PARTICLE SIZE DISTRIBUTION

Light Microscopic Determination of Particle Size Distribution in an Aqueous Gel

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INTRODUCTION

The need for particle size control in the manufacture of pharmaceuticals is becoming increasingly apparent as the pharmaceutical industry attempts to capitalize on some APIs with less-than-ideal solubility profiles. Also, significant advances in drug delivery have been made in which a finely divided API, with the concomitant increase in specific surface area, has resulted in increased bioavailability. Precise particle size control technologies have also assisted in the development of drug delivery platforms for the delivery of a medicament to the lung. As these trends have occurred, the need for highly reproducible particle size assessment techniques has grown significantly in the past decade. The interest in particle size measurements will remain high, particularly in view of FDA trends toward recommending more thorough descriptions of particle size distributions in submissions in which the emphasis of a drug product claim is based in a tightly controlled particle size.

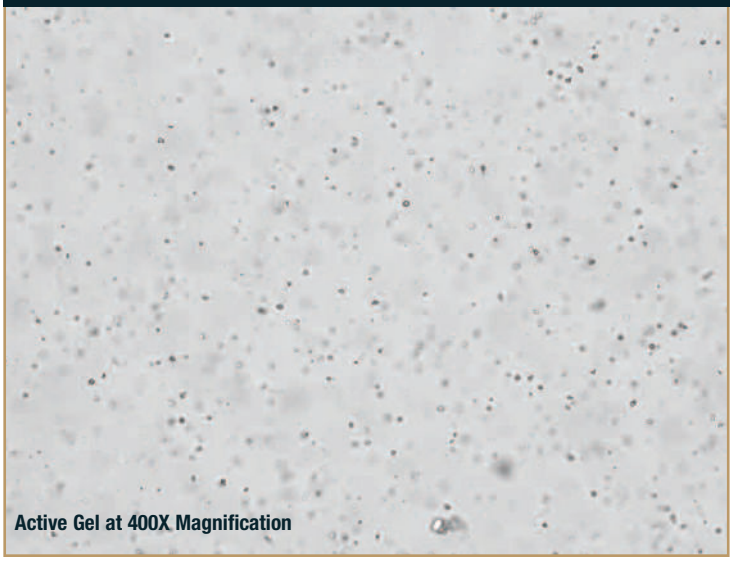
COMPARISON OF METHODS TO MEASURE PARTICLE SIZE DISTRIBUTION

Particle sizing of dispersion can be accomplished using laser scattering or diffraction techniques or by disc centrifuge techniques if high resolution of the size distribution is required. Laser scattering requires very low particle concentrations, usually requiring significant sample dilution. The particles in the sample must be below 1 micrometer in size and free to undergo Brownian motion. For laser diffraction methods, dilution is again often required to optimize the intensity of diffracted light at the detectors, though dilution requirements are not as stringent as for scattering techniques. These methods give weight-average particle size, and although these can be

FIGURE 1

Placebo at 400X Magnification

FIGURE 2



Active Gel at 400X Magnification

mathematically converted to number-weighted distributions, the conversions can produce misleading artifacts.

Disc centrifuge methods rely on the ability of the particles to move through the sample under the influence of a centripetal force generated in a spinning disc containing the sample; so the sample viscosity must be low enough that the force overcomes viscous resistance to particle movement in the field. While some drug product formulations can be diluted without significant change to the particle size distribution (allowing appropriate sample concentrations and viscosities for the aforementioned methods) for the development of highly viscous gel-based products, whose API particle size distribution may be affected by significant sample dilution, standard methods may not be not appropriate. As a pharmaceutical contract research organization (CRO), Particle Sciences routinely develops such viscous systems for clients (especially for topical products), and to enable useful particle sizing of such products, has developed two methods to determine the particle size distribution of suspended API in a viscous aqueous gel that involve a minimum of sample preparation and can analyze samples with broad particle size distributions.

The methods are based on laser diffraction using a specifically designed cell for viscous paste analysis, and image analysis of optical photomicrographs using image analysis software to identify particles and numerically bin them according to shape and size.

TABLE 1

Sample	Magnification	# Objects	d10	d50	d90
Placebo Alone	400X	106	0.73	1.4	3.2
Placebo Spiked w/ 1.9 µm Latex Beads	400X	23031	2.5	3.5	4.4
Placebo Spiked w/ 5.3 µm Latex Beads	400X	4090	2.8	6.1	6.9
Placebo Spiked w/ 20.9 µm Latex Beads	400X	482	1.8	22.1	26.1
Placebo Spiked w/ 43.3 µm Latex Beads	100X	622	16.2	45.2	63.9
Active Gel	400X	7197	2.2	4	7.1

Spiked Placebo Gel Linearity Results

TABLE 2

Added Particle Size (µm)	Determined Size (d50 in µm)	% Deviation
1.90	3.5	84.2
5.34	6.1	14.2
20.9	22.1	5.7
43.3	45.2	4.4

Accuracy of Method

TABLE 3

Added Particle Size (µm)	Determined Size (d50 in µm)	% RSD (n=4)
1.90	3.5	1.6
5.34	6.1	2.7
20.9	22.1	2.6
43.3	45.2	0.3

Precision of Method

TABLE 4

Sample	Magnification	No. Objects	d50
1 mil Thickness (25 µm)	400X	10806	2.2
2 mil Thickness (50 µm)	400X	10282	2.2
5 mil Thickness (125 µm)	400X	10513	2.2

Gel Thickness Study

The method of particle size distribution determination by optical microscopy and image analysis is a technology-intensive method requiring the capacity to automatically acquire and analyze a large number of photomicrographs. Particle Sciences uses a powerful optical microscope fitted with a dedicated digital camera and automated stage and focusing movement, controlled by software that also handles the analysis of the images collected. This enables automatic collection of the large number of image objects required for statistically relevant analysis, which includes measurement of length, width, area, circle diameter, roughness, etc.

With careful selection of objectives and camera, the technique also has a broad dynamic range in which the upper limit is several millimeters at low magnification, and the lower limit that is close to 1 micrometer, which is correlated to the resolution inherent in the use of white light illumination. A significant advantage of microscopy over laser diffraction is verifiable and calibrated accuracy, as calibration of the instrument may be carried out with the use of NIST traceable stage micrometers and verified by the use of the monodisperse latex microspheres. This is opposed to that available for laser diffraction, which is based on first principles, and the measurement may only be verified with the use of monodisperse latex microspheres but no corrections or "calibrations" may be performed to modify the

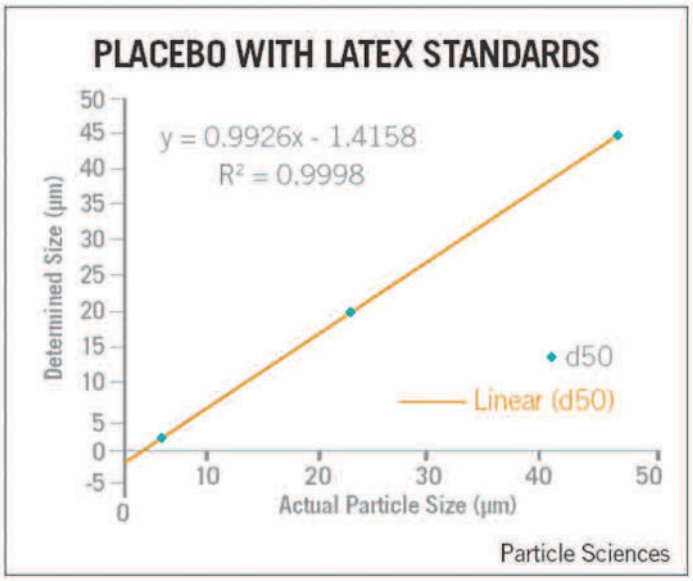
instrument result if an inaccurate result is observed. Optical microscopy, however, suffers in the need for a very large number of observations. ISO 13322-1 contains a guide for the number of particles required at a 95% confidence based on the width of the particle size distribution. The shortcoming in this of course is the analyst has no knowledge of this fact a priori, and the range of distribution widths described by ISO 13322-1 are too few to describe many real-world particle size applications even though the largest of the distributions requires an extraordinarily high number of observations.

Sample preparation in the case of microscopy is very simple, requiring only the sandwiching of ~100 microliters of sample between a slide and a cover slip with gentle pressure to achieve a sample thickness of ~25 micrometers.

Particle size standards are assorted monodisperse polystyrene latex standards ranging from 1.0 micrometers to 43.6 micrometers, and commercially available polydisperse glass microbead standards of 1 to 10 micrometer, 3 to 30 micrometer, and 10 to 100 micrometer size ranges.

The method described herein was developed for the analysis of a gel based on hydroxyethyl cellulose (HEC) and containing 0.05% (w/w) micronized API. The placebo was prepared in the same fashion as the active, but without API. The monodisperse standards were prepared at approximately 0.001% (w/w) by adding approximately 1 microliter of 1% (w/w) dispersions of latex microspheres to 10 g of placebo, mixed thoroughly by hand, centrifuged at 150 g for 30 minutes to remove entrained air bubbles. Polydisperse standards were prepared at 0.005% (w/w) by the addition of 0.1 g standard beads to 20 g of placebo gel, mixed thoroughly by hand, and centrifuged at 150 g.

FIGURE 3



Placebo With Latex Standards

CHALLENGES TO VALIDATION OF PHYSICAL CHARACTERIZATION METHODS

If particle size is to be a quality control criteria for a given product or claims of product stability are to be made based on a specific particle size, the method of particle size distribution determination will have to be validated. The US FDA cGMP section 211.165(e) requests methods to be validated. The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with section 211.194(a). These requirements include a statement of each method used in testing the sample to meet proper standards of accuracy and reliability, as applied to the tested product[1].

Laser methods are incapable at this time of API specificity. An optical system is currently available that incorporates near infrared spectroscopy as a detection method (SyNIRgi, Malvern Instruments, Ltd.). This system should be able to discern between API and excipients or impurities. However, for this discussion, all particles will be included in the microscopic and laser-sizing techniques regardless of the identity of the particle.

Often ignored in particle sizing method validation are the parameters of detection limit and quantitation limit of which only the detection limit may be addressed by reference to the instrument manufacturers claims. Range and linearity can be examined in the same way as in all other techniques in which placebo or vehicle is spiked with standard material, and measurement is carried out in the intended fashion. In the case of automated image analysis techniques, the lower bound of the range will be defined by the area inscribed by a minimum number of pixels that are capable of carrying any size/shape information.

In this case, the lower limit was defined in the image analysis routine as a 5 x 5 pixel square or a diameter of approximately 1 micrometer. Accuracy and precision can be assessed by the proximity of the experimental values to those published for the standard material, and the coefficient of variation calculated from repeated measurements of the spiked sample, respectively. Intermediate precision or analyst-to-analyst variation can be seen by

the examination of multiple preparations of the material by multiple analysts and calculation of the resulting RSD between analysts and the RSD of all samples pooled. Resolution of the method, defined as the ability of a technique to differentiate between discrete monodisperse particle sizes, can be addressed in the linearity/range examinations. If the definition is based on an instrumental ability to resolve monodisperse particle sizes that are mixed, the assessment becomes more difficult.

PRELIMINARY VALIDATION OF A MICROSCOPY TECHNIQUE

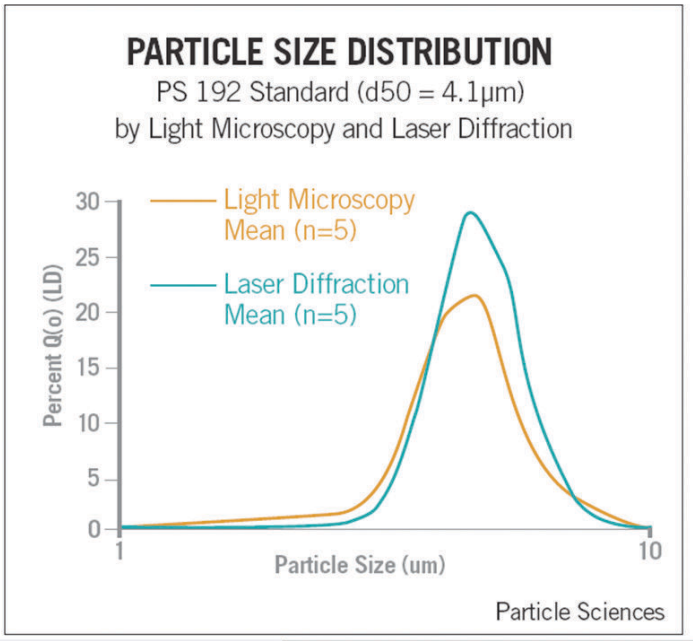
The determination of particle size by microscopy requires the use of defined routines that rigorously control the collection of photomicrographs and the binary processing of the image results. Figures 1 and 2 are example photomicrographs of the placebo and active HEC gels, respectively, at 400X magnification.

Confirmation of the counting/image analysis technique for particle sizing was accomplished by mixing placebo gel with latex beads of known size, performing the proposed sample preparation, and analyzing the slide via the counting/acquisition routine. The results of spiked placebo gel determinations agree very well with expected values, as shown in Figure 3.

For measuring the largest particles (43 micrometers), the result was generated by the use of the 100X microscope objective to allow more particles per field to be captured. If required, the method could be modified so that all results will be generated using the lower magnification. This would be contingent on the linear range required of the final method.

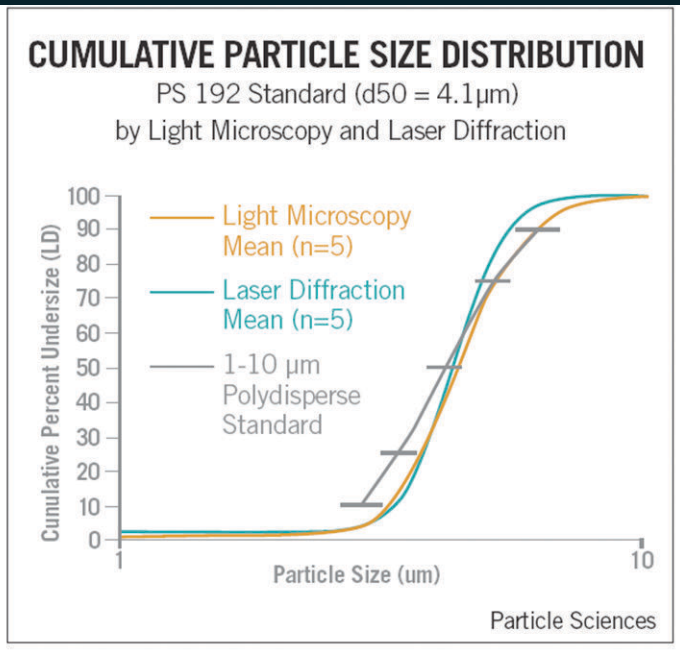
The accuracy and precision results of the method are collated in Tables 2 & 3, respectively. Acceptable precision is demonstrated with a maximum RSD of 2.7% for four determinations performed on the 5.34 micrometer latex beads spiked into the placebo. A minimum RSD of 0.3% is demonstrated for the four determinations of the 43.3 micrometer beads spiked into the placebo. The accuracy of the method shows significant deviations. A deviation of 84.2% was found for placebo gels spiked with 1.9 micrometer latex beads. The larger spiked particle diameter results were more accurate with minimum deviation of 4.4% demonstrated for 43.3 micrometer latex beads spiked into the placebo, and a maximum deviation of 5.7% demonstrated for 20.9 micrometer beads spiked into the placebo. The absolute error of ~1 micrometer is quite small, to be expected, and not considered an issue for the tracking of change in particle size, for

FIGURE 4



Particle Size Distribution

FIGURE 5



Cumulative Particle Size Distribution

which this method was developed, nor for relating particle size to other system analysis, such as in vitro release testing (IVRT).

A further indication of method "robustness" was investigated in which an active gel was assessed using the counting routine, and the gel thickness was varied. This was accomplished by using spacer tapes of 1, 2, or 5 mil in thickness to separate the cover slip from the slide to create a gap for the sample. The results in Table 4 indicate that the sample thickness had little effect on the determined median (d50) particle size of the active gel.

CORRELATION OF LASER DIFFRACTION METHOD WITH LIGHT MICROSCOPY

Ideally, the ultimate verification of any analytical techniques involved in particle size analysis would be the exact agreement with another technique. To this end, the two techniques discussed here were used to examine samples of a polydisperse particle size standard suspended in water. Presented in Figure 4 are overlaid microscopic particle size distribution and laser diffraction particle size distribution of the 1 to 10 micrometer polydisperse standards (PS192). The laser diffraction data are the average of 5 distribution measurements. The distribution results from each method agree well with the 95% confidence intervals provided with the certified standard values (Figure 5). The results of similar determinations performed with the HEC gel showed an upward shift (~2 micrometers) in the particle size distribution on estimation by microscopy.

We have shown that optical microscopy can be used to monitor the particle size of API in an aqueous gel. The method is able to be validated, robust, and reliable as can be seen by the establishment of linearity, precision, and accuracy, with minimal sample preparation. In addition, because only very small volumes of gel are required, microscopy presents no challenge when only small volumes of sample are available.

REFERENCE

1. US FDA - Guidance for Industry (draft) Analytical Procedures & Methods Validation: Chemistry, Manufacturing, and Controls and Documentation; 2000.

BIOGRAPHIES

Mr. Philo Morse joined Particle Sciences in early 2008. With several years experience in the development of testing methods for inhaled pharmaceutical dosage forms, he has accepted the responsibility of managing Particle Sciences' Physiochemical Characterization Laboratory. Mr. Morse has over 15 years of experience in academic research and development, pharmaceutical industry research and development, and quality control laboratories. He earned his MS in Chemistry from SUNY College of Environmental Science and Forestry in Syracuse, NY.

Dr. Andrew Loxley is Director of New Technologies at Particles Sciences Inc., a contract research organization in Bethlehem, PA, specializing in pharmaceutical formulation development. He leads a variety of projects, many based on novel and proprietary nanotechnologies, in fields from HIV vaccine and microbicide development to gene-silencing siRNA delivery. Prior to joining Particles Sciences, he led the development efforts in next-generation lithium ion batteries at A123 Systems Inc, electrophoretic displays at EINK Corp., and latex-based adhesives at Synthomer Ltd. British-born, he earned his BSc in Chemistry from the Univeristy of Sussex and his PhD in Physical Chemistry focusing on Microencapsulation from the University of Bristol.