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A SIMPLE INDEX FOR MEASURING PARTICULATE CONTAMINATION IN PARENTERAL SOLUTIONS

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SUMMARY

Concern over the recent introduction of limits for allowable particulate contamination in small volume parenterals by the United States Pharmacopeia XXI has drawn attention to the physics of systems containing small numbers of particles of varying (random) size and random identity dispersed in an aqueous medium. In general, a size distribution of parenteral particulate characteristically consists of large numbers of small particles and relatively few particles at larger sizes. In parenteral solutions, the size distributions found tend to fit a linear power law curve of the type

$$\ln N = C - M \ln D$$

where N = cumulative number of particles/unit volume at size threshold D, M is the slope of the distribution and C is a constant. A numerical index of contamination can be obtained by integrating this curve between limits, effectively an area under the curve, AUC.

The limits are defined as the number of particles/unit volume (mL) at a size of 1.0μm, and the size at which the count falls to 1 per mL. By simple geometry, the area of a triangle,

$$AUC = 1/2[(\ln N_{1.0}) \times (\ln D_{1.0})]$$

Use of this AUC is illustrated by examples taken from the literature, experimental data generated for the purpose and archived quality control data. Differences on the same materials examined by different instrumental principles may be explained by shape factors inevitably involved when analyzing samples contaminated with random identity particulate. The AUC may provide a tool for exploring this feature of parenteral solutions. Some initial experimental evaluations of the concept are provided.

INTRODUCTION

Instrumental methods for the objective determination of suspended particulate matter in parenteral solutions were introduced in the 1973 British Pharmacopoeia following earlier investigations using the Coulter Counter[1-5]. As experience developed, it became evident that different results could be obtained on the same solution according to the method of determination[7]. The next edition of the B.P. recognized this effect by specifying different limits by Coulter Counter or the HIAC light blockage method. The recent introduction of a small volume parenteral limit test on allowable particulate matter in the USP XXI has caused some controversy[6] but is based on the use of the HIAC instrument. The present compendial situation would justify a re-examination of the underlying physical situation to be found in most parenteral solutions since it is unique.

Pharmaceutically, solutions intended for parenteral administration are characteristically repeatedly passed through filters, usually mambranes with pore sizes of 0.4 μm or less. In addition, containers and seals are washed thoroughly to remove particulate and the filling process is designed to minimize particulate contamination. Although intrinsic and extrinsic contamination does occur the purpose of a Good Manufacturing Procedure is to ensure that the contamination is minimal.

Physically insoluble particulate matter, contamination, in parenterals is characterized by large numbers of particles at small size thresholds and low numbers at larger sizes. This situation has been extensively reviewed elsewhere[8] but the size distribution encountered experimentally is a power equation in the form

$$\ln N = C - M \ln D \qquad \text{Equation (1)}$$

where N is the cumulative number of particles per unit volume at a size threshold of D, M is the slope of the distribution and C is a constant. The validity of this approach has been amply confirmed by others[3-12].

Both the British and United States Pharmacopeias define limits on allowable particulate by specifying allowable numbers of particles at each of two size thresholds. These depend critically on the accuracy and precision of the calibration procedures used for the determinations. They also involve extrapolation procedures since calibration standard reference materials are not available at exactly the thresholds specified by the respective compendia.

There are likely to be some advantages to be gained by reducing a complex multipoint analysis to a simple numerical index, providing that the index was based on an understanding of the realities of the situation. In the event other, more subtle, advantages may also be gained, as we hope to demonstrate in this present communication.

Attempts to provide suitable indices of contamination have been described in the literature[7,8,10,12] and are all based on some form of transformation of the log log distribution plot described by equation 1.

CALCULATION OF AN AUC

A log log plot has some interesting conceptional problems since, on a cartesian coordinate system, it is infinite in any direction and has no intercepts. Intercepts may be arbitrarily defined and, for practical purposes we have recently[13] suggested that two subset axes may be defined by choosing $X = \ln (N=1/\text{mL})$ and $Y = \ln (D=1\mu\text{m})$. Logarithms to any base may be used and we have employed natural logarithms, base (e), here. Since $\ln 1 = 0$, these axes now form true cartesian coordinates and numbers in the quadrant can be regarded as real. An integral of a log log plot between the limits $N = 1/\text{mL}$ and $D = 1\mu\text{m}$ is the area of the triangle under the curve, AUC, between these limits, calculated by

$$\text{AUC} = 1/2\ [(\ln N_{1.0}) \times (\ln D_{1.0})] \qquad \text{Equation (2)}$$

where $N_{1.0}$ is the cumulative number of particles/mL at a size threshold of 1.0μm and $D_{1.0}$ is the particle size threshold at which the cumulative count falls to 1/mL.[12] The values of $N_{1.0}$ and $D_{1.0}$ are readily calculated by standard statistical methodology, inserting averaged experimental counts at known size thresholds using the relationship $y = c - mx$. Clearly at least two, preferably more, thresholds are required to establish the parameters of the line, but it should be noted that it is not necessary to determine counts at the thresholds specified by the pharmacopeial test methods. The criteria are determined by the need for accuracy and precision of the method and the test materials. Ideally the counts can be determined by using the thresholds of the instrument corresponding to the standard reference materials. It should also be noted that an estimate of "goodness of fit" to a log log plot is obtained as a correlation coefficient. This may be a valuable indication of the nature of the contamination[13].

The calculations are readily made using most handheld scientific calculators. Different results will be obtained when using logarithms to different bases.

This concept is discussed in more detail elsewhere[13].

PHARMACOPEIAL LIMITING AUC'S

The count/unit volume particulate limit test used in the British Pharmacopoeia 1980 correspond to AUC's of 14.89 when determined by Coulter Counter or 14.44 if measured using the HIAC/Royco instrument. This may seem to be in reasonable agreement although, as noted later, the agreement may be coincidental.

The USP XXI uses the concept of limiting the allowable counts per container fill volume. In this case, the limiting AUC will be a function of the fill volume. Results calculated over the range 1 - 100 mL, Table One, show that the relationship between limiting AUC (Y) and container fill volume (X) is approximated by

$$\ln Y = 3.82 - 0.16 \ln X$$

The AUC could be used directly as a number or, following the general suggestion by Muhlen[12], as a percentage of the allowable limit. This would have the effect of removing the differences due to container fill volume.

EXPERIMENTAL METHODOLOGY

Data was generated for the purpose of evaluation of the AUC's of standard pharmacopeial parenteral solutions. Instruments used were

(i) Spectrex Prototron, Model ILI 1000 laser particle counter fitted with computer and small vial attachment.

TABLE ONE: THE LIMITING AUC's CALCULATED FOR VARIOUS SIZES OF CONTAINER FILL, BASED ON THE USP XXX LIMITS OF NOT MORE THAN 10,000 PARTICLES AT 10μm AND 1000 PARTICLES AT 25μm PER CONTAINER

Container volume (mL)	Limiting AUC
1	44.75
2	40.71
5	35.66
10	32.06
20	28.66
25	27.60
50	24.45
100	21.49

fits $\ln Y = 3.819 - 0.158 \ln X$

Y = AUC

X = container fill volume

correlation coefficient 0.999

(ii) HIAC/Royco Counter, Model 4103 fitted with a HR-120 STD Sensor and operated in accordance with the USP XXI monograph description as modified in Pharmacopeial Forum.

In addition, data published in the literature were recalculated, in particular the invaluable comparative information presented by Taylor and Spence[9]. Finally, some routine quality control data obtained during regular production of different product codes were kindly provided to us by a manufacturer who wishes to preserve anonymity.

EXPERIMENTAL DATA

A comparison of AUC's obtained on glass ampoules from a lot of sodium bicarbonate injection USP are shown in Table Two and data obtained on products using the USP XXI HIAC method in Tables Three and Four. These data indicate that size distributions can be measured that approximate to linear log log plots, defined by a correlation coefficient of 0.90 or better.

DATA COMPARING HIAC AND COULTER METHODS

The earliest comparative investigation of Coulter and HIAC methods was that of Groves and Wana[7]. It was pointed out that the two methods measured different paramaters of the suspended particles. The Coulter effectively reports the size as the diameter of a sphere of equivalent volume and is, therefore, relatively unaffected by the shape of the particle and especially the orientation. Taking the data of Fig 2 in Groves and Wana, the calculations shown in Table Five indicated an AUC ratio Coulter:HIAC of 1.17.

We have examined quality control data provided by a producer of parenteral products that had been measured by both types of instrument. More to the point, the products, operators, laboratories and instruments were all different, in effect a type of data randomization. The calculated AUC's, Table Six, had an averaged ratio Coulter:HIAC of 1.20.

The comparative instrument trial organized by the American Society of Testing Materials, Johnson and Swanson[14], involved instrument calibration with spherical particles followed by a sizing of A.C. Fine Test Dust as an example of an irregular shaped particulate. These workers noted that it was necessary to transpose the size axis by a ratio of 1.30 to allow HIAC data to be superimposed upon that obtained by Coulter. This would suggest that for an irregularly shaped particle, AC Fine Test Dust, the HIAC is effectively undersizing by 30%.

TABLE TWO: AVERAGED AUC's DETERMINED BY PROTOTRON AND THE HIAC (USP) METHODS ON 30mL AMPULES OF SODIUM BICARBONATE INJECTION USP

Ampule Number	HIAC AUC	Correlation Coefficient	%USP Limit	Prototron AUC	Correlation Coefficient
1	12.14	0.75	45.6	8.48	0.75
2	14.17	0.85	53.2	15.10	0.90
3	14.27	0.91	53.6	21.90	0.95
4	15.65	0.91	58.8	4.90	0.92
5	13.46	0.90	50.6	5.70	0.87
Average	13.4	(SD 1.15)	52.4	11.22	(SD 6.44)

TABLE THREE: HIAC (USP) COUNTS ON 10 mL GLASS AMPULES OF AN EXPERIMENTAL DRUG PRODUCT

Ampule	AUC	%USP Limit	Correlation Coefficient
1	10.64	33.2	0.72
2	20.16	62.9	0.61
3	13.72	42.8	0.90
4	14.58	45.5	0.87
Average	14.78	(SD = 3.44)	

Accordingly, AUC's for the data shown in Table Six were recalculated, transposing the HIAC size axis by 30% in the manner suggested by Johnson and Swanson[14]. Recalculated ratios are shown in Table Seven, from which it will be noted that the Coulter: HIAC ration has now been adjusted to an average of 1.05 (SD 0.08).

TABLE FOUR: HIAC (USP) COUNTS ON 20 mL VIALS OF WATER FOR INJECTION USP

Unfiltered Vial	AUC	% USP	Correlation Coefficient
1	11.64	40.6	0.94
2	11.10	38.7	0.91
3	9.92	34.6	0.97
4	16.02	55.9	0.75
5	10.78	37.6	1.00
6	8.05	28.1	0.96
Average	11.89	(SD 2.14)	
Filtered			
1	9.27	32.3	0.97
2	4.32	15.1	1.00
3	8.24	28.8	0.98
4	5.81	20.3	0.92
Average	6.91	(SD 1.95)	

TABLE FIVE: DATA TAKEN FROM FIG 2, GROVES AND WANA[7] SHOWING COMPARATIVE COUNTS FROM COULTER AND HIAC MEASUREMENTS ON THE SAME CONTAINER

Coulter		HIAC	
Size (μm)	Count/mL	Size (μm)	Count/mL
5	190	5	100
8	25	8	30
10	8	10	18
13	2.5	15	6
AUC = 17.449		AUC = 14.901	
Correlation Coefficient 0.9995		Correlation Coefficient 0.9997	

ratio Coulter/HIAC = 1.17

TABLE SIX: COUNTS OBTAINED ON LARGE VOLUME PARENTERALS. DIFFERENT PRODUCT CODES ALL COUNTED AT DIFFERENT SITES BY DIFFERENT OPERATORS USING DIFFERENT INSTRUMENTS (SEE TEXT)

Product		Counts/mL						Ratio
		> 2	> 5	> 10	> 20	> 25	AUC	C/H
1	H	2,007	630	181	26	15	21.98	1.18
	C	4,287	842	340	78	38	26.00	
2	H	3,225	419	13	0	0	17.09	1.35
	C	7,282	943	128	26	18	23.15	
3	H	2,129	287	26	1	1	16.48	1.20
	C	3,879	463	44	12	8	19.85	
4	H	805	262	62	2	1	15.94	1.30
	C	1,893	343	108	27	12	20.73	
5	H	462	78	21	0	0	14.49	1.10
	C	840	133	66	4	2	15.99	
6	H	266	84	31	6	2	13.98	1.24
	C	891	94	61	12	8	17.33	
7	H	483	72	38	16	0	16.77	1.00
	C	904	113	48	14	4	16.85	
8	H	341	58	19	2	0	12.98	1.21
	C	639	84	37	11	3	15.67	

Average C/H ratio
= 1.20 (SD = 0.103)

The comparative data obtained by Taylor and Spence[9] was obtained by single instruments but on a wider range of products. Taking their numbers (Table 2, ref 9) and calculating AUC's provided the ratios shown in Table Eight. Transposition of the size axes, as above, resulted in a lowering of the average HIAC AUC's and the Coulter/HIAC ratio, also shown in Table Eight.

DISCUSSION

The AUC as a method of unitizing a size distribution for a system containing few particles/unit volume of random numbers of random identity

TABLE SEVEN: AUC's TAKEN FROM TABLE SIX IN WHICH THE HIAC SIZE AXIS IS TRANSPOSED BY 30% IN THE MANNER SUGGESTED BY JOHNSON AND SWANSON[14]

Product	HIAC AUC Adjusted		new ratio (Coulter/HIAC Table 6)
1	24.49		1.06
2	20.02		1.16
3	19.30		1.03
4	18.49		1.12
5	16.51		0.97
6	15.93		1.09
7	18.65		0.09
8	15.02		1.04
		Average	1.05 (SD 0.077)

would appear to be rational and effective. The method appears to have some advantages, including

(a) a simplified means of presenting data from a complex size distribution.

(b) a means of providing a statistical goodness-of-fit for underlying presumption of a log-log plot.

(c) the AUC provides a single numerical parameter useful in quality control and production charts, correlations of lot-to-lot variations and general collection of data.

(d) a numerical parameter which would enable a product to be reported as a percentage of an allowable compendial limit test.

(e) the AUC is a better indicator of contamination than current compendial threshold limits.

(f) the AUC effectively provides a limit on the total numbers of particle present within a system, effectively between 1μm diameter and approximately 30-40μm diameter, the diameter of the largest particle present in the system.

TABLE EIGHT: AUC's CALCULATED FROM THE DATA PROVIDED BY TAYLOR AND SPENCE[9], TABLE 2

Product	Coulter AUC	HIAC Original AUC	Original C/H ratio	HIAC Adjusted AUC*	New C/H ratio
Gentamycin	20.98	19.95	1.052	22.46	0.934
Syntometrine	18.12	17.47	1.037	19.57	0.926
Valoid	21.33	23.31	0.915	25.86	0.825
Lincocin	20.19	17.67	1.143	19.61	1.030
Maxolon	18.23	23.09	0.790	25.78	0.707
Colomycin	26.56	27.84	0.954	30.78	0.863
Crystapen	16.93	15.91	1.066	17.98	0.943
Endoxana	21.97	18.135	1.211	20.85	1.054
Soframycin	27.37	24.50	1.117	27.49	0.996
Aerosporin	24.37	18.87	1.291	21.10	1.155
Ampliclox	19.28	18.28	1.055	20.89	0.923
Breidil M	18.24	18.50	0.986	20.80	0.877
			1.0514		0.9361
			(SD = 0.1280)		(SD = 0.1111)

(*by 30%)

To amplify some of the points, the underlying assumption of a log-log plot, (b) is clearly invalid if the correlation coefficient is less than some defined value. We suggest, quite arbitrarily, if a system does not have a correlation coefficient better than 0.90, that there may be some underlying cause for this failure. In this case, the analyst should determine if a single species is contaminating the product by some suitable alternative method, for example, the microscope. A poor fit may be due to a number of other causes however, insufficient sampling being one.

Turning to accuracy and precision, (e), the point has already been made that the need to measure particulate at the size thresholds specified is removed by using this form of data manipulation. Any number (two or more) calibration points can be taken to define the size distribution of the contamination with greater precision since instrument thresholds do not have to be adjusted after calibration to correspond to the compendial thresholds. Experimentally the numbers of particles/mL encountered in a good quality parenteral at the USP XXI thresholds of 10 and 25μm are low, thereby increasing the uncertainty of the method. Better precision is obtained at lower size thresholds where the counts are higher and more readily determined.

As a simple index, the AUC may provide a working tool for an examination of the effects of particle shape. As noted, Groves and Wana[6] examined the effect of particle shape factors on the response of the HIAC instrumental principle relative to that of the Coulter and demonstrated that the HIAC under sizes. This effect, quantitatively measured by Johnson and Swanson[14] for AC Fine Test Dust can be corrected for. Assuming the 30% undersizing determined for AC Fine Test Dust enabled a Coulter/HIAC ratio close to unity to be obtained for different products, Table Seven. However, the same effect was not noted for the extensive tabulation of data of Taylor and Spence[9], Table Eight, the 30% correction apparently overcompensating. The reason for this is less than clear. These authors do not provide details of calibration procedures used for their instruments and the explanation may be connected with this factor. The HIAC principle is affected by particle shape but it must remain an open question as to whether or not AC Fine Test Dust offers an approximation to the many and varied shapes that might be anticipated to be present on a randomly contaminated parenteral solution. This concept requires further evaluation.

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REFERENCES

1. Groves, M. J. and Major, J. F. G., Pharm. J., 193, 227 (1964).
2. Groves, M. J., Pharm. Pharmacol., 18, 160 (1966).
3. Groves, M. J., Analyst, 9(4), 992 (1969).
4. Vessey, I. and Kendall, C. E., Ibid, 91(4), 273 (1966).
5. Vessey, I. and Kendall, C. E. and Peters, F. E., Med. J. Austral, 1(8), 293 (1966).
6. DeLucca, P. P., Boddapati, S., Haack, D. and Schroeder, H., J. Parent. Sci and Technol., 40(1), 2 (1986).
7. Groves, M. J. and Wana, D., Powder Technol., 18, 215 (1977).
8. Groves, M. J. "Parenteral Products", Heinemann Medical Books, London (1973).
9. Taylor, S. A. and Spence, J., J. Pharm. Pharmacol, 35, 769-773 (1983).
10. VanWyk, C. J. and Goosens, A. P. G., S.A. Pharm. J., 47(12), 550 (1980).
11. Alexander, D. M. and Veltman, A. M., J. Pharm. Pharmcol., 37, 13 (1985).
12. Muhlen, K. Ph.D. Thesis (1984).
13. Groves, M. J. and Wong, J., Pharm. Technol., (in press) (1986).
14. Johnston, P. R. and Swanson, R., Powder Technol., 32, 119 (1982).