Preparation of AgBr Quantum Dots via Electroporation of Vesicles

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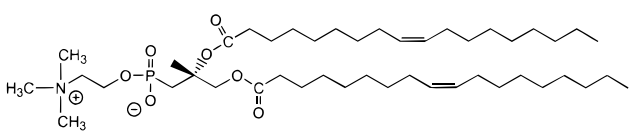
Abstract: Electric field-induced transient pore formation (reversible electroporation) in the bilayer membrane of synthetic large unilamellar vesicles (LUV) is used as a novel method for the preparation of angstrom size quantum dots of the indirect band gap semiconductor AgBr. With Ag^+ ions encapsulated in 178 nm diameter LUVs of dioleoylphosphatidylcholine (DOPC) and Br^- ions placed in the bulk medium, the reaction $Ag^+ + Br^- \rightarrow$ AgBr and subsequent clustering of the product are initiated by the application of a 500 μs long high-voltage ($E = 6$ kV/cm) electric square pulse to the vesicular suspension. The slow growth of clusters (taking several hours) on the exterior surface of the vesicles is monitored through the blue-shift followed by a red-shift of their UV absorption band. At the turn-around point (269 nm) of the spectral band-shift, the size of the AgBr clusters is estimated to be ∼5 Å, the smallest achieved by colloid chemical methods.

Introduction

The creation of quantum dots in solution represents an experimental challenge because the spontaneous self-aggregation of the constituent molecules must be controlled and halted at the quantum size level. In the case of the indirect band gap semiconductor AgBr quantum dots, arrested precipitation[1] and synthesis by stopped-flow mixing in reverse micelles[2] resulted in minimum cluster sizes of 35 and 30 Å, respectively. Due to quantum size effects, the growth of semiconductor clusters in general is associated with a red-shift of their UV−vis absorption band(s), and from a size of ∼30 Å up to crystallite sizes the decreasing transition energy is well described by Brus's formula.[3] In the present paper, we describe a novel method for the reproducible production of AgBr quantum dots utilizing the electroporation of synthetic unilamellar vesicles. The method seems to be superior to other colloid chemical methods, concerning the smallest cluster size achievable. Beyond this advantage, the rate of cluster growth in the presence of vesicles is immensely reduced which allows, for the first time, the observation of also the early, molecular stage of self-aggregation that, according to our finding, is associated with a blue-shift of the absorption band.

Experimental Section

Unilamellar vesicles of mean hydrodynamic diameter $\langle D_h \rangle = 178$ nm were made from the synthetic phospholipid dioleoylphosphatidylcholine (DOPC, Avanti Polar Lipids):



Details of their preparation and characterization[4] as well as the procedure of loading the vesicles with silver ions[5] have been described previously. Prior to electroporation, the concentrations of relevant species in the compartmentalized system were the following: [DOPC] = 2 mg/mL, [Ag^+ inside] = 0.01 M, which corresponds to [Ag^+ global] = 1.5×10^{-4} M, [Br^- outside] = 0.01 M. Freshly prepared solutions were used in the experiments, although they tested free of spontaneous transmembrane reactions for up to a week. All manipulations were carried out under exclusion of light, except for inevitable brief illuminations in spectral and birefringence measurements. Typically, a 500 μs long 1.5 kV electric square pulse was applied to the solution between gold-plated electrodes (2.5 mm apart). The corresponding applied field strength is $E = 6$ kV/cm. Under these conditions no fusion of DOPC vesicles occurs.[4] Details of the instrument (Figure 1) and experimental procedure were similar to that reported previously.[4,6] The absorption spectra were recorded on a computerized Gilford Response-II UV−vis spectrophotometer that allows determination of the wavelengths of absorption maxima with 0.5 nm accuracy. Quartz cuvettes with 1 cm path length were used. Polyoxyethylene 23 lauryl ether (Brij-35, Sigma), utilized for lysing the vesicles, was used as received.

Results and Discussion

Electroporation, a method routinely used in molecular biology (e.g., for transfection), is the fully reversible transient pore formation in the bilayer of cells or vesicles, induced by the application of a homogeneous high-voltage electric field pulse to the aqueous suspension.[7] With Ag^+ ions originally entrapped in the interior compartment of vesicles and Br^- ions placed in the bulk medium, their reaction

$$Ag^+ \text{ (inside)} + Br^- \text{ (outside)} \rightarrow AgBr \text{ (outside)} \quad (1)$$

can only occur if the separating bilayer is temporarily punctured, i.e., electroporated. (Alternatively, each of the Ag^+ and Br^-

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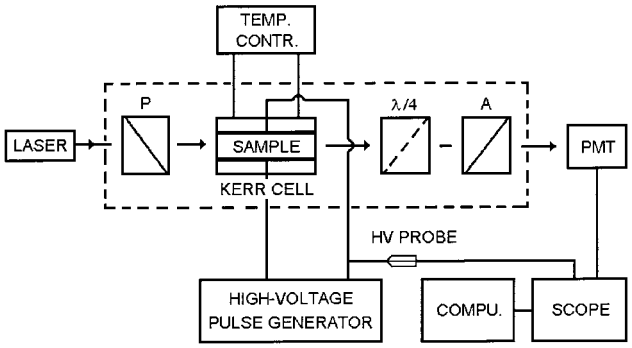

Figure 1. Block diagram of the instrument used for electroporation of vesicles, with transient electric birefringence monitoring of the evolution of induced anisotropy: laser (He−Ne, 10 mW), P (polarizer), $\lambda/4$ (quarter-wave plate), A (analyzer), PMT (photomultiplier tube), scope (digital oscilloscope). The rise and fall times of the high-voltage square pulse delivered to the electrodes of the Kerr cell are <100 ns.

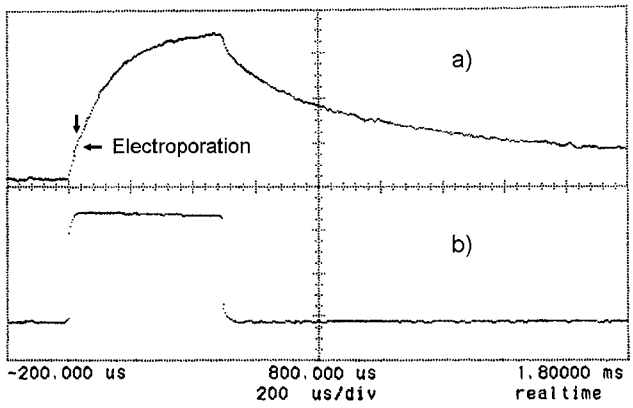

Figure 2. Oscilloscope traces of a typical birefringence signal of loaded unilamellar DOPC vesicles (a), and the attenuated corresponding perturbation square pulse (b). The point of electroporation is indicated by arrows.

reactants can be encapsulated in different populations of vesicles, and the electroporation with the proper pulse length performed on their mixture.) Depending on the concentrations and experimental conditions used, the subsequent net aggregation and cluster growth processes

$$n\mathrm{AgBr} \rightarrow (\mathrm{AgBr})_n \text{ and } (\mathrm{AgBr})_n + m\mathrm{AgBr} \rightarrow (\mathrm{AgBr})_{n+m} \quad (2)$$

can be monitored through the UV absorption spectrum of the particles formed. For large values of $n + m$, the species $(\mathrm{AgBr})_{n+m}$ represents fully developed crystallites approximating the properties of bulk AgBr.

The primary effects of the applied electric field $\boldsymbol{E}$ are the global polarization and elongation (in the direction of $\boldsymbol{E}$) of the time average spherical vesicles, and the concomitant alignment of the resultant of the permanent and induced dipoles parallel to $\boldsymbol{E}$.[4] The arising structural anisotropy of the solution is manifested in the observed transient birefringence of the solution (Figure 2). In the loaded vesicle systems (with Ag^+ inside and Br^- outside), in addition, electroporation occurs above threshold values of the applied field strength (2 kV/cm) and pulse length (30 μs), which permits the inception of reactions 1 and 2. Subsequent monitoring of the time evolution of the UV absorption spectrum (starting 6 min after electroporation) reveals the presence of angstrom size $(\mathrm{AgBr})_n$ clusters and their slow growth over a period of 11 h (Figure 3, A−C). Even though there is an almost 90-fold excess of the Br^- ions in the bulk solution after electroporation, the absorption bands observed are not due to negatively charged clusters. Such transient species would absorb around 230 nm,[8] which we were unable to detect.

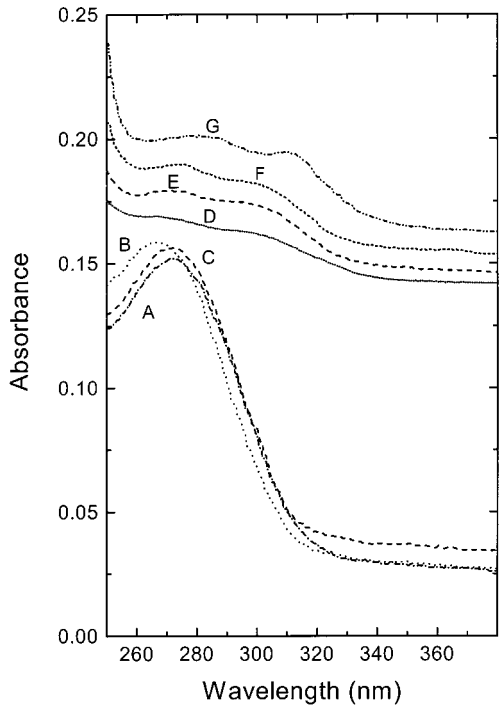

Figure 3. Absorption spectra of AgBr quantum dots. Curves A−C: Clusters produced from Ag^+ (trapped in DOPC vesicles) and Br^- ions (in the bulk solution). Using electroporation, the growth of the clusters is restricted to the "molecular regime": 0.1 (A), 5 (B), and 11 h (C) after application of the high-voltage square pulse. The corresponding shifts of absorption maxima are 274 nm → 269 nm → 273 nm. Curves D−G: Clusters of the "crystallite regime" are formed if the vesicles are lysed instead of using electroporation (0.1 (D), 1 (E), 3 (F), and 20 h (G) after addition of Brij-35). The reference for spectra A−C was the same as the sample prior to electroporation. For spectra D−G, the reference was a vesicle solution (with Ag^+ both inside and outside, lysed with Brij-35) of the same lipid concentration and light scattering properties as the sample.

Although our current experimental setup does not permit direct detection of the initially formed AgBr monomer and dimer $(\mathrm{AgBr})_2$ (with absorption bands at 295 and 285 nm, respectively),[9] we do observe the entire blue-shift followed by red-shift of the absorption band (274 nm (5 h) → 269 nm (6 h) → 273 nm) associated with the slow growth of the clusters through the molecular size to the typical quantum size regime. Density functional calculations at the B3P86/LB level suggest that the turn-around point (269 nm) in the band-shift occurs at the trimer $(\mathrm{AgBr})_3$ or tetramer $(\mathrm{AgBr})_4$ stages of cluster growth, at which point the particle size is ~5 Å.[10] At the same point of growth, TEM (Figure 4) reveals particles with a typical size of 19 Å on the exterior surface of the vesicle, which has shrunk and become shriveled during drying of the sample. Accumulation of clusters in the crevices, and in the contact area between two vesicles, is also evident from the micrograph. Since the clusters undergo a substantial amount of growth during evaporation of the solvent, we expect the actual size of clusters in solution absorbing at 269 nm to be close to the theoretical estimate.

The success of the application of electroporation of vesicles for the production of quantum dots is based in part on the metered admission of only very small amounts of one of the

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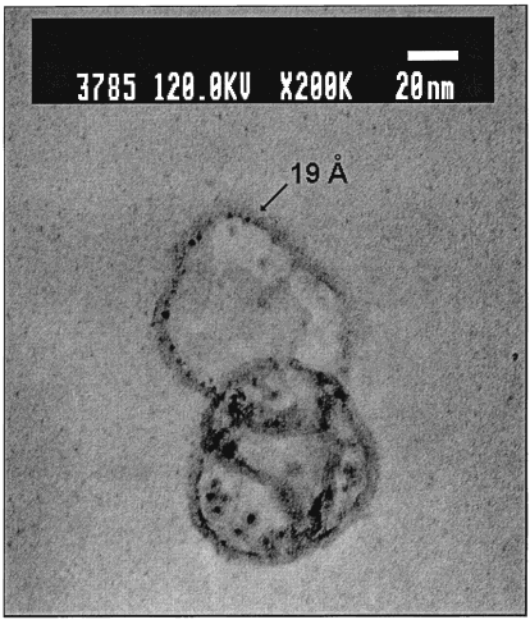

Figure 4. TEM micrograph of AgBr quantum dots prepared via electroporation of DOPC vesicles. The sample was taken from the solution that absorbs at the turn-around point (269 nm) of the spectral shift.

reactants (Ag^+) throughout into a solution of the other reactant (Br^-) during the limited lifetime of the pores. Namely, the early, field-induced elongation of the spherical vesicle to prolate ellipsoid results in an increased pressure in its interior compartment which leads to the ejection of approximately 75% of the entrapped Ag^+ ions when the pores open ~30 μs later[5] (Figure 2). These correspond to $\sim 1.3 \times 10^4$ Ag^+ ions per vesicle transiting through the membrane during a single pulse. The small size and limited lifetime of the pores and the outward flux of Ag^+ solution prevent the larger Br^- ions from entering the vesicle. This conclusion is in accord with the finding that a 2-fold dilution of the solution immediately after electroporation reduces the bulk concentrations below the level necessary for cluster growth, and no associated spectral shift of the initial band (at 274 nm) is observed. If reactions 1 and 2 took place in the protected interior compartment, dilution would have no effect on their progress.

The choice of the pulse length applied is dictated by the following considerations. The minimum pulse length at which electroporation of the loaded DOPC vesicles is detectable through the appearance of the characteristic absorption band at 274 nm is ~30 μs (Figure 2).[5] Under the given experimental conditions, the time of pore opening (~30 μs) counted from the leading edge of the pulse is not an experimental variable. The number of Ag^+ ions ejected from the vesicle depends to a certain extent on the pulse length applied which, of course, must be greater than ~30 μs. With a short pulse, however, due to the small number of Ag^+ ions ejected and AgBr formed, the resulting absorbance is too small for reliable monitoring of the cluster growth through the time-dependent shift of the absorption band. Thus we used a large pulse length (typically 500 μs), while safely avoiding the field-induced pearling/fusion of the vesicles which starts to occur with ~800 μs long pulses.[4,5]

Another contributing factor to the utility of electroporation of vesicles is the availability of their contiguous exterior layer (consisting of the zwitterionic phosphatidylcholine headgroup of the surfactant) as adsorption site for all the species involved in the cluster growth process. Although the approach of anions to the positive tetramethylammonium sites is sterically limited, the access to the PO_3^- sites for cationic species is wide open. The positively charged species are Ag^+ and the $Ag_{n+1}Br_n^+$ ($n \geq 1$) intermediates of the cluster growth. Their adsorption on the vesicle surface is responsible for the very low rate of cluster growth, i.e., for the stability of the small clusters.

The importance of the bilayer surface for stabilizing the molecular size AgBr clusters is demonstrated by the following findings. If the vesicle (with Ag^+ inside and Br^- outside) is destroyed through lysis by the addition of the nonionic surfactant Brij-35 (that forms mixed micelles with DOPC) *instead* of using electroporation, all Ag^+ ions are released for reaction, and immediately the split exciton bands at ~272 and ~300 nm appear which are characteristic of large AgBr crystallites[11] (Figure 3D). Upon standing, both of these bands are red-shifted with further growth of the crystallites. The final spectrum (Figure 3G) is similar to that obtained when reactions 1 and 2 occur in pure water in the absence of any surfactants. In contrast, the crystallite state (split bands) is not attained even with repeated application of electric pulses to the loaded vesicles, even though essentially all Ag^+ ions are ultimately successively released. Of course, in electroporation, the bilayer skeleton remains intact.

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