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Application News

No. Q120

Powder Property Analysis

Rapid Quantification of Blood Particles: Application of qLD Method to Blood Samples

Because particulate substances in blood (e.g. erythrocytes, leucocytes, thrombocytes, apoptotic bodies, microvesicles, and exosomes, see Fig. 1) have diverse types of bioactivity in the body, information concerning their concentrations is important in disease risk prediction and judgment of health condition.

Although automatic blood cell measurement devices and techniques such as the electrical sensing zone method, ELISA, and flow cytometry are used as quantification methods for particles in blood, there are problems affecting throughput when analyzing large numbers of samples, in that the antibody reaction requires time and the objects of measurement and target particle size range are limited.

The Shimadzu Aggregates Sizer™ is an analytical device which measures the size and concentration of particles in liquids by the quantitative laser diffraction (qLD) method. Since pretreatment by the antibody reaction is not necessary, and the particle size distribution is calculated from the total information obtained when the laser is irradiated once on a large number of particles, it is possible to acquire data with high reproducibility in one measurement requiring only a few seconds. Because the measurable particle size range is wide, from 0.1 µm to 10 µm, if the sizes of the particles are different, the concentrations of the respective particles can be obtained without separation. (However, quantification accuracy decreases in the case of mixed samples of unknown density and refractive index. This is not a problem in relative comparisons of the same types of particles.)

This article introduces an example of measurement of blood with the Aggregates Sizer and quantitative evaluation of the particles in blood.

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Materials and Methods

Fig. 2 shows the sample preparation procedure. Whole blood was divided into two samples, and the supernatant of one was sampled by centrifugal separation at 250 G × 15 min as platelet-rich plasma (PRP). The supernatant of the other was sampled by centrifugal separation at 600 G × 1 min + 10,000 G × 20 min as plasma. Particle size distribution and concentration measurements were carried out using the Aggregates Sizer. Table 1 shows the measurement conditions.

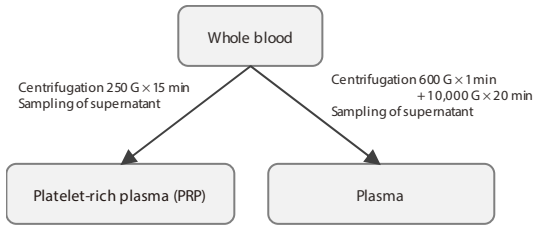

Fig. 1 Sample Preparation Procedure

Table 1 Measurement Conditions

Instrument	: Aggregates Sizer
Method	: Quantitative laser diffraction method (qLD method)
Measuring unit	: Microcell
Measurement range	: 0.1 µm -10 µm
Refractive index	: 1.41-0.10i *1
Density	: 1.37 g/cm^3 *1

*1 If the composition of the particles is known, more accurate quantitative measurement is possible by using that information in calculation of this parameter.

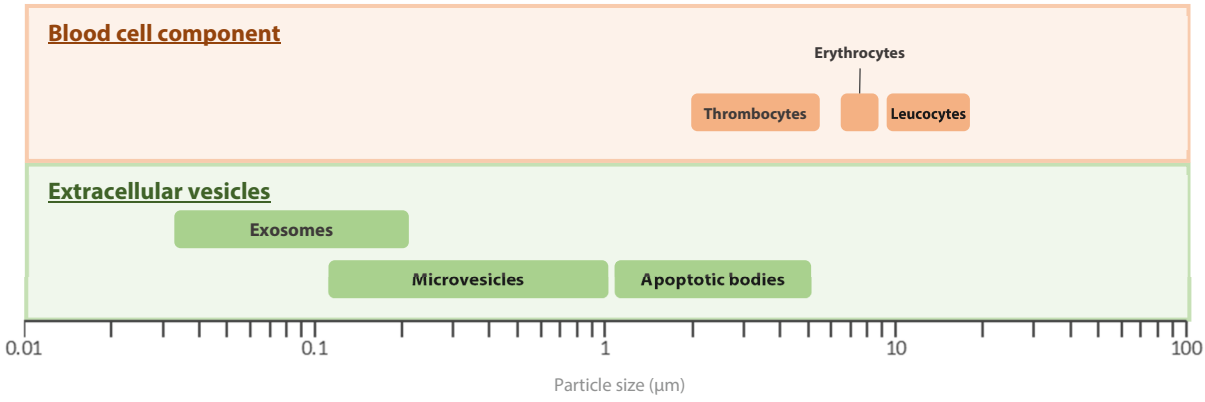

Fig. 2 Sizes of Particles in Blood

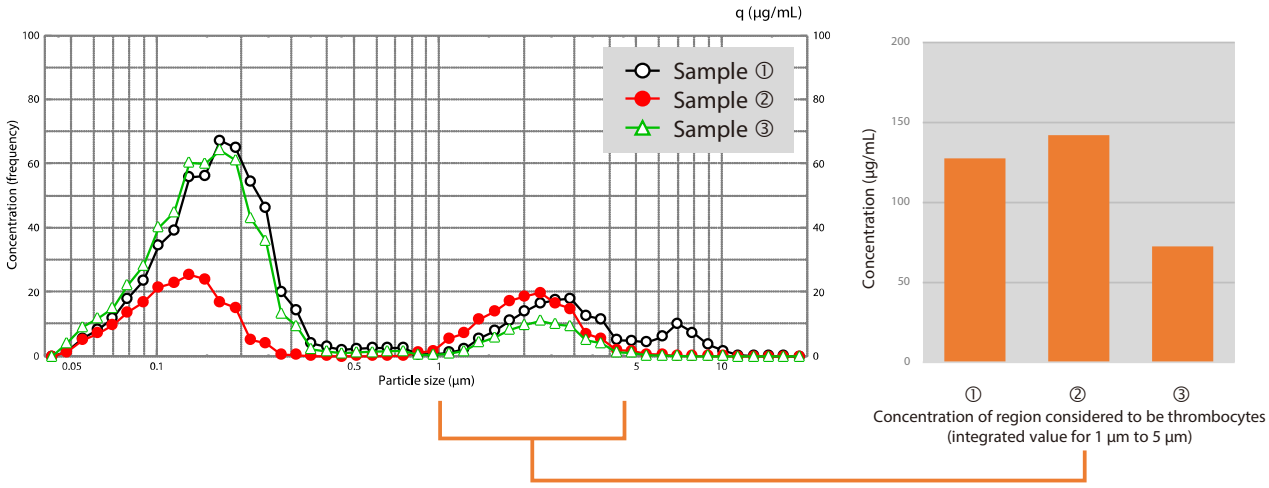

Fig. 3 Measurement Results of PRP

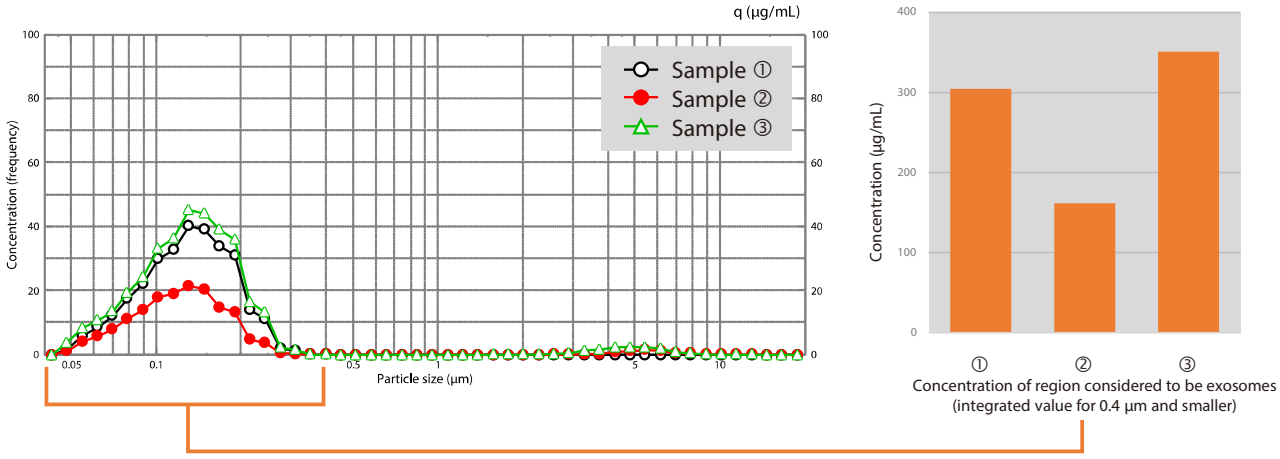

Fig. 4 Measurement Results of Plasma

Measurement Results

Fig. 3 and Fig. 4 show the measurement results of PRP and plasma, respectively. The figures show the particle size distributions of three samples and the concentrations of particles that were estimated to be thrombocytes (Fig. 3) or exosomes (Fig. 4).

Although the substances cannot be identified by this instrument, it can be estimated from their size that the peaks around 2 µm and 8 µm in the particle size distribution of the PRP sample are thrombocytes and erythrocytes, respectively, and the peak around 100 nm in the distribution of the plasma sample is exosomes.

As the particle concentrations, for the PRP sample, the particle concentration is shown for the size range of 1 µm to 5 µm, which is estimated to be thrombocytes, and for the plasma sample, the concentration is shown for the range of 0.4 µm and smaller, which is estimated to be exosomes. The differences in the amounts of the respective particles in each sample can be confirmed from these results.

Conclusion

As shown above, quantitative evaluation of the concentration difference between samples of blood particles with sizes of 0.1 µm to 10 µm with less separation operation than with the conventional technique is possible by using the Aggregates Sizer.

The product described in this document can be used only in research applications.
It is not approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan.
It cannot be used for the purpose of medical diagnosis, treatment, or related procedures.

First Edition: Oct. 2019

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