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La contaminazione da particelle di vetro durante l'apertura delle fiale

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Summary: Particulate matter contamination in injections and in perfusional liquids consists of foreign matter other than gas bubbles, which is not dissolved, mobile and not purposely present in such preparations. This subject is dealt with in the USP24-NF19 and in the Appendix XIII of the British Pharmacopoeia 1998, where specific monographs are reported. We therefore thought its aspects worth studying in greater detail, in order to provide useful information to improve the total quality of injectables. The first aspect to be observed is the fact that pharmaceutical industries, also owing to the more and more rigorous GMPs, have mostly solved the part of the problem they can be responsible for, i.e: primary contamination, where the polluting material is present before the vial is opened. However, the problem of secondary contamination, caused by material which is formed when the vial is actually used, i.e. at its opening, is still partially unsolved.

The aim of our work is the quantitative identification of both the polluting material and the contamination source, so as to avoid or limit such occurrences and to stop the potential pathological consequences.

Riassunto: La contaminazione particellare delle preparazioni iniettabili e dei liquidi perfusionali consiste nelle particelle estranee - diverse dalle bolle di gas - non disciolte, mobili ed involontariamente presenti in tali

preparazioni. La problematica è presa in considerazione in varie farmacopee (quali la Farmacopea Italiana X Edizione, La United States Pharmacopoeia 24, la British Pharmacopoeia 1998) dove sono riportate monografie specifiche. Gli AA. hanno ritenuto interessante approfondirne gli aspetti correlati con l'obiettivo di ottenere informazioni utili per il miglioramento della "qualità totale" dei preparati iniettabili. Il primo aspetto da considerare è rappresentato dal fatto che le industrie farmaceutiche, anche a causa delle sempre più rigide GMPs, hanno risolto quasi completamente la parte di loro competenza, vale a dire la contaminazione primaria, dove il materiale inquinante è già presente prima dell'apertura del contenitore primario. Tuttavia, il problema della contaminazione secondaria, causata dal materiale che si forma al momento dell'uso del contenitore (cioè all'atto dell'apertura) è ancora parzialmente irrisolto.
Scopo di questo lavoro è l'identificazione quantitativa sia del materiale inquinante sia della fonte di contaminazione così da evitare o, quanto meno, limitare tale eventualità e bloccare le potenziali conseguenze patologiche.
Key words: Vials, Ampuls, Particulate matter analysis, sealing glass.
Parole chiave: Contenitori primari, fiale, contaminazione particellare, sigillatura delle fiale.

Introduction

Because of their special way of administration injectable preparations need special technological requirements to be fulfilled, among which the absence of foreign particles is a crucial one. The importance of this condition is shown by the existence of newly published or updated monographs devoted to the subject in several pharmacopoeias[1–3]. By "particulate contamination of injections and parenteral infusion" we indicate extraneous, mobile, undissolved particles, randomly-sourced - other than gas bubbles - unintentionally present in these preparations.

One method of classification of foreign particles concerns the possibility, or impossibility of identifying them with the naked eye. Anyway, they cannot usually be quantitatively analysed by chemical methods, owing to their small amounts, and, equally important, to their heterogeneous composition (metal, rubber, cellulose fragments, glass, etc.).

Specific literature information and the knowledge of the various

sources of pollution make it possible to identify the contribution of two major sources to the overall pollution: the particle charge due to the production cycle of the injectable preparation, and the one related to the modes of injection, e.g. syringes, administration sets and procedures, vial opening[4,5]. It is interesting to emphasize from the beginning that a significant number of potentially injectable particles comes from sources which are outside the producers' operational control.

Therefore, it can be concluded that so far the "Quality Assurance" philosophy of the pharmaceutical industries has substantially solved the aspects of the problem it can be held responsible for, i.e. primary contamination, the presence of polluting material in the sealed vials, while secondary contamination, occurring when the injectable material is actually used, is still partially unsolved.

We therefore thought it would be worth while to analyse the problems concerning particle contamination of glass material which can be attributed to the opening procedures of primary glass containers, in order to provide information potentially to be related to the well-known injuries, such as micro-clots, thrombuses, granulomas, to which animals[6] as well as man[7,8] can be subject as a consequence of this kind of pollution, with the ultimate aim to find ways of preventing their occurrence.

Materials and methods

- Hardness test apparatus HT300 of PELUI AG, Zurich, modified in order to allow the opening of empty vials and the collection of the outgoing glass material. The collection was performed by means of a suitable purposely built device, which was kept at a constant negative pressure of 50 kPa with respect to the surroundings, and possessed of Millipore filtering membranes, SK-114, Ø=mm 47, pores 1.2 μm, white and grid-shaped to make the outgoing particle count easier. No rigorous working conditions were adopted concerning the surroundings, as the blank air aspiration test from the apparatus without vial opening did not result in any glass contamination, as expected. The tester footstock opening was fixed at mm 36 in all, symmetrically divided, i.e. 18 mm for each footstock, with special attention to the correct positioning of the vials. The apparatus described above allowed a correct evaluation of the force necessary to the opening of each vial[9].

- Horizontal laminar flow hood (STERIL, Oasis model) in the particle count.

- Millipore microanalyses filter support, with spring collet, HAWG, Ø=mm25 pores 0.45 μm vacuum flasks, white and grid-shaped for washing liquid filtration, collection and analysis of the contaminants present in the tested solution.

- Washing solutions and/or liquids (Triton X-100, isopropanol, water) are all of reagent grade or purer and 0.45 μm filtered before use.

- Petri plates Ø=cm 10 to keep the material clean.

- Particle counting system, consisting of Reichert Wien optical microscope, with a viewer to make reading easier, and suitably equipped with a double lighting system (the first consisting of an episcopic brightfield internal to the microscope and an external one to give an incident oblique illumination at an angle to about 20°. The system allows easier identification and counting of glass (vitreous) material; a 100x magnifying eyepiece. A calibrated micrometric scale, (the notches on the micrometer corresponding each to 8.47 μm), but not certified for the purposes of this work. The longest dimension from edge to edge (length) of the counted particles oriented parallel to the ocular scale was taken into account. The optical microscope was chosen for our analysis under controlled environmental conditions, in spite of the fact that it is a manual and more cumbersome device with respecto to other analytical methods, as it is a primary counting method, and above all, as it allows vitreous particles to be qualitatively differentiated[10].

- Empty vials with sealed top (point), pre-breaking "One-Point Cut" (OPC) and "Colour Break" (CB), nominal volume 5mL, for the evaluation by means of a hardness test device of the glass particles given off when the vials are opened. OPC pre-breaking kind was chosen because of the advantages it possesses which make it a widespread method[11], while CB pre-breaking kind, though not as widespread as the first one, was chosen because of the irrefutable identification it allows of the glass particles which are doubtlessly given out at hand vial opening, as proved by the presence of coloured glass particles belonging to the ring.

- Commercially available vials containing water for injectable preparations, pre-breaking, "Colour Break" (CB), nominal volume 2mL. The outer surface of the hand-opened vials was cleansed with distilled water, and filtered. The vials were opened by exerting a manual pressure along the pre-breaking line, and in such a way as to produce the lowest possible number of particles. The vial content either directly or previous aspirations with a needle was poured into the funnel of the filtering device, and *vacuum* was applied until the whole liquid was filtered. Two units at a time were tested throughout the experiment.

Results and discussion

Vial opening before use is only apparently an obvious procedure, as many problems can arise in the process, both for the user (as the ideograms provided by some firms indicating the correct operations to be performed as part of the general instructions clearly show) and for the patient who is given the contents. The exchange of letters between anaesthetists discussing solutions in order to avoid accidents when opening vials during surgical operations may be worth mentioning by way of example[a].

We first investigated whether the number of particles given out at the opening of the vial could be related to the force needed to open it by means of HT 300 apparatus described above. Figure 1 represents the dimensional distribution of the glass particles after the automatic opening of OPC vials. The results represent the mean and the corresponding SD of ten independent tests. The presence of such a number of glass particles of irregular form definitely proved that our doubts were reasonable, and stimulated further research. The values of the opening force, which are all largely within those admitted by ISO 9187-2[9], are reported in Table 1, and these are the values that led us to discard this kind of approach.

Figures 2 to 4 show the number of potentially injectable particles which are found in the injectable liquid after vial opening. In particular, Fig. 2 indicates the glass particle contamination in a solution as it is, i.e. not needle aspirated.

Figures 3 and 4, on the other hand, show the number of glass particles counted after aspiration of liquids, by means of needles for aqueous and oily media, respectively. As expected, the number of

[a] cf, e.g., the letters "Glass ampoules another approach" in Anaesth. Analg. *78*: 809-810, 1994, and "Risk of injury and inections from broken glass ampoules", in Health Devices *23*, 56, 1994.

particles flowing through the needle is considerably lower than that of the potentially injectable ones before aspiration.

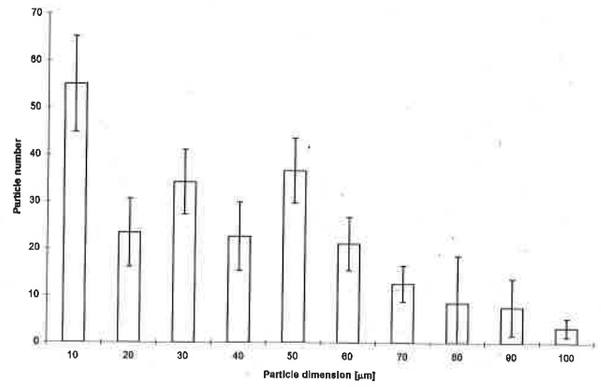

Figure 1 — Dimensional classification of glass particles at the opening of empty vials with OPC pre-breaking. Five units at a time were opened. Results represent the mean±SD of ten independent tests.

Table 1 — Opening force, F, values.

F(in Newton)	45.9±0.8
Fmax	57.1 N(70.0 N)
Fmin	37.4 N (30.0 N)

Besides, it is interesting to remark that vitreous particles of small diameter, but much longer than 25μm (acicular particle shape) are able to pass through the needle. Moreover, many are of coloured glass, deriving undoubtedly from the pre-breaking ring. This is important from a toxicological point of view, as well, if we think that the paints most frequently employed could be based on lead and cadmium[12,13].

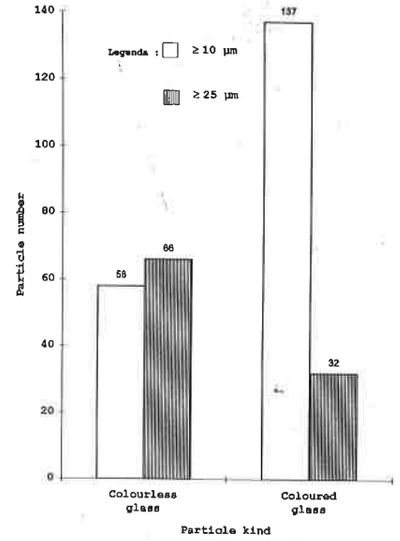

Figure 2 — Vials, with CB pre-breaking, containing WFI. Potentially *in vivo* injectable glass particles; no needle aspiration of contents. Two units at a time were tested. Results represent the mean of three independent tests (NOTE: SD very wide, so it is not reported).

We therefore thought it would be interesting to analyse the glass particles outgoing at the opening of vials cooled (+4°C) and heated (+40°C) for three days in a suitable thermostat, and manually opened immediately after having been taken out of the thermostat. The rationale of the test derives from the fact that a mild cooling might cause a slight negative pressure to occur within the vial and there might be an implosion of glass particles at the opening. On the other hand, mild heating might cause a slight overpressure to occur, and an explosion of particles would then be likely to take place. The results are reported in Table 2. Although the comparison of the results might show statistically significant differences the data do not follow a logical pattern. The great differences between one vial and another, rather than external factors or the method employed are the responsible for the results. We are running additional experiments and data analysis according to procedure indicated by the American Society for Testing and Materials in order to eliminate anomalous data[14].

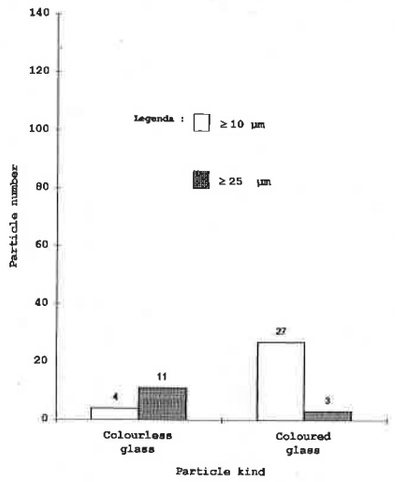

Figure 3 — Vials, with CB pre-breaking, containing WFI. Potentially *in vivo* injectable particles; content aspiration with needle for aqueous vehicle. Two units at a time were tested. Results represent the mean of three independent tests (NOTE: SD very wide, so it is not reported).

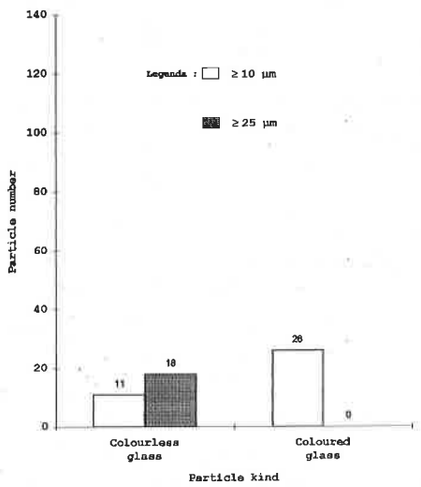

Figure 4 — Vials, with CB pre-breaking, containing WFI. Potentially *in vivo* injectable particles; content aspiration with needle for aqueous vehicle. Two units at a time were tested. Results represent the mean of three independent tests (NOTE: SD very wide, so it is not reported).

Table 2 — Glass particles generated by manual opening of ampoules after storage at 4 °C, 25 °C and 40 °C.

Glass particle dimension (μm)	Number of glass particles (mean ± SD) 4 °C	25 °C	40 °C
≥ 10	193.6±32.0	107.0±28.6	165.3±15.3
	p* = 0.025		p* = 0.036
	p = 0.239		
≥25	210±42.6	93.7±73.8	255.3±76.0
	p = 0.077		p = 0.057
	p = 0.418		

Note: Commercially available vials containing WFI. Type o pre-breaking: colour break. Nominal volume: 2 mL. Number of vials for each experiment: 2. Number of independent tests: 5. Statistical evaluation: Paired t-test.

However, these differences does not make the problem of secondary contamination due to vial opening any less important, and we believe it should be taken into the most serious consideration, as the results here reported clearly show.

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