DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 15(14-16), 2441-2454 (1989)

COUNTING OF MICROSPHERES IN ELECTROLYTES AND PARENTERAL SOLUTIONS

Thau-Ming Cham*, Huey-Mee Yu and Loong-Chyau Tung
School of Pharmacy , Kaohsuing Medical College,
Kaohsiung, Taiwan, R.O.C.

ABSTRACT

The counts of microspheres in 20 testing solutions were determined using 2 Coulter Counters (electrical resistance) and 2 HIAC/Royco (light blockage) particle analysers. The mean cumulative number of microspheres ($\geq 2\mu$ m) in unit volume of the solutions determined using the Coulter Counters was higher than those determined by HIAC/Royco particle analysers. The mean cumulative counts obtained by the two Coulter Counters are similar; however, t-test analysis shows significant difference for the counts of 8 different types of solutions. The mean cumulative counts obtained by the two HIAC/Royco Counters varied greatly and t-test analysis showed that 18 out of 20 of the solutions gave significant difference.

INTRODUCTION

Due to their advantages, automatic electronic particle counters are preferred for counting particles in parenteral solutions. The commercial instruments are either based on electrical resistance principle (Coulter Counter) or light blockage principle (HIAC/Royco).

During the past decade a number of publications concerning with the determination of particles in parenteral solutions showed great discrepancies between the results obtained by these two techniques.

In a comparision study Groves and Wana[1] showed that the particle count in normal saline ($\geq$ 2 µm) obtained using the Coulter was higher than that obtained using the HIAC. They attributed the differences to the shape factor of the particles present in the solutions. The particulate matter present in amino-acid solutions was studied by Dawes et al[2] . They found that the counts obtained with a HIAC would be much lower than those obtained with a Coulter Counter. This difference was attributed to the elongated shape of the particles and the different parameters measured by the two methods.

However, Haines-Nutt and Munton[3] pointed out that shape factor might not be the only reason responsible for the differences in particle count obtained by these two techniques. They believed that the refractive indices of the particles and of the medium would also be responsible for the lower count of particles obtained by the HIAC.

To minimize the effects of shape factor and refractive index of the particles in electrolytes and parenteral solutions, standard latex spheres (nominal diameter, 5.96 µm, microspheres latex suspension, Coulter Electronics Ltd.) were added to 20 different solutions. The number of microspheres in each solution was determined by two Coulter Counters (model TA II and Multisizer) and two HIAC/Royco particle analysers respectively. The purpose of this study was to evaluate the counting and sizing ability of the two techniques. T-test analysis of the results enabled us to understand the instrument to instrument variability for the two techniques.

MATERIALS

P.D.V.B. microspheres latex suspension (nominal diameter, 5.96 µm) and Isoton II (1% w/v saline), (Coulter Electronics Ltd., U.K.); sodium chloride and sodium azide (Osaka Hauashi Pure Chemical Industries, Ltd., Japan); large volume parenteral (LVP) solutions: Compd. sodium lactate, 0.9% Saline, Velip, Ringer solution, 5% Glucose, 10% Glucose, 50% Glucose, Xylitol, Maltose, Gurocan, Fructose, 2.5% Glucose in 0.45% Saline, 5% Glucose in 0.33% Saline, 5% Glucose in 0.9% Saline, Promin, Aminol-k, Aminol-s, Conamin and Aminogen-x (Nan Kuang Pharmaceutical Co., Ltd., Taiwan, R.O.C.); 0.22 and 0.45 µm diameter filter membranes (Millipore Corporation, U.S.A.).

METHODS

Preparation of Testing Solutions Containing Microspheres

1% sodium chloride was added to poor or non-conductive LVP solutions and then 0.1% sodium azide was added to all 20 solutions which were then filtered through filter membranes (pore size 0.45 and 0.22 µm). The background count of these solutions was not more than 50 particles per ml at 2 µm level, when examined with a Coulter Counter model TA II.

Approximately 6-8 drops of microsphere latex suspension (nominal diameter, 5.96 µm) were added to each of the filtered solutions and then stirred vigorously to ensure that the distribution of microspheres was uniform. The suspensions were transferred to 500 ml containers and ultrasonicated for 1 min to eliminate the air bubbles present in the solutions subsequently.

Counting of Microspheres in the Testing Solutions

(i) Electrical Resistance Principle

Coulter counters model TA II and Multisizer fitted with a 70 μm orifice tube and calibrated with the 5.96 μm microsphere latex suspension were used.

(ii) Light Blockage Principle

Two HIAC/Royco (NTUH and CCPC) analysers fitted with HR-120HA and HR-60HA Sensors respectively were used. Their flow rates were set at 20 ± 10% and 9 ± 10% ml/min respectively. The calibration of these two instruments was done by the distributor (Sunway Corporation, Taipei, Taiwan.).

(iii) Each bottle of the testing solutions containing microspheres was sampled by the four instruments. Procedures and precautions stated in B.P[4] for the determination of particulate matter in LVP solutions were used. The counting by an instrument was repeated 6 times for each solution and made at four size ranges $\geq$ 25, $\geq$ 10 , $\geq$5 and $\geq$2 μm.

Scanning Electron Microscope Examination

Electronic micrographs of the microspheres latex suspension which was filtered through a filter membrane and allowed to air dry in a laminar flow hood were made using a scanning electron microscope (JEOL JSM-35CF SEM, Japan.). Prior to examination, the samples were dried in a vacuum and coated with a sputtering device.

RESULTS AND DISCUSSION

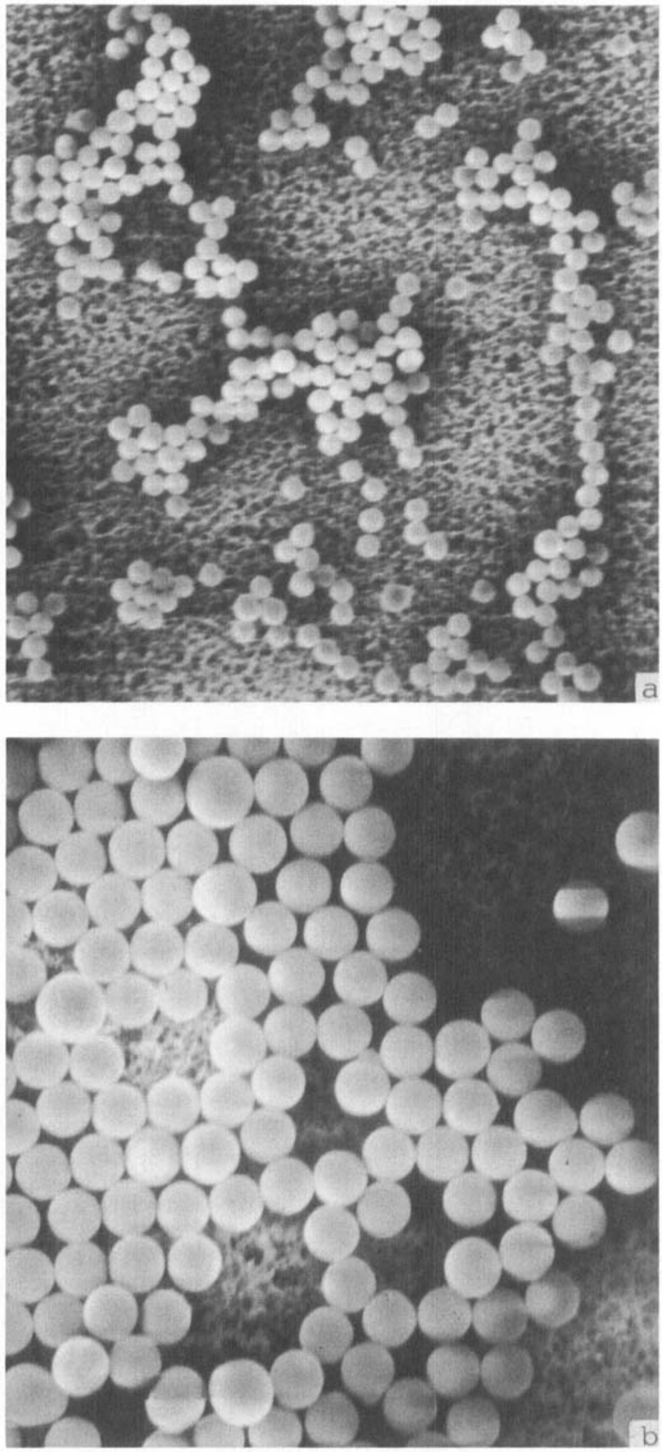
Scanning Electron Micrographs of P.D.V.B. Microspheres

The P.D.V.B. microsphers having a nominal diameter 5.96 μm are shown in the scanning electron microscope photomicrographs in photographs 1a, b and c. The photomicrographs clearly show that the microspheres are spherical in shape. Only a few of them are in slightly ellipsoidal shape. Based on the longest dimension the size of the microspheres is essentially in the range 3-7 μm. Some of the microspheres are slightly larger than 10 μm.

Comparison of Coulter Counters and HIAC/Royco Analysers

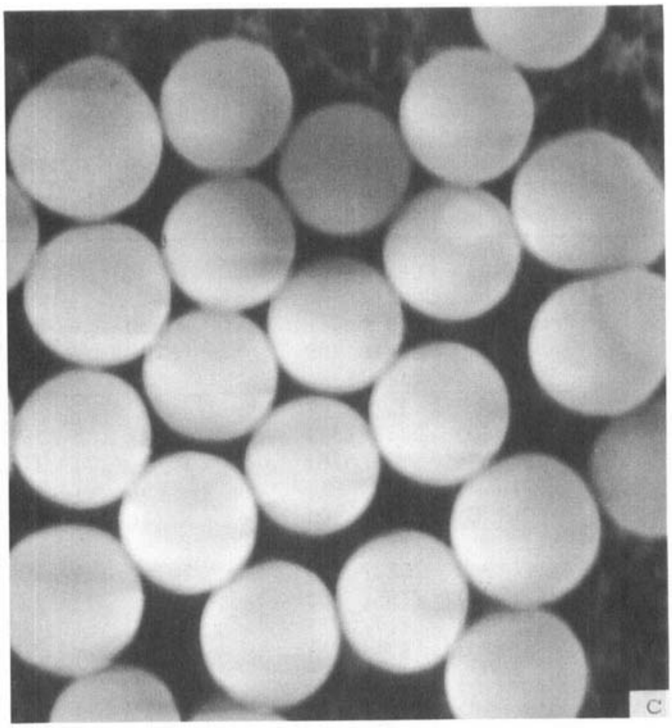
Tables 1, 2 and 3 show the cumulative counts of microspheres in 20 testing solutions determined using 2 Coulter Counters and 2 HIAC/Royco analysers at four size levels. The Coulter Counters illustrated that there was not any microsphere at $\geq$ 25 μm level. At higher size levels ($\geq$ 10 and $\geq$25 μm) the cumulative counts obtained using the HIAC/Royco analysers are higher than those obtained using the Coulter Counters.

The Coulter Counters sized particles in 3 dimensions and expressed as the equivalent volume spherical diameter. On the other hand the HIAC/Royco analysers sized the largest light-obscuration (shadow) caused by the particles passing/rotating through the sensor . For an ellipsoidal microsphere the HIAC/Royco analyser will give a larger projected area diameter. However, the ellipsoidal shape factor may not affected the equivalent volume diameter determined by the Coulter Counter . The microphotographs showed that some of the microspheres are in slightly ellipsoidal shape. This may explain the higher cumulative counts obtained by the HIAC/Royco analysers at the higher



PHOTOGRAPH 1

Photomicrographs of P.D.V.B. microspheres latex suspension
Magnification: (a) x 500 (b) x 1300 (c) x 3000



PHOTOGRAPH 1C

size levels (≥10 and ≥25 μm). Another possible cause of the higher counts at these two size levels might be due to "coincidence effect". The coincidence is defined as the presence of two or more particles in the optical view volume of a particle analyser at the same time. Chrai et al have expressed that the accuracy of the data determined by HIAC/Royco analysers can be affected by "coincidence effect".

Groves and Wana[1] demonstrated that particle count in normal saline (> 2 μm) obtained using the Coulter was higher than that obtained using the HIAC. They attributed the differences to the shape factor of the particles in the solutions. Dawes et al[2] also showed that the Coulter would give a much higher count than the HIAC for

TABLE 1

Cumulative Particle Count per ml (mean of 6 determinations and standard deviation) and t-Test analysis.

Solution	Size Range ≥; µm	Coulter Counter TA II	Coulter Counter Multisizer	t-Test	HIAC/ Royco (NTUH)	HIAC/ Royco (CCPC)	t-Test
Compd. Sod. Lactate	2	2328(40)	2338(133)	N	1652(16)	1789(39)	-
	5	2011(52)	2065(76)	N	1517(19)	1639(23)	-
	10	9(4)	12(4)	N	55(2)	45(2)	-
	25	0(0)	0(0)	N	2(1)	1(1)	N
0.9% NaCl	2	2515(96)	2622(82)	N	2014(19)	1395(42)	-
	5	2232(94)	2185(30)	N	1885(18)	1239(39)	-
	10	11(5)	17(7)	N	56(5)	94(4)	-
	25	0(0)	0(0)	N	2(1)	2(0)	N
Velip	2	2619(48)	2653(63)	N	1461(23)	1927(10)	-
	5	2213(42)	2118(48)	N	1192(6)	1611(13)	-
	10	16(7)	17(9)	N	157(5)	60(5)	-
	25	0(0)	0(0)	N	1(1)	1(1)	-
Ringer Solution	2	2581(108)	2562(101)	N	1531(30)	1484(20)	-
	5	2337(102)	1969(40)	-	1416(24)	1393(10)	N
	10	10(3)	17(9)	N	171(4)	203(13)	-
	25	0(0)	0(0)	N	2(2)	2(1)	N
Isoton II	2	3437(91)	3355(58)	N	2432(187)	2285(36)	N
	5	2635(36)	2459(40)	-	2116(99)	2101(19)	N
	10	31(6)	28(7)	N	308(35)	160(8)	-
	25	0(0)	0(0)	N	8(3)	2(1)	-
5% Glucose	2	2462(58)	2386(52)	-	1597(23)	1595(35)	N
	5	2157(0)	2055(44)	-	1512(21)	1405(14)	-
	10	10(5)	7(3)	N	38(1)	103(2)	-
	25	0(0)	0(0)	N	8(4)	1(1)	-
10% Glucose	2	2380(65)	2545(79)	-	1945(11)	1880(18)	-
	5	2074(38)	2125(49)	N	1808(10)	1633(12)	-
	10	16(5)	22(13)	N	45(4)	107(9)	-
	25	0(0)	0(0)	N	2(1)	2(1)	N

N:P>0.05; -:P<0.05; NTUH:National Taiwan University Hospital; CCPC:China Chemical & Pharmaceutical Co., Ltd.

TABLE 2

Cumulative Particle Count per ml (mean of 6 determinations and standard deviation) and t-Test analysis.

Solution	Size Range ≥; µm	Coulter Counter TA II	Coulter Counter Multisizer	t-Test	HIAC/ Royco (NTUH)	HIAC/ Royco (CCPC)	t-Test
50% Glucose	2	2335(49)	2309(102)	N	1732(14)	1682(15)	-
	5	2066(60)	1857(134)	-	1607(70)	1588(10)	N
	10	8(2)	21(4)	-	52(5)	29(3)	-
	25	0(0)	0(0)	N	1(1)	1(1)	N
Xylitol	2	2505(42)	2439(53)	-	1848(15)	1997(11)	-
	5	2161(50)	2157(63)	N	1615(17)	1817(8)	-
	10	22(6)	8(4)	-	133(6)	89(5)	-
	25	0(0)	0(0)	N	3(1)	2(1)	N
Maltose	2	2474(55)	2544(50)	-	1905(25)	2063(30)	-
	5	2122(64)	2266(44)	-	1744(14)	1905(15)	-
	10	6(3)	7(4)	N	51(2)	71(6)	-
	25	0(0)	0(0)	N	2(1)	1(1)	N
Gurocan	2	2537(56)	2574(101)	N	1373(13)	1857(17)	-
	5	2158(64)	2265(61)	-	1287(20)	1807(15)	-
	10	8(5)	10(4)	N	183(9)	124(4)	-
	25	0(0)	0(0)	N	1(1)	1(1)	N
Fructose	2	3428(72)	3314(69)	-	2210(8)	2053(25)	-
	5	3105(58)	2960(52)	-	1946(10)	1904(10)	-
	10	13(5)	11(3)	N	194(7)	138(8)	-
	25	0(0)	0(0)	N	3(1)	1(1)	-
2.5% Glucose	2	2696(52)	2683(76)	N	1538(24)	1758(19)	-
+	5	2333(37)	2214(75)	-	1407(11)	1635(19)	-
0.45%	10	21(7)	12(0)	-	152(3)	73(8)	-
NaCl	25	0(0)	0(0)	N	3(1)	2(1)	N
5% Glucose	2	2578(47)	2461(51)	-	2194(49)	1870(10)	-
+	5	2169(34)	2066(63)	-	2028(25)	1799(12)	-
0.33%	10	11(6)	15(2)	N	57(6)	91(12)	-
NaCl	25	0(0)	0(0)	N	2(1)	2(1)	N
5% Glucose	2	2790(35)	2781(53)	N	1847(14)	2191(25)	-
+	5	2473(3)	2412(56)	N	1629(23)	1995(22)	-
0.9%	10	10(4)	7(4)	N	109(2)	37(2)	-
NaCl	25	0(0)	0(0)	N	2(1)	1(1)	N

N:P>0.05; -:p<0.05; NTUH:National Taiwan University Hospital; CCPC:China Chemical & Pharmaceutical Co., Ltd.

TABLE 3

Cumulative particle Count per ml (mean of 6 determinations and standard deviation) and t-Test analysis.

Solution	Size Range ≥; µm	Coulter Counter TA II	Coulter Counter Multisizer	t-Test	HIAC/ Royco (NTUH)	HIAC/ Royco (CCPC)	t-Test
Promin	2	3069(44)	3039(51)	N	1869(21)	1452(33)	-
	5	2613(67)	2698(46)	-	1763(14)	1376(33)	-
	10	11(3)	10(3)	N	138(5)	91(7)	-
	25	0(0)	0(0)	N	1(1)	2(1)	N
Aminol-K	2	2539(65)	2703(171)	-	1459(10)	1704(17)	-
	5	2295(56)	2260(106)	N	1342(10)	1549(12)	-
	10	9(7)	20(6)	-	180(6)	185(8)	N
	25	0(0)	0(0)	N	4(1)	2(1)	N
Aminol-s	2	2721(64)	2861(151)	N	1328(11)	2254(27)	-
	5	2419(68)	2321(40)	-	1257(12)	1949(19)	-
	10	11(5)	12(4)	N	94(5)	97(8)	N
	25	0(0)	0(0)	N	1(1)	4(2)	-
Conamin	2	2875(52)	2876(87)	N	1220(11)	2013(26)	-
	5	2662(45)	2341(50)	-	1142(8)	1920(18)	-
	10	6(3)	21(7)	-	184(3)	123(13)	-
	25	0(0)	0(0)	N	1(1)	1(1)	N
Aminogen -X	2	3691(55)	3882(116)	-	1865(13)	2188(16)	-
	5	2839(64)	3111(73)	-	1777(13)	2119(14)	-
	10	45(12)	68(13)	-	226(4)	88(9)	-
	25	0(0)	0(0)	N	1(1)	1(1)	N

N:P>0.05; -:P<0.05; NTUH:National Taiwan University Hospital; CCPC:China Chemical & Pharmaceutical Co., Ltd.

determining elongated particles (crystals) in amino-acid solutions.

Haines-Nutt and Munton[3] also showed that the counts of particulate matter in a number of parenteral solutions by HIAC are lower than those obtained by Coulter. They believed that other than particle shape factor, the refractive indices of the particles and the medium would also be responsible for the lower count of particulate matter by the HIAC.

Tables 1, 2 and 3 illustrated that the Coulter Counters saw more of the total microspheres than the HIAC/Royco did. This is quite surprising. Since the microspheres were essentially in spherical shape. The microspheres added to the 20 different solutions were come from the same bottle of latex sphere suspension. The microsphere concentration of the testing solutions was below the maximum concentration set by manufacturer for the two sensors used in the present study. Therefore, it is believed that the effects of particle shape factor, particle refractive index and coincidence effect shall not be responsible for the lower counts obtained by the HIAC/Royco analysers at the lower size levels.

However, Seville et al[9] expressed that the response of a single particle counter is sensitive to both refractive index and shape of the particles sampled. The ratio of the cumulative counts obtained by the HIAC/Royco (NTUH) to those obtained by the Coulter Counter (TAII) ranged from 0.4-0.8. These variation could be due to the effects of relative refractive indices between the microspheres and the solutions.

Comparison of Two Coulter Counters

Comparatively little data have been published on the comparison study between Coulter Counters. Johnston and Swanson[8] reported the results of multi-lab tests on the particle size distribution in a test dust sample by 3 different investigators using Coulter Counters. The curves of the cumulative count per ml vs particle diameter for the 3 sets of results are virtually superimposable. This indicates that the cumulative counts obtained using Coulter Counters in the three different laboratories are in good agreement.

Tables 1, 2 and 3 showed that the cumulative counts obtained by the 2 Coulter Counters at 4 different size levels are similar. t-Test analysis showed that 8 out of the 20 solutions gave significant difference for the cumulative counts at 2 μm level. These might be attributed to the differences in precise size levels set for the TA II and Multisizer after calibration.

Comparison of Two HIAC/Royco Analysers

Groves and Wana[1] confirmed that a HIAC PC 320 gave a higher count than a Royco model 345 at 4 different size levels when a standardized D.V.B. latex suspension was presented to the two instruments. They expressed that it was difficult to explain the differences because the test material was a spherical D.V.B. latex. The type of flow, turbulent or laminar can affect the HIAC/Royco determination of particulate matter in solutions. Only spherical particles are unaffected. Based on this ground it is also difficult to explain the differences between the cumulative counts obtained by the two HIAC/Royco analysers at the ≥2 μm level.

Chrai et[7] al had classified the limitations in the use of HIAC/Royco analyser in five different categories. They stressed that the sensitivity of sensors varied from model to model and unit to unit. In the present study the main difference between the use of the two HIAC/Royco analysers was the two different sensors. We believed that this difference would be responsible for the variation of the cumulative counts obtained by the two HIAC/Royco analysers.

SUMMARY AND CONCLUSION

The purpose of this study was to compare the ability of 2 Coulter Counters and 2 HIAC/Royco analysers in sizing and counting of the microspheres (nominal diameter, 5.96

μm) in parenteral solutions. The lower cumulative counts (≥2 μm) obtained by the HIAC/Royco analysers could be attributed to instrumental limitations of this technique. Therefore, it may be said that HIAC/Royco analyser is not good for the determination of absolute count of particulate matter in LVP solutions. On the other hand a Coulter Counter seems to be more suitable for the determination of absolute count of particulate matter in LVP solutions and also the comparison of results obtained between different laboratories.

ACKNOWLEDGEMENTS

This work is partially financed by the Department of Health, Executive Yuan (DOH-77-67) and National Science Council (NSC 77-0606-B037-05). The authors wish to thank the Department of Dispensing Pharmacy, National Taiwan University Hospital and China Chemical and Pharmaceutical Co., Ltd. for the use of their HIAC/Royco particle analysers; Research Center, Yuen Foong Yu paper MFG. Co., Ltd. for the use of their Coulter Counter Multisizer; and Miss L.J. Lee for typing the manuscript.

REFERENCES

1. M.J. Groves and D. Wana, Powd. Technol., 18, 215, (1977).

2. V.H. Dawes, J. Erawati and J. Eluemuno, J. Pharm. Pharmacol., 35, suppl. 53p, (1983).

3. R.F. Haines-Nutt and T.J. Munton, Ibid., 36, 534, (1984).

4. British Pharmacopoeia, Volume II, Appendix XII, A 120, (1980).

5. H.G. Schroeder and P.P. Deluca, J. Parenter. Drug Assoc., 34, 1983, (1980).

6. Personal Communication, Joung Y.H., Sunway corporation, Taipei, Taiwan, April, (1988).

7. S. Chrai, R. Clayton, L. Mestrandrea, T. Myers, R. Raskin, M. Sokol and C. Willis, J. Parenter. Sci. Technol., 41, 209, (1987).

8. P.R. Johnston and R. Swanson, Powd. Technol., 32, 119, (1982).

9. J.P.K. Seville, J.R. Coury, M. Ghadiri and R. Clift, Part. Charact., 1, 45, (1984).