp having an almost gaussian shaped emission spectrum with a peak at 350 nm, and approximate half power bandwidth of 40 nm.) Assuming that such a source is employed in an illuminator for all bags studied in this work, maximum UVA energy transfer to the cells contained in a bag will be possible if FEP based plastic film is used. This is true for non-treated as well as PUVA treated plastic bag samples. In all cases, UVB energy transfer is small (primarily due to the emission spectrum of the UVA tube), and it is further reduced if EVA and PVC bags are irradiated by such a source.

In the earlier independent photopheresis studies the fluence rate of UVA radiation was measured with a UV radiometer. Here we measure UVA–vis spectra using a wavelength scanning spectrophotometer, which gives more detailed information about the wavelength sensitivity of photodegradation within the UV spectrum.

It was noted that wetting the samples of the bags in distilled water (by keeping them in a beaker for 20 min) has no significant influence on their spectral performance as referenced to non-illuminated dry samples.

It should be pointed out that in this study the term “percent transmission” rather than “transmittance” is used to describe UV spectra. The reason for this preference is the fact that in ECP one is more interested in “transmission of UV energy at a particular wavelength” than “coefficient of transmittance” of a given plastic material, the latter term being a material constant which is independent of film thickness. When comparing UVA transmission properties of bags which have different chemical compositions, it is more advantageous to discuss the transparency of one bag with respect to another one, although their thicknesses may not be identical.

It is observed in this study that there exists an inverse relationship between the use of psoralen and optical UVA transmittance of this kind of bags. PUVA treatment of bags affects their optical behavior, and causes reduction of transmittance of the material in UV range of the spectrum. Therefore, in order to effectively irradiate the leukocytes in a bag (for a given concentration of psoralen), the bag needs to be irradiated at higher levels of UVA light or requires longer ECP time, while keeping the optical path length within the bag as short as possible. In order to provide a small and relatively homogenous optical path length (therefore to obtain more effective UVA irradiation of the cells), cell concentrate obtained during the third step of the off-line ECP procedure can be distributed into more than one irradiation bag. These bags can be simultaneously PUVA treated, because most of the commercially available UVA irradiators have enough internal space for such a procedure. Alternatively, a single large sized bag (e.g. 20 × 35 cm) can be implemented to reduce the effective thickness.

Regarding their higher and more uniform UVA transmission values, FEP based bags perform superior to the others. On the other hand, considering its UVB absorption and relatively high UVA transmission properties, EVA bag is a good choice, while Polyimide Kapton–FEP plastic bags should not be considered for use in ECP. Besides their relatively poor UVA transmission, PVC–DEHP based bags are not recommended for use in ECP due to their plasticizer leaching (migration) and thermally or UV generated dehydrochlorination problems.

Although much less amount of UVA radiation density has been used in this study as compared to earlier ones [2,3,12], the results of experiments demonstrate valuable information regarding the influence of PUVA radiation on several commercially available plastic bags.

Acknowledgements

The author would like to thank Yener Koc, Professor of Hematology in Oncology Department, for his useful discussions, and the staff of the Biochemistry Laboratory of Yeditepe University. The author is also indebted to Demet Keles (Kansuk Labs), Guldeniz Mese (Eczacibasi-Baxter Healthcare), Elif Merdanogullari (Amphi Ltd), Sutude Aktan (Liba

References


