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COMMUNICATIONS

Particulate contamination in ampoules

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The particulate contamination in 19 formulations of solutions in ampoules supplied by eight South African manufacturers, thirty-three batches in all, was analysed using a HIAC PC 320 light blockage particle analyser linked to a CMB 60 sensor. Results showed that the level of contamination was generally low and that, where comparisons could be made, manufacturers both of the ampoules and the solutions maintained similarly high standards. Problems in this field appeared to be related to the formulation or the quality of the raw material.

Standards limiting particulate contamination in Large Volume Parenterals have been in force for many years and modifications of these standards have also been proposed (Groves 1969; Groves & Wana 1977; Hailey et al 1982; van Wyk & Goossens 1980). However there is still much confusion about reasonable levels of contamination in Small Volume Parenterals (Hayashi 1980; Spence 1981; Haines-Nutt 1983). With these facts in mind and using a method of opening ampoules without introducing particulate contamination (Alexander 1983) we have undertaken a particle size analysis of particles present in 19 different formulations (33 batches) of locally manufactured ampoules.

Method and materials

Thirty-three batches of ampoules from eight different manufacturers using both locally manufactured and imported ampoules were analysed (Table 1). The mean count from 10 ampoules per batch was recorded but in circumstances where fewer than 10 ampoules were available the number is shown in Table 1.

The ampoules were opened using a single flame burner, placed in a Branson Ultrasonic Cleaner bath for 5 mins[1] and then a particle size distribution analysis on the particulate contamination was carried out using a HIAC PC 320 Light Blockage Particle Analyser linked to a CMB 60 sensor. For the first five analyses the HIAC was set to count the total number of particles in the size ranges ≥2·5, 3·5, 5, 7·5, 10, 15 and 18 μM. Thereafter the settings were standardized to the size ranges ≥3, 4, 5, 6, 7·5 and 10 μm.[2] Where the number of particles of ≥10 μm exceeded 10 per ml the size range was extended to ≥15, 20, 25 and 30 μm.

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Results

Of the 33 ampoule solutions tested, only 8 ampoule solutions contained more than 80 particles per ml ≥5 μm, the BP limit for LVPs. These are listed in Table 2.

Table 1. Ampoule solutions included in survey (M = manufacturer).

Preparation	No. of batches	M	No. of ampoules examined per batch
Ascorbic acid 500 mg 5 ml [1]	2	D	10
Atropine sulphate 0·6 mg ml [1]	2	D	(9) (10)
Atropine sulphate 1 mg ml [1]	3	D	(9) (10) (9)
Ergometrine 0·2 mg ml [1]	1	D	10
Ipradol 2 ml	1	D	10
Lanoxin 2 ml	1	G	20
Lasix 2 ml	1	C	10
Lignocaine HCl 1% 1 ml	1	D	10
Mepyramine 2 ml	1	D	9
Mersalyl 2 ml	1	D	3
Pyridoxine 1 ml	1	D	3
Remicaine 2% 5 ml	1	E	10
Sodium bicarb. 8·5% 50 ml	1	D	10
Sodium chloride 0·9% 5 ml	1	D	8
Sodium chloride 0·9% 10 ml	1	D	9
Syntometrine 1 ml	1	F	10
TED IV 10 ml	3	D	(9) (9) (10)
Valoid 1 ml	1	G	20
Vitamin B Co 2 ml	2	D	(5) (10)
Water for Inj. 2 ml	1	B	10
Water for Inj. 2 ml	1	D	10
Water for Inj. 5 ml	1	D	10
Water for Inj. 10 ml	1	B	10
Water for Inj. 10 ml	1	D	10
Water for Inj. 20 ml	1	A	10
Water for Inj. 20 ml	1	D	10

[1] Deaeration of the ampoules was undertaken to disperse the air bubbles that were incorporated when the liquid was shaken down from the necks of the ampoules. These bubbles, which took a long time to disperse, would be counted as particles by the analyser. Sonification has the advantage of removing the air bubbles, suspending the particles and standardizing the method. The USP Convention has since recommended this technique. However they have received queries regarding the possible disintegration of certain types of particles. As this possible disintegration is product-dependent, each preparation should be individually tested. Large scale studies are being undertaken in the USA and it would be advisable to await their results before standardizing the method.

[2] A particle size of 3 μm was finally chosen as the lowest setting because the accuracy of the counts below that level were not assured.

The level of contamination in various batches of Water for Injection supplied by different manufacturers were compared and the results are tabulated in Table 3.

Levels of contamination in similar preparations using ampoules supplied by different manufacturers were also compared and the results are shown in Table 4.

Finally levels of contamination in three batches of locally manufactured ampoules were compared with the results of Taylor & Spence (1983). These workers counted in the size ranges 1, 2, 5, 10, 20 and 40 μm so that only counts in the 5 and 10 μm ranges could be compared (Table 5).

Table 2. Ampoule solutions containing more than 80 particles ≥5 μm ml^{-1}.*

Ampoule soln	Batch no.	No. of particles x̄	s
Atropine sulphate 0·6 mg ml^{-1}	2510	122	163
Atropine sulphate 1·0 mg ml^{-1}	3110	526	275
	3410	917	482
	4210	719	266
Vitamin B Co. 2 ml	3510	131	124
	9009	3080	1171
Ergometrine 0·2 mg ml^{-1}	2403	191	109
Syntometrine (Ergometrine 0·5 mg ml^{-1}, Oxytocin 5 units ml^{-1})	376E1	108	101

* Details of other solutions examined for particles from 2–18 μm are available from the authors.

Table 3. Levels of particulate contamination in water for injection ampoules supplied by three different manufacturers (M).

M	Amp. vol. (ml)	Cumulative no. of particles ml^{-1} ≥3 μm x̄	s	5 μm x̄	s	7·5 μm x̄	s	10 μm x̄	s
B	2	180	165	50	53	10	10	2	1
D	2	24	10	4	2	1	1	<1	
B	10	7	5	1	<1	<1		<1	
D	10	76	68	13	12	1	1	<1	
A	20	11	7	4	3	1	1	<1	
D	20	9	4	3	2	1	<1	<1	

Table 4. Level of particulate contamination in solutions packed in ampoules supplied by three different suppliers (S).

Ampoules soln	(S)	Cumulative number of particles ml^{-1} ≥ 3 μm x̄	s	5 μm x̄	s	7·5 μm x̄	s	10 μm x̄	s
Ascorbic acid 500 mg 5 ml^{-1}	a	107	70	31	24	8	7	2	2
	c	100	52	33	15	9	4	3	3
Thiamine ethylene-diamine i.v. 10 ml	a	7	3	4	2	2	2	1	2
	b	13	9	6	6	3	4	2	3
Water for injection 20 ml	a	11	7	4	3	1	1	<1	
	c	9	4	3	2	1	1	<1	
Sodium chloride 0·9% 10 ml	c	13	9	5	4	2	2	<1	
5 ml	b	6	4	3	3	2	3	1	2

Table 5. Cumulative number of particles ml^{-1} in ampoule solutions of similar formulations manufactured locally and overseas as found by I, Taylor & Spence* and II, Alexander**.

	I Mean of 5 ampoules manufact. overseas ≥5 μm x̄	s	≥10 μm x̄	s	II Mean of 10 ampoules manufact. locally ≥5 μm x̄	s	≥10 μm x̄	s
Ampoule soln								
Lanoxin 2 ml	943	348	202	55	18	15	2	5
					18	16	1	1
Lasix 2 ml	603	281	110	49	10	8	2	3
Valoid 1 ml	1254	848	328	212	16	6	3	3
					22	10	7	5

* Ampoules snap-opened.
** Ampoules opened using single flame burner.

Discussion

The first point that becomes apparent is that the level of contamination in a broad cross section of locally manufactured ampoules is, in most cases, very low and is not necessarily dependent on the manufacturer or the source of supply of the ampoule. It must however be stressed that only products released by the manufacturers Q.C. laboratory are included in this study and no account is taken of the individual manufacturer's reject rate. In the one instance where a direct comparison could be made. Water for Injection, three manufacturers were involved (Table 3). The 20 ml ampoules supplied by manufacturers A and D both showed very low levels of contamination. However on comparing the 10 ml and 2 ml ampoules of manufacturers B and D, it is seen that B produced 10 ml ampoules with very low counts but the 2 ml product had a relatively high count. On the other hand D's 2 ml ampoules had a low particle count and their 10 ml ampoules a higher count. On balance there does not seem to be a significant difference.

Regarding the similarity between the particle counts among the ampoule manufacturers leaves no doubt that in these particular instances the ampoules are not a significant source of particulate contamination (Table 4).

The eight batches of ampoules with counts in the ≥5 μm range higher than those laid down for LVPs by the BP, were made up of 4 lots of atropine sulphate injection (1 ml), 2 lots of vitamin B injection (2 ml) and 2 lots containing ergometrine (1 ml) (Table 2). The atropine sulphate 0·6 mg ml^{-1} formulation included sodium chloride whereas the 1 mg ml^{-1} formulation did not. Two batches of the former were analysed; one had a low level of contamination (39 particles ≥5 μm) and the other was considerably lower than 1 mg ml^{-1} (Table 2). There is a strong possibility that the inclusion of the sodium salt prevents the leaching out of barium from the glass with consequent formation of barium sulphate crystals (Boddapati et al 1980).

There was also a marked difference between the levels of contamination in the two batches of vitamin B Co injection; it was subsequently learnt that the quality

of the nicotinamide in the heavily contaminated batch was such that its solubility was a problem.

The reason for the particulate contamination in the preparations containing ergometrine has not been elucidated but it is possible that it is also a formulation problem.

The analysis of particulate contamination by Taylor & Spence (1983) does not give a comparable indication of the number of particles present in the solutions because they snap-opened the ampoules and did not use an ultrasonic bath to remove any air bubbles. A comparison between the level of contamination in their ampoules compared with locally manufactured ampoules would be interesting. Certainly the latter showed a commendably low level of contamination (Table 5).

Conclusion

It can be concluded that, in most instances, the level of particulate contamination in locally manufactured ampoules is low. In this limited study it appeared that where problems arise they appear to be due to the formulation or the quality of the raw materials rather than to the manufacturer of the ampoule solutions or the supplier of the ampoules.

With regard to setting a standard for limiting contamination in ampoules, the problem is complicated by lack of knowledge about the size of particle or the number of particles that constitute a danger. The USP Sub-Committee on Parenteral Products (1983, 1984) favours limiting the number of particles per injection rather than per ml. This appears to be a realistic approach and takes into account the problem of increasing the particulate contamination of LVPs when relatively large volumes of SVPs are included as additives. However it is obvious that it is possible to produce ampoules with a low level of particulate contamination and that particle numbers would appear to be related to the formulation, the purity of the raw material and to GMP; therefore a reasonable limit would help to ensure a well formulated high quality product.

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A carrier-mediated transport system for benzylpenicillin in isolated hepatocytes

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The transport mechanism of benzylpenicillin was studied in freshly prepared rat hepatocytes. The initial uptake rate followed both saturable and unsaturable transport processes. The Arrhenius plot of the initial uptake rate gave an activation energy of 16.8 kcal mol^{-1} (69 kJ mol^{-1}). The benzylpenicillin uptake by hepatocytes was significantly inhibited by antimycin A, sodium cyanide, rotenone, 2,4-dinitrophenol, phenoxymethylpenicillin, probenecid and taurocholic acid. No significant inhibition was observed by acetylaminohippuric acid and several kinds of amino acids and dipeptides. The present study provides the first evidence for the the existence of a carrier-mediated and energy-dependent transport system of benzylpenicillin in the liver.

Hepatic membrane permeation is the most important step in the process of metabolism and biliary secretion in the liver. Since some derivatives of β-lactam antibiotics are known to be rapidly and exclusively eliminated from the liver while others are eliminated from the kidney after administration (see Bergan 1978; Brogard et al 1978), the reason why these different elimination routes were characteristic of certain derivatives needs clarification. Though the mechanism of renal β-lactam antibiotic elimination has been studied (Hori et al 1982; Inui et al 1983), there is little knowledge about the hepatic transport process. In this communication, we describe the basic characteristics of the transport process of benzylpenicillin, the most fundamental β-lactam antibiotic, in hepatic parenchymal cells of rats. Our present study provides the first evidence for the existence of a carrier-mediated and energy-dependent transport

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