Mechanism of rhodamine 6G molecular aggregation in montmorillonite colloid

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Abstract: Stopped-flow mixing device and visible absorption spectroscopy were used for the analysis of dye rhodamine 6G (R6G) molecular aggregation in the colloids based on Na-saturated montmorillonite. Two stages of the reaction were identified: The first stage was very short and taking only several seconds, involving the adsorption of R6G cations and their initial aggregation on the surface of colloid particles. The initially formed J-aggregates exhibited similar spectral properties as monomeric form of R6G. In the second stage, initially formed aggregates converted to sandwich-type H-aggregates absorbing light at significantly lower wavelengths and adsorbed monomers. The aggregate rearrangement took several hours. Monomers, with the spectral properties identical to R6G solution, were also identified as a component in complex spectra using principal component analysis (PCA) and multivariate curve resolution (MCR). Partial bleaching of the dye was also proven. Reaction kinetics of the rearrangement of the aggregates followed the model considering a complex mechanism of the molecular aggregation. Data fits using stretched-exponential function led to the determination of rate constants, which had been in the range $10^{-4}$ - $4 \times 10^{-3}$ s⁻¹.

Keywords: Rhodamine 6G • Molecular aggregation • Dye adsorption • Montmorillonite • Colloid

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1. Introduction

Interaction of cationic organic dyes with layered inorganic compounds is important from several points of view:

1. Hybrid materials based on layered inorganic hosts and organic dyes are prospective candidates for new photonic materials, such as solid lasers, sensors, non-linear optics, detectors and photocatalysts [1-3]. The parameters of inorganic host significantly affect the properties of adsorbed dye molecules. This influence can be partially due to the changes of chromophore properties upon the adsorption, such as structural changes, molecular aggregation, dye activation or stabilization or due to photophysical interactions, e.g. resonance energy transfer. One should understand mechanism of the formation of hybrid materials from their components in order to design their synthesis to achieve optimal photophysical properties of the products.

2. Another important and perspective application of organic cationic dyes is their use as molecular sensors for the characterization of the properties of clay minerals or similar inorganic layered compounds [4,5]. The method for the characterization of negative layer charge density of inorganic solids is based on dye molecular aggregation [6]. Recently, the molecular aggregation of cationic dyes was applied for a very sensitive and selective detection of expandable clay minerals in aqueous colloids reaching low detection limits up to sub-ppm concentrations [7]. Basically, application of the method can be expanded to the analysis of any types of layered nanoparticles with a surface charge. It would be very useful especially of such materials, which cannot be detected by other, alternative methods. One has to understand basics of the dye molecular aggregation in colloids, in order to develop the way to quantitatively determine the properties of analyzed particles.

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3. Colloids of clay minerals with organic dyes are good models for studying the interaction of complex colloidal systems in general. Only high sensitivity of dye sensor molecules allows to selectively detect phenomena such as molecular aggregation, self-assembly of organic dye molecules to larger supramolecular systems and their rearrangement and redistribution with time, etc. Knowledge gained from these systems can be applicable to other studies, such as self-assembly of organic molecules in biological colloids, intermolecular interaction induced by bio-polymeric templates, etc.

1.1. Dye molecular aggregation
Molecular aggregation of planar dye molecules often results in significant changes of spectral properties. These changes are result of the electrostatic interactions between the transitional moments and depend much on intermolecular distances and orientations. Relation between the structure of the molecular aggregates and their spectral properties has been interpreted using an exciton theory [8]. Two main types of the molecular aggregates have been recognized: H-aggregates, which exhibit light absorption at significantly larger energies with respect to the non-aggregated species (monomers), and J-aggregates, characterized by a red-shifted absorption band. Aggregate structure, size and amount depend on dye molecular geometry and concentration, and reaction conditions. In heterogeneous systems, dye concentration can be enhanced by an adsorption. Both the H- and J-aggregates could be formed via an association of coplanar molecules. In the structure of H-aggregates, a plane-to-plane intermolecular association occurs. The structure of J-aggregates is based on head-to-tail association. Another specific type of molecular aggregates are so called oblique J-aggregates. According to exciton theory, the oblique J- aggregates have two allowed electronic transitions to both higher and lower energy excited states while the coplanar J-aggregates display the transition only to the lower energy excited state.

1.2. Dye molecular aggregation in clay mineral colloids
Mixing rhodamine 6G (R6G) solution with montmorillonite colloid leads to an immediate adsorption of the dye cations and aggregation [9]. Slow formation of dye species absorbing at around 450-460 nm has also been observed [10]. This form was assigned to H-aggregates typical of the structure based on a sandwich-type intermolecular association [11,12]. The H-aggregates always coexist with the second type of the species of spectral properties similar to non-aggregated dye cations (band at 530 nm) [13,14]. Therefore, this form was mostly assigned to adsorbed monomers. Some older studies had claimed two forms of adsorbed R6G monomers at both the external or internal surface of clay mineral particles. These forms exhibited only slightly different spectral properties, but no alternative method, complementary to the spectroscopy studies, has proved their existence or identified in detail these species. The formation of R6G H-aggregates after longer reaction time is in contrast to the systems based on clay minerals and another cationic dye, methylene blue (MB) [15,16], where H-aggregates were initially formed and slowly changed with time to monomers or J-aggregates. Thus the spectral evolution for R6G/clay mineral colloids seems to be opposite to the systems based on MB.

The formation of R6G J-aggregates in clay mineral colloids had not been much considered. Spectral shifts of the initially formed species to longer wavelengths were negligible. The spectral change has mostly been attributed to the effects of the polarity of colloid particles surface. Later, several studies identified the formation of structurally variable forms of R6G in heterogeneous systems, including also both the oblique and coplanar J-dimers or higher aggregates. One could mention materials based on porous silica [17,18], various forms of organically-modified clay minerals [19,20] and solid crystals of the dye itself [21]. Interestingly, R6G J-aggregates which could not be detected by absorption spectroscopy, were proven by fluorescence measurements in highly concentrated solutions or adsorbed on silver colloid particles [22]. Strong overlap between the absorption and fluorescence spectra does not allow to spectrally distinguish specific R6G aggregates from monomers [23]. The interpretation of spectra can be dramatically complicated by the processes of energy transfer, as has been observed for similar systems [24]. The formation of R6G molecular aggregates, which are difficult to be recognized from monomers was, observed using spectrophotometric titrations [25]. The absorption band only slightly red-shifted with respect to the position of the main band of monomers was assigned to J-aggregates. The intensity of the bands at higher wavelengths increased with the loading of the dye up to the loading point equal to the cation exchange capacity (CEC). The formation of just monomers at the highest loadings after reaching titration endpoint would be improbable. Thus the band at longer wavelengths can be definitely assigned to J-aggregates.

Molecular aggregation of cationic dyes in montmorillonite colloids occurs also at very low bulk concentrations. Surface concentration of R6G cations is significantly enhanced by adsorption. Considering a theoretical surface area of montmorillonite...
The mechanism of R6G adsorption and aggregation in clay mineral colloids has not been fully understood. The first stage reaction likely includes the diffusion (or more precisely electrodiffusion) of the dye cations on the particle surface of an opposite charge. Fast adsorption of R6G has been already proven by specific conductivity measurements [9]. Titrations of montmorillonite colloid with the solution of cationic dyes showed molecular aggregates exhibit similar properties at both the extremely low and high loadings [25]. Such phenomenon has been observed for various systems based on clay minerals and cationic dyes [15]. Although the adsorption is a very fast process, spectral equilibrium is achieved in much longer times.

The objectives of this work concern the identification partial steps of the mechanism of R6G adsorption and dye species formed during the reaction. Stopped-flow rapid mixing device was applied in the combination with UV/Vis absorption spectroscopy. Spectral data were analyzed using conventional spectral analysis, derivative spectroscopy, chemometry methods and reaction kinetics analysis.

2. Experimental procedure

Montmorillonite Kunipia F (Kunimine Industries, Japan) was used without purification. Colloid of concentration 0.2 g L⁻¹ was prepared by stirring for 24 h. R6G purchased from Lambda Physics was used in concentration 10⁻⁵ mol L⁻¹. Both the components were prepared in pure deionized water and their temperatures were fixed before use. The components were mixed using RX2000 Rapid MixingStopped Flow Unit in 1:1 volume ratio also kept at constant temperature. Temperature of the reaction mixture in a spectroscopy cell was additionally controlled by 89054A thermostat cell holder (Agilent). The spectra were recorded using Agilent 8453 UV-visible Spectroscopy System in full range (180-1000 nm). Integration time and initial cycle times were fixed to 0.1 s. Only the visible part actively absorbed by R6G was used for further processing the data. Recording the spectra had been started a few seconds before initializing stopped-flow device. Complete delivery of the dye to the cell was recognized and a formal beginning of the reaction was assigned to the first ‘full scale spectrum of the dye in an early series of recorded spectra.

Spectroscopy data of absorbance values as a function of wavelength and time were arranged into matrices. The matrices were decomposed to a new set of data using the method of singular value decomposition (SVD) run by Pro-K Global Analysis Software (Applied Photophysics, Ltd.). This software was used also for preliminary simulations of reaction kinetics. However, a more complex model had to be used, which is not possible with this software. The matrices were analyzed also using principal component analysis (PCA) and multivariate curve resolution (MCR) performed by Unscrambler (Camo). Principles of the methods are briefly shown in Supplementary data 1 (SD1). At first, PCA based on both the algorithms using SVD and NIPALS were tested, which led to practically equal results. Then, the calculations using the former way algorithm was used and these data are presented in this work. The baselines were subtracted from the data and all the calculations were performed for the wavelength range 440-580 nm. Pure variables and natural constraints are usually used to avoid problems with rotational ambiguity. We expect that the signal at the lowest wavelengths contribute to only H-aggregates, so this part can be considered as so called ‘pure variables’. Additionally, natural constraints were used to minimize rotational ambiguities. Non-negativity constraints for both the spectral and concentration profiles were used. Unimodality constraint was applied only for the concentrations profiles. The closure constraint was firstly included, but led to false results (see discussion below) and, therefore, this constraint was rejected in final calculations. Inclusion of the spectrum of dye solution as the spectrum related to ‘a zero time measurement’ was included in the calculation to verify the performance of MCR method for the analyzed series of spectra. MCR was able to identify the solution of the dye as the system with a single component. Basics and more information on the used chemometry methods is presented in SD1, more complex information appeared in the literature, e.g. [26-28]. Fitting of reaction kinetics were done using Gnuplot version 4.4 software by means of the nonlinear least-squares Marquardt-Levenberg algorithm.

3. Results and discussion

Absorption spectroscopy using a stopped-flow mixing device was able to distinguish two main processes. No apparent spectral changes were observed for a very short time (several seconds) after complete mixing of
the components. This stage of the reaction is assigned to “first stage”. After longer reaction time, significant changes in the spectra indicated the rearrangement of J-aggregates, absorbing at longer wavelengths, in favor of the formation of H-aggregates. The second stage took hours to approach a spectral equilibrium. Although the first stage was very short, it could be formally distinguished from the second one. In the attempts simulating simple kinetic models by Pro-K software, significantly different results were obtained if either both the stages, or just the second stage, were used for the simulation of kinetics (not shown). If the first stage was included, unrealistic differences between the molar absorptivities of the components was observed achieving several orders of magnitude.

3.1. Basic characterization of the reaction stages

Pre-stage. As described in the experimental section, the starting point of the reaction was chosen at the time, when mixed components were completely delivered to a spectroscopy cell and a full spectral absorption of the dye was detected. At earlier times, spectrophotometer recorded the spectra either without detectable dye or with significantly reduced intensities. Before complete delivery of the components to spectroscopy cell, absorbance changed significantly by tens or hundreds percents between each couple of measurement steps (0.1 s sampling).

First stage. After the reactants were fully delivered and mixed in the cell, the spectra did not change significantly for a relatively longer time (several seconds). This period was assigned to the first stage of the reaction, which is characterized by rather negligible changes. This stage might have included migration or adsorption of the dye on montmorillonite particles, as well as first steps of aggregation at the zones of electric double layer colloid particles. We tried to formally estimate the start and the end of this stage.

In order to track the spectral changes more sensitively, the difference spectra were calculated by the subtraction of the spectra with the one in a previous record:

\[ \Delta A = A_{\text{t}} - A_{\text{t-0.1}} \] (1)

The results represented by selected time-difference spectra (TDS) obtained at 20ºC are shown in Fig. 1. TDS labeled as “1” represents the difference between the first spectrum of the first stage and the last one of a pre-stage. The components had not been delivered completely to the spectroscopy cell when the spectrum of the pre-stage measurement was recorded and, therefore, TDS “1” exhibits the most significant spectral change. In contrast, selected TDS labeled as “2” and “14” exhibited rather negligible changes. All the TDS between “2” and “14” exhibited a similar shape (not shown) and absorbance difference profiles (\( \Delta A \)) were almost constant and unchanged with time. After 13.3 s, significant changes were detected by time-difference spectroscopy. This time formally represents the start of the second stage of the reaction. A more illustrative presentation of the borderline between the two stages of the reaction is shown in Fig. 2, which presents time evolution of the two signals in TDS representing absorption by H-aggregates and J-aggregates (partially overlapped by monomers) at 466 and 545 nm, respectively. The signals taken from TDS helped to roughly estimate the length of the first stage of the reaction, which seemed to be completed in a bit more than 10 s. Similar plots for the estimation of the first stage reaction were verified also for the reactions measured at higher temperatures (25, 30 and 35ºC) and the plots are shown in SD2-SD5. Time, until the second stage of the reaction starts, seems to be a little shorter (< 10 s) at higher temperatures, which could have been due to the influence of temperature on kinetics of the first stage processes. However, the ends of the first stage should be considered only as a rough and illustrative estimation. It helped to decide, however, which timescale assigned to the second stage should be analyzed in modeling the processes which are characterized by more significant spectral changes. This borderline formally presents a starting point of a significant rearrangement of the dye species on the surface of colloid particles.

Second stage. The second stage took significantly longer time than the previous one, and apparently included two main processes:

1. The formation of the H-aggregates (near 465 nm) at the expenses of other forms (negative bands at 535, 550 nm) (Figs. 1a, 1b).

2. The reduction of initially formed J-aggregates absorbing at longer wavelengths (550 nm) was observed mainly at early times (see Fig. 1a, TDS 18); later the reduction of other species prevailed (a negative band at 535 nm in TDS “22”).

The spectra recorded during the second stage are shown in Fig. 3. Similar trends were observed with similar colloidal systems based on R6G [29-31], but also with other rhodamine dyes [32-34]. There is a problem to identify all the species absorbing near 530-550 nm spectral range. Probably, the bands related to the transitions of J-aggregates and monomers are significantly overlapped. The presence of more types of any form, either monomers or aggregates, cannot be
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The presence of J-aggregates with similar spectral properties to monomers is supported by recent observation based on spectrophotometric titrations, which showed the formation of J-aggregates hardly distinguishable from monomers [25]. In that study, spectral bands only slightly red-shifted with respect to those of solution were observed at full CEC loadings. In order to formally recognize the species absorbing at longer wavelengths, R6G with the peak centered at <540 nm is assigned to monomers and >540 nm to the J-dimers or higher aggregates.

Selected TDS obtained at the beginning and at the end of the second stage showed a partially different character (Fig. 1a, TDS “18” to “22”). At the beginning of the reaction, mainly J-aggregates (negative band at 550 nm) were converted to the H-aggregates (positive band at 465 nm). Later, the H-aggregates seemed to be formed mainly due to the expense of the species absorbing at 535 nm. At this reaction time, the amount of the J-aggregates absorbing near 550 nm had already significantly decreased, which may obscure interpretation of the trends in TDS spectra (Fig. 1a). This evolution is illustrated more clearly in Fig. 1b. For the first series of TDS (“17”-“20”), the values of negative peaks at 550 nm, related to the decomposition of J-aggregates, increased with approximately the same increment in this series of spectra. This increase expressed as the

Figure 1. Selected time-difference spectra related to the reaction of rhodamine 6G and montmorillonite in colloid at 20ºC. a. The numbers of the measurement denote that from the n° measured spectrum (n-1)° spectrum was subtracted. Due to the arbitrary chosen times the numbered time-difference spectra represented: 1: \(A_1 - A_0\); 2: \(A_2 - A_0\); 14: \(A_{14} - A_{12}\); 18: \(A_{18} - A_{16}\); 22: \(A_{22} - A_{20}\). b. \(\Delta A\) of the time difference spectra were calculated as following: 17: \(A_{17} - A_{15}\); 18: \(A_{18} - A_{16}\); 19: \(A_{19} - A_{17}\); 20: \(A_{20} - A_{18}\); 21: \(A_{21} - A_{19}\); 22: \(A_{22} - A_{20}\).

Figure 2. The \(\Delta A\) signals taken from time-difference spectra at 466 and 545 nm for the reaction at 20ºC. The absorbance changes after about 10 s denote the start of the second stage of the reaction. (Until 0.7 s, cycle time was 0.1 s, and then the cycle time increment was increased by 100%: 0.6, 0.7, 0.9, 1.3, 2.1, 3.7, 6.9 s...).
bands in TDS just reflected the arbitrary chosen times for the spectra recordings, and alone, do not provide any significant information. However, interestingly, TDSs “20” and “21” exhibit very similar amplitudes of the negative peaks near 550 nm. On the other hand, the negative peak of TDS “21” is partially shifted to lower wavelengths, which may indicate a different type of species played the role in the reaction after longer reaction times or another process took a significant role, which affects the trends in TDS. One may assume that the concentration of the J-aggregates absorbing at 550 nm had been approaching zero or very low values and a parallel process started to dominate the reaction at that time. The last TDS shows clearly the shift of the reactant absorption to lower wavelengths (535 nm). The main conclusion from the trends observed in TDS is the fact that the re-arrangement of J-aggregates to H-aggregates is not the only one process taking place. Another process took part, which significantly affected the trends in TDS.

An important feature was observed in TDS related to the formation of H-aggregates, if it is compared to the concentration decrease of the reactants, J-aggregates and/or monomers. Whereby a negative peak reflecting the decomposition of the J-aggregates changed significantly in the series of TDS (Fig. 1b), the positive bands of the formed H-aggregates showed more or less similar (positive) values. The comparison of the two evolutions related to positive and negative values may provide valuable information on the stoichiometry of the reaction. It seems that much more reactants decomposed, in particular at the end of the reaction, than would be expected from the amount of produced H-aggregates. One possible explanation would be based on the consideration of a more complex character of this stage, which would include another reaction or more processes. Large negative bands, which are not compensated with positive ones in TDS (Fig. 1b) could indicate the formation of non-colored spirolactone form of R6G. The presence of this form has been repeatedly observed for various reaction systems [35].

3.2. Mathematical analysis of spectral data

Principal component analysis. PCA was performed for the series of spectra representing the reaction at 20°C. Using this method one can identify a minimum number of factors necessary to reconstruct the matrix of the spectral data within an experimental error, i.e., to identify the number of uncorrelated species. The number of the components may relate to the number of present chemical species, but specific cases should be considered. For example, more species that do not change with time contribute to only a single component determined by PCA. This could be a part of non-reacting monomers, the signal from light scattering baseline related to the presence of colloid montmorillonite particles, etc. Leuco-forms of dyes, e.g. a lactone form in the case of rhodamines [35], whose formation is hypothesized (see discussion above), would not be detected and recognized at all. If the concentration of one species is linearly proportional to another (e.g. those of reactant and product), then both the species may be identified as a single component with positive and negative absorbance values. If two different species have very similar spectra, they may not be distinguished and are considered by PCA as a single component. Due to experimental errors, generally all eigenvalues determined by PCA are nonzero. However, only first significant components having the highest values were considered. The results of PCA presented for the second stage of the reaction are presented in Fig. 4 and additional information is shown in SD6. Explained variances of the PCA analysis (SD6) confirm that the spectra can be described with a relatively small number of components. Only two components are needed to achieve relatively high values of explained variances (above 98%). This fact can be explained by the similarities of the spectra in this series and a relatively small contribution of additional components. Optimal
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number of the components came from the comparison of variance from the calibration and validation of the model (SD6). The smallest difference is for the calibration and validation model starts with four components: PC0, presenting the mean profile of the whole series of spectra, and PC1-PC3, showing maximal orthogonal variables with respect to previous component(s). The loadings of PCs have negative and positive values indicating possible relation of some of the species which either decomposed or were formed during the reaction. PC1 presents a positive band at 547 nm which is in relation with a negative band at 460 nm. These two bands are apparently related to J- and H-aggregates, respectively. The “opposite sign relation” may be explained in terms of a direct transformation from the J- (547 nm) to the H-aggregates (460 nm). It confirmed the assumption based on trends observed in TDS. PC2 indicates the presence of another type of J-aggregates absorbing at longer wavelengths (negative loading at 556 nm). The decomposition of this species might have been related to the formation of H-aggregates (467 nm) and monomers with the peak at 530 nm. PC3 presents a relatively noisy profile with hardly recognized bands of both the J- and H-aggregates. Very complex character of PCs does not allow easy interpretation of the processes. Another strategy was to calculate singular value decomposition (SVD) vectors and their kinetic profiles. SVD led to similar results and are shown in SD7-10. Valuable information was obtained from kinetic profiles. Increasing value of SVD vector S[1] with time, representing negative spectrum of the mean spectrum profile, might indicate the overall decomposition of the dye or decreasing mean absorptivity during the reaction. The decomposition may relate to the formation of a non-visible spirolactone form of the dye [35]. S[2] vector presents the formation of the H-aggregates from the J-ones. Monomers seem to be another product of this reaction (S[2], S[3]). The interpretations of S[3] and further vectors and their evolutions with time are non-trivial. Nevertheless, similar S[1]-S[2] vectors and similar evolutions with time were observed for the measurements recorded also at higher temperatures (SD7-10).

Multivariate curve resolution. Neither PCA nor SVD represent real spectra of the components of the reaction mixtures. They just may indicate relationships between hypothesized species based on absorption maxima and their changes in course of the reaction. On the other hand, MCR is the method, which may lead to the real spectra and concentrations. Unfortunately, mathematical solutions by this method are not unique due to a rotational ambiguity. To avoid or minimize this problem, one has to use constraints to approach the unique solution. Both the concentration and spectral non-negativity were used as basic constraints in our calculation. The closure constrain was applied initially to consider closed reaction system. Under these conditions, MCR led to large spectra residuals (not shown), which perfectly fitted to the profile of dye monomers. This finding has two important consequences:

1. The closure constraint cannot be used in MCR model for R6G/montmorillonite reaction.
2. The reaction is not closed system for spectrally detectable species.

These conclusions are in agreement with the hypothesis considering the formation of non-absorbing

Figure 4. Loadings of principal components obtained by PCA using the spectra series measured at 20ºC for the second stage of the reaction.
species during the course of the reaction, most likely based on a spirolactone form. After the closure constrain was removed from the model, MCR led to the results with significantly smaller and rather uniform residuals. Nevertheless, the non-uniquity of the methods can still be the problem. In order to check the correctness of the model, the spectral data of a dilute R6G solution was added to the data matrix and involved to the MCR calculation. R6G solution was expected to contain practically only monomers. The MCR results are shown in Fig. 5. Interestingly, MCR could recognize the spectrum for the dye solution as a pure monomer system with zero concentrations of other components. Moreover, the spectral shapes of other components are realistic. Although MCR is the method which may not always lead to a unique and real solution, the correctness of the analysis of known dye solution spectrum indicates a high relevance of the results. Small amounts of R6G, with spectral properties similar to free dye monomer in solution, were also found in the colloids (about 10% of total dye concentration). There could be equilibrium between dye, which was electrostatically bound to the surface, and free monomers. Such dye monomeric species was present either free in the bulk or rather in hydrated form in the zone of electric double layer of colloidal particles.

Figure 5. Spectra (upper) and concentrations (lower) of the components determined by multivariate curve resolution. Calculated from the data obtained by the experiment at 20°C and pure rhodamine 6G spectrum.
More than one species were detected for each spectrum recorded for the R6G/montmorillonite colloid during the course of the reaction. Besides monomers, the method could recognize the spectrum of J-aggregates, with the band maximum centered at 540 nm. The spectral profile of the J-aggregates is very similar but slightly shifted to longer wavelengths with respect to that of monomers. The H-aggregates present a more complex spectrum. It has two maxima at both the shorter and slightly longer wavelength, respectively (467 and 532 nm). The component spectrum might not be related to a single species. It may include the H-aggregates and monomers, whose amounts were linearly correlated. This assumption is supported by the results of older studies related to linearly-polarized spectroscopy of R6G/montmorillonite films [13,36-41]. A dichroic transition at lower wavelength was assigned to R6G H-aggregates but did not include another band near 530 nm. Concentration profiles with time indicate the increase of the concentrations of the H-aggregates and monomers at the expense of that of the J-aggregates. The changes of the concentrations of these species seem to be of similar extents, although MCR results do not allow comparing the species to each other. Based on all information, the main reactions can be summarized to the following scheme:

Dominant reaction:
J-aggregates $\rightarrow$ H-aggregates + monomers \hspace{1cm} (R1)

Partial reaction:
Non specified form (monomers?) $\rightarrow$
$\rightarrow$ spirolactone form (?) \hspace{1cm} (R2)

Reaction R2 reflects a non-specific decomposition of the dye monomers. The decomposition product may be a spirolactone form of the dye. A neutral molecule of this form may be released from the colloid particles due to the absence of electrostatic positive interaction between a leuco-form of the dye and the surface of the particles. The reaction is described as a slow decrease of R6G monomers with time.

Reaction R1 is in principal similar to the reaction observed for methylene blue [16] and other dyes [42]. It leads to the decomposition of initially formed molecular aggregates produced in the first stage of reaction to another type of the aggregates with monomers as a side product. The difference to MB is that H-aggregates are initially formed and slowly decompose to J-aggregates and monomers. The question is why there are two different types of the aggregates. One is formed very fast and another slowly when system approaches a spectral equilibrium. The answer could come from the concept describing the reaction mechanism. Fast reaction kinetics of dye adsorption does not allow complete mixing of the components and homogeneous initial adsorption of dye molecules over the all clay mineral surface. Dye cations are adsorbed very fast and cover the surface heterogeneously. Molecular aggregates are formed immediately after dye molecules achieve the zones of electric double layer of the first contact. The structure and properties of the aggregates formed at the first stage of reaction are probably influenced by the parameters of the electric double layer of colloid particles. One should note the clay mineral particles are unsaturated with dye cations with respect to the CEC. Due to the processes of desorption - re-adsorption and/or collisions between colloid particles, distribution of dye molecules over the whole surface of present colloidal particles takes place. Possible pathways of the decomposition of initially formed J-aggregates are shown in Scheme 1. These processes lead to the formation of new assemblies with different structural and spectral properties. Due to the re-distribution of the molecules over the large surface, a local dye concentration at the surface decreases which results to a partial de-aggregation. In the case of R6G, the formation of adsorbed monomers, represented by the band at 532 nm is, therefore, quantitatively related to the amount of newly formed H-aggregates, absorbing at 467 nm.

MCR method does not provide accurate quantitative data. The concentrations can be compared only in the series of the same component. However, the results could be analyzed qualitatively or semi-quantitatively. The bands of the components in the spectra absorbing at the longest wavelengths present similar values. Molar absorption coefficients of these forms can be estimated as of relatively similar magnitudes. Concentration profiles of the components (J- and H-aggregates) seem to be quantitatively related. An approximate sum of their concentrations is a nearly constant value over the whole reaction time.

3.3. Molecular aggregation kinetics

Chromometrics presents soft models used for the analysis of complex spectral data, which are purely mathematical, but do not consider laws of chemistry, such as chemical equilibrium or reaction kinetics. The last objective of this work was modeling of the aggregation kinetics. For simplification, formation of H-aggregates and monomers from J-ones was considered as a single process. It is assumed that aggregate re-arrangement proceeds via de-aggregation of the J-aggregates to monomers or smaller aggregation units. The formation of the H-aggregates would then run from monomers or smaller aggregates as reaction intermediates.
In the first step, we tried to find a relevant kinetic model for spectral data for H-aggregates formation (Fig. 6). Absorbance at 465 nm was chosen for the first series of fits. The band of the H-aggregates absorption should not be significantly overlapped with other spectral species. Using the model of simple reaction mechanisms based on the first or the second order reaction kinetics failed (not shown), because these models do not reflect a complex character of the dye molecular aggregation. We applied another model which has been repeatedly used for similar systems [43-46]. According to this model, reaction kinetics obeys the 1st order law; however, the rate constant is time-dependent, increasing with a power of time reflecting increasing

**Scheme 1.** Possible pathways of the redistribution of dye cations from initially formed J-aggregates over free clay mineral surface. J-aggregates are formed in an early stage of the reaction, probably in the zones of electric double layer. It is followed by much slower process of dye cations rearrangement and redistribution over free surface. Left - Migration on the surface of the same clay mineral particle. Right - Redistribution upon the collision between two clay mineral particles in the colloid.

**Figure 6.** Kinetic data fits obtained using stretched exponential function.
Mechanism of rhodamine 6G molecular aggregation in montmorillonite colloid. The concentration and hence the absorbance of a product then changes in time following a “stretched exponential” function Eq. 2:

\[
A = a + b(1 - e^{-(kt)^n})
\]  

Constants \(a, b\) can be expressed by the absorbance at the beginning (zero time) and its increase to an infinity time \((a = A_0; b = A_\infty - A_0)\). The sum \(a + b\) denotes \(A_\infty\) value in an infinity time. Constant \(n\) is the measure of two limiting mechanisms being involved in the aggregation. For the first limiting case, the larger value of \(n\) means the growth of the molecular aggregates occurring mainly through the association of monomers (or rather smaller aggregates). Limiting value \(n = 1\) expresses the association of purely monomers, which results in the first order reaction kinetics. On the other hand, negative \(n\) values would mean the aggregation is controlled via an association of rather very large molecular assemblies. The association of monomers would be negligible in the case of negative \(n\) values. Nevertheless, in most cases published in the literature \(n\) parameter is positive and near the range 0.3-0.7 [43].

Fitting the model function based on Eq. 2 to absorbance values at 465 nm led to the results shown in Table 1 and Fig. 6. The fits were performed for three series of measurements carried out at the temperatures 20, 25 and 30°C, each taking 1 h. Surprisingly, a complex effect of temperature is observed when the kinetic profiles are compared. The largest amount of H-aggregates as estimated from absorbance values seemed to be formed at medium temperature, 25°C (Fig. 6). This might be explained by the role of the changes in chemical equilibrium with temperature thus affecting the extent of the reaction. However, fitting the curves led to no maxima of the parameters for the experiment carried out at 25°C (Table 1). The extents of the reaction expressed by the parameter \(b_{465}\) increased with reaction temperature, although the difference of this parameter for 25 and 30°C reaction \((b_{465,25} \text{ and } b_{465,30})\) was rather negligible. Both the \(k_{465}\) and \(n_{465}\) parameters contribute to a final shape of the function (SD11). A moderate increase of the rate constant \(k_{465}\) with temperature was observed. This parameter primarily affects an initial increase of the function. The parameter \(n_{465}\) decreases with increasing temperature. Its largest value was obtained at 20°C \((n_{465,20} = 0.66±0.05)\). More monomers or smaller molecular aggregates were involved in the reaction at 20°C. At higher temperatures (25 and 30°C), \(n_{465,25}\) and \(n_{465,30}\) parameters were lower, which was reflected in a more sigmoid-like shape of the function with a steeper increase at the beginning of the reaction (see SD11) [44,46]. Reactions at enhanced temperatures involved relatively more molecular assemblies vs. monomers as reactants. The trend of molecular aggregation in clay colloids is opposite to that observed for solution. This might be explained by an increased association between clay mineral particles in colloids with increasing temperature, which would significantly promote the molecular aggregation.

The comparison of the fits for different temperatures neglecting \(a\) and \(b\) parameters are shown in SD12. Initial increase is much related to the rate constant \(k_{465}\) which is proportional to reaction temperature. Similar curves are observed for the reactions at 25 and 30°C. The highest \(k_{465,30}\) value was reflected in a steeper increase of the aggregates at the beginning of the reaction. After longer times, the reaction rate at this temperature turned to relatively slower than that observed for 25°C due to the faster consumption of monomers as the main reactants or intermediates, for the H-aggregates formation.

Table 1. Parameters of the kinetic model obtained for the data measured at 465 nm. \(R\) - correlation coefficient of non-linear fits.

| \(\theta / ^\circ\text{C}\) | \(a_{465}\) | \(b_{465}\) | \(k_{465} / \text{s}^{-1}\) | \(n_{465}\) | \(R\) |
|-----------------|-------|-------|-----------------|-------|-------|
| 20              | 0.019 | 0.040 | 0.0019          | 0.66  | 0.9994|
|                 | ±0.001| ±0.002| ±0.0002         | ±0.05 |       |
| 25              | 0.016 | 0.052 | 0.0032          | 0.58  | 0.9997|
|                 | ±0.001| ±0.002| ±0.0004         | ±0.05 |       |
| 30              | 0.010 | 0.054 | 0.0037          | 0.49  | 0.9993|
|                 | ±0.001| ±0.002| ±0.0003         | ±0.03 |       |

The model based on stretched exponential function was also used for fitting concentration profiles obtained from the MCR method. Both the formation of H-aggregates (Eq. 3) and the decomposition of J-aggregates at 20°C (Eq. 4) were fitted:

\[
c = c_1 + c_2\left(1 - e^{-(kt)^n}\right)
\]  

\[
c = c_1 + c_2 \cdot e^{-(kt)^n}
\]
The curves are shown in SD13 and parameters are listed in Table 2. Parameters $c_1$, $c_2$ are in arbitrary concentration scale obtained by MCR, which is different from $a$ and $b$ parameters expressed as absorbance values. Concentrations as obtained by MCR have no physical meaning and provide only a relative change of the concentration in arbitrary units within a single component. Scale of the concentration profile obtained by the calculation using MCR could affect both $c_1$ and $c_2$ parameters, but reaction rate $k_{\text{MCR,20}}$ and parameter $n_{\text{MCR,20}}$ should be concentration independent. Determined value of the rate constant $k_{\text{MCR,20}}$ (0.0016±0.0001 s$^{-1}$) for H-aggregates formation was slightly lower than that obtained with a single wavelength data ($k_{465,20} = 0.0019±0.0001$) as well as the parameter $n_{\text{MCR,20}}$ (0.53±0.03) compared to $n_{465,20}$ (0.66±0.05). One should consider that MCR method recognized the formation of H-aggregates together with the formation of adsorbed monomers (see Fig. 5, band 532 nm). The effects of $k_{20}$ and $n_{20}$ parameters on the shape of the function when neglecting the effect of $c_1$, $c_2$ parameters are presented in SD14. Dye bleaching, effects of chemical equilibrium or different accuracy of the fits obtained at single wavelengths or from MCR data could contribute to the observed difference.

Small differences are observed when H-aggregates formation is compared with J-aggregates decomposition, both obtained from the MCR data (SD14). Slower decomposition of J-aggregates is confirmed by lower reaction rate constant $k_{\text{MCR,20}}$. The extents of the reactions are similar at the end, but relatively different at the beginning of the J-aggregate rearrangement to H-ones, when bleaching of the dye is relatively fast. Thus lower extent of the decomposition of J-aggregates could be influenced by this side reaction. The formation of monomers as another product of the rearrangement of J-aggregates to H-ones could also contribute to this difference.

### 4. Conclusions

Complex process of the molecular aggregation of rhodamine 6G in montmorillonite colloids can be characterized by the following:

1. Dye adsorption and initial aggregation is a very fast process. Due to the fast kinetics, initial adsorption and aggregation could not be sufficiently monitored with a stopped-flow technique used in this study.

2. Two main stages of the reactions were recognized. First stage was characterized by the formation of dye J-aggregates adsorbed on colloid particles. During this stage, colloids exhibited spectral stability with duration of several seconds. The first stage was followed by the second stage, which was characterized by large spectral changes due to rearrangement of the initially formed J-aggregates of the dye.

3. Rearrangement of the J-aggregates to H-aggregates producing adsorbed monomers as a side product was confirmed as the main process of the second stage. Another reaction included conversion of the dye to its leuco-form, which was most likely based on a spirolactone form.

4. The conversion of the J-aggregates to H-ones and monomers followed kinetic laws based on stretched-exponential equation. It reflects the mechanism, which has been observed for the aggregation of other dyes. Rate constants increased with the temperature, but also other parameters significantly contributed to final shapes of the kinetic curves, such as aggregation size of reaction intermediates (reflected in parameter $n$) and extents of reactions.

5. Specific properties of cationic dyes are known to sensitively probe the properties of clay minerals, especially layer charge. The method for the layer charge characterization can be potentially expanded to probing other nanomaterials with charged particles of similar structures. Detail analysis of the spectral changes could help to improve the currently used method, and in
Mechanism of rhodamine 6G molecular aggregation in montmorillonite colloid.

general to understand complex processes taking place in the colloids based on charged layered particles and organic cations. Future experiments are still needed applying other spectral methods aimed at investigation of the systems based on various types of dyes and inorganic colloids.

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Supplementary material

Supplementary data associated with this article can be found both in the online and print version.

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