

# Kinetic study of the initial stages of agglutination process with scanning flow cytometer

Ivan V. Surovtsev<sup>\*\*a</sup>, Maxim A. Yurkin<sup>b</sup>, Alexander N. Shvalov<sup>a</sup>, Galina F. Sivolobova<sup>1</sup>,

<sup>a</sup> State Research Center for Virology and Biotechnology VECTOR, Koltsovo, Novosibirsk region, Russia, 630559

<sup>b</sup> Institute of Chemical Kinetics and Combustion, Institutskaya 3, Novosibirsk, Russia 630090

## ABSTRACT

The main disadvantage in ordinary agglutination immunoassay is difficulties to distinguish between specific and non-specific particle aggregation. We proposed to use Scanning Flow Cytometry to the kinetic study of the initial stages of agglutination process. The main advantage of the Scanning Flow Cytometry is a possibility to measure angular dependency of the light scattered by a single particle, an indicatrix. The most promising field for application of the indicatrix technology is a characterization of non-spherical particles. Validity of proposed method was verified by simultaneous measurements of the light scattering and fluorescence signal. We used Wentzel-Kramers-Brillouin approximation to simulate light scattering from two glued spheres and to explain the results obtained from measured indicatrices. To show an applicability of the proposed technique, the kinetic experiments were performed on latex particle covered with BSA (diameter 1.8  $\mu\text{m}$ ). Kinetics of dimer fraction growth initiated by mixing BSA-covered latex particles with anti-BSA immunoglobulins IgG was studied. In order to evaluate kinetic rate constant simple kinetic model involved only dimer growth reaction was applied for data treatment. Two kinetic rate constant for dimer fraction growth  $k_B=2.88 \cdot 10^{-12} \text{ cm}^3 \text{ s}^{-1}$  and  $k_A=0.85 \cdot 10^{-12} \text{ cm}^3 \text{ s}^{-1}$  were evaluated for two samples with the same origin but with different prehistory.

**Keywords:** agglutination immunoassay, kinetics, light scattering, flow cytometry

## 1. INTRODUCTION

Agglutination is one of the oldest serological reactions that results in clumping of a cell suspension by a specific antibody directed against an antigen<sup>1</sup>. Nowadays this phenomenon is widely used in different ways. Although there is a number of high sensitive and resourceful techniques and methods, for example PCR, ELISA, that have already been developed by present time for diagnostics purposes, the interests to rapid simple involving non-hazardous reagents and inexpensive tests has still been keeping. For this reason immunoagglutination tests has a new birth now. Enhanced latex particle immunoagglutination tests are widely used in the diagnosis of various infections, especially for bacterial invasion, and to detect biomarkers or some chemical compounds in biological fluids<sup>2-4</sup>. On the other hand, agglutination reaction attracts strong attention because it is involved in different biological processes like a platelets or red cells aggregation<sup>5</sup>. Further understanding of agglutination reactions features may provide substantial opportunities to apply this knowledge for diagnostic and therapeutic benefits<sup>6-7</sup>.

Standard agglutination tests are usually read by eye and lead to some insensitive, non-quantitative and difficulties in interpretation of results. Moreover, the main disadvantage of the custom immunagglutination tests is difficulty in discrimination between specific and non-specific particle aggregation. Various techniques for quantitative assays have been proposed for quantitative that rely on turbidimetric methods<sup>4</sup>, ultrasound instruments<sup>8</sup>, electrophoretic mobility<sup>6</sup>, centrifugations<sup>7</sup> and light scattering<sup>9-10</sup>. Experimental methods based on light scattering have been used mainly with the purpose to measure bulk properties of colloidal dispersions. While working with polydisperse samples, it becomes much more difficult to characterize the distribution using these methods. As a result, single particle techniques that provide the measurement of distribution itself have been emerged.

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\* [surovtsev@ns.kinetics.nsc.ru](mailto:surovtsev@ns.kinetics.nsc.ru); phone 7-3832-366592; fax 7-3832-367409

The most powerful and widely spread technology that analyzes properties of an individual particle is flow cytometry. In 1985 Bowen and coworkers developed an instrument based on light scattering and flow system allowing to investigate particle aggregation when particles are small relative to the wavelength of illuminated light<sup>11-12</sup>.

Over the past decade the light scattering indicatrix technology based on Scanning Flow Cytometry (SFC) has been developed for particle characterization in our group<sup>13</sup>. We proposed to use SFC for kinetic study of agglutination process at initial stages. The main undoubtful advantage of the Scanning Flow Cytometry is a possibility to measure angular dependency of the light scattered by a single particle, an indicatrix, in range from 5° to 100° for scattering angle.

An indicatrix is a unique characteristic of the particle. The appearance of the indicatrix depends on size and refractive index, shape and structure of particle<sup>14</sup>. Indicatrix technology provides researcher more detailed information about the particle than custom wide angle forward and side scattering concepts. The most promising and attractive field for application of indicatrix technology is a characterization of non-spherical particles<sup>15</sup>. Since clusters of two, three and so forth spheres arising from single beads at the initial stages of agglutination process are sufficiently non-spherical, using of SFC for quantitative study of immunoagglutination is promising.

In present work we propose light scattering indicatrix based technique as a novel powerful means of kinetic study of the immunoagglutination initial stages. To verify proposed method we compared results obtained for particle discrimination by light scattering and fluorescence. To demonstrate applicability of SFC to this problem we investigated initial stages of agglutination of particles covered with BSA and mixed with anti-BSA antibody. Our data show that apparent rate of the process of dimer sphere growth during immunoagglutination are slower than diffusion limited aggregation. In order to explain the obtained results detailed mathematical analysis has been applied for data treatment in order to obtain kinetic rate constant for particle dimerization.

## 2. MATERIAL AND METHODS

### 2.1 Experimental setup

In this work we used an optical setup and data acquisition system similar to one described previously. The Scanning Flow Cytometer (SFC) concept differs from conventional flow cytometry in two essential points<sup>14</sup>. 1) the laser beam (441 nm, 50 mW, Kimmon, Japan) is coaxial with the flow and 2) the optical system allows to measure angular dependence of light scattered by individual particle, an indicatrix. For each single particle the time-dependent light scattering signal, so called native SFC trace, is obtained at ADC, while the particle is moving through testing zone. The measured native SFC traces could be transformed into the indicatrices using transfer functions as described in ref.15 SFC allows measurements of indicatrices at polar angle ranging from 5° to 100° with integration over the azimuthal angles from 0° to 360°. The angular resolution of the indicatrix measurement is better than 0.5°. As in custom flow cytometry, fluorescence signal excited by (441 nm, 50 mW, Kimmon, Japan) from individual particle could be measured simultaneously with light scattering. Accurate measurements of the indicatrices and fluorescence are available with a rate 300 particles/s. To simulate the experimental indicatrix, we used the Mie theory and Wentzel-Kramers-Brillouin (WKB) approximation.

### 2.2 Latex particles and antibodies

All chemicals used in this study were analytical grade and were used without additional purification. The carboxylated fluorescent latex microspheres 1.8 μm in diameter from Polysciences (USA, PA) was used in our work. Carboxylate-modified particles have pendent carboxylic acids, making them suitable for covalent coupling of protein or other amine-containing biomolecules. Two sets of latexes were employed in the experiments: initial uncovered particles and particles covered with bovine serum albumin (BSA, Sigma). We employed wide spreading method using a water-soluble carbodiimide, namely 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), to activate the surface carboxyl groups on the microsphere particles and to attach BSA to particle surface covalently. The procedure of coupling has been described in details<sup>16</sup>. This method is a simple one-step method with good results referenced to laboratory coupling avidin, streptavidin, BSA and immunoglobulins of different origin. After washing procedure latex particles covered with BSA were resuspended in 50 mM PBS (pH=7.4). Upon addition of the 2 mM sodium azide as a preservative covered latex particles had been stored in dark place at 4°C without freezing until using in experiments. Concentration of BSA-covered latex particles was  $6.0 \cdot 10^8 \text{ cm}^{-3}$  prior experiments. Anti BSA polyclonal rabbit immunoglobulins IgG were kindly supported by Dr. G.M. Ignatiev (SRC VB VECTOR). The antibodies were used to initiate agglutination process in our study by adding to the particles which carried corresponding antigen. The IgG concentration was determined by Louri method<sup>17</sup> and was 1.6 μM.

### 2.3 Kinetic measurements of latex agglutination

Initial stages of agglutination of the BSA coated latex particles mixed with anti-BSA antibodies were studied in series of experiments with different initial concentration of the latex particles in reaction tube after mixing with antibody ranging from  $0.38 \cdot 10^8 \text{cm}^{-3}$  to  $1.0 \cdot 10^8 \text{cm}^{-3}$ . All experiments were carried out at room temperature ( $25^\circ\text{C}$ ). Buffered saline (PBS,  $\text{pH}=7.4$ ) was used as solvent for all reagents. The experiments were performed in the following manner. Prior to undertaking our studies, latex particle suspension was sonicated in an ultrasonic bath to break up initial aggregates. 150 ml of the anti-BSA antibodies dissolved in buffered saline were mixed in reaction plastic tube with equal volume of the preliminary sonicated BSA-coated particles. Then, during up to 2 hours aliquots ( $1 \mu\text{l}$ ) were taken away at different time points and dissolved in  $50 \mu\text{l}$  of the PBS to stop agglutination reaction. Further, obtained sample was measured with the Scanning Flow Cytometer. In order to examine this stopping procedure, some reaction samples were measured again 2 hours later. There were no noticeable distinctions in these two data sets. As for the procedure of sample taking, the captured aliquot was  $y$  small in comparison with reaction volume; so, influence of this operation could be neglected.

### 3. KINETIC MODEL

In order to interpret experimental kinetic data a mathematical analysis of the kinetics of the agglutination at initial stage were performed. In accordance with useful approximation<sup>10</sup> we suppose that only monomer and dimer concentration are predominant at the earliest stages of the agglutination. So, only two processes are significant to be taken into account at these stages. First reaction is a binding of solute antibodies to antigen immobilized on spheres. Thus, some antibodies become immobilized on sphere surface by this reaction, but they still have a free second antigen-binding site. Second reaction corresponds to a dimer formation caused by binding of immobilized antibody to antigen anchored with another particle.



Here  $A$ ,  $Y$ , and  $AY$  are an antigen (immobilized on particle), an antibody (dissolved in medium), and an antigen-antibody complex respectively.  $L$  and  $L_2$  mean single ("monomer") and two linked particles ("dimer"). Corresponding rate constants are denoted as  $k_+$  for antigen to antibody binding,  $k_-$  for antigen-antibody complex dissociation, and  $k$  for particle to particle coupling ("dimerization rate constant").

We assume that first reaction is quick in comparison with the second. Hence, first reaction is in equilibrium, and leads to distribution of free and bound antigen site on spheres in accordance with equilibrium constant or affinity constant  $K_A = k_+/k_-$ . This assumption is valid if the rate of particle to particle binding is small relatively to antibody to antigen binding. It is believed that this assumption is justified in wide range of experimental conditions. We consider reaction (2) as an irreversible process, because we consider only the earliest agglutination stage, far from equilibrium, and rate of the backward reaction in eq.(2) is small in comparison with forward reaction, and therefore it could be neglected.

The rate constant  $k$  formally introduced in eq.(2) depend both on antigen-antibody interactions and reaction conditions, that is  $k$  depend also on antigen and antibody concentrations. The relation between these concentrations and  $k$  could be established using the equilibrium of the initial reaction (1) of antibody to antigen binding. It is obviously, that for agglutination reaction influence of diffusion rate on particle to particle coupling is to be taken into account in the general case. It has been shown that two limiting regimes of the agglutination process exist: the diffusion controlled latex agglutination (or DLA) and the reaction controlled latex agglutination (RLA). In the case of the DLA spheres binding proceeds with the rate constants  $k_d$  that is independent on coverage of spheres with antibodies and on properties of antigen-antibody interaction. Value of the diffusion limited rate constant  $k_d$  is defined by Smoluchowski theory. Following the work it can be derived as

$$k_d = 4\pi RD = \frac{4kT}{3\eta} \quad (3)$$

Here  $R$  is a “reaction” radius of colliding particles, and  $R=2R_L$ , where  $R_L$  is a latex particle radius.  $D$  means diffusion coefficient for particle in solution medium and could be calculated from Stokes-Einstein law as

$$D = \frac{k_b T}{6\pi\eta R_L} \quad (4)$$

where  $T$  is temperature,  $k_b$  is Boltzman constant,  $\eta$  is a viscosity of the liquid. For room temperature ( $T=298$  K) and water  $k_d=5.5 \times 10^{-12} \text{cm}^3 \text{s}^{-1}$ .

Let us focus on the other limiting case when the particle coupling rate is limited by reaction rate. In this case the constant rate for particle coupling can be represented in following manner:

$$k = k_0 f \quad (5)$$

In this expression is defined dimensionless factor  $f$  which depend only on affinity constant of the investigated antigen and antibody pair and on their concentration. Proportionality coefficient  $k_0$  has dimensions as rate constant and includes collision rate constant, properties of antigen-antibody interactions, and geometrical parameters of particles, antigens and antibodies. But this one is independent on initial concentrations of either antigen or antibody.

In the assumptions of the model kinetic equations for reaction (1) and (2) is provided by mass-action law

$$\frac{dn_1}{dt} = -2kn_1^2 \quad (6)$$

$$\frac{dn_2}{dt} = kn_1^2 \quad (7)$$

where  $n_1$ ,  $n_2$  are concentration of spheres and bispheres correspondingly. Using equations (6) and (7) kinetic equation for dimer fraction  $f_2=n_2/(n_1+n_2)$  at initial stages could be derived using conservation of the total amount of the spheres in sample:

$$\frac{df_2}{dt} = kn_0(1-f_2)^2 \quad (8)$$

Here  $n_0=n_1+2n_2$  is total concentration.  $f_2$  means dimer fraction in the sample. This equation more convenient for data interpretation than eqs. (6)-(7) because it involves dimer fraction and determination of absolute particle concentration are not necessary during kinetic measurements. We use the equation (8) for treatment of the initial apparent rate  $w_0$  (or the initial slope) of our kinetic curves:

$$w_0 = kn_0(1-f_2^0)^2 \quad (9)$$

It is to be noted that initial dimer fraction  $f_2^0$  will appear in eq.(5) for initial time point. So, if the dimer fraction at start point is not negligible, a factor  $(1-f_2^0)^2$  should be taken into account for estimation of the coupling rate constant  $k$ .

## 4. RESULTS AND DISCUSSION

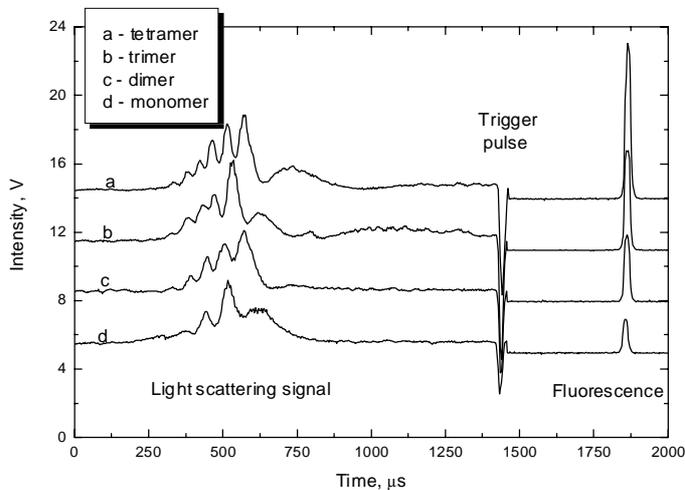


Fig. 1. Native SFC records of the single particles obtained in the uncovered non-sonicated latex sample. Intensity on ADC inlet is pointed versus time. The signals are shifted on intensity for convenient view. Each signal consists on three parts (from left to right on the picture): light scattering signal, trigger pulse, and fluorescence signal from particle. Number of spheres in a cluster was determined with fluorescence intensity'

consists of three separate parts. First part is a light scattering signal. Second part is a trigger pulse necessary for data acquisition and channel switching. The third following the trigger pulse is a signal from fluorescence photodetector. The intensity of the fluorescence pulse is proportional to the number of dye molecules in (or on) measured particle. So the value of fluorescence signal allowed to determine number of particles in measured clusters. Light-scattering indicatrix of the same particles are shown on Fig. 2.

Indicatrix of a single particle is unique. Both Fig.1 and Fig.2 confirmed that both light scattering signals and indicatrices differ for different clusters. Hence, light scattering indicatrix can be used for "monomer", "dimer" and "trimer" identification in a sample.

Size and refractive index of homogeneous spherical particle could be determined from light scattering indicatrix using the FLSI-method developed by Maltsev<sup>13</sup>. Figure 3 shows these parameters revealed from measured indicatrix in non-sonicated sample. It should be noted, that

### 4.1 Light scattering indicatrices arising at initial stages of agglutination.

First of all, preliminary non-kinetic experiments with uncovered fluorescent latex particles were carried out in order to verify proposed method and to demonstrate potential of Scanning Flow Cytometer in discrimination of the clusters of spheres. Sample with uncovered fluorescent latex particles without sonication but after only mild pipettation was studied with SFC in these preliminary experiments. A non-specific aggregation of particles resulted in formation of some of the particles into the clusters of two and more was occurred in these conditions. So, single spheres ("monomers"), clusters of two spheres ("dimers"), and so forth should be obtained in measured sample. Both light scattering indicatrix and fluorescence signals were recorded simultaneously for each measured particle in the sample.

Four different experimental signals, native traces of the Scanning Flow Cytometer, from four different particles obtained in the samples are presented at Fig. 1. Each signal

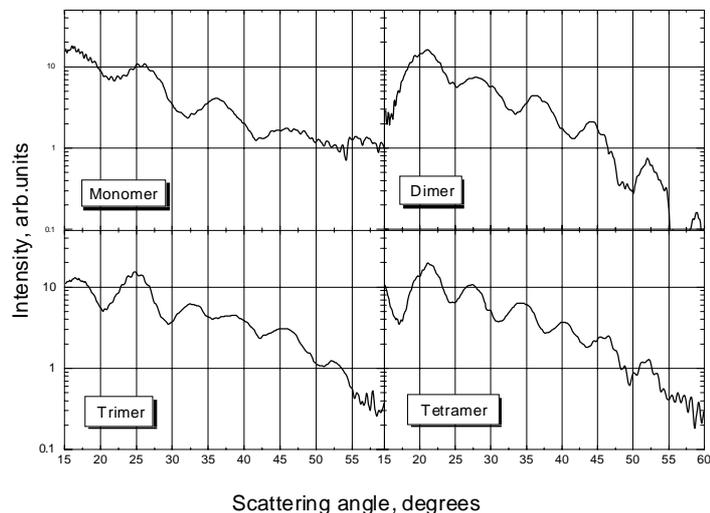


Fig. 2. Light-scattering indicatrices for particles the same in Fig.1. Native SFC records from Fig.1 were transformed into angular dependency using the transfer function.

this method was developed for spherical particles. Therefore parameters obtained for non-spherical clusters of spheres with the method are not real size and refractive index. But we made an attempt to apply this method to the non-spherical clusters, and we defined a “size” obtained from this procedure as an effective size of clusters.

There is a map on this figure where refractive index versus size for each measured particles is plotted. As shown by map, the most of the particles can be attributed to one of the two groups. The left group, more numerous, consists of single spheres confirmed by their size (1.8 microns as stated for single particles), and the right, not so numerous, corresponds to the clusters of two spheres.

The Figure 3b confirms the supposition about the origin of two groups on SFC maps. The values of fluorescence signal of single particles versus its sizes are shown at the figure. As in previous case each point describes parameters of one measured particle. Good correlation between these two measured values allowed us to conclude that SFC discriminates one from two glued spheres very well by light scattering.

It is interesting to consider the mean value of “dimer” size determined by method developed for spherical particles. This value approximately amounts to 2.68  $\mu\text{m}$ . But the sphere with volume that equal to volume of two spheres of diameter 1.8  $\mu\text{m}$ , has a diameter  $d=2.27 \mu\text{m}$ . Hence, non-spherical geometry of the object plays an important, and size of dimer determined in this manner should be interpreted only like an effective size.

To explain this variance we simulate light scattering indicatrices for two linked sphere of diameter 1.8  $\mu\text{m}$  and spheres of diameter 2.68  $\mu\text{m}$  and diameter 2.27  $\mu\text{m}$ . Unfortunately, the exact solution of the light scattering properties can be obtained by Mie theory only for single and concentric spheres, and for infinite cylinders. Another way to the exact solution bases on T-matrix method developed by Barber and Yeh (1) but demands a high computing power and a long time. Therefore we use a Wentzel-Kramers-Brillouin (WKB) approximation to model the light scattering properties of the bisphere. It is believed that this approximation describes well minima location of the indicatrix, but it is not so well in determination of the minima depth. The FLSI-method developed by Maltsev uses two parameters of indicatrix, namely distance between minima after boundary angle 15° and visibility of indicatrix. The size of particles is determined by distance between indicatrix minima after boundary angle. Therefore, indicatrix calculated using WKB approximation should give the FLSI size close to one of the measured indicatrix.

The figure 4 shows the simulated light scattering indicatrices. There are indicatrices for bisphere with single sphere of diameter 1.8  $\mu\text{m}$ , for a single sphere of diameter 2.68  $\mu\text{m}$ , and for sphere of diameter 2.27  $\mu\text{m}$ . For bisphere, as for any non-spherical object,

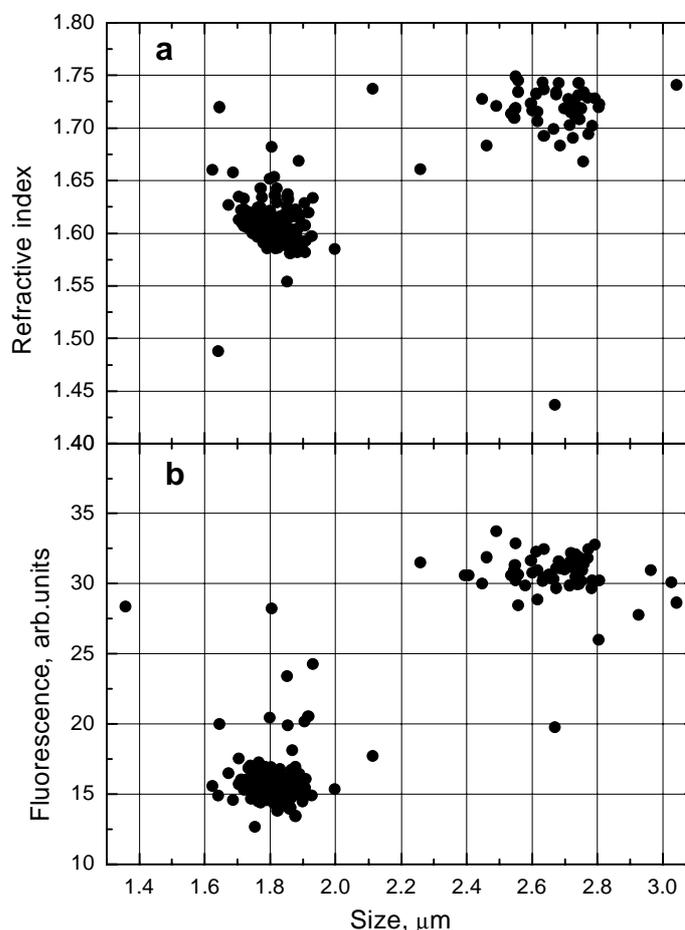


Fig. 3. Parameters of particles obtained in the non-sonicated fluorescent latex sample. Each point represents one measured particles. A: refractive index versus size map, the parameters obtained from light scattering indicatrix with the FLSI-method. B: Fluorescence versus size map depicted for the same particles

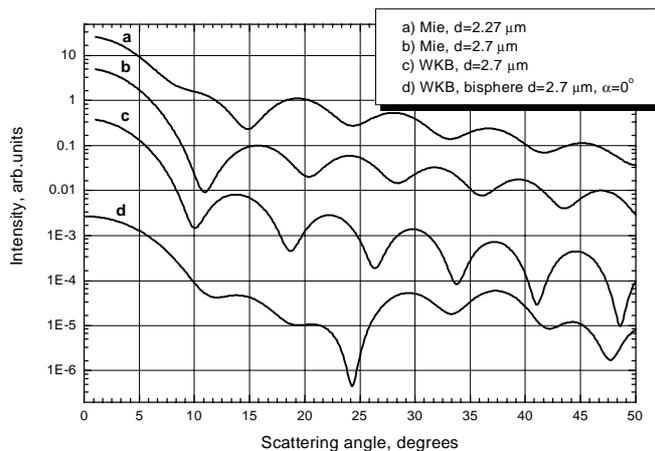


Fig. 4. Light-scattering indicatrices simulated by WKB approximation and Mie theory. Two spheres, one of the equivalent diameter ( $d=2.27 \mu\text{m}$ ) and one of the FLSI-determined diameter ( $d=2.7 \mu\text{m}$ ) was calculated using Mie theory for spherical particles. WKB approximation was applied for calculation sphere of FLSI determined diameter ( $d=2.7 \mu\text{m}$ ) and cluster of two spheres of diameters  $d=1.8 \mu\text{m}$  with orientation angle  $\alpha=0^\circ$ . All particles had the same refractive index  $n=1.6$ . The signals are shifted on intensity for convenient view.

“dimers” using a light scattering indicatrices. Therefore we can conclude that SFC is an applicable tool for kinetic study of the initial stages of agglutination process where “dimers” of initially single spheres arise as a consequence of the antibody to antigen binding.

#### 4.2 Kinetics of the initial stages of agglutination

After preliminary measurements kinetic experiments for study of the initial stages of the agglutination process were carried out. Kinetic measurements of the dimer fraction following the mixing the latex particles covered with BSA and anti-BSA immunoglobulins IgG were performed during up to 90 minutes after start point in manner described in the methods. Kinetics data obtained in experiments with different initial concentration of the latex particles and the same concentration of the antibody are presented on Figure 5. Points represent the dimer fraction measured at varying time points as described in Methods for the initial concentration of the antigen presenting latex particles ranged from  $0.38 \cdot 10^8 \text{ cm}^{-3}$  to  $1.0 \cdot 10^8$ .

As expected and illustrated in Figure 5 dimer fraction increases monotonically on time. Important to note that initial dimer fractions were

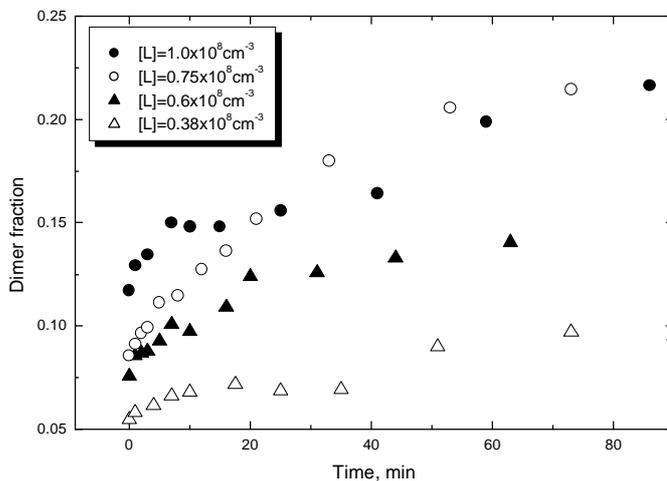


Fig. 5. Kinetics of dimer fraction growth at initial stages of the agglutination process for BSA covered latex particles initiated by adding anti-BSA IgG.

orientation of particle in incident laser beam plays a considerable part. In our previous works we have shown both theoretically and experimentally that SFC setup has orientating effect for elongated particles (Ошибка! Залка не определена.). Therefore indicatrix only for orientation angle  $\alpha=0^\circ$  between incident light beam and long axis of the bisphere has been shown on the Fig.4. A single sphere of diameter  $2.68 \mu\text{m}$  was calculated both by WKB approximation and Mie theory for comparison of the indicatrix structure obtained in approximation and exact theory. One can see that distance between minima after boundary angle  $\theta_0=15^\circ$  for orientated bisphere is in agreement with the the same parameter for single sphere of diameter  $2.68 \mu\text{m}$ , and considerably disagrees with the sphere of diameter  $2.27 \mu\text{m}$ . This comparison explains the obtained value of the obtained effective size. For explanation of the shift in “effective refractive index” for bisphere light scattering should be modeled with theory well described a minima depth.

These preliminary experiments shown that Scanning Flow Cytometer allows exact discrimination between “monomers” and

observed in all kinetic experiments, nevertheless that all particles samples were sonicated in ultrasonic bath just before the mixing with antibodies. This circumstances is to be taken into account during data treatment procedure.

At initial period apparent rate of sphere coupling process is about constant independent of time. So, initial slope of the dimer fraction time profiles can be approximated with linear function using Eq. (5) initial rate of particle coupling  $w_0$  is defined as

To evolve initial apparent rate initial experimental points were fitted with linear function. As mentioned above, the initial dimer fraction should be taken into account as formulated by Eq. (9).

Experimental values obtained for two samples of the anti-BSA immunoglobulins are presented in Table 1 . The sample was the same origin from one serum sample but have different history. Sample marked as A had been defrozen-refrozen several times prior kinetic experiments. The sample B had been defrozen only one time just prior measurements. Both sets of experimental points (not shown) are well described by linear relationship between latex concentration and apparent rate. But the slopes of the lines are distinctive for either of them.

In accordance with Eq.(9) linear dependence of the apparent rate on particle concentration means that coupling rate constant  $k$  is independent of particle concentration in our experiments. Using Eq. (9) the value of the rate constant  $k$  can be evolved via line fitting to the experimental data. The best fitting results are shown as solid lines. The slope of the line is a product of the rate constant  $k$  and initial particle concentration  $n_0$ . So, the fitting procedure yielded the values for coupling rate constants  $k_1=1.89 \cdot 10^{-12} \text{ cm}^3 \text{ s}^{-1}$  and  $k_2=0.74 \cdot 10^{-12} \text{ cm}^3 \text{ s}^{-1}$ .

The values for the coupling rate constants obtained in the kinetic experiments differed from diffusion limited rate constant  $k_d=5.5 \cdot 10^{-12} \text{ cm}^3 \text{ s}^{-1}$  not so large. So, a question about validity of the reaction-limited regime for agglutination kinetics arises. But for diffusion limited kinetics  $k$  should be replaced with the  $k_d$  determined in Eq.(3). Hence, in the diffusion limited regime coupling rate constant  $k_d$  is independent of particle concentration and the apparent rate constant also depends on particle concentration linear. Since, apparent rate constant  $k_{eff}$  for the coupling process in intermediate regime, when diffusion and reaction limited rate constant are comparable, can be written as

$$k_{eff} = \frac{k \cdot k_d}{k + k_d} \quad (10)$$

linear relation between apparent rate constant and particle concentrations is remained. It allows to exclude from  $k_{eff}$  the calculated value for  $k_d$  yielding the real value for  $k$ . thus corrected values for rate constants was  $k_B=2.88 \cdot 10^{-12} \text{ cm}^3 \text{ s}^{-1}$  and  $k_A=0.85 \cdot 10^{-12} \text{ cm}^3 \text{ s}^{-1}$ .

One of the most interest results observed in the experiments is dependence of kinetic constant on antibody properties. How can be explained this fact? Some assumptions can be proposed for the explanations. Two ways are seemed likely. First, recurring changes in external conditions (namely in temperature) led to conformational alterations of the antibody molecules that resulted in affinity constant differences for the antibodies samples. Another possible explanation accounts for observable changes as changes in number of “working” antibodies molecules. Observed results can be treated with the proposed kinetic model for agglutination process. This problem will be investigated in our following work.

## 5. CONCLUSIONS

To sum up, the presented work demonstrates potential of the use of the Scanning Flow Cytometer for the quantitative study of the non-spherical particles. The kinetic measurements during agglutination with light scattering from individual particles are available with the SFC techniques. The proposed method was verified by simultaneous measurements of light scattering and fluorescence of individual particle. Although preliminary demonstrative experiments were performed with fluorescence measurement, this is not necessary in the method. This experimental method is valid for particle larger then wavelength of laser used in SFC. It seemed to be very important when cell agglutination caused by interaction with different antigens (virus, for example) is aim of an investigation. Also we supposed the experimental

Table 1. Obtained rate constants for particle coupling

	Sample A	Sample B
Apparent rate constant, $10^{-12} \text{ cm}^{-3} \text{ s}^{-1}$	0.74	1.89
Corrected with diffusion, $10^{-12} \text{ cm}^{-3} \text{ s}^{-1}$	0.85	2.88

method based on light scattering indicatrix will be useful for kinetic study of the red blood cell and platelets aggregation.

Kinetic measurements for initial stages of agglutination initiated by mixing latex particles covered with BSA and ant-BSA antibodies were made. The simple mathematical

model for initial stages of agglutination was used to explain the obtained experimental results. In spite of the simplicity, the proposed model described well obtained dependences for initial apparent rates. Using the model kinetic rate constants were determined for antibody and particles under investigation. Obtained values are less than diffusion rate constant, but not so large to neglect the diffusion process. Mathematical analysis based on proposed kinetic model allows to exclude one diffusion influence from measured kinetic constants. Moreover, the analysis of the efficiency factor  $f$  allows one to discriminate possible explanations of the measured linear dependencies. However, the analysis shown that performed experiments can not give exact answer to explain the different slope in apparent rate versus latex concentration and additional kinetic investigation should be made. Also the model displays the possibility to quantitative characterization of the antibody concentration via kinetic measurements of the initial stages of immunoagglutination.

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