J. Pharm. Pharmacol. 1983, 35: 769–773
Received May 18, 1983

Particles in small volume injections

S. A. TAYLOR AND J. SPENCE*

The Wellcome Foundation Ltd., Pharmaceutical Research & Development Laboratories, Temple Hill, Dartford, Kent DA1 5AH, U.K.

The level of particulate contamination in small volume injections has been examined using the light blockage (HIAC) and electrical sensing zone (Coulter counter) techniques, the HIAC system being found to be the more suitable. Particle counts on the same batch of injection showed a large and variable difference between the HIAC and the Coulter counter results, especially below 5 μm. None of the injections examined complied with the British Pharmacopoeia limits for particulates in large volume parenterals, suggesting the unsuitability of the limits for small volume parenterals.

It has been widely reported that particles introduced into the circulation by intravenous (i.v.) injection could result in adverse effects such as granulomas, thromboses etc. (see bibliog. by Turco 1978). The British (1980) and United States (1980) Pharmacopoeias specify limits for particulate contamination of intravenous parenterals over 100 cm^3.

Adverse effects from particles in intramuscular (i.m.) and subcutaneous (s.c.) injections have been documented to a lesser extent and there are no official limits for injections below 100 cm^3 apart from visual inspection, although standards would be a useful guide for quality assurance (Spence 1981). However, there is an increasing interest in the quantitative measurement of sub-visible particles in injections contained in ampoules and vials. An examination of small volume injections available in Japan was carried out by Hayashi (1980) who discussed quality control standards.

Several instrumental techniques are available for the determination of particles in liquids. For the large volume parenteral solutions the British Pharmacopoeia specifies either the Electrical Sensing Zone method (ESZ) or the Light Blockage method (LB). The United States Pharmacopeia uses microscopic examination after filtration. In his study of Japanese injections, Hayashi used the LB method. The microscope and LB method were compared by Delly (1980) and the microscope method was used by Hammer (1974) to examine particles in solid products for reconstitution, and by Longe (1980) for powders and solutions. The LB method has been used by Davies & Smart (1981) and by Tsuji & Lewis (1978) for small volume injections. Groves & Wana (1977) compared the LB and ESZ instruments for measuring particles in large volume parenterals.

* Correspondence.

This paper reports the results of an investigation of the particulate contamination of a range of small volume injections available in the U.K., using the LB and ESZ methods. The total particles which would be injected into a patient were counted, including the particles generated on snapping the ampoules. The contribution of the particles from snapped ampoules has been examined by Turgang (1974), Katz (1973), Spence (1981) and Tsuji & Lewis (1978). With vials, in clinical use insertion of the needle would introduce particles from the rubber plug, and with multi-dose vials this would occur several times. This source of contamination was not included in this study.

METHODS

Instruments

Light blockage. The instrument used was an HIAC PC320 with a small volume sampling system and a 1·0–60·0 μm sensor. The size levels examined were particles greater than or equal to 1, 2, 5, 10, 20 and 40 μm. The flow rate was adjusted to 1 cm^3/7·5 s for each preparation. The characteristics of the instrument are: (a) the particles can be suspended in any liquid that is transparent, having a refractive index different from that of the particles; (b) the probe is a thin robust metal tube, external diameter approx. 1·7 mm, ensuring easy entry into the smallest ampoules; (c) approximately 1 cm^3 of liquid is required for sampling; and (d) it is easy to use.

Electrical sensing zone method (ESZ). A Coulter counter (model TA II with the population accessory) was used. A 70 μm orifice tube of narrow bore was attached and the external electrode was wrapped around the tip of the tube just above the sapphire wafer. The characteristics of ESZ are: (a) the

Table 1. Products examined

Name & Manufact.	Contents	Batch
	1 cm^3 Ringsnap ampoules	
Betnesol	Betamethasone (as sodium	OFP 009
Glaxo	phosphate) 4 mg in 1 cm^3	Ex Aug 81
Gentamicin	Cidomycin intrathecal inj.	088234.E12
Roussel	40 mg cm^{-3} as sulphate	Ex. Dec 82
Syntometrine	Ergometrine & oxytocin	337FO
Sandoz	inj. 500 μg 5 u cm^{-3}	Ex Jun 82
Valoid	Cyclizine lactate	A53367
Wellcome	50 mg cm^{-3}	Ex Jul 84
	2 cm^3 Ringsnap ampoules	
Kemadrin	Procyclidine hydrochloride	D51606
Wellcome	10 mg in 2 cm^3	Ex Sep 85
Lanoxin	Digoxin 250 μg cm^{-3}	D51600
Wellcome		Ex Apr 86
Lasix	Frusemide 10 mg cm^{-3}	090147/2
Hoechst		Ex June 85
Largactyl	Chlorpromazine Inj.	M2C7
May & Baker	25 mg cm^{-3}	DM2308
		Ex Apr 86
Lincocin	Lincomycin (as hydro-	23558
UpJohn	chloride) 300 mg cm^{-3}	Ex Dec 83
Maxolon	Metoclopramide hydro-	47161/A
Beecham	chloride 5 mg cm^{-3}	Ex Jul 83
	10 cm^3 vials	
Insulin—Nuso	40 u ml^{-1}	P A164
Wellcome		Ex Jul 82
	80 u ml^{-1}	DA 234
		Ex Sep 82
	Freeze dried preparations & sterile powders in glass vials with rubber seals & metal collars	
Aerosporin	Polymyxin B Sulphate	A53756
Wellcome	500 000 u vial	Ex Jun 86
Calmic		
Colomycin	500 000 u vial Colistin	B 3654
Pharmax	sulpho methate Na	Ex Dec 83
Crystapen	Benzylpenicillin (300 mg)	IGP 716A
Glaxo	500 000 u	Ex June 84
Endoxana	Cyclophosphamide 100 mg	BN 7392M8
WBP		
Soframycin	Framycetin Sulphate	093251-2
Roussel	500 mg	Ex Mar 84
	Ringsnap ampoule containing freeze dried preparation	
Brevidil M	Suxamethonium Bromide	L14
May & Baker	67 mg	DL5527
		Ex Dec 85

particles have to be suspended in an electrolyte. For injections with a non-conducting vehicle it is necessary to add an electrolyte, this may give mixing problems in small containers, leading to interference of the pulses; (b) the thin bore glass tube suitable for use in ampoules and vials is delicate, being 3·5 mm in diameter and with the external electrode requires a larger entry hole than the HIAC probe; (c) the external electrode has to be wrapped around the aperture tube, making the entry into the ampoule a delicate operation; (d) volumes of 2, 0·5 and 0·05 cm^3 can be counted, but a minimum volume of 1 cm^3 is required to cover the electrode; (e) more experience is required to operate the Coulter than the HIAC counter; (f) electrolysis of the solution may occur with certain formulations and give spurious results; (g) viscous media give problems.

Preparation of samples

Glass ampoules were snapped open as they would be in normal use. For the vials containing solid material, the metal collars were removed using a Fermpress cap remover. A measured volume of filtered 0·9% w/v sodium chloride (saline, of known particle count) was added to each vial. The rubber closures were replaced and each vial shaken, until the solid had dissolved, and left to stand for at least 10 min to allow air bubbles to rise. Before the particles were counted, the vials were gently inverted twice to suspend the particles and the HIAC probe or Coulter tube inserted directly into the ampoule or vial.

For Coulter counting, the glassware was primed with filtered aqueous saline, and the resistance of each injection was measured across the electrodes. The controls were set to match the resistance, the tube having been calibrated for each resistance setting. Where the resistance was too large, attempts were made to add saline to the injections, but two difficulties occurred which made this technique impracticable, there was insufficient room in the ampoule to add enough saline solution, and homogeneous mixing in the ampoule was impractical.

Volumes of 0·5–2·0 cm^3 were used for the HIAC, depending on the volume in the ampoule, and 0·05 cm^3 for the Coulter. Replicate determinations were made where possible. Five ampoules or vials were examined for each method, and mean and standard deviations were calculated (see Tables 2–4).

Table 2. Ampoules

		Particles cm^{-3} oversize			
	Size	HIAC		Coulter	
Injection	μm	mean	s.d.	mean	s.d.
Betnesol	1	1346	1252	see *	
1 cm^3 ampoule	2	690	646		
	5	226	191		
	10	56	40		
	20	15	14		
	40	3	5		
Gentamycin	1	6022	2575	27 740	7 384
1 cm^3 ampoule	2	2887	1381	5 376	2 307
	5	479	216	568	238
	10	107	38	108	64
	20	22	14	12	18
	40	2	3		

Tables 2. Ampoules—continued

Injection	Size µm	Particles cm^{-3} oversize HIAC mean	HIAC s.d.	Coulter mean	Coulter s.d.
Syntometrine 1 cm^3 ampoule	1	960	474	15 456	10 359
	2	700	371	2 756	1 908
	5	320	164	344	250
	10	115	52	60	32
	20	24	15	4	9
	40	1	1		
Valoid 1 cm^3 ampoule	1	5498	2470	53 024	68 200
	2	3679	1880	6 172	1 891
	5	1254	848	876	232
	10	328	212	74	49
	20	51	31	0	0
	40	4	4		
Kemadrin 2 cm^3 ampoule	1	2412	126	See †	
	2	1937	169		
	5	673	70		
	10	142	25		
	20	16	10		
	40	1	1		
Lanoxin 2 cm^3 ampoule	1	3718	1357	see ‡	
	2	2387	881		
	5	943	348		
	10	202	55		
	20	33	6		
	40	4	1		
Largactyl 2 cm^3 ampoules	1	3182	1868	see §	
	2	2077	1605		
	5	602	405		
	10	114	49		
	20	16	6		
	40	2	1		
Lasix 2 cm^3 ampoules	1	2884	1595	see ¶	
	2	2264	1209		
	5	603	281		
	10	110	49		
	20	9	6		
	40	1	—		
Lincocin 2 cm^3 ampoules	1	796	482	14 156	9 210
	2	482	241	2 792	1 236
	5	221	104	520	230
	10	73	39	148	92
	20	20	18	8	13
	40	3	3		
Maxolon 2 cm^3 ampoules	1	6907	619	18 772	8 536
	2	5023	471	5 478	1 210
	5	2029	478	576	117
	10	459	156	54	30
	20	41	13	2	4
	40	2	1		

* The resistance of the injection is too great for Coulter counting. The addition of salt solution is impractical due to lack of space and problems with mixing.

† Electrolysis appeared to be occurring at the Coulter electrode, giving slight opacity and a very noisy trace.

‡ A non electrolyte injection, insufficient room and uneven mixing made Coulter counting with added saline impracticable.

§ A red precipitate occurred around the Coulter electrode. This dissolved when the ampoule was shaken but made Coulter counting impractical.

¶ A brown precipitate formed around the electrode.

Table 3. Vials and ampoules containing powder

Injection	Size µm	Particles cm^{-3} oversize HIAC mean	HIAC s.d.	Coulter mean	Coulter s.d.
Colomycin Powder in vials reconstituted to 8 cm^3	1	18 429	423	792 921	8 538
	2	15 795	83	272 417	22 816
	5	7 705	628	9 136	1 480
	10	1 005	246	385	125
	20	19	5	4	6
	40	0	0		
Crystapen Powder in vials reconstituted to 5 cm^3	1	1 148	212	20 793	12 862
	2	601	171	2 393	1 542
	5	164	90	148	82
	10	73	47	32	12
	20	10	7	0	0
	40	1	1		
Endoxana Powder in vials reconstituted to 10 cm^3	1	8 966	2490	313 695	45 940
	2	5 211	1617	36 461	13 888
	5	807	317	997	427
	10	46	22	57	44
	20	2	1	0	0
	40	1	0		
Soframycin Powder in vials reconstituted to 10 cm^3	1	17 891	464	600 555	271 924
	2	12 801	900	25 756	5 262
	5	3 068	1148	1 199	674
	10	781	501	653	457
	20	70	41	56	54
	40	1	1		
Aerosporin Freeze dried reconstituted to 10 cm^3	1	2 232	987	57 734	20 377
	2	1 516	584	11 196	6 272
	5	379	183	1 467	1 050
	10	84	37	273	216
	20	9	3	23	18
	40	0	0		
Ampliclox Powder reconstituted to 5 cm^3	1	6 737	1363	131 437	38 616
	2	3 950	891	8 411	2 483
	5	628	216	307	101
	10	54	27	44	17
	20	5	2	0	0
	40	1	1		
Brevidil M Powder in ampoules reconstituted to 5 cm^3	1	2 270	2189	17 065	4 569*
	2	1 612	1672	2 041	1 012
	5	570	642	254	135
	10	99	103	47	27
	20	5	3	0	0
	40	0	0		

* Possibly slight precipitation at the Coulter electrode.

RESULTS AND DISCUSSION

Techniques. All the injections were examined satisfactorily using the HIAC light blockage technique. The use of the Coulter counter was limited by the need for an electrolyte solution, which was difficult to obtain with non-conducting injections due to the limited space available for addition of electrolyte and the poor mixing obtained. With some injections a

reaction occurred, resulting in bubbles or a precipitate. The Coulter tube with electrode was difficult to introduce into ampoule necks giving rise to the possibility of glass particles being introduced.

Table 4. Vials containing liquid

Injection	Size μm	Particles cm^{-3} oversize HIAC mean	s.d.
Nuso Insulin 40 u cm^{-3} vials	1	2 201	977*
	2	1 553	754
	5	489	303
	10	87	60
	20	8	6
	40	1	0
Nuso Insulin 80 u cm^{-3} vials	1	12 089	1484†
	2	11 011	1499
	5	5 712	1165
	10	1 623	812
	20	81	82
	40	1	3

With the Coulter:
* Some precipitation at the electrode occurred when Coulter counting, Bubbles were also seen at the electrode.
† Bubbles and a white precipitate formed at electrode.

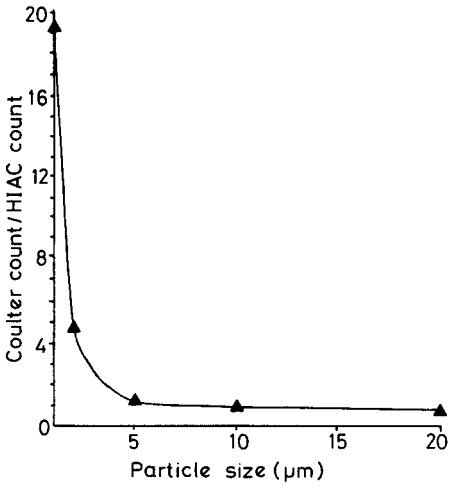

FIG. 1. The relation between Coulter counts and HIAC counts at various size levels.

The light blockage system was considered preferable to the electrical sensing zone system for counting particles in small volume parenterals.

The variation in particle counts between containers of the same batch was large, but when mean counts were calculated, as expected, the differences between the HIAC and Coulter values were small at the larger particle sizes above 5 μm but diverged widely at the smaller sizes. A cross over point at 6 μm was found (Fig. 1). This was in agreement with the findings of Groves & Wana (1977).

At 5 μm the ratio between the counts agreed approximately with that in the B.P. standard (1980), but it was much greater at 2 μm. However, as the variation in the ratio for different injections was large, to predict the relationship for any given injection would be unwise (Table 5).

Table 5. Ratio of Coulter count/HIAC count values at each size level

Size μm	Ratio: Coulter count value / HIAC count value mean	s.d.	B.P.
1	19·4	12·8	
2	4·6	4·5	2·0
5	1·2	1·0	1·25
10	0·9	0·9	
20	0·7	0·9	

Injections

None of the injections examined passed the British Pharmacopoeia limits (B.P. 1980) for particulates in large volume parenteral solutions at either the 2 μm or 5 μm size levels. Most of the powders, when reconstituted, gave particle counts which were similar to the injection solutions, indicating that the powder injections can be produced to a similar standard of particle contamination as the solutions.

We have shown that for small volume injections in ampoules and vials the HIAC light blockage system is more suitable than the Coulter electrical sensing zone system for determining particle contamination. Particle counts on the same batch of injection showed a large and variable difference between the HIAC and Coulter results, especially at the sizes below 5 μm. A standard permitting the use of either instrument with a fixed ratio between the counts is undesirable. The injections examined showed a large variation in particle counts, and none complied with the British Pharmacopoeia limits for large volume parenteral solutions. The application of this limit to small volume parenterals may be seen to be inappropriate since the total number of particles and the nature of the particles administered parentally are not taken into account.

REFERENCES

British Pharmacopoeia (1980) Volume II, Appendix XIII, A120

Davies, P. J., Smart, J. D. (1981) Int. J. Pharmaceut. Techn. Prod. Mfr 3: 53–58

Delly, J. G. (1980) Pharm. Forum 6: 357–376

Groves, M. J., Wana, D. (1977) Powder Technol., 18: 215–223

Hammer, H. F. (1974) Bull Parenteral Drug Assoc 28: 205–216

Hayashi, T. (1980) Yakuzaigaku 40: 133–136

Katz, (1973) Ibid. 39: 354

Longe, R. L. (1980) Can. Anaesth. Soc. J. 27: 62–64

Spence, J. (1981) Anal. Proc. 18: 522–525

Tsuji, K., Lewis, A. R. (1978) J. Pharm. Sci. 67: 50–55

Turco S. J. (1978) The Clinical Effects Of Particulate Matter. Burron Medical Inc.

Turgang, (1974) Anaesthesiology 41: 325

United States Pharmacopeia (1980) XX: 863