Aggregation of Methylene Blue Adsorbed on Starburst Dendrimers

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ABSTRACT: The adsorption and aggregation of methylene blue (MB) on anionic starburst dendrimers (*n*.5 SBD) was studied by UV–vis and fluorescence spectroscopy. The aggregation of MB depends strongly on the generation of SBD. For SBDs of later generation the aggregation occurs more readily and the number of MB molecules involved in one aggregate is larger than for earlier generations. It is proposed that the MB molecules stack perpendicular to the surface of the SBDs and not as a pile parallel to the surface. UV–vis and fluorescence experiments give identical results.

The poly(amido amine) family of starburst dendrimers (SBDs)[1] is a novel class of macromolecules which possess a definite molecular composition and constitution. The SBD structure is created by stepwise attachment of layers, termed generations, on an ammonia core.[2,3] The half-generation (*n*.5 SBD), terminated with carboxylate salts represent a novel class of anionic polyelectrolytes whose structure has been examined by various probe techniques.[4–8] Each of these techniques has revealed that the binding (adsorption) of small cations to the anionic dendrimer surface is qualitatively different from the earlier generations (2.5 SBD or less) relative to the later generations (3.5 SBD or greater). Typically, the earlier generations behave as weak electrolytes rather than polyanions, whereas the later generations behave as polyanions capable of organizing bound cations to form self-organizing supramolecular structures. The formation of self-organizing aggregates between polyions and small molecules of opposite charge is of great interest in colloid, polymer, and surface science. Because of the potential importance of the mechanism of binding, the dynamics of binding and the nature of the supramolecular structures formed are important in fields as diverse as enhanced oil recovery, isolation of membrane-bound proteins, polymer solubilization, conformational changes, flocculation, gene therapy, etc.[9] The supramolecular structures formed by interactions of SBDs and cationic surfactants have been investigated by fluorescence[8] and ESR spectroscopy.[4] In this work we report a simple UV–vis spectroscopic method to study the adsorption of cationic organic dyes (methylene blue,[10] acridine orange,[11] pyronine G,[12] and phenosafranine[13]) on the anionic surfaces of *n*.5 SBDs.

When cationic organic dyes are adsorbed on negatively charged polyelectrolytes, aggregation of the dyes is sometimes observed. In addition to the electrostatic forces which bind the opposite charges of the polyanion and cation to one another, the self-organization of dye aggregates is promoted by two forces: dispersion forces due to the interaction between the π-systems of the dyes[14] and hydrophobic effects which may involve the details of the polyanion.[15] For aggregates to form and be stable, the sum of these forces must be larger than the electrostatic repulsion between the positive charges on the dye molecules.

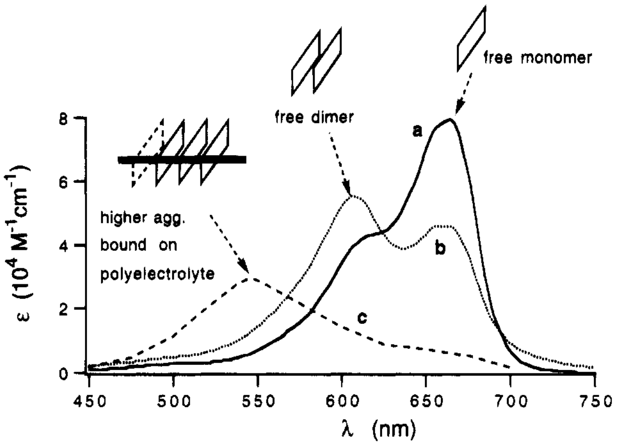

Figure 1. UV–vis spectra of aqueous solutions of MB (1×10^{-5} (a) and 4×10^{-4} M (b)) measured on a HP 8452A diode array spectrophotometer using quartz cells with path lengths of 1.0 cm at 293 K. Spectrum c presents data of ref 17 which indicate the aggregation of MB (1×10^{-5} M) in the presence of potassium poly(vinyl sulfate) ([SO_4^-] = 2×10^{-4} M).

The dimerization of organic dyes in aqueous solutions is a well-studied phenomenon and in the case of methylene blue (MB) occurs at relatively high dye concentrations (dimerization constant: 4.71×10^3 M^{-1}).[16] Monomers and dimers are easy to distinguish by UV–vis spectroscopy, as shown in Figure 1.[16] The aggregation of MB has been shown[17] to occur in the presence of polyanions to form large aggregates of MB bound to the surface of the polyanion and readily detectable by a hypsochromic shift of the absorption to the monomers and dimers (Figure 1). We take advantage of this technique to probe the dimerization and aggregation of MB in the presence of 1.5 SBD through 8.5 SBD carboxylate-terminated dendrimers.[18]

At [MB] = 1×10^{-5} M, MB does not show significant dimerization or aggregation in aqueous solutions; however, dimers and aggregates of MB on the surface of SBD were detected by UV–vis spectroscopy in the presence of SBD (Figure 2). The optical density at 666 nm, where the monomer dominates the absorption (Figure 1), show the most significant change; therefore this wavelength was used to monitor the dimer/aggregation behavior. Figure 3 shows the dependence of the dimerization/aggregation on the concentration of SBD molecules. In all cases, the monomer absorption of MB decreases with increasing concentration of SBD.

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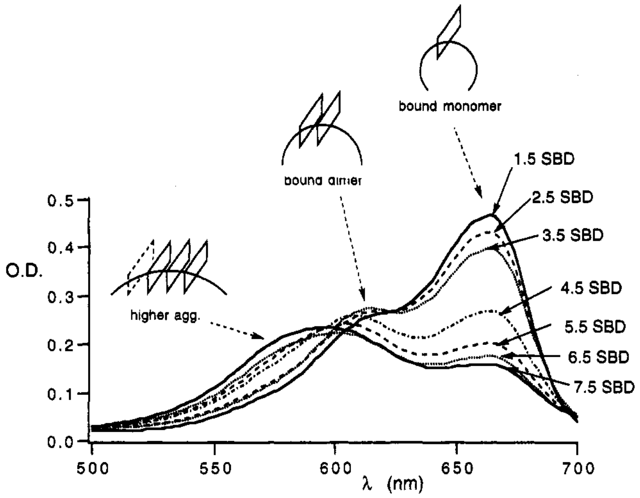


Figure 2. Optical absorption of MB ([MB] = 10^{-5} M) at the concentration of SBDs (generations 1.5 to 7.5) of the minimum of optical density at 666 nm (see Figure 3).

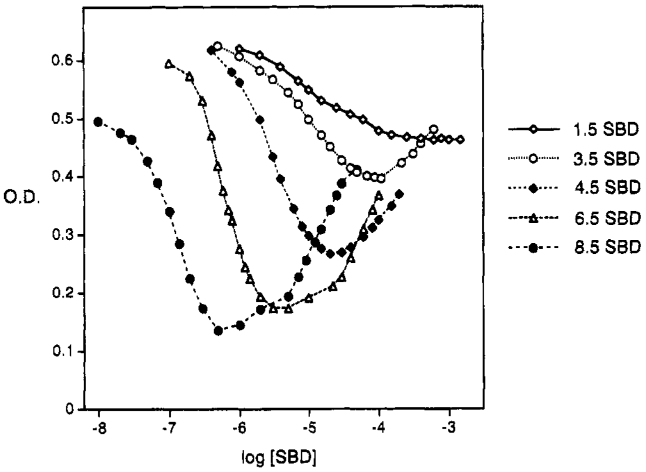
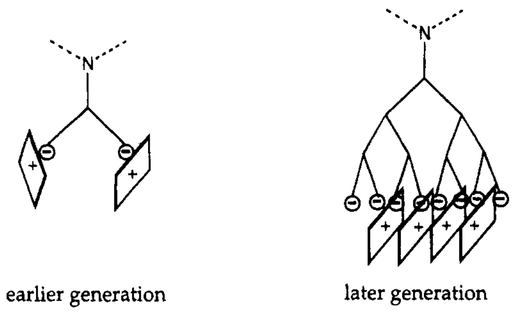


Figure 3. Optical density at 666 nm of the MB/SBD system for different generations of SBD; [MB] = 1 × 10^{-5} M.

Simultaneously, the optical density at wavelengths lower than 590 nm increases, where dimers/aggregates of MB absorb. Accordingly, MB forms aggregates on SBDs. At the higher concentrations of SBD, MB becomes distributed on the SBD molecules at the expense of the aggregates of MB. This causes an increase in optical absorption at 666 nm.

Figures 2 and 3 also reveals not only that MB associates with SBD but that aggregation occurs and also that *this aggregation between the dye molecules depends strongly on the generation of the SBD.* As anticipated from other investigations,[4-8] the aggregation of MB on SBDs of later generations occurs more readily than for earlier generations. For example, the minima of the plot of optical density *versus* [SBD] are deeper for SBDs of later generations than for the earlier generations, indicating that the extent of aggregation is larger for later generation SBDs. In addition, the values of the minima in Figure 3 provide information about the concentration of SBDs when aggregates of MB were formed. This concentration depends on the generation of the SBDs (Figure 3). An increase of the generation of SBDs is accompanied by an increase in the size of the SBD and in the number of surface groups (COO^-) (supporting information: see paragraph at end of paper). However, since the [SBD] and the [COO^-] show parallel behavior on the observed aggregation (supporting information), the effect is not simply due to a larger number of surface groups.

Scheme 1



Two structures of the MB aggregates formed on the dendrimer surface can be readily envisioned: a stacked aggregate sited parallel to the dendrimer surface or a stacked aggregate sited perpendicular to the dendrimer surface.[10] In the first case, the number of MB molecules involved in one aggregate is not expected to depend on the generation of SBD; furthermore, it is not obvious what the origin of the driving force for aggregation would be in this case, since the stabilizing effects of the anionic surface would be minimal and since MB does not show any tendency to aggregate in aqueous solution at the concentrations employed. *Therefore, we postulate that the more plausible aggregate structure is that for which the MB molecules are stacked perpendicular to the dendrimer surface* (Scheme 1). From this model, interaction between the aggregated MB molecules and the surface should be enhanced as the dendrimer surface becomes flatter, i.e., as the size of the dendrimer increases and the curvature of the surface decreases. This expectation is consistent with the greater ability of the later generations to induce aggregation, since the larger the size of the dendrimer, the flatter the surface. In addition, the separation of the anionic head groups becomes more congested as the generation increases (supporting information). The earlier generations possess more open and curved surfaces, both structural features binding the efficient formation of aggregates (Scheme 1). The UV–vis spectra show a larger hypsochromic shift of the aggregates on SBDs of the later generations (Figure 2). The intensity of the hypsochromic shift is expected to be a function of the number of dye molecules comprising the aggregates; i.e., the aggregation number for a single dendrimer/MB supramolecular system increases with the extent of the hypsochromic shift.[10] Thus, the experimental results indicate that on larger SBDs, larger MB aggregates are formed. This conclusion is easily consistent with the aggregate proposed.

It is almost certain that the dye aggregation occurs outside the surface of the SBDs, because the cavities inside the SBD are too small to be hosts of dye aggregates of the MB. Furthermore, the later generations, where aggregates were readily observed, possess a closed surface structure, which should inhibit or prevent the dyes from entering the SBD interior. In addition, the aggregates can be readily destroyed by adding NaCl. An increase of the ionic strength should not significantly influence the aggregates inside the SBD.

The spectra of Figures 2 and 3 show a relatively large break in the observed effects measured at 3.5 SBD. The ability of SBDs to adsorb small-molecule cations has been observed by other methods.[4-8] In addition, molecular simulation of the *n*.0 SBD structures have shown a dramatic change in the dendrimer morphology occurring around the third generation.[19] Generations lower than 3.0 were found to form an asymmetric shape and

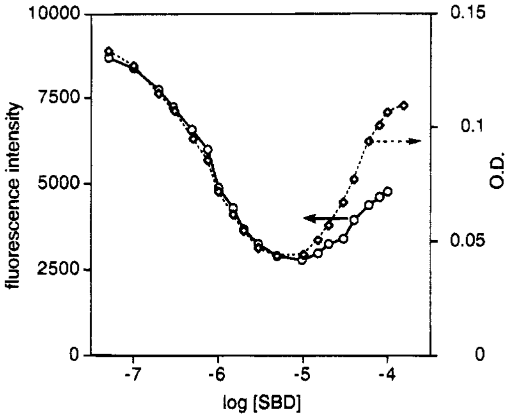


Figure 4. Quenching of the fluorescence of MB by adding 6.5 SBD ([MB] = 1×10^{-5} M, λ_{ex} = 600 nm). Intensity of the fluorescence (arbitrary units) at 684 nm of MB (left) and optical absorption at 666 nm (right) vs the concentration of 6.5 SBD. The fluorescence spectra were recorded with a SPEX Fluorolog spectrofluorimeter.

open structure, whereas generations higher than 3 were found to be nearly spherical in shape and to possess an external densely packed structure.[19]

The aggregation of MB on SBDs is expected to achieve an equilibrated state involving free dye molecules in solution with dyes adsorbed on SBDs as monomers, dimers, and higher aggregates. Thus, the concentrations of both SBD and dye will influence the composition of the equilibrium. Because of the complex equilibria involved, no attempt was made to calculate the equilibration constants.

The structural model proposed for aggregation, stacking of MB molecules, is expected to be detectable by a second analytical technique, fluorescence spectroscopy, because MB fluorescence is self-quenched upon formation of dimers and aggregates.[20] As a monomer in aqueous solution, MB exhibits a fluorescence maximum at 684 nm. From steady-state measurements the fluorescence of the free MB in the bulk solution and the fluorescence of nonaggregated MB adsorbed on the surface of SBD can be detected. The steady-state fluorescence of the dimers and higher aggregates is negligible because of self-quenching. Figure 4 shows that the amounts of monomer and aggregates by UV−vis and fluorescence measurements follow essentially identical trends. That both graphs do not show an overlay at concentrations of SBD larger than 10^{-5} M is probably caused by a different fluorescence quantum yield of MB in the bulk solution and MB bound to the SBD surface. Since both fluorescence and UV−vis measurements give parallel results in the view of the aggregation effects, no further discussion of the fluorescence results will be given.

In summary, the aggregation of organic dyes on surfaces of SBDs may be determined by UV−vis absorption and may be developed into a useful method to study the adsorption of small cationic organic dyes on the surface of SBDs. In addition to methylene blue, thionine and thiopyronine were tested and gave analogous results; however, MB was found to be the most suitable dye, because of its strong tendency to form aggregates on SBDs and because the differences in optical absorption of the different forms of MB aggregates are large. As noted in previous studies, the ability of the later generations to cause aggregation is greater than that of the earlier generations. We speculate that the more open structure and larger separation of the surface anions of the earlier generations hinder the aggregation of MB, whereas the closed structure and smaller separation encourage aggregation. However, the effect appears to "saturate" at the highest generations because the separation of the head groups approaches a constant value.

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Supporting Information Available: A table containing a brief description on the *n*.5 SBDs used in this work, and two figures, one showing the influence of the concentration of 7.5 SBD on the UV−vis spectra and the other demonstrating the different aggregation behavior of MB on SBDs in earlier and later generations (the latter figure also shows that the different aggregation is not caused by the increasing number of surface groups (COO^-) at increasing generations) (3 pages). Ordering information is given on any current masthead page.

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