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Particle release and surface characteristics of polyvinylchloride perfusional containers after steam sterilization

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The loss of the plasticizer di(2-ethylhexyl) phthalate (DEHP) from the inner surface of polyvinylchloride (PVC) bags and the surface characteristics of the PVC containers after steam sterilization of bags filled with sterilized water for injectable preparations were studied. Particulate contamination level was obtained using the Coulter counter method. All the sterilized bags showed an acceptable particle number according to the limits established by several pharmacopoeias. These results were confirmed by gas chromatographic and mass spectroscopic (GC-MS) analyses in which low levels of DEHP ranging from 2.4 to 17.2 µg/l were measured. Surface characteristics were determined by atomic force microscopy. Limited changes in the inner surface of the PVC bags after steam sterilization were determined.

Key words: Di(2-ethylhexyl) phthalate – Polyvinylchloride – Perfusional containers – Particle release – Surface characterization – Steam sterilization – Tensiometry – Atomic force microscopy.

Pharmaceutical grade containers for parenteral administration of drugs or nutrients are commonly made of polyvinylchloride (PVC), a material used due to its known chemical inertness and low cost. However PVC is made flexible by the presence of various plasticizers such as di-(2-ethylhexyl) phthalate (DEHP), which may constitute up to 40% of the finished medical devices. The present plasticizer is extracted from PVC and leaches into the parenteral liquids [1, 2]. There is great concern about the toxicity of DEHP [3].

Particulate contamination measurement is an important test in large volume parenteral formulations. Several pharmacopoeias defined the contamination limits for extraneous particles. The European Pharmacopoeia, the Italian Pharmacopoeia and USP 24 state that the average number of particles per milliliter should not exceed 25 with a 10-μm diameter and 3 with a 25-μm diameter using the light obscuration test. Recently, an experimental protocol to evaluate the effects of storage of different liquid contents on the characteristics of the inner wall of bags made of plastic materials such as PVC, widely used in medical devices, has been carried out by using tensiometry and atomic force microscopy (AFM) techniques [4].

Bags containing perfusional solutions often undergo physical treatment (i.e. sterilization) that may alter the properties of PVC and, as a consequence, may induce an undesired contamination of their content by the auxiliary agents. The aim of this investigation was to evaluate the effect of different industrial protocols of steam sterilization on the inner surface characteristics of PVC bags and the level of particles and DEHP contamination of the solutions they contain. The experimental protocol based on tensiometric measurements and atomic force microscopy (AFM), devised for controlling the quality of any plastic device, was used to evaluate the inner surface characteristics of the bag. The Coulter counter technique was used to determine the level of particle contamination while gas chromatography, coupled with mass spectrometry (GC-MS), was performed to quantify chemical contamination.

I. MATERIALS AND METHODS

1. Materials

Untreated, unsterilized tubular PVC and PVC bags filled with sterile water for injectable preparations were provided by Sifra-Est Group (Trieste, Italy). All the reagents and buffers were of analytical grade and used as received.

2. Methods

2.1. Steam sterilization protocols of PVC bags

Four different steam sterilization protocols, shown in *Table I*, were followed for sterilizing PVC bags.

Table I - Experimental conditions of steam sterilization.

Method	t (°C)	Steam exposition time (min)	Pressure (bar)	Cooling process	Opening t (°C)
1	118	50	1.4	air	80
2	118	18	2.9	controlled*	60
3	121	17	2.9	controlled*	53
4	119	50	2.3	air	70

*Cooling rate (with air): 5°C/min until reaching 80°C.

2.2. Determination of particle contamination

A Coulter counter (Multisizer IIe, Instrumentation Laboratory, Milan, Italy), fitted with a 70-μm orifice tube and calibrated with 10-μm diameter latex microspheres (Coulter CC Size Standards, Beckman Coulter), was used. This orifice tube was used to measure particle diameters ranging from 1.4 to 42 μm. Samples were prepared by mixing 100 ml of distilled water from each PVC bag with a previously filtered (2x; 0.22 μm) NaCl solution (1.8%, 100 ml) of known background count. The number of particles larger than 2, 5, 10 and 25 μm was determined in 2 ml of sample, corresponding to 1 ml of distilled water from the PVC bag. Ten determinations were performed on each sample and the mean and standard deviation values were calculated. Two aliquots of distilled water from the same bag were analyzed. All instruments and glass containers were carefully washed with distilled water twice filtered.

2.3. Determination of DEHP

The amount of DEHP in the sample solution was determined by GC-MS (FinniganMat GCQ System, ThermoQuest Italia, Milan,

Italy). A GC column (CP-SIL 8 CB-MS, 30 m x 0.25 mm, 0.25 μm internal diameter, Superchrom, Milan, Italy) was used. The temperature program was set between 40-300°C with a heating rate of 40°C/min. The injector temperature was 260°C.

Standard solutions of DEHP in n-hexane (0.25-20 μg/ml) were prepared to obtain a calibration curve by plotting the area under peak of DEHP against the corresponding concentration.

Two aliquots (250 ml) of distilled water from each bag were added to 30 ml of acetonitrile and 20 ml of saturated NaCl aqueous solution, then extracted twice with 50 ml of n-hexane. The extraction process was repeated five times, then the collected organic phase was evaporated to dryness, the residue re-dissolved in 0.5 ml of n-hexane and injected (1 μl) in GC-MS.

The amount of DEHP released from PVC bags was determined by measuring the area-under-curve corresponding to the peak of DEHP.

2.4. Surface characterization by tensiometric measurements

Tensiometric measurements were carried out by a tensiometer G40 (Kruss GmbH, Hamburg), equipped with a G1041 microcamera, a manual dosimeter for surface and interface tension measurements, a manual dosimeter for contact angle, a G1023 automatic dosimeter for dynamic contact angle, a TD-211 temperature monitor with a Hamilton 1750 TLL syringe. The G402.06 software was used.

Four samples (75 x 25 mm) were taken from different areas of each bag and dried carefully with a nitrogen stream and stored in closed, sterile containers until they were tested. The outer surface of each sample was made to adhere to a microscopy glass slide. Water and diiodomethane were used as standard liquids [5]. A drop of a standard liquid (diameter 2-6 mm) was applied to the surface of the sample and the contact angle was measured [7]. The acquired angle values were elaborated using the Wu method [6] to obtain the polar (PC) and dispersed (DC) components the sum of which gives the surface free energy (SFE).

The tensiometric characteristics of the material can be represented in terms of TVS (Tensiometric Versus Skin) Index according to the parametric model which represents integrated "tensiometricprints" and surface free energy (SFE) contextually with its related dispersed (DC) and polar (PC) components of a material or biological substrate and their tensiometric affinity [4, 8-9].

The SFE, PC and DC values reported in this paper are the mean values obtained from the measurements carried out on four samples of bags.

Four bags for each sterilization protocol were tested. Four unsterilized bags filled with sterile water were tested as a control.

2.5. Surface characterization by atomic force microscopy (AFM)

AFM measurements were carried out at room temperature using an Autoprobe CP atomic force microscope (Park Scientific Instruments, Sunnyvale, CA, USA), equipped with a contact head, an intermittent contact head, and a 5-μm scanner.

All the samples were investigated in air using contact or tapping mode techniques. Silicon Ultralevers type C-D (Park Scientific), with resonance frequency of 300-400 kHz, were used for tapping mode. Silicon nitride (Si_3N_4) microlevers, type A, with a nominal spring constant of 0.05 N/m, were used for contact mode. Scan rates were in the range of 2-4 Hz. Four samples (10 x 10 mm) were cut from each bag. The samples were dried carefully with a nitrogen stream and stored in closed, sterile containers until they were mounted on the AFM sample holder. A set of nine images of the inner surface was acquired for each sample analyzed, usually at three different positions, namely at the center, at the upper left corner and at the lower right corner of the sample. The surface mean height and the surface mean roughness (R_{rms}) were used as parameters to characterize the surfaces of the samples (*Equation 1*):

$$R_{rms} = \sqrt{\frac{\sum_{i=1,N} (z_i - \langle z \rangle)^2}{N-1}}$$

where N is the number of pixels in the image, z_i are the heights of each pixel, and $\langle z \rangle$ is the mean height calculated over 512 points per scan line and 512 scan lines per image.

All the images, and the mean heights and roughness values reported refer to those obtained using the contact mode technique and Si_3N_4 probes (microlevers, type A).

Four bags for each sterilization protocol were tested. Four unsterilized bags filled with sterile water were tested as a control.

III. RESULTS AND DISCUSSION

1. Particle contamination and chemical composition of the contaminants

Particle contamination in distilled water was measured on two bags after each sterilization protocol. Results are shown in *Table II*. All samples showed a particle number within the limits allowed by the different pharmacopoeias considered. Only small differences were observed between sterilized and non-sterilized bags, indicating a limited effect of the sterilization process on particle contamination. Interestingly, different levels of contamination were sometimes observed among bag contents treated with the same sterilization method. This might be due to a non-uniform thermal stress effect.

The nature and amount of chemical contaminants was measured by GC-MS. As shown in *Figure 1*, several peaks were detectable corresponding to epoxidized vegetal oil, calcium and zinc stearate stabilizers (peaks 1-5) and DEHP (peak 6).

The amount of DEHP in sterilized samples, shown in *Table III*, was between 2.4-17.2 μg/l, indicating only a low contamination le-

Table II - Particle contamination of bags.

Sterilisation method	Particle number			
	> 2 m	> 5 m	> 10 m	> 25 m
Not sterilised	321±17	12 ± 4	1 ± 1	0
1	185±15	9 ± 2	< 1	0
1	309±20	10 ± 3	< 1	0
2	230±15	8 ± 3	< 1	0
2	424 ± 24	20 ± 4	2 ± 1	0
3	544 ± 22	17 ± 4	< 1	0
3	634 ± 43	22 ± 5	< 1	0
4	248 ± 15	6 ± 2	< 1	0
4	333 ± 23	10 ± 4	1 ± 1	0

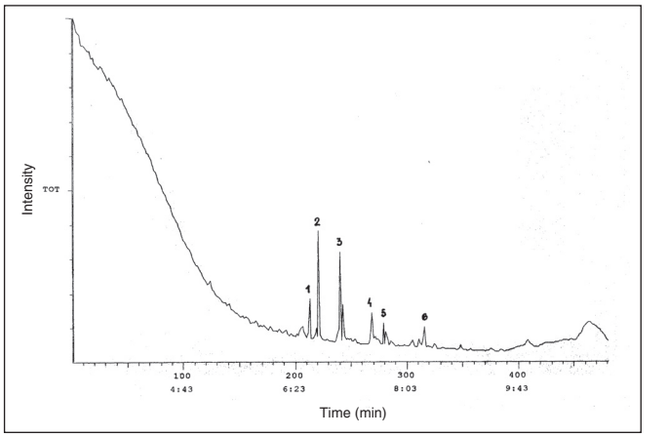

Figure 1 - Chromatographic sample showing peaks of several contaminants. Peak No. 6 corresponded to DEHP.