

Light absorption study of aggregating porphyrin in aqueous solutions

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UV-visible absorption measurements have been applied to the study of the aggregation kinetics of the porphyrin $t\text{-H}_2P_{\text{agg}}$ in aqueous solution. The temporal evolution of the spectra can be related to the time dependence of the monomer concentration and the mean cluster size. Results from the absorption experiments agree with the findings of previous light scattering measurements, and with the current theoretical models and molecular dynamics simulations of diffusion-limited aggregation kinetics. [S1063-651X(98)04605-4]

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I. INTRODUCTION

The formation of clusters through spontaneous self-aggregation is a process of both theoretical and experimental interest in physics, chemistry, biology, and engineering. This focus is particularly true in the complex fluids, as, for polymers, gels, and colloidal and biological molecules, where the spontaneous or induced buildup of large supramolecular assemblies have significant effects on the basic properties of the system [1]. Statistical physics have proven quite successful for the description of many properties of these assembling phenomena through the use of fractal geometry and scaling concepts [2].

A number of different growth processes [i.e., percolation, diffusion-limited aggregation (DLA), reaction-limited cluster aggregation (RLA), diffusion-limited cluster-cluster aggregation (DLCA), etc.] have been proposed for the formation of these assemblies. The impetus for considering such models comes from the recognition that the large clusters formed in these processes exhibit a scale-invariant fractal structure. In fractal aggregates, the various growth mechanisms relate the mass M of the cluster to the corresponding radius R via the well known scaling form $M \propto R^{d_f}$ [3], where the fractal or Hausdorff dimension d_f differs from the Euclidean dimension d . This fractal parameter has become a subject of considerable interest—as implied, it serves as a signature for the mechanism of cluster growth—and a variety of experimental techniques as well as computer simulations have been used to determine d_f . The fractal dimensionality, however, conveys only limited information about the aggregation process, since it refers to the geometrical properties of only a particular cluster and it is not useful in describing the ensemble and the dynamics of the clusters in the system. A more deep understanding of the dynamical properties of these systems

is achieved through a consideration of statistical quantities, as, for example, the dynamic cluster size distribution function $n_s(t)$, which represents the number of clusters, consisting of s particles, in a unit volume at time t .

Scattering techniques, initially used to probe geometrical aspects of aggregates, have provided a great deal of information on the properties of these structures, e.g., detailed relationships between hydrodynamic and static properties. The second moment of the cluster size distribution was measured via light scattering on colloids and gels [4] indicating a “scaling” time behavior. Many attempts ensued to determine the temporal evolution of the cluster size distribution theoretically [1,2,5]. Quantitative comparisons with experimental data have been achieved using molecular dynamics (MD) simulations and models based on the von Smoluchowski equation [6] by assuming specific mathematical forms for the inter-particle reaction rates. These comparisons have been made possible through the use of a dynamic-scaling description, and a knowledge of precise relationships between rate constants and the dynamics of aggregation.

In a previous study [7], we have shown (using both elastic and quasielastic light scattering experiments) that aqueous solutions of a particular porphyrin display precise fractal properties. The aim of the present study is to provide insight into the aggregation kinetics, and, in particular, on the quantities directly related to the MD simulations alluded to above. In addition, we show that the aggregation kinetics can be successfully studied through UV-visible absorption experiments. These findings can be considered as being of general applicability in aggregation investigations and especially in biological systems where the clustering process generally takes place with concomitant changes in the electronic properties of monomers. It is common for biologically relevant species like DNA or proteins to show fluorescence and/or optical density changes upon aggregation. Analogous attempts have been exploited in the past in the study of polymerization under high pressure and association kinetics [8].

Porphyrins constitute an important class of biochemical

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compounds. An important application of synthetic cationic porphyrins (and their metallo-derivatives) in biophysics is their use as probes of the dynamics and the structure of nucleic acids and helical polypeptides [9]. These chromophores can interact and bind to DNA and RNA by intercalation, external groove binding and external binding with self-stacking. Under appropriate conditions, depending on porphyrin structure and polynucleotide composition, they are also able to induce conformational changes of the double helix (e.g., converting *Z*-DNA to the *B*-form [9]). The rapid uptake of these molecules by DNA, accompanied by the simultaneous breaking of adjacent hydrogen bonds, supports the idea of a “collective motion” model for nucleic acids.

Here we report on the salt induced aggregation of the dicationic porphyrin *trans*-bis(*N*-methylpyridinium-4-yl)diphenylporphyrine (*t*-H₂P_{agg}) which forms supramolecular assemblies [9]. This phenomenon has been evidenced by resonance Rayleigh light scattering [10] and, in the presence of helical templates by circular dichroism spectroscopy [9]. Recently, we have reported a characterization of the late aggregation stage of the porphyrin by using elastic and quasielastic light scattering [7]. From the measured intensity profile of elastically scattered light we obtained a clear indication that aggregation—driven by diffusion-limited aggregation kinetics—produces large rigid monodisperse clusters having a fractal structure (with $d_f \cong 2.5$). From the q^2 dependence observed in the mean decay rate of the intensity-intensity correlation function and the effect on this dynamical quantity of anisotropies in the cluster structure, we confirmed such a picture giving a hydrodynamic radius R_H consistent with the radius of gyration R_g measured via elastic scattering.

II. EXPERIMENTAL DETAILS

Aqueous solutions of porphyrin were prepared by using the free base as chloride salt. The concentration used in our experiments ($5 \times 10^{-6} M$) was determined spectrophotometrically using $\epsilon_{420} = 2.24 \times 10^5 M^{-1} \text{ cm}^{-1}$ at the Soret maximum in water [7]. Aggregation was induced at 298 K by adding NaCl up to a final concentration of 0.1M. The kinetic runs were performed on a Hewlett-Packard model 8452A diode-array spectrophotometer by repetitive scanning of the spectrum at suitable time intervals. Measurements were begun immediately after salt addition in the range 200–800 nm. The deconvolution of the spectra as Gaussian contributions (centered at 365, 420, and 452 nm, respectively) in the region 320–500 nm was achieved by performing a fitting procedure based on Marquadt algorithm.

III. RESULTS AND DISCUSSION

In neat water, the *t*-H₂P_{agg} porphyrin exhibits an intense Soret maximum at 420 nm in the absorbance spectra [9,10]. Under these conditions, resonance light scattering spectra indicate that the porphyrin has not formed extended aggregates. This spectral feature can be associated with monomeric porphyrin. Upon salt addition, the spectra change dramatically with time (Fig. 1) showing the buildup of a more complex structure. At least two new contributions centered at 365 and 452 nm (more intense) are detected in ad-

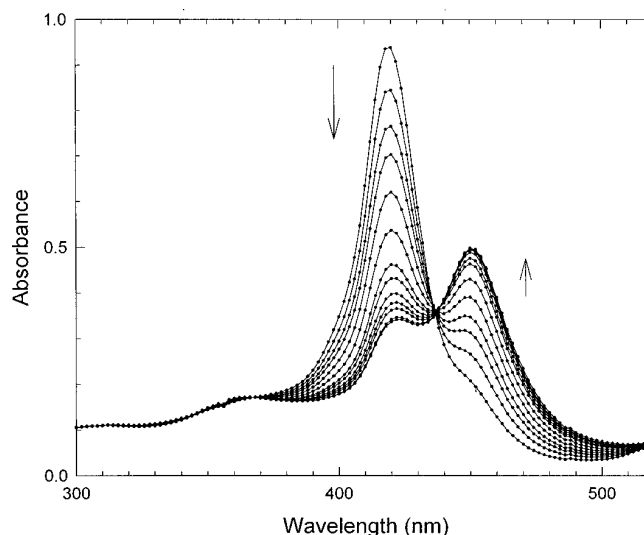


FIG. 1. UV-visible spectral changes relative to the aggregation process of the porphyrin solution. Different spectra are measured at different times after salt addition. Arrows indicate the time behavior of the two mean contributions 420 nm (decreasing) and 452 nm (increasing).

dition to the 420 nm Soret band. There is a well-defined isobestic point at 434 nm. As time proceeds, the 420 nm band decreases in intensity while the new peaks increase. The spectra corresponding to a late stage of aggregation show that both monomeric and aggregated porphyrins are present. The new spectral features are assigned to porphyrin in the aggregated form. Whereas the 420 nm peak is clearly due to the absorbance of the monomeric species, the 452 nm band can be attributed to “extinction effects” [12] due to resonant light scattering of the aggregated form as already reported [9,10].

Clusters arise from the relatively strong dispersive forces acting between these molecules. Porphyrins are compounds possessing an extended π -electron system in which the so-called π - π or stacking interactions take place. Such interactions lead basically to two different spatial arrangements [11]: (i) offset π stacked, in which two molecules overlap each other with a lateral offset and (ii) a T-shaped geometry, in which one molecule is orthogonal with respect to the other one. As is well known, the absorbance of the system is directly connected with its electronic properties, and the observed changes in the spectra can be related to the presence of these strong π - π molecular interactions. They are able to originate completely different electronic configurations for the monomeric and the aggregated chromophores.

In order to analyze the absorbance data, we considered the time dependence of the two major peaks related to the monomeric and aggregated species, respectively. We decided to use the areas of the corresponding bands performing a deconvolution in the spectral range of interest [Fig. 2(a)]. In this way, we obtained quantities related to the concentration of porphyrin molecules in the monomeric form (420 nm) and to the mass of the aggregates (452 nm). In Fig. 2(b) the time evolution of the areas relative to the two bands is shown in a logarithmic plot. It is evident that porphyrins belonging to the monomeric and aggregated species exhibit different time dependences. These behaviors can be explained considering

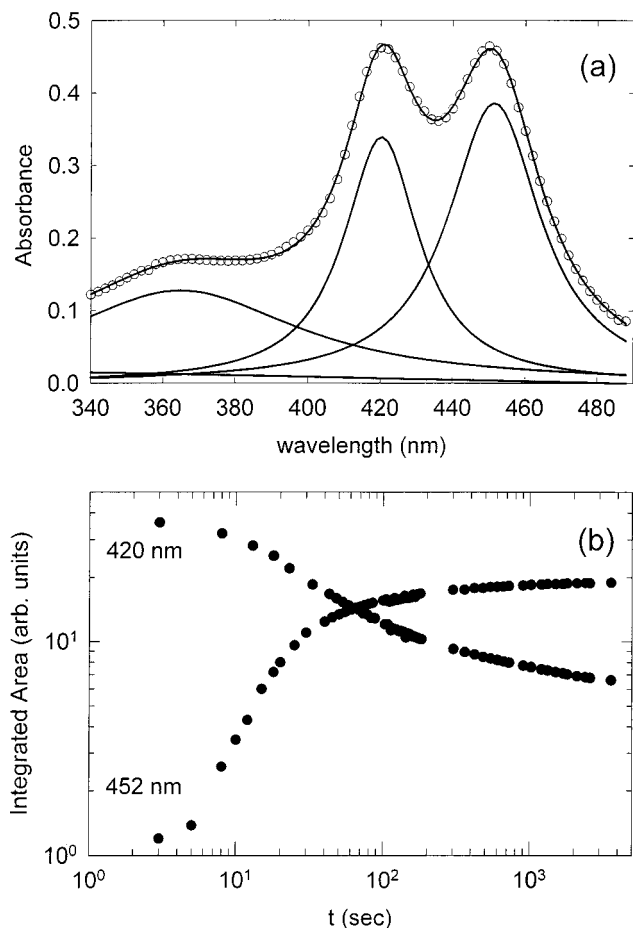


FIG. 2. (a) Typical deconvolution procedure applied to an UV-visible spectrum. The spectral region between 325 and 500 nm is treated as three Gaussian contribution centered at 365, 420, and 452 nm. (b). Logarithmic plot of the calculated area of the 420 nm and 450 nm peaks vs time.

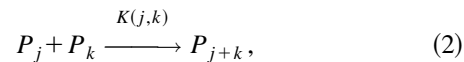
results coming from current models that have been shown to account well for the DLA aggregation kinetics [13–15]. More precisely, by considering results of MD simulations [16] and mean-field theoretical approaches based on the von Smoluchowski equation [17], we are able to show that the quantities obtained from UV-visible spectra agree well with these theoretical analyses, and in addition, correspond to quantities considered in a direct way in MD studies.

Scaling concepts describing fractal aggregations are based on the dynamic cluster size distribution function $n_s(t)$ [1,2]:

$$n_s(t) \sim k^{-\theta} f(s/t^z), \quad (1)$$

where $f(x)$ is a scaling function with rapid decay at large x . From mass conservation follows $\theta=2$. More precisely, $f(x) \sim x^\delta$ for $x \ll 1$, and $f(x) \ll 1$ for $x \gg 1$. According to Eq. (1), for $x \ll 1$, $n_s(t) \sim t^{-w} s^{-\tau}$ with $w = z\delta$ and $\delta = 2 - \tau$, i.e., the cluster size distribution decreases as a power law in s and t and the exponents satisfy the scaling relation $w = (2 - \tau)z$. Another consequence of Eq. (1) is that the mean cluster size $S(t) = \sum s^2 n_s(t)$ diverges for large t , and in terms of Eq. (1) is $S(t) \sim t^z$.

In general, aggregation processes in which cluster structures are originated by monomeric units can be described by following the reaction scheme:



where P_j denotes an aggregate containing j monomeric units and $K(j,k)$ is the rate at which the reaction proceeds and is model dependent. From a general point of view, in self-similar processes the reaction rates $K(j,k)$ are characterized only by two exponents λ and ν and are written as

$$K(a_j, a_k) = a^\lambda K(j, k), \quad (3)$$

$$K(1, j) \approx j^\nu,$$

with $\lambda \leq 1$ and $\nu \leq 1$ [17] (for $\lambda \geq 1$ gelation will occur). Under these conditions the mean cluster size $S(t)$ is determined by the rate at which two aggregates of this size interact, and the time dependence of its concentration is expressed by the equation $S(t) \sim t^z$, with $z = 1/(1 - \lambda)$ [16,17].

To determine the exponents τ and w it is necessary to know the behavior of the monomer concentration $C_1(t)$. The time behavior of such a quantity depends on the exponent ν , and is strongly related to the physics of aggregation, i.e., to the relative reactivity of the monomer with itself or with a cluster of size S . Rate equations have been developed for different possible situations [17] depending on the value of the quantity $\mu = \lambda - \nu$. For example, $\mu > 0$ implies that large aggregates react preferentially with equally large ones. We limit our considerations to the picture proposed for the system under investigation by light scattering data: the DLA aggregation phenomenon originates large monodisperse clusters (i.e., $\lambda < \nu$). In such a case, the growth mechanism is dominated by large growing clusters depleting the solution of the smaller ones. In these conditions, the number of smaller aggregates decreases exponentially with time. It can be shown that the monomer concentration $C_1(t)$ is given by

$$C_1(t) = \alpha \exp(-t^{\bar{\tau}}) = \alpha \exp(-t^{-(\lambda - \nu)/(1 - \lambda)}). \quad (4)$$

The application of scaling concepts to DLA aggregation implies also that the diffusion coefficient D_s of a cluster of mass s (s is the number of particles in the cluster) scales as

$$D_s \sim D_0 s^{-\gamma}, \quad (5)$$

where D_0 is a constant and γ is the diffusion constant exponent [16]. Furthermore, by considering that the studied objects are fractals, then their radius is $R(s) \sim s^{1/d_f}$, and we have the following relations [17]:

$$\lambda = \left(\frac{d-2}{d_f} \right) - \gamma \quad (a) \quad \text{and} \quad \nu = \left(\frac{d-2}{d_f} \right) \quad (b), \quad (6)$$

where d represents the Euclidean space dimension.

Under the assumption that the area of the bands, measured in the present experiment, is proportional to the concentration of species in solution, Eq. (4) can be written as $C_1(t) = A_0 + A_1 \exp[-(kt)^{\bar{\tau}}]$, where k is an observed time constant (sec^{-1}) and $\bar{\tau} = -(\lambda - \nu)/(1 - \lambda)$. The best fit of the experimental data to this equation [Fig. 3(a)] gives $A_0 = 7.5 \pm 0.2$, $A_1 = 48.1 \pm 3.9$, $k = 0.064 \pm 0.012 \text{ sec}^{-1}$, and $\bar{\tau} = 0.45 \pm 0.03$ (standard error 0.61). In such terms, as can be observed, the data analysis does not give satisfactory results.

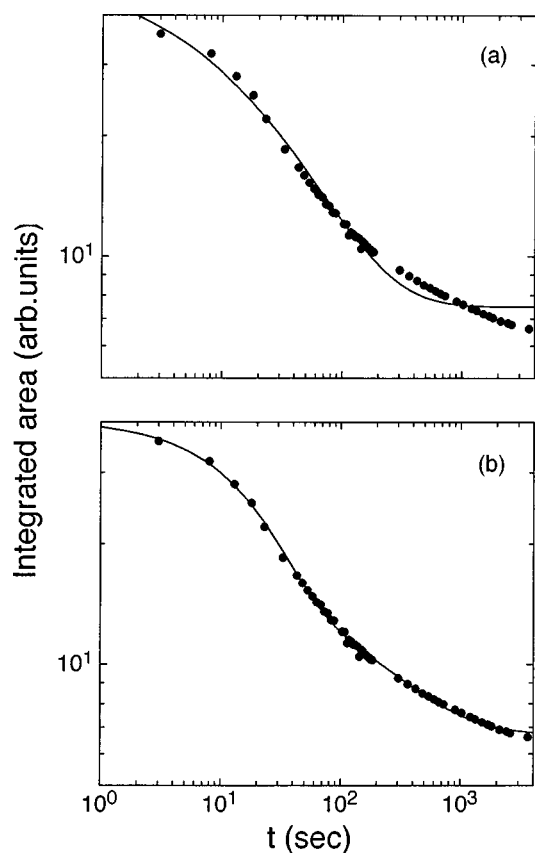


FIG. 3. (a) Logarithmic plot of the calculated area of the peak at 420 nm vs time; the continuous line represents the best fit by using Eq. (4). (b) Logarithmic plot of the calculated area of the peak at 420 nm vs time; the continuous line represents the best fit by using Eq. (7).

Following results coming out from recent theoretical MD simulation studies [18] we consider that a better description of the measured time profile of porphyrin molecules in the monomeric state (absorption band centered at 420 nm) can be achieved by introducing an additional short time exponential contribution. On these bases (i.e., considering the possible presence of a RLA early stage) we use the form

$$C_1(t) = A_0 + A_1 \exp[-(k_1 t)^{\bar{\tau}}] + A_2 \exp(-k_2 t). \quad (7)$$

The continuous line in Fig. 3(b) represents the best fit of the experimental data with Eq. (7) that provides a fairly good description of experimental results in the measured time range. From such a fit we obtain $A_0 = 6.7 \pm 0.2$, $A_1 = 14.0 \pm 3.2$, $A_2 = 20.8 \pm 1.5$, $k_1 = 0.01 \pm 0.004 \text{ sec}^{-1}$, $k_2 = 0.044 \pm 0.001 \text{ sec}^{-1}$, and finally $\bar{\tau} = 0.46 \pm 0.08$ (standard error 0.24).

The additional exponential term on passing from Eq. (4) to Eq. (7) deserves an additional comment; it is explained by considering that the porphyrin monomers are not the real starting units for the studied aggregation phenomenon. This hypothesis is supported by the recent MD simulation study on colloidal solutions [18] suggesting that the DLA aggregation process is, in general, preceded by a very early reaction limited aggregation between monomers leading to small clusters. Present results give an experimental indication of the validity of this MD suggestion.

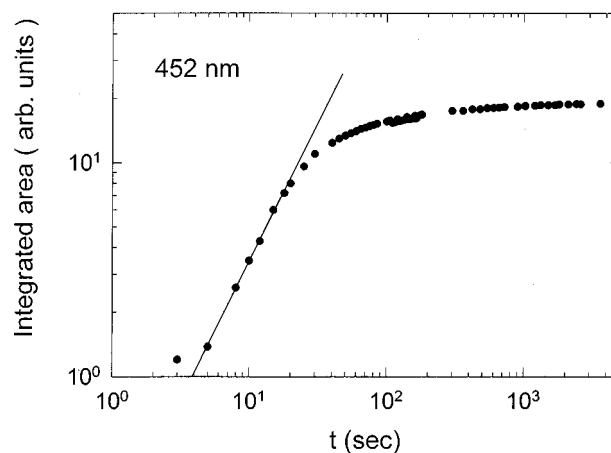


FIG. 4. Time evolution of the increasing spectral contribution (452 nm) ascribed to porphyrins in the aggregated form, the continuous line gives $z = 0.9 \pm 0.1$.

Furthermore, it is well known that dimerization of porphyrins [9] is a very fast process and, in the case of the porphyrin under investigation, a recent ^1H NMR study has pointed out the presence of dimers in aqueous solutions [19]. In addition, elastic light scattering data on unsalted porphyrin samples confirm the presence of a detectable wave vector dependent intensity contribution (two orders of magnitude smaller than the corresponding salted solution). On these bases, we can suppose that the fast initial exponential decay is a contribution due to basic units forming a threshold size aggregate. However, the intriguing issue of an RLA early stage in a DLA process seems to be confirmed by preliminary data of a proper investigation performed in our laboratory [20].

The consistence of the present data analysis in the framework of current models and MD simulations for DLA aggregation kinetics is confirmed by the measured $\bar{\tau}$ value. Considering the measured values of d_f and $\bar{\tau}$, Eqs. (6), and by using the relation connecting $\bar{\tau}$ with the scaling terms λ and ν , we obtain $\gamma = 0.512 \pm 0.04$, i.e., the value expected and measured in an aggregating system in which the diffusion process is controlled by the shear viscosity [16]. Such a result is usually obtained, for $d > 2$, when the DLA aggregation process originates a sharply peaked well-shaped cluster-size distribution, and it is in complete agreement with the findings of light scattering measurements [7].

Finally, we consider the time evolution of the increasing spectral contribution (452 nm) ascribed to porphyrins in the aggregated form (Fig. 4). On the basis of the previous discussion, we can relate such a peak to the mean cluster size $S(t)$. As previously discussed, this quantity scales as t^z with a diverging behavior for $t \rightarrow \infty$. However, in a real system this power-law behavior is expected to level off due to finite concentration constraints. A careful MD simulation for DLA [16], performed with different concentrations and γ values, confirms that in the time evolution of the mean cluster size there is a crossover from the t^z behavior to an asymptotically finite value. The observed time dependence is qualitatively similar to that obtained in this MD simulation. A more quantitative study is actually in progress in our laboratory. We conclude by observing that just after the early stage of the aggregation the power law t^z well fits the experimental data

(continuous line in Fig. 4). In this case, again, the obtained $z = 0.9 \pm 0.1$ value agrees with the above reported exponents characterizing the scaling laws and reproduces qualitatively the results of the cited MD simulation.

IV. CONCLUSIONS

In summary, we have showed that UV-visible absorption spectroscopy can give detailed information on the fractal aggregation kinetics of the $t\text{-H}_2P_{\text{agg}}$ porphyrin in aqueous solution. The obtained results are in complete agreement with light scattering measurements performed on the same system, showing that the aggregation phenomenon follows a simple DLA giving rise to rigid monodisperse clusters. This spectroscopic technique provides useful information on the temporal evolution of the aggregation kinetics. In particular, it describes fairly good, in a direct way, the time dependence

of the monomer concentration during the aggregation. These experimental findings are completely in line with scaling models and numerical simulations. In conclusion, we outline that UV-visible measurements can constitute a powerful tool in studying aggregation of biological system in which changes of their absorption is a frequent phenomenon as a consequence of the interaction between monomers.

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