

Analysis of particulate contamination in ampoules using a light blockage particle analyser

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A method of opening ampoules without introducing particles has been developed and the level of particulate contamination in a number of ampoule solutions using a light blockage particle analyser (HIAC) has been determined. Low levels of contamination were found and a method of setting limits of particulate contamination in ampoules is suggested.

Standards limiting the level of particulate contamination in large volume parenterals (LVPs) have been in operation for many years (BP 1973, 1980; USP 1980) but there are no official standards for small volume parenterals (SVPs).

We report an initial investigation into a method of setting such standards. It was undertaken in two phases; in phase A experiments were carried out to: (i) identify the nature of the contaminant to determine which of the two materials, AC Fine Test Dust (ACFTD) or latex spheres, should be used to calibrate the light blockage particle analyser. ACFTD is the material of choice for contaminants such as dust and other non-organic materials, while latex spheres should be used for undissolved, precipitated or crystallized ingredients of a formulation (Lieberman, A., personal communication). (ii) Devise a method of opening ampoules without introducing contamination. Many particles are generated in the snap-opening of ampoules (Katz et al 1973; Davies & Smart 1982; Tsuji & Lewis 1978) and it is necessary to exclude them for the purposes of control of the solution.

The results obtained were used in phase B to analyse different ampoule solutions.

MATERIALS AND METHODS

A. Preliminary experiments

I. Seventy-five 2 ml ampoules were washed in detergent and rinsed twice with twice filtered water in a 100 W cleaning power ultrasonic cleaner; they were rinsed twice more in the Laminar Air Flow cabinet using a filter gun fitted with a 0.22 μm membrane or cartridge type filter. These ampoules were randomly assigned to 3 groups. Group 1 was left unsealed. Group 2 was sealed and opened using a single flame ampoule sealer (as follows: the flame was directed to a few mm below the ampoule tip until

the pressure inside the ampoule caused the glass to balloon and burst. Heat was maintained on this spot, thus increasing the size of the hole until the glass was sufficiently molten to flip the tip back using particle-free forceps.)

Group 3 was sealed, rinsed twice with filtered water, dried using particle-free dichlorodifluoromethane and file-opened at the neck. In another experiment, ampoules of 10 ml water for injection supplied by a local manufacturer were also opened using the flame method.

A HIAC PC 320 particle analyser, linked with a CMB 60 Sensor (range 1–60 μm) which had been calibrated with both latex spheres and ACFTD, was set at the channels shown in Table 1. In the small particle size range, the particle appears larger when the calibration material was spheres rather than ACFTD; but at $\pm 2.5 \mu\text{m}$ the position was reversed, the particle appearing larger when ACFTD was the calibration material (Fig. 1).

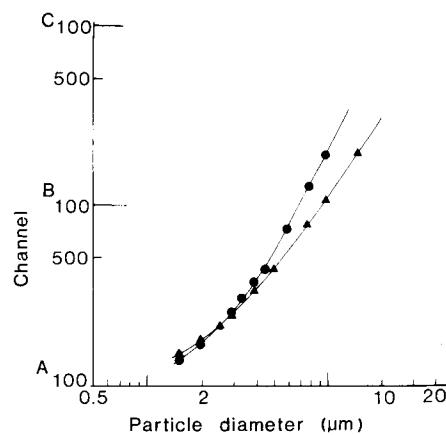


FIG. 1. Calibration curves of CMB 60 Sensor using latex spheres and ACFTD.

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Table 1. Particle size analysis of ampoules of filtered water and water for injection.

HIAC channel number	Particle size μm calibration method		Particle counts ml^{-1}							
			2 ml ampoules filtered water (1 ml samples)						10 ml W.F.I. (5 ml sample)	
	ACFTD	Sphere	Group 1 unsealed		Group 2 flame-opened		Group 3 file-opened		Group 4 flame-opened	
			\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s
A 175	2	2.2	22	(17)	5	(6)	500	(647)	9	(7)
235	3	2.8	16	(14)	3	(5)	365	(473)	5	(4)
315	4	3.15	12	(11)	3	(3)	252	(338)	3	(2)
410	5	4.2	9	(9)	2	(2)	177	(249)	2	(2)
620	7	5.2	6	(6)	1.5	(2)	92	(135)	1	(1)
B	10	7.4	3	(3)	1	(1.5)	36	(50)	1	

II. Twenty 2 ml ampoules, a 5 ml syringe and a 250 ml beaker were washed and rinsed as described in I. A 20% solution of potassium chloride was prepared, twice filtered through a 0.22 μm membrane and the filtrate collected in the beaker. Two 2 ml ampoules were filled from each syringe of filtrate and assigned to two separate groups. One group was left unsealed and one group sealed and opened using the flame method. A particle size analysis covering a wider size range was made on the ampoule solutions.

III. One of a rejected batch of potassium chloride 20% ampoules was filtered through a 0.22 μm membrane filter which had been rinsed with particle-free water. The membrane was dried, examined using a Scanning Electron Microscope (SEM) and the particle subjected to Energy Dispersion X-ray (EDAX).

B. Analysis of particulate contamination in ampoules
Five ampoules from the following batches were tested using the flame method of opening and the latex sphere calibration curve:

10 ml ampoules potassium chloride 20% from a batch rejected by manufacturer.

10 ml ampoules sodium chloride 20% from a batch rejected by manufacturer.

10 ml ampoules potassium chloride 20%.

10 ml ampoules potassium chloride 15% supplied by Cape Provincial Administration Quality Control Laboratory. Also tested were single ampoules of Calcium chloride 10% (10 ml), Sodium bicarbonate 4% (20 ml), and absolute alcohol (50 ml) provided by C.P.A. Quality Control Laboratory. Although results from only one ampoule are suspect they do give an indication of the level of particulate contamination.

RESULTS AND DISCUSSION

I. Table 1 shows that no particles are introduced into the ampoule when it is flame-opened. The slightly higher level of contamination in group 1 compared with groups 2 and 4 could be due to particles being detached from the tip of the ampoule when the needle was inserted. Heat fuses any particles onto the flame-opened ampoules.

II. Although the heat of the flame evaporated the film of liquid at the tip of the ampoules, leaving a deposit on the glass, the results in Table 2 prove that no particles fell into the solution during the flame-opening procedure.

Table 2. Particle size analysis of sealed and unsealed ampoules of potassium chloride 20%.

HIAC channel number	Particle size μm Calibration method		Particles per ml^{-1}			
			Unsealed ampoules		Sealed and flame-opened ampoules	
	ACFTD	Sphere	\bar{x}	s	\bar{x}	s
A 175	2	2.2	16.0	(5.7)	12.9	(6.7)
410	5	4.2	3.4	(2.6)	2.8	(2.9)
B 105	10	7.4	0.5	(0.8)	0.6	(0.8)
195	15	10.0	0.2	(0.4)	0.0	
450	25	15.0	0.0		0.0	
C 135	50	25.0	0.0		0.0	

III. The only particles found to be present on SEM examination were proved to be potassium chloride by EDAX. Therefore the latex sphere calibration curve was used for further work.

The results in Table 3 show that the rejected batch of 20% potassium chloride had a very high level of contamination and although the rejected batch of 20% sodium chloride was not as heavily contaminated it did contain a greater number of particles than the other ampoule solutions. There was considerable variation among the individual ampoules.

In setting standards for particulate contamination in large volume parenterals van Wyk & Goossens (1980) introduced the Z value. This was an extension of the S value (Groves 1969) and C Index (Groves & Wana 1977) and it depended on the intercept of the slope of the log log graph of particle size vs. cumulative number of particles. Any large volume parenteral solution with a value higher than 0.4 should be rejected.

The results in Table 3 were therefore used to prepare the log log graphs and these confirmed previous findings, using the HIAC particle analyser, that: (a) the graph tailed off in the lower particle size range (Johnston & Swanson 1982; Alexander 1982), (b) counts of less than 10 per ml frequently deviated from the straight line (Alexander 1982) (Fig. 2).

Taking these factors into account, the Z values of the ampoule solutions were calculated and it was found that the rejected batch of potassium chloride had a value of 0.88 which was well above the accepted range and therefore failed, whereas the passed batch had a value of 0.4 which was just acceptable. The rejected batch of sodium chloride 20% ampoules had a Z value of 0.47 and would also have failed.

From application of the same standard to the ampoules from Cape Town Quality Control Laboratory, it can be seen (Table 3) from the low Z values

that the potassium chloride 15% and sodium bicarbonate 4% ampoules had very low levels of contamination. Calcium chloride 10% and absolute alcohol were more heavily contaminated but their Z values still fell within the limit recommended for LVPs.

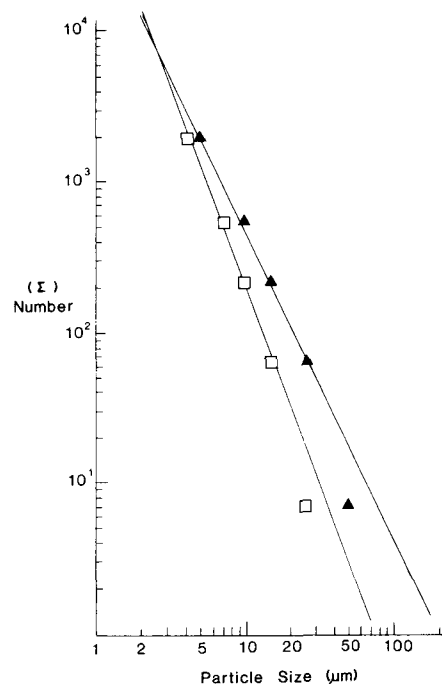


Fig. 2. Particle contamination in rejected potassium chloride ampoules.

In view of the small volumes introduced into the body and the different routes of administration it is reasonable to maintain a flexible approach when setting limits for particulate contamination in SVPs. For this reason it is necessary to examine a large variety of formulations to discover where the problem areas lie and we are presently extending the

Table 3. Particle size analysis on ampoule solutions.

Particle size μm Latex sphere calibration	Particles ml^{-1} ampoule solution										
	5 ampoules KCl 20% Rejected		5 ampoules KCl 20% Passed		4 ampoules NaCl 20% Rejected		5 ampoules KCl 15%		1 ampoule Na_2HCO_3 4%	1 ampoule CaCl_2 10%	1 ampoule absolute alcohol
	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s			
2.2	5123	(1138)	374	(173)	779	283	57	(24)	20	162	243
4.2	1907	(237)	102	(49)	223	68	13	(4)	7	43	79
7.4	529	(45)	21	(8)	72	37	2.5	(0.6)	3	6	19
10.0	217	(24)	6	(1.5)	30	28	1	(0.4)	2	1	5
15.6	64	(19)	1.2	(0.2)	14	26	1		1	1	1
25.0	7	(4)	1		12	26	0.1		1	0	1
Z Values	0.88		0.40		0.48		0.11		-0.64	0.33	0.39

scope of our study in this direction. The preparations tested in this preliminary study were simple solutions with low levels of particulate contamination for which the *Z* value proved a useful parameter. However Taylor & Spence (1983) found high levels of particulate contamination in several proprietary preparations.

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