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Particulate contamination of Australian ampoules

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Theoretical predictions and computer simulations indicate that it may be impossible to achieve values of the coefficient of variation usually found in large volume parenterals (LVP) when the intrinsic particulate contamination in small volume parenterals (SVP) is investigated. Snap-opened ampoules from Australian manufacturers containing 5, 10 or 20 ml Water for Injections or Sodium Chloride Injection had a high level of particulate contamination, although within the USP XXI SVP limits. Heat-opened ampoules had much lower levels of contamination which were generally below the official LVP limits. Counts ml^{-1} were typically <10 and <1 for 5 and 20 μm particles, respectively. Coefficients of variation of the 5 μm data from an ampoule in any batch examined, typically ranged over 30–70%. Statistical analysis of the 5 μm data indicates significant differences between batches. Occasional ampoules had higher 5 μm counts than others in the same batch. At no time were the particulate contamination levels considered to be clinically important.

Since Garvan & Gunner (1963) first highlighted the problem of particulate contamination of parenteral products, there has been considerable interest in the cleanliness of large-volume parenterals (LVP). Particulate matter, inadvertantly administered along with the LVP solution, has been implicated in the formation of foreign body granulomae, phlebitis, thrombophlebitis, platelet aggregation, infarction and pulmonary emboli. Robinson et al (1984) have recently shown that LVP on the US and UK markets can cause a progressive increase in coronary vascular resistance when a LVP is infused intracoronarially.

Particulate contamination standards exist for LVP in both the British Pharmacopoeia (BP) and the United States Pharmacopeia (USP). The National Biological Standards Laboratory (NBSL) in Australia has issued a draft standard which the local industry accepts. These standards represent a compromise between the clinically ideal zero level of contamination and the practical, achievable limits on routine cleanliness of manufacture. Table 1 summarizes these LVP standards.

Table 1. Upper limit on particles ml^{-1} for LVP. ($\bar{X}$ = mean; S = std. dev.)

	BP 1980	USP XX/XXI	NBSL	
	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}+2S$
≥ 2 μm	1000	—	—	—
≥ 5 μm	100	—	100	200
≥10 μm	—	50	—	—
≥20 μm	—	—	2	4
≥25 μm	—	5	—	—
Visible particles	0	0	—	—

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The linking of the standard deviation with the mean by NBSL is an attempt to produce a standard which stipulates a distribution for permitted contamination rather than a point standard, which is a simple cut off point. There are a number of other unofficial ways of characterizing and limiting the extent of particulate contamination in LVP (Blanchard et al 1977; Hailey et al 1982).

The particulate contamination of small-volume parenterals (SVP) has only recently been considered in detail. It is well known (Davies & Smart 1982) that snap-opening of ampoules leads to contamination of the contents with glass fragments. The USP XXI has for the first time included a monograph on the particulate limits for SVP. These limits have been set on a 'per container' basis to provide a maximum particulate load administered to a patient, assuming all the contents of the container is administered. Table 2 shows these limits on a 'per ml' basis for two extreme SVP situations. As the size of the SVP decreases, it becomes increasingly more contaminated when compared with acceptable LVP products.

Whilst studies have been reported on the intrinsic particulate contamination of ampoules on the US, South African and UK markets (USP Pharm.

Table 2. USP XXI SVP particulate contamination limits calculated on a 'per ml' basis.

Product	Volume (ml)	≥10 μm	≥25 μm	Ratio of USP SVP/LVP limits
1	100	100	10	2
2	2	5000	500	100

Forum 1983; Alexander & Veltman 1985; Taylor & Spence 1983), no detailed study has been published on those of Australian manufacture. We present such a study and compare the results to the standards for LVP and to the standard for SVP in USP XXI. A critical evaluation of the method used to obtain and interpret these results is also presented.

METHODS

Ampoules of Water for Injections and Sodium Chloride 0·9% were investigated in the sizes of 5, 10 and 20 ml. Ampoules for investigation were randomly selected from batches within the stock commercially purchased by the hospital for clinical use. A sufficient number of ampoules were selected so that a minimum of 100 determinations of particulate contamination could be recorded on each batch, provided always that at least 10 ampoules were included. For the smaller ampoule sizes such as 5 ml, 20 to 25 ampoules were required to ensure at least 100 readings were obtained. Four different batches of each ampoule size from each manufacturer were investigated.

The ampoules were opened either by the usual clinical snap procedure or by the application of heat. The snap-opened ampoules were cleaned before opening in a laminar flow cabinet using the principles described by de Luca et al (1980).

The heat opening procedure is a variation of the gas torch method developed by Tsuji & Lewis (1978). The ampoules were shaken for 30 s and, whilst in the laminar flow cabinet, the small pointed flame from the torch was applied directly to the ampoule wall at a part about 5 mm from the base. Increasing internal pressure caused the melting glass to deform into a bubble which exploded to create a 2–4 mm diameter hole. The ampoules were then placed vertically in a cleaned stainless steel grid stand in a cleaned 2000 ml glass vacuum dessicator. The products were degassed at about 50 kPa absolute pressure for 4 min. Air was returned to the dessicator through a 0·22 μm Millex filter.

A HIAC 420 light blockage counter connected to a CMB 60 sensor was used to determine the extent of particulate contamination of each ampoule. A 20G stainless steel needle had its Luer fitting removed and the resultant 8 cm long tube was inserted into the end of the plastic probe of the sensor. The needle and sensor combinations were thoroughly cleaned with 0·22 μm filtered Water for Injections until no contamination was recorded. The sensing equipment was similarly cleaned between batches of ampoules.

The liquid flow rate through the sensor was established at 8 ml min^{-1}. A 20 litre container was connected downstream from the sensor. Its internal pressure was reduced by a Bell & Gossett 30 litre min^{-1} vacuum pump and the pressure adjusted by controlled bleeding of air into the container so that it was held at about 15 kPa below atmospheric pressure. This was the pressure required to give the 8 ml min^{-1} flow rate through the sensor.

The counter settings were adjusted to correspond to 5 and 20 μm and the number of particles equal to or greater than these sizes were counted in aliquots of 0·66 ml over 5 s.

All ampoules were placed in the laminar flow hood. The snap-opened ampoules were held vertically in a cleaned stainless steel grid and the needle inserted so that it reached to within 1·0 mm of the base of the ampoule. The heat-opened ampoules were initially held horizontally so that the contents were not in contact with the hole. The needle was placed in the hole. As the readings progressed, the top of the ampoule was slowly raised until the ampoule was at approximately 10° to the horizontal. At no stage did the contents come into contact with the hole. However, the liquid in the top of the ampoule readily drained into the body of the ampoule and was withdrawn into the sensor.

RESULTS AND DISCUSSION

Table 3 compares the contamination found in 20 ml Water for Injections ampoules which were either heat- or snap-opened in the laminar flow cabinet.

Table 4 lists the contamination found in heat-opened Water for Injections ampoules from a number of batches made by the three Australian manufacturers of such ampoules.

Table 5 lists the contamination found in heat-opened Sodium Chloride 0·9% ampoules.

All the results in Table 3–5 were derived from particle counts in an 0·66 ml aliquot over a 5 s sampling period. The coefficient of variation (CV_X) of our results is defined by

$$CV_X = 100s/\bar{x} \quad (1)$$

where s is the standard deviation and $\bar{x}$ is the mean.

Whilst we used a convenient size and time for our work, other people may choose a different sampling procedure. The volume used to sample the contents of the ampoules was chosen for experimental convenience. We had to achieve a reasonable balance between having a large enough sample volume to ensure we counted some particles most of the time and having enough sampling points, particularly from the small 5 ml ampoules. At a flow rate of

Table 3. Contamination per ml in ten heat-opened and ten snap-opened 20 ml Water for Injections ampoules (David Bull Laboratories, DBL, Batch B067605).

	≥5 μm		≥20 μm	
	$\bar{X}$	$\bar{X}+2S$	$\bar{X}$	$\bar{X}+2S$
Heat-opened	6·68	13·67	0·29	1·64
Snap-opened	192	488	0·89	3·17

Table 4. Contamination per ml in ten heat-opened ampoules from each batch of Water for Injections.

Size	Manufacturer	Batch	≥5 μm $\bar{X}$	≥5 μm $\bar{X}-s2S$	≥20 μm $\bar{X}$	≥20 μm $\bar{X}+2S$
5 ml	Astra	1580/1	5·90	10·07	0·23	1·31
		1341/1	5·58	9·57	0·15	1·05
		1580/2	2·31	7·74	0·69	2·88
		1598/1	6·57	18·51	0·95	3·89
	Boots	8TT	8·39	12·23	0·15	1·05
		56RR	8·43	11·76	0·20	1·22
		54RR	5·04	9·42	0·21	1·41
		16TT	4·79	8·30	0·15	1·05
	DBL	B167603	3·90	7·17	0·12	0·93
		B177603	5·40	9·48	0·12	1·02
		C047603	3·87	7·89	0·15	1·05
		C077603	5·73	9·36	0·29	1·49
10 ml	Boots	27SS	6·87	11·40	0·20	1·34
		19TT	6·53	10·13	0·18	1·38
		42TT	4·82	8·54	0·09	0·90
		56TT	8·91	14·79	0·14	1·01
	DBL	C077604	5·40	9·42	0·17	1·22
		C097604	5·00	9·35	0·12	1·02
		C127604	5·36	10·37	0·17	1·19
		C137604	5·90	11·30	0·12	1·05
20 ml	Boots	19MM	8·03	19·13	1·17	5·85
		46RR	3·72	7·95	0·14	1·07
		64SS	3·36	7·38	0·09	0·78
		37SS	3·87	7·53	0·12	1·05
	DBL	B077605	5·94	13·38	0·98	4·43
		B047605	4·94	8·72	0·18	1·53
		A027605	8·07	14·28	0·63	3·15
		B067605	6·68	13·67	0·29	1·64

Table 5. Contamination per ml in ten heat-opened Sodium Chloride Injection ampoules.

Size	Manufacturer	Batch	≥5 μm $\bar{X}$	≥5 μm $\bar{X}+2S$	≥20 μm $\bar{X}$	≥20 μm $\bar{X}+2S$
5 ml	Boots	81SS	6·92	14·96	0·68	2·57
		16SS	9·60	22·41	0·63	2·55
		86WW	15·83	41·42	1·49	5·78
		103VV	11·45	38·66	2·09	11·18
	DBL	C096153	4·38	12·36	0·38	1·88
		C106153	2·24	7·04	0·47	2·03
		C146153	3·04	9·11	0·63	2·64
		C116153	3·35	9·53	0·60	2·79
10 ml	Boots	52TT	2·93	8·12	0·26	1·58
		69TT	2·79	7·41	0·44	2·66
		31VV	2·99	8·30	0·60	3·15
		24WW	5·55	17·46	1·13	4·58
	DBL	C166154	3·18	9·15	0·15	1·05
		C186154	4·46	14·54	0·38	2·06
		C196154	2·16	5·83	0·23	1·31
		C206154	2·40	6·84	0·12	1·05
20 ml	Boots	82TT	3·23	8·78	0·42	2·73
		44VV	2·04	5·79	0·14	1·10
		80TT	2·10	7·77	0·36	2·64
		53VV	4·83	16·95	1·40	6·86
	DBL	C046155	3·50	7·82	0·14	1·04
		C066155	6·48	12·30	0·32	1·76
		C056155	2·64	6·63	0·32	1·97
		C086155	3·66	9·60	0·42	2·37

8 ml min^{-1} through the sensor, the volume sampled corresponds to an elapsed time of 5 s. The sampling size is not a characteristic of the HIAC 420. There will be a different CV for each experiment on the same product depending on the sampling procedure.

The effect of sampling duration (aliquot volume) on the CV of the particle count can be predicted as follows. For n independent random variables $X_1, X_2 \ldots X_n$, the mean and variance of their sum is given by

$$\mu_{X_1+X_2+\ldots+X_n} = \mu_{X_1} + \mu_{X_2} + \ldots + \mu_{X_n} \quad (2)$$

$$\sigma^2_{X_1+X_2+\ldots+X_n} = \sigma^2_{X_1} + \sigma^2_{X_2} + \ldots + \sigma^2_{X_n} \quad (3)$$

If the variables $X_1, X_2, \ldots X_n$ have a common mean (μ) and variance (σ^2), equations (2) and (3) become

$$\mu_{X_1+X_2+\ldots+X_n} = n\mu \quad (4)$$

$$\sigma^2_{X_1+X_2+\ldots X_n} = n\sigma^2 \quad (5)$$

Let $X_1, X_2, \ldots X_n$ represent the particle count for 0·66 ml aliquots drawn from a container using 5 s sampling periods, the count coefficient of variation (CV_{nX}) of a continuous sampling period of 5n seconds is obtained from equation (4) and (5)

$$CV_{nX} = CV_{CX_1+X_2+\ldots+X_n} = CV_X/\sqrt{n} \quad (6)$$

So a log–log plot of CV_{nX} versus n has an intercept of CV_X and a slope of −0·5. Hence equation (6) could be used to compare results obtained under different sampling procedures.

An examination of the results in Tables 4 and 5 shows that for the ≥5 μm size results, a representative mean particle count could be 5 with a representative standard deviation of 2·5. Hence the CV for this single 5 s sampling period is 50%.

To test the applicability of equation (6), Monte Carlo simulation was used. Sets of particle counts were generated, similar to those measured experimentally, using

$$X = \text{absolute [integer (Z)]}$$

where Z is normally distributed with $\mu = 5$ and $\sigma = 2{\cdot}5$. This distribution has a value of $CV_X = 48{\cdot}5\%$, determined by simulation. The coefficient of variation of linear combinations of the sets (CV_{nX}) was calculated for various n. Fig. 1 shows that the theoretical equation (6) and the results of the sampling simulation are in close agreement. Hence, using equation (6), it will be a simple matter for any controller of quality to predict what sample volume is required to achieve any CV, given the results of a trial experiment using a small sampling volume. It is also equally clear that for small volume parenterals, there may be insufficient volume in any given unit ampoule to enable a predetermined relatively low

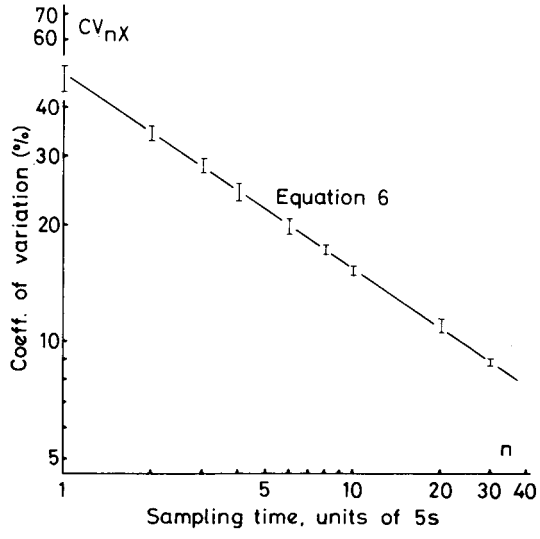

FIG. 1. Monte Carlo simulation to show the effect of sampling time (aliquot volume) on the count CV. The line represents equation 6 drawn through the intercept CV_X = 48·5 and the error bars the range of eight values of CV_{nX} resulting from the simulation.

CV to be obtained without combining the contents of ampoules. Such a combination would be hard to do without further contaminating the product.

Table 3 shows that snap-opened Australian ampoules are more heavily contaminated than heat-opened ampoules. The adoption of special cleaning procedures and care with the snapping as well as opening the ampoules in a laminar flow cabinet does not ensure that the opened ampoules have a low level of contamination. In a clinical setting, the extent of contamination found in snap-opened ampoules is likely to be even higher. In agreement with the report by Taylor & Spence on UK products (Taylor & Spence 1983), the snap-opening procedure can lead to the product failing the LVP standards shown in Table 1. Much of the variation in the contamination level reported in the literature is on snap-opened ampoules.

However, the heat-opened product had a greatly reduced level of contamination. This extent of contamination is the sum of the inherent contamination of the product and that introduced in the heat-opening procedure. One of the manufacturers allowed us to view the particulate contamination data from the bulk, filtered water used to fill their products in Tables 4 and 5. The two estimates of contamination were very similar. So we believe that our heat-opening procedure contributes very little to the particulate load of the product. Hence, the heat-opened ampoules have no trouble in meeting the LVP standards. Our procedure has an advantage over that of Alexander & Veltman (1985) in that we did not use forceps or probes to open up the hole.

The increase in contamination upon snap-opening the glass ampoules means that such containers are unsatisfactory for use in the clinical setting. The large variation in the particulate level reported in literature on snap-opened ampoules reflects the variation in skill in opening the ampoule coupled with the variation in the quality and properties of the glass ampoules rather than problems in the manufacture of the ampoules. Certainly the imposition of the LVP standards to SVP is illogical if SVP continue to be marketed in containers producing gross contamination upon opening. However, the heat-opening procedure is not routinely possible in the clinical setting, nor can it be used for ampoules with a high solute content (e.g. Potassium Chloride 20% Injection) or for those containing a thermally unstable solute (bicarbonates or vitamins).

Tables 4 and 5 show that Australian manufacturers are able to make the liquid contents of a variety of ampoules with very low levels of particulate contamination. The counts used to generate the summary 5 μm results presented in Table 4 for the 20 ml Water for Injections and in Table 5 for the 5 ml Sodium Chloride Injection, were individually subjected to analysis of variance investigation using the statistical package MINITAB.

For the 20 ml Water for Injections data, we first showed, using a 2 way ANOVA (Table 6), that the sampling had no time-dependent effects. This permits the data to be treated as replicates. Experimentally this means that particle settling during the analysis of any individual ampoule is not a problem. The gentle movement of the liquid down the ampoule as the analysis proceeds would assist in maintaining a uniform particulate dispersion.

In the five batches indicated in Table 6, there is a significant difference in the level of contamination between the ampoules. These tend to be the more highly contaminated batches. The batch labelled b in Table 6 had all the large outlying counts for that manufacturer. We believe that our opening procedure contributes little to the particulate load and any such contribution should randomly occur in each ampoule. So the ampoule variation is probably due to a small increase in background contamination in those ampoules.

A two-way ANOVA with replication (Model II) was performed on the four batches from the two manufacturers. The results, given in Table 7a/b,

Table 6. Two-way ANOVA effect of sampling time using 10 ampoules (≥5 μm, 20 ml Water for Injections data used).

Batch	Sampling time F value	*P*	Between ampoules F value	*P*
B027605[a]	0·91	>0·25	2·07	<0·05
B077605[a]	0·87	>0·25	4·24	<0·001
B067605[a]	0·67	>0·25	2·97	<0·01
B047605	0·50	>0·25	1·57	>0·1
B19MM[a,b]	1·20	>0·25	11·9	<0·001
B37SS	0·71	>0·25	0·99	>0·25
B46RR[a]	1·08	>0·25	4·43	<0·001
B64SS	1·17	>0·25	1·03	>0·25

[a] Indicates that the levels of contamination in the ampoules sampled in that batch are significantly different.
[b] Indicates some ampoules from that batch had all the outlying counts from that manufacturer.

Table 7. Two-way ANOVA with replication (Model II), variation between ampoules and batches (≥5 μm, 20 ml Water for Injections data used).

Source	DF	SS	MS	F	*P*
		(a) Bulls data			
Ampoules	9	93·82	10·42	2·57	<0·03
Batches	3	546·89	182·30	45·0	<0·001
Interaction	27	109·39	4·05	1·58	<0·04
Error	360	920·30	2·56		
Total	399	1670·40			
		(b) Boots data			
Ampoules	9	186·05	20·67	1·40	≈0·24
Batches	3	593·57	197·86	13·4	<0·001
Interaction	27	399·02	14·78	4·36	<0·001
Error	400	1356·73	3·39		
Total	439	2535·37			

show significant differences between the batches for each manufacturer ($P < 0·001$). The ampoule–batch interaction was also significant for both manufacturers, due to the higher levels of contamination in certain ampoules from a particular batch. For example, the sample from batch 19MM (Boots) contained 3 ampoules with significantly higher levels of particulate contamination (see Tables 4, 6). However, it is emphasized that *none* of the products from any manufacturer had 5 μm contamination levels which might cause rejection under any of the criteria summarized in Table 1.

Very similar conclusions were drawn from the same analysis of the data for 5 ml Sodium Chloride Injection (Table 5). For both manufacturers, the sampling had no time-dependent effects, permitting the data to be treated as replicates. There was a significant difference in the level of contamination between all the ampoules, and also between the batches from each manufacturer, although the contamination in all batches was low. The ampoule–batch interaction was significant for both manufacturers.

It is appropriate to consider these results in terms of the USP XXI SVP limit test. If we take the worst case for a 20 ml Water for Injections ampoule of 10 particles > 5 μm ml^{-1}, there would be 200 particles > 5 μm in the ampoule. Similarly, there would be about 30 particles > 20 μm in the 20 ml ampoule. Before the USP XXI SVP standard is exceeded, two or more orders of magnitude of particles of each size would have to be introduced by the snap-opening procedure. In our experience this is very unlikely to occur. Examination of Tables 4 and 5 also shows that the products tested generally met the stringent NBSL LVP standard given in Table 1. Hence we conclude that Australian SVP products are being manufactured to a very high standard. The glass ampoules used are unlikely to contribute sufficient contamination to cause the opened ampoules to fail the USP XXI SVP limit test. The addition of multiple lots of opened ampoules to LVP is not recommended practice in Australian hospitals and hence any such particulate contamination problem, leading to the 1/5 rule as advocated by the USP, is a theoretical one. Consequently we believe that the USP XXI test will not be useful to the Australian manufacturer, the user or the recipient of such products.

CONCLUSIONS

Computer simulations show that it may be impossible to achieve values of coefficient of variation usually found in LVP when the intrinsic particulate contamination in SVP is investigated. The relationship between the CVs obtained using different sampling times (aliquot volumes) is given by equation 6. The validity of this equation was demonstrated by Monte Carlo simulation.

The snap-opening of glass ampoules creates particles which contaminate the contents to an extent such that they would fail accepted standards for LVP. However, the USP XXI SVP test would not reject such snap-opened products. Heat-opened glass ampoules generally easily pass the LVP standards and the excellent product made by the Australian pharmaceutical industry clearly is not supported by a container of equal standard. Industry should be concerned with the search for a more appropriate and versatile container which has a simple 'particle free' opening technique which would be acceptable in clinical practice.

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