METHOD OF LIGHT SCATTERING SPECTROSCOPY FOR READING THE PRECIPITATION TEST

I. M. Aref'ev, A. P. Es'kov,
M. Ya. Korn, and V. A. Chibisova

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A method of light scattering spectroscopy was used to read the precipitation test. The system investigated consisted of human serum globulin and rabbit antiserum against human globulins. By light scattering spectroscopy the precipitation test can be read in much higher dilutions of antigen and antibody than by the ordinary visual method.

KEY WORDS: precipitation test; light scattering spectroscopy.

The possibilities of the method of light scattering spectroscopy (LSS) for problems in medical microbiology have been discussed previously [1]. The use of this method is promising fot the detection and study of antigen–antibody reactions. It has been shown [2] that antibodies can be detected by a change in the electrophoretic mobility of latex particles with antigen adsorbed on them when they combine with antibody. It has also been shown [3] that measurement of the quasielastic scattering spectrum can be an accurate and sensitive method of studying agglutination tests in very early stages.

The object of this investigation was to study the possibility of using the LSS method for reading the precipitation test.

LSS is a method of measuring narrow lines of quasielastic scattering of light [4]. Such lines arise through the scattering of monochromatic laser light in solutions or suspensions as a result of the brownian movement of the particles (biological molecules). The width of the line is proportional to the coefficient of diffusion of the particles. In turn, the coefficient of diffusion is inversely proportional to the size of the particles. If particles of this type are present in the suspension, the width of the quasielastic scattering spectrum determines the mean coefficient of diffusion. If an antigen–antibody reaction takes place in a suspension and leads to the appearance of larger particles than those of each of the components of the reaction, the mean coefficient of diffusion of the system will be reduced and the scattering spectrum narrowed.

By using the LSS method both the spectrum of scattered light and the correlation function or correlogram, which is a linear function of the spectrum, can be measured. The narrower the spectrum, the wider the correlogram.

The Malvern Model 4300 (England) correlation spectrometer was used. A block diagram of the spectrometer is shown in Fig. 1. Simultaneously with the correlogram, the integral intensity of light scattered at an angle $\theta = 90°$ was recorded in the experiments.

The precipitation test was studied in a system consisting of human globulin and rabbit antiserum against human globulin, using a series of dilutions of antigen with a constant concentration of antiserum (1:16) and also different dilutions of antiserum with a constant concentration of antigen (1 mg/ml). Corresponding dilutions of antigen and antiserum were mixed in precipitation tubes or titration plates and left for 45 min at room temperature. The reaction was then read visually and the correlograms recorded.

With dilutions of antigen of 1:8 and 1:16 the reaction could be read visually as strongly positive (++++); in a dilution of 1:128 as doubtful (±), and in higher dilutions of antigen the test was negative. Meanwhile, widening of the correlograms was observed down to the lowest antigen concentration used, namely 1:2048. Reduced correlograms R (nt) of antigen (AG) (1:1), of antiserum (AS) (1:16) and of mixtures of AS+AG in the

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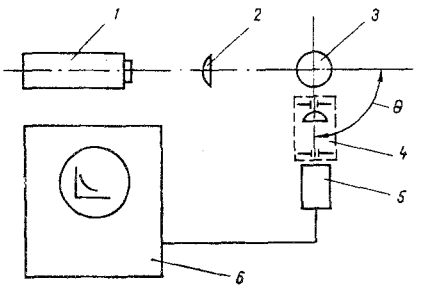

Fig. 1

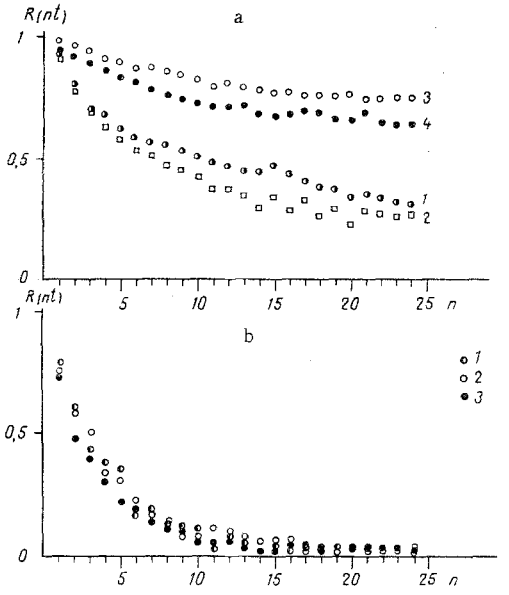

Fig. 2

Fig. 1. Block diagram of correlation spectrometer: 1) helium–neon laser (emission wavelengths 0.63 μ, output power 5 mW; 2) focusing lens; 3) cuvette with specimen (temperature 22°C); 4) optical receiving system; 5) photoelectric multiplier; 6) correlator; θ) angle of scatter.

Fig. 2. Reduced correlograms of scattered light R (nt). Abscissa, No. of correlator channel, n; ordinate, value of correlation function R (nt), where t is the sampling time. a) In system with reaction: t = 100 μsec; 1) AG 1:1; 2) AS 1:16; 3) AS 1:16/AG 1:1024; 4) AS 1:16/AG 1:2048. b) In system with no reaction: t = 200 μsec; 1) AG 1:16; 2) AS 1:16; 3) AS 1:16/AG 1:32.

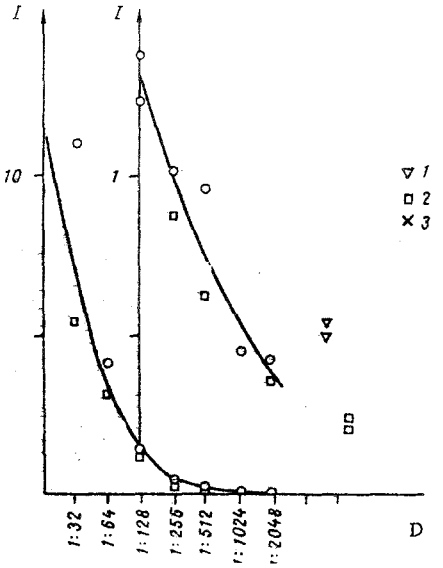

Fig. 3. Integral intensity of scattered light I (in relative units) in AS/AG system with different dilutions of antigen D. 1) AC 1:16; 2) AG 1:1; 3) AG 1:4.

ratios of 1:16/1:1024 and 1:16/1:2048 are illustrated in Fig. 2a. Absence of the antigen–antibody reaction would lead to additive summation of the curves for AG and AS, and for low AG concentrations the resultant curve would be close to the curve for AS (1:16). The striking widening of the correlograms 3 and 4 is evidence of the appearance of agglutinated particles in the mixture, i.e., of the presence of an antigen–antibody reaction.

These arguments are confirmed by the results of one of the experiments in which, when rabbit antiserum kept for a long time was mixed with antigen no precipitation reaction could be observed visually whether with the maximal concentration of antigen and antiserum or with different dilutions of the components. No widening of the correlograms likewise was observed in this case (Fig. 2b). No antigen–antibody reaction evidently occurred in this case.

Similar changes in the correlograms also were observed when the test was carried out with different dilutions of antiserum and a constant concentration of antigen (1 mg/ml). A reaction could be detected visually when the antiserum was used in dilutions of not more than 1:16 (both after 30 min at room temperature and also after 3 h), whereas according to the correlation curve and the intensity after 30 min the titer was 1:64 and after 3 h it was 1:256.

The results of measurement of the integral intensity of scattered light (two series of experiments) are given in Fig. 3. The region of the curve corresponding to AG concentrations below the limit of visual observation of the precipitation test (1:128) is also given on a scale ten times larger. On the right of Fig. 3, on the same enlarged scale values of the intensity of scattered light in AS (1:16) and in AG solutions (1:1 and 1:4) are plotted. Clearly although the method of measurement of the integral intensity of scattered light is more sensitive than the visual method, it is however less sensitive than the spectral method, for the intensity of scattering in AS (1:16) corresponds to the intensity of scattering in a mixture of AG+AS when the dilution of AG was 1:1024. The fact is that, despite the high sensitivity of the system of measuring the integral intensity of scattered light, this method is inferior to the spectral method, mainly because of the presence of dust in the solutions under ordinary conditions of observation of the reaction. The presence of dust has a far greater effect on the integral intensity than on spectrum formation. The LSS method thus has effectively greater sensitivity than the method of measurement of integral intensity.

The results indicate that the precipitation test can be read by means of LSS and that this method is more sensitive under certain conditions than the visual method.

Further investigations are necessary to assess the possibility of detecting and studying other immunologic reactions by the LSS method.

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