J. Pharm. Pharmacol. 1984, 36: 534–536
Communicated February 28, 1984

COMMUNICATIONS

Particle size measurement in intravenous fluids

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The level of particulate contamination in a range of large volume injections has been measured using electrical resistance (Coulter) and light blockage (HIAC) techniques. Particle counts showed large variations between the two techniques and although a correlation could be shown for ionic solutions no such correlation could be found for sugar containing solutions. Shape factors alone cannot explain these discrepancies but other differences fundamental to the physics of the two measuring techniques play an important part. We conclude that results obtained using one technique cannot be correlated, theoretically or actually, with those obtained from the other technique.

A recent article by Dawes et al (1983) was concerned with the counting of particles in large volume parenteral fluids. In the abstract reference was made to a talk given by one of the authors (RFH-N) to the Joint Pharmaceutical Analytical Group reported in Pharm. J (1983). The results presented at that talk have not been published and we believe that their wider dissemination will be helpful in the debate on whether particle measurement by both electrical resistivity and light blockage principles should be allowed in official compendia (the British Pharmacopoeia 1980).

A standard for subvisible particles in intravenous fluids was introduced in the 1973 British Pharmacopoeia. The test could be performed by using 'an instrument capable of counting the numbers of particles having equivalent sphere diameters equal to or greater than 2 μm and equal to or greater than 5 μm'. Suitable instruments were those 'based on changes in electrical resistance, light scattering or the obstruction of a light beam'. Originally, most tests were performed using instruments based on the electrical resistance principle but increasingly, instruments based on the light blockage principle were coming into use. Groves & Wana (1977) noted this trend and compared results from different instruments using a contaminated saline solution. They showed that the instrument based on the electrical resistance principle (Coulter) was 'not in complete agreement' with the other instruments based on the light blockage principle (HIAC and Royco). They postulated that the differences in the counts were due to the different particle parameters the instruments were measuring, the Coulter measuring the equivalent volume diameter whereas the HIAC measured the equivalent projected cross-sectional area diameter. As particles would not be perfect spheres, these two diameters would not be equivalent and hence the particle count would be different using different instruments. This would depend upon the actual shape of the particle. The shape can be defined in terms of a shape factor and Groves and Wana calculated that the HIAC would give counts of 730, 252 and 215 at 2 μm, equivalent to 1000 particles measured on a Coulter Counter, when the shape factors were 1, π and 4 respectively. The equivalent counts for 5 μm were 95, 80 and 76 respectively, all counts being based on a 6 μm cross- over.

In view of this paper and other information sent to the B.P. Commission, including information from our laboratories, the 1980 British Pharmacopoeia included dual standards for subvisible particulate matter. These standards, although reflecting the differences found when measuring saline type solutions, do not necessarily reflect the differences found when investigating sugar or sugar derivative containing solutions.

Materials and methods

Electrical resistance method. A Coulter Counter Model ZB fitted with a 70 μm orifice tube was used. A volume of 0.5 cm^3 was sampled. This was repeated at least 4 times for each solution.

Light blockage method. A HIAC Model 420 fitted with a 1–60 μm probe was used. The flow rate was adjusted to 8 $cm^3\ min^{-1}$ for each preparation and 5 cm^3 sampled. This was repeated ten times for each solution.

Samples and their preparation. A range of sterile products shown in Table 1 were analysed using both a Coulter and a HIAC counter. Results were obtained using fresh bottles for each determination. All samples for the HIAC determinations and those for the Coulter which contained an electrolyte were used without further manipulation. Solutions not containing an electrolyte had an appropriate amount of 30% sodium chloride solution added through a 0.22 μm filter to produce a final concentration of 0.6% sodium chloride before analysing using the Coulter Counter. In this case a background count was performed upon the filtered concentrated saline solution. Calibrations were performed with each solution using 2.03 μm diameter polystyrene latex.

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Table 1. Comparison between Coulter and HIAC counts for particles ml^{-1} when sampling different bottles from the same batch.

Solution	Coulter 2 μm	Coulter 5 μm	HIAC 2 μm	HIAC 5 μm
Dextrose 40%	2250	120	134	19
Mannitol 20%	3265	194	120	38
Dextrose 10% NaCl 0.18%	3065	36	280	18
Mannitol 20%	2760	171	163	29
NaCl 0.9%	620	40	110	23

Table 2. Comparison between Coulter and HIAC counts for particles ml^{-1} when sampling the same bottle.

Solution	Coulter 2 μm	Coulter 5 μm	HIAC 2 μm	HIAC 5 μm
Mannitol 5%	384	19	309	37
Dextrose 10% NaCl 0.225%	417	36	286	33
Sorbitol 30%	1166	46	327	78
Mannitol 20%	412	16	113	43
Dextrose 10% and electrolyte	2936	198	268	61
Dextrose 10% and electrolyte	834	18	172	31
NaCl 0.45%	877	79	249	31
$NaHCO_3$ 8.4%	792	125	305	69
NaCl 0.9%	42	2	15	2
NaCl 0.9%	366	20	128	31
NaCl 0.45%	504	12	159	22

In a second set of experiments, a further range of products shown in Table 2 were used. In this case each bottle was sampled by the two instruments. Procedures and precautions discussed above were used, except that all non-conducting solutions had filtered concentrated saline added before counting, using either the Coulter or the HIAC Counters.

Results and discussion

Average results using five fresh bottles for each instrumental determination are shown in Table 1. Table 2 shows the mean results from individual bottles sampled using both instruments. Comparison of all the results show a correlation co-efficient for both instruments of 0.12 at 2 μm and 0.35 at 5 μm. If, however, the results are separated into those solutions containing only non-sugars or non-sugar derivates (salt solutions) and those containing sugars or sugar derivatives (sugar solutions) then a correlation co-efficient of 0.93 is obtained for salt solutions at 2 μm and 0.91 at 5 μm. The figures for the sugar solutions are 0.11 and 0.27 respectively. Hence when using salt solutions these results support the finding of Groves & Wana (1977), i.e. if a certain count is obtained using a Coulter counter then a proportionate count will, on average, be obtained using a HIAC counter. If, however, sugars are present in reasonable concentration then this correlation does not hold and there is no relationship between Coulter and HIAC results, except that the Coulter count was always considerably higher than the HIAC count (up to eleven times as high for the dextrose and electrolyte solution at 2 μm).

It would seem, therefore, that differences in particle shape alone do not adequately explain the lack of correlation when sugar solutions were investigated. Groves and Wana showed that as the shape factor varied so did the equivalent counts of the Coulter and the HIAC. In the case of sugar solutions, however, one would have to believe that the shape factor of the particles in each bottle varied even when solutions of the same composition were investigated, however, in the case of salt solutions this does not seem to occur!

The basic detection processes employed by both instruments are entirely different, the Coulter counter depends upon changes in electrical conductivity between two electrodes. Any particle will affect this conductivity, including air bubbles, although precautions should be taken to minimize this possibility. Electrical interference can also occur, however, this is usually obvious particularly when a comparatively small number of particles are being counted and action can be taken to eliminate this source of error.

The light blockage method depends upon a light beam being scattered by a particle and not reaching the detector. In fact, the light scattering will be affected by the individual particle shape, size and surface texture, the wavelength of the incident light, the refractive index of the particle and of the media, reflection and diffraction. The relationship is complex and various types of scattering are well described in text books, e.g. Bayvel & Jones (1981) and Jelinek (1970). Mie's theory deals with very dilute, monodisperse particles in the size range up to and including 2 μm. The theory gives the following relationship:

$$C = \frac{4\pi No}{\lambda r} f(a.m.)$$

where C is the specific turbidity co-efficient when concentration $\rightarrow 0$. No = the refractive index of the medium; λ = the wavelength of the light in a vacuum; r = the particle density; $a = (\pi \text{ No } d)/\lambda$; where d = the particle diameter; and m = Np/No, where Np = the refractive index of the particle.

Hence, if a particle is much bigger than the wavelength of the illuminating radiation, rear and lateral scattering will approach 100%. However, as the particle size approaches that of the illuminating radiation, this figure decreases and forward light scattering increases thus increasing the disparity between real and measured size. An elongated particle, such as that described by Dawes et al (1983) would be invisible if presented side on to the light beam. Furthermore, in a liquid Mie's theory predicts that the amount of scattering will depend upon the ratio of the refractive indices of the particle and that of the solution. As the refractive index of the particle approaches that of the medium, the scattering will

approach zero and the particle will be invisible. In the case of sugar solutions, many of the particles will be degraded sugar particles or sugar particles which may be crystallizing out. In these cases the refractive index of the particle will be similar to that of the solution and hence they will be invisible when using optical detection methods but countable by electrical conductivity measurements. We believe that these effects rather than differences in particle shape, are the major causes of the discrepancies found in both our results with sugar solutions and those of Dawes et al (1983) using amino acid solutions.

REFERENCES

Bayvel, L. P., Jones, A. R. (1981) Electromagnetic Scattering and its Applications. Applied Science Publishers, London

British Pharmacopoeia (1973 and 1980) HMSO London, p A123 and p A120 respectively

Dawes, V. H., Erawati, J., Eluemuno, J. (1983) J. Pharm. Pharmacol. 35: Suppl. 53P

Groves, M. J., Wana, D. (1977) Powder Technol. 18: 215–233

Jelinek, Z. K. (1970) translated by Bryce, W. A. J. Particle Size Analysis Ellis Henwood, Chichester, pp 35–42

Pharm. J. (1983) 2nd April: 373

J. Pharm. Pharmacol. 1984, 36: 536–538
Communicated January 26, 1984

An in-vivo – in-vitro correlation for the bioavailability of frusemide tablets

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The dissolution behaviour of four commercial and two experimental formulations of frusemide tablets has been investigated using the USP rotating basket apparatus and pH 5·0 buffer at 37 °C as the test medium. There is a linear relationship between the percentage dissolution in 30 min and the bioavailability relative to an oral solution of frusemide over the bioavailability range 76–97%. Predicted bioavailabilities differed by no more than 2% from the measured values.

We have reported (Eggers et al 1983) a bioavailability trial of four commercial tablet formulations whose performance was assessed by comparing urinary recovery of frusemide and chloride excretion with the values obtained after administration of a frusemide solution. One of the tablets showed significant reduction of frusemide recovery.

This work has been extended by determinations of the bioavailabilities of two further batches of tablets, formulated to have impaired bioavailability, and use of the six tablet batches to develop a dissolution test using the rotating basket system (United States Pharmacopeia 1975).

Dissolution specifications or attempts at in-vivo/in-vitro correlations for tablets commonly involve either the time for a specified proportion of dose content (usually 50%) to dissolve, or the proportion of the dose content that dissolves in a specified time. The former approach suffers from the need to ignore the shape of the dissolution curve subsequent to the selected point and particularly the possibility that a proportion of the dose may dissolve only with great difficulty. The latter approach comes close to the clinicial situation.

If bioavailability problems exist with a drug, this commonly reflects the fact that absorption takes place for a limited time after ingestion. This places a time limit on the dissolution process in the gut, if good bioavailability is to be achieved. Thus the present study was directed towards finding the critical time at which the percentage dissolution should be measured.

Previous studies (Rubinstein & Price 1977; Rubinstein & Eastwood 1978; Rubinstein 1980; Marvola et al 1979; Stuber et al 1982) suggest that pH 5·0 might be suitable for the dissolution medium but cast little light on the critical time for dissolution. To facilitate identification of the critical time, a decision was made to fit our data to the Rosin-Rammler-Sperling-Weibull (RRSW) distribution (Langenbucher 1976; Gurny et al 1976; Goldsmith et al 1978; Christensen et al 1980) and use an iterative procedure to locate the desired time. The RRSW distribution has the following form:

$$W(t)/W(\infty) = 1 - \exp(-((t - T)/\alpha)^{\beta}) \quad (1)$$

Equation (1) expresses the amount dissolved in time t (W(t)) in terms of the dose content (W(∞)), the lag time for dissolution (T), the time for 63·2% dissolution (α) and the parameter, β which controls the curve shape.

Methods

The experimental tablets were prepared by Mr C. J. Budgen of the New Zealand School of Pharmacy. Batch 57 comprised frusemide (40 mg), dicalcium phosphate (100 mg), wheat starch (3·5 mg), magnesium stearate

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