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Particulate contamination from siliconized rubber stoppers

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Summary

Elastomeric closures are an essential packaging for parenteral drug products and they represent a possible source of particulate matter. The purpose of this study was to analyze the particulate contamination of siliconized rubber closures and to evaluate how different siliconization processes influence stopper quality. Sixteen types of rubber stoppers from the same manufacturer, differing in rubber formulation, shape, size, method and degree of siliconization were analyzed. Particulate analyses were performed on aqueous stopper eluates and on stoppers in the conditions of use, using two methods: light blockage and optical microscopy. The amount of silicone extractable from the closures was quantified by I.R. spectrophotometry. Results show that the siliconization method and the degree of siliconization can affect the particulate burden of pharmaceutical rubber closures.

Introduction

Elastomeric closures are an essential packaging for parenteral drug products; they represent a possible source of particulate matter. Therefore it is of great importance to minimize leaching of particulate from these closures.

Problems arising from the use of rubber stoppers are extensively discussed in the literature (Lachman et al., 1964, 1966; Hopkins, 1965; Boyett and Avis, 1975, 1976; Tchao et al., 1977; Beaumont et al., 1982; Danielson et al., 1983, 1984; Knapp et al., 1984; Preston and Anderson, 1984; Whyte, 1987; Townsend et al., 1989). They result from the complex composition of elastomeric materials and from closure stability with respect to treatments during product packaging.

Particles adhering to the surface of rubber closures can arise from manufacturing processes, from surface scratching during manufacturing and shipping, and from the environment. Therefore particles leached from rubber closures can originate from external (exogenous) or internal (endogenous) sources.

Exogenous particles may come from foreign material that accumulates on the stopper surface in the various manufacturing steps.

Endogenous particles are organic or inorganic substances. They originate from constituents of rubber formulations that migrate to the surface during moulding or during subsequent processing and storage. The chemistry of elastomeric materials is very complex and the number of substances constituting a 'rubber formulation' is quite high,

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even though it has been restricted by current legislation.

A careful cleaning of completed stoppers, in a particulate-controlled environment, is required by pharmaceutical companies and it is essential to minimize exogenous contamination. However, stoppers are usually siliconized after being cleaned, and this treatment can affect their quality (Anschel, 1977; Dean, 1985; Whyte, 1987; Doelcher, 1990; Verdegan et al., 1990). Siliconization can be an optional operation but a silicone film prevents the generation of rubber particles caused by friction and reduces stopper tackiness. However, silicone can migrate from the stoppers' surface into parenteral solutions, or can trap particulates which are then transferred to the solutions following shaking. This increased particulate burden in a parenteral solution is highlighted, above all, by instrumental counters (Coulter Counter, HIAC/ROYCO), since silicone droplets are not visible under the microscope because they pass right through the filter or get absorbed into the composition of the filter membrane itself (Groves, 1990). Silicone oil droplets are considered the same as particulate from the standpoint of contamination in parenteral products (Groves, 1990), and Italian regulations prescribe qualitative standards for particulate in large volume parenterals (Farmacopea Ufficiale della Repubblica Italiana, 1985). Therefore, the purpose of this study was to analyze the particulate contamination of pharmaceutical rubber closures arising from different siliconization processes.

Sixteen different types of rubber stoppers produced by the same manufacturer were analyzed. The stoppers differed in rubber formulation, shape, size, method and degree of siliconization. Siliconization was obtained by two different techniques: by adding silicone emulsion to the wet rubber stopper (type e) after the cleaning cycle, or by addition of silicone oil during the drying cycle (type o). Some types of stoppers were siliconized with silicone emulsion and silicone oil (types e + o).

Particulate analyses were performed, on aqueous stopper eluates and on stoppers in the conditions of use, using 2 methods: light blockage by an HIAC/ROYCO counter and optical microscopy by a polarizing research microscope. The amount of silicone extractable from the closures was quantified by I.R. spectrophotometry.

An evaluation of the different siliconization processes was made with respect to the particulate contamination levels determined.

Materials and Methods

(1) 16 types of siliconized chlorobutyl and bromobutyl stoppers with different degrees of siliconization, from less than 5 $\mu g/cm^2$ (level I) to a maximum of 40 $\mu g/cm^2$ (level V), and of different external diameter sizes (32 mm, 26 mm, 20 mm, 16 mm).

(2) Silicone oil used to siliconize the stoppers.

(3) Infusion bottles; glass type I, 250 ml, 100 ml volume (Bormioli Rocco, Italy).

(4) A HIAC/ROYCO model 3000 counter equipped with a HR120HA sensor (2–120 μm analysis range), flow rate 20 ml/min, and with a large sampler probe. The instrument was supplied by the manufacturer already calibrated with standard spherical latex.

(5) Optical microscope Laborlux Leitz model K with vertical incident illumination and ocular reticle.

(6) Gridded Millipore membrane filter (0.8 μm).

(7) I.R. spectrophotometer Perkin Elmer model 257 (wave number range 4000–625 cm^{-1}) equipped with a 0.05 mm thickness NaCl liquid cell, for liquid measurements.

(8) Carbon tetrachloride and isopropyl alcohol Carlo Erba R.P.E.

(9) Bi-distilled water filtered through 0.45 and 0.22 μm Millipore membrane.

The particulate control test, performed by the light blockage method, on the bi-distilled water, before the analyses on stopper eluate, always revealed less than 10 particles/ml ≥ 5 μm and no particles/ml ≥ 20 μm.

Sample preparation for particulate matter analysis

All sample preparation and filtration operations were performed under a laminar flow hood.

The samples were prepared using three different methods.

Method a. To highlight the global leaching from the materials, eluates in water were prepared from a number of stoppers equivalent to a surface of 100 cm^2.

The stoppers were inserted in a 500 ml Erlenmeyer flask, 200 ml of distilled water were added and the stoppers were soaked under continuous stirring for 15 min at room temperature. The procedure was replicated for each batch of stoppers.

Method b. To highlight the presence of silicone, eluates were obtained after soaking a number of stoppers equivalent to a surface of 100 cm^2 in a filtered solution of isopropyl alcohol 1% v/v for 15 min at room temperature. The alcohol solution was discarded, the stoppers were rapidly rinsed with 200 ml of bi-distilled filtered water, and finally 200 ml of bi-distilled water were added and the stoppers were treated as before (Method a). This procedure was replicated for each batch of stoppers.

Method c. To evaluate the influence of the autoclaving step on particulate leaching from stoppers, a number of stoppers equivalent to a surface of 100 cm^2 were inserted in a 500 ml Erlenmeyer flask and 200 ml of distilled water were added. All flasks were autoclaved for 0.5 h at 121° C and then analyzed at room temperature. This procedure resulted in such high particulate contamination values that the measurements made by light blockage apparatus were unreliable.

Hence the preparation method was modified: samples were prepared using infusion bottles previously autoclaved twice for 0.5 h at 121° C, to minimize leaching from glass. Each stopper was fixed with a metal ring on the top of an infusion bottle containing bi-distilled filtered water. The bottles were autoclaved for 0.5 h at 121° C. After autoclaving, each bottle was inverted 20 times at room temperature and analyzed for particulate leaching. Three stoppers were analyzed from each batch.

Analysis by HIAC / ROYCO

70 ml of eluate prepared by Methods a and b were withdrawn twice, from each flask, for analysis. The infusion bottles were directly analyzed. All samples (70 ml eluate or the whole bottle) were degassed using ultrasonication for 1 min and allowed to stand for 10 min before analysis. The analyses were performed, under continuous stirring, at the following size levels: ≥ 5 μm, ≥ 10 μm, ≥ 20 μm, ≥ 25 μm, ≥ 50 μm. Four readings of 10 ml each were made for each sample, discounting the first one. The contamination values detected for the eluate samples of each batch of stoppers were averaged out. The particulate matter results from the infusion bottles of each batch were also averaged out.

Analysis by microscope

Samples of 50 ml of eluate or sterilized water from the infusion bottles were filtered through a gridded Millipore membrane filter (0.8 μm), following U.S.P. XXII (1990). Filters were air-dried under a laminar flow hood and completely analyzed under the microscope with vertical incident illumination, at 100 ×. Analyses were performed on exactly the same stoppers as those examined by the light blockage method.

The particles were catalogued as: ≥ 10 μm–< 25 μm; ≥ 25 μm–< 50 μm; and ≥ 50 μm. Particulate matter results were averaged out using the same method as for those obtained by the light blockage method.

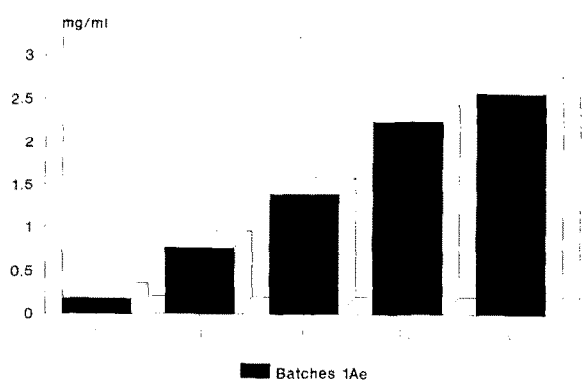

Fig. 1. Amount of silicone extracted from stoppers with different degrees of siliconization.

TABLE 1

Cumulative number of particles per cm² of stopper surface, detected by the light blockage method [a]

Batch no. [b]	≥ 5 μm	≥ 10 μm	≥ 20 μm	≥ 25 μm	≥ 50 μm
1A e I	909	124	4	1	0
1A e II	760	143	6	2	0.4
1A e III	591	133	5	1	0.3
1A e IV	2943	431	12	3	0
1A e V	1742	281	17	6	0.7
2A e IV	2014	321	7	1	0
2A e V	3073	474	26	10	0.7
3B e I	1855	294	23	10	0.6
1B e II	2518	424	14	5	0.3
4Ce + o III	4678	816	17	5	0.4
5De + o V	1875	360	13	4	1
6E o I	768	159	11	3	0.2
6E o II	622	69	2	1.1	0.1
6E o III	496	103	7	0.9	0.4
6E o IV	986	120	3	1.1	0.2
6E o V	982	122	2	1.1	0.5
2A	230	17	2	1	1

[a] Each figure is the mean of 4 samples.

[b] 1–6, type of stopper; A–E, rubber formulation; e, silicone emulsion; o, silicone oil; I–V, degree of siliconization.

Analysis by I.R. spectrophotometer

Analyses by I.R. spectrophotometer were performed at 1260–1270 cm^{-1} (polysiloxanes absorption wavelength) to detect the amount of silicone oil in the elastomeric closures.

Silicone was extracted from the closures as follows: a number of closures, corresponding to 100 cm^2 of surface area, were put into a beaker; 50 ml of CCl_4 were added and the stoppers were stirred for 2 min after which the solvent was poured into a second beaker and the stoppers were rinsed with another 25 ml of CCl_4. The two portions of solvent were collected and completely evaporated at 80 °C, taking care not to reach the solvent boiling point. The solution obtained, adding 2 ml of CCl_4 to the stoppers' extracts, was injected at room temperature into the I.R. cell. The whole spectrum was slowly scanned (to highlight all the possible absorptions), and the absorption of polysiloxanes was quantified at 1260–1270 cm^{-1}.

Silicone oil solutions of different concentrations in CCl_4 were used as standards. The quantitative determination of the silicone oil in the closures was made by mathematical interpolation of the standard curves.

Results and Discussion

Table 1 lists particle counts per cm^2 obtained by the light blockage method from eluates prepared with Method a. Particulate matter contamination because of bi-distilled water used has been subtracted in all measurements. Samples 6Eo, siliconized with silicone oil during the drying cycle, resulted in the cleanest stoppers, while the highest number of particles originated from sample 4Ce + o, siliconized with silicone oil and emulsion. Samples 1Ae showed on average higher contamination levels as the siliconization degree increased. Particle counts obtained by microscope analyses are listed in Table 2. The particulate contamination levels were extremely low and almost equivalent to those of control blanks. These results confirmed our initial hypothesis that the particulate contamination registered by the light

TABLE 2

Mean number of particles per cm² of stopper surface, detected by microscopy [a]

Batch no. [b]	≥ 10 μm < 25 μm	≥ 25 μm < 50 μm	≥ 50 μm
1A e I	1.7	0	0
1A e II	0	0	0
1A e III	0	0	0
1A e IV	0	0	0
1A e V	4	1.7	0
2A e IV	0	0	1
2A e V	0	0	0
3B e I	1.2	1.2	1
1B e II	3	1.2	1
4Ce + o III	0	0	1
5De + o V	0	0	0
6E o I	1.2	0	0
6E o II	3	1.2	1
6E o III	0	0	0
6E o IV	1.2	0	0
6E o V	1.7	1	6
2A	0	0	0

[a] Each figure is the mean of 4 samples.
[b] For explanation of symbols, see Table 1.

blockage method is mainly formed by silicone oil droplets not detectable by microscopy.

Table 3 lists the particle counts per cm² taken before and after rinsing with isopropanol (Method b). The data show that after isopropanol treatment the particulate matter values decreased between 70% and 97% for all siliconized stoppers. No change in contamination levels was noticed for non-siliconized stoppers (2A), showing that isopropanol treatment is not just rinsing but also acts as a silicone extraction method.

Table 4 lists the particle counts obtained by light blockage analysis on the stoppers in the conditions of use. The counts are given as mean cumulative measurements per ml so that they can be compared to the official standard limits for large parenteral solutions. In all counts particulate matter contamination because of the bi-distilled water used has been subtracted. This shows that the particulate burden increases with the degree of siliconization for stoppers siliconized with emulsion (samples Ae), while for particulate

TABLE 3

Particulate matter contamination detected by light blockage method before and after rinsing with isopropanol. Cumulative number of particles per cm² of stopper surface [a]

Batch no. [b]	≥ 5 μm	≥ 10 μm	≥ 20 μm	≥ 25 μm	≥ 50 μm
1A e III	591	133	5	1	0.3
	196	41	2	0.5	0.2
2A e IV	2014	321	7	1	0
	328	47	1	0.3	0.1
3B e I	1855	294	23	10	0.6
	215	31	0.6	0.3	0
1B e II	2518	424	14	5	0.3
	503	57	1	0.1	0
4Ce + o III	4678	816	17	5	0.4
	532	80	2	0.3	0
6E o V	982	122	2	1	0.5
	168	26	1	0.5	0
2A	230	17	2	1	1
	224	16	2	1	1

[a] Each figure is the mean of 4 samples.
[b] For explanation of symbols, see Table 1.

TABLE 4

Particles leached from a single stopper after sterilization. Cumulative counts / ml of solution determined by the light blockage method [a]

Batch no. [b]	≥ 5 μm	≥ 10 μm	≥ 20 μm	≥ 25 μm	≥ 50 μm
1A e I	24	1.5	0	0	0
1A e II	83	5	0.1	0	0
1A e III	160	12	0.5	0	0
1A e IV	192	28	2	0.5	0
1A e V	688	80	6	2.5	0.3
2A e IV	57	2.4	0	0	0
2A e V	233	16	0.7	0.4	0
4Ce + o III	296	18	0.2	0	0
6E o I	101	8	0.3	0	0
6E o II	79	6	0.5	0.2	0
6E o III	77	5	0.2	0	0
6E o IV	85	6	0.2	0	0
6E o V	107	6	0.1	0	0
2A	26	1.6	0	0	0
F.U. IX	100		4		

[a] Each figure is the mean of 3 samples.
[b] For explanation of symbols, see Table 1.

matter from closures treated with silicone oil (samples Eo) it is independent of their degree of siliconization. Stoppers siliconized with emulsion are generally more contaminated than those siliconized with oil, and above degree III they greatly exceed F.U. (1985) standard limits. In the conditions of use, the stoppers siliconized with silicone oil were on average the cleanest at every siliconization degree analyzed. This is in keeping with the results of Table 1 even if results are not directly comparable. Table 5 lists the contamination levels obtained with microscopy for the same samples as Table 4. Even in these conditions, counts are extremely low if compared with those of the light blockage method.

Table 6 lists the silicone oil concentrations (mg/ml) determined by I.R. spectrophotometer. The amount of silicone extracted seems to be related to the siliconization degree, but also depends on the type of closure (rubber formulation and shape), and type of siliconization.

I.R. analysis did not detect any leaching of silicone oil from stoppers siliconized with oil (6Eo) during the drying cycle.

The histogram in Fig. 1 shows that, for the same type of closures (1Ae), the amount of silicone oil extracted increases with the degree of siliconization.

TABLE 5

Particles leached from a single stopper after sterilization. Mean counts / 50 ml of solution, detected by microscopy [a]

Batch no. [b]	≥ 10 μm < 25 μm	≥ 25 μm < 50 μm	≥ 50 μm
1A e I	48	7	4
1A e II	20	3	3
1A e III	11	2	1
1A e IV	156	15	3
1A e V	150	18	3
2A e IV	7	2	1
2A e V	26	1	0
1B e II	19	3	1
4Ce + o III	12	0	0
6E o I	3	0	0
6E o II	38	7	3
6E o III	10	1	0
6E o IV	38	6	1
6E o V	30	4	3
2A	3.7	0.44	0.16
U.S.P. XXII	50	5	

[a] Each figure is the mean of 3 samples.
[b] For explanation of symbols, see Table 1.

TABLE 6

Amount of silicone detected by I.R. spectrophotometer

Batch no. *	mg/ml
1A e I	0.175
1A e II	0.761
1A e III	1.392
1A e IV	2.248
1A e V	2.563
2A e IV	1.662
2A e V	3.464
3B e I	0.004
1B e II	0.176
4C e + o III	8.915
5D e + o IV	9.185
6E o I	0
6E o II	0
6E o III	0
6E o IV	0
6E o V	0

* For explanation of symbols, see Table 1.

Conclusions

The investigation shows that the siliconization process greatly affects particulate contamination leached from pharmaceutical rubber stoppers. Particulate contamination leached by closures after sterilization appears to be a good parameter with which to indicate a correct siliconization process. Hence the instrumental analysis (HIAC/ROYCO) of particulate matter coming from closures in the conditions of use (closure fixed on the bottle), is the method giving the best indications on the actual contamination of the product and on the merits of the siliconization process.

The lowest results in terms of particulate burden are obtained by addition of silicone oil during the drying cycle; low contamination levels, even at high siliconization degrees, can be obtained by this process.

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