of charge separation at low temperature, where dielectric relaxation can no longer stabilize the radical ion pair, suggests that charged functional groups are important in stabilizing photosynthetic charge separation in vivo. In photosynthetic organisms charge separation proceeds efficiently even at cryogenic temperatures. Model donor-acceptor systems with good distance and geometry constraints need to incorporate charged groups to better simulate the natural environment of photosynthetic reaction centers.

Acknowledgment. We wish to thank Peter Rentzepis, Danny Huppert, and Steve Milton for helping us with some of the fluorescence measurements. This work was sponsored by the Division of Chemical Sciences, Office of Basic Energy Sciences, of the Department of Energy. We wish to thank the National Science Foundation for providing the VAX 11/780 at the University of Illinois.

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Optical Absorption, Electron Spin Resonance, and Electron Spin Echo Studies of the Photoionization of Tetramethylbenzidine in Cationic and Anionic Synthetic Vesicles: Comparison with Analogous Micellar Systems

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Received February 10, 1983

Abstract: The photoionization of *N,N,N',N'*-tetramethylbenzidine (TMB) in dihexadecylphosphate anionic vesicles and in dioctadecyldimethylammonium chloride cationic vesicles has been studied by optical absorption and electron spin resonance in liquid and frozen solutions. The TMB cation has been observed to be stabilized in both types of vesicles. The photoionization efficiency is about twofold greater in the cationic vesicles compared to the anionic vesicles. Shifts in the optical absorption maximum between micellar and vesicle solutions indicate that TMB is in a less polar environment in the vesicle systems. Electron spin echo modulation spectrometry has been used to detect TMB cation-water interactions that are found to be weaker than in previously studied micellar solutions. This is consistent with the optical absorption results and with an asymmetric solubilization site for TMB and TMB^+ within the vesicular structure. A new absorption in the photoionized vesicles is assigned to a nonparamagnetic diamine-diimine charge-transfer complex between two TMB cations in the same vesicle. This complex is not formed in micellar systems.

Photoinduced charge separation and photosensitized electron transport in organized molecular assemblies such as micelles and vesicles are being widely studied as models for artificial photosynthetic systems.[1-6] To optimize the charge separation efficiency in such systems, it is necessary to understand the role of the geometry and structural location of the photoactive molecule in the organized molecular assembly. We have recently shown how electron spin resonance (ESR) and electron spin echo modulation (ESEM) can be successfully used to deduce the surrounding structure of paramagnetic species in frozen solutions.[7,8] Most recently we have shown how ESR and ESEM can be used to obtain structural information about photoproduced aromatic amine cations in micellar systems.[9,10] In this work we extend such investigations to studies of photoproduced cations in synthetic cationic and anionic vesicle systems. We find interesting contrasts with micellar systems that give new information about the structural requirements for optimum photogenerated charge separation in organized molecular assemblies.

Experimental Section

The anionic surfactant dihexadecylphosphate (DHP) was obtained from Sigma Chemicals, the cationic surfactant dioctadecyldimethylammonium bromide (DODAB), and the aromatic amine *N,N,N',N'*-tetramethylbenzidine (TMB) were obtained from Eastman Chemicals. DHP and DODAB were purified by recrystallization from acetone. DODAB was exchanged by chloride ion to produce dioctadecyldimethylammonium chloride (DODAC).[11] This was done by passing a solution of DODAB in MeOH-$CHCl_3$ (70:30, v/v) solvent through a polystyrene exchange resin type AG2-X8 from Bio-Rad Laboratories. The DODAC was purified by recrystallization from acetone/water mixtures (95/5 volume ratio).

The vesicle solutions were prepared by dissolving the synthetic surfactants in triply distilled water (over alkaline permanganate and acid dichromate) heated to 80 °C followed by sonication with a Fisher Model 300 sonic dismembrator operated at 30 W with a 4-mm o.d. microtip for 1 h at 80 °C. The initial aqueous solution is a cloudy suspension, but after sonication it becomes a clear solution with a slight bluish color. Typical concentrations used are 9 mg of DODAC, 6 mg of DODAB, and 6 mg of DHP, each in 1.5 mL of water. For some of the electron spin

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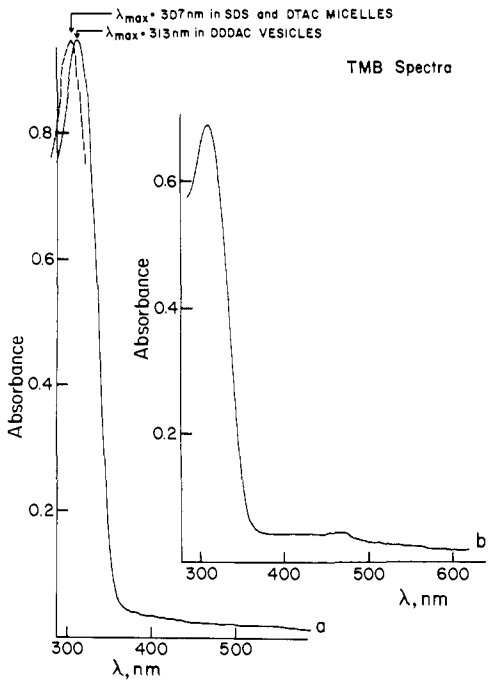

Figure 1. Optical absorption spectra of 3.7×10^{-4} M TMB in a 0.1-cm path length cell in (a) 0.1 M SDS micelles, 0.1 M DTAC micelles, and 0.01 M DODAC vesicles at room temperature and (b) 0.01 M DODAC vesicles at room temperature *after* 100-s exposure to 350-nm light. Note the increased absorption in the 425–475-nm range after light exposure.

resonance experiments samples were prepared in D_2O. Approximately 0.1 mL of 17 mM stock solution of TMB in benzene was added to the sonicated solutions with a 100 λ micropipette. The mixture was then vigorously shaken for 20 min by using a vortex mixer. It was then bubbled with nitrogen gas in a glovebag for 2 h in order to evaporate the benzene and to eliminate the dissolved air in the solution. This procedure was shown to eliminate the benzene by carrying out a control experiment in which the TMB solution was added to pure water. After evaporation of the benzene by nitrogen gas the TMB precipitated out and no optical absorption of TMB in the water solution was observed.

The concentration of TMB in the vesicle solutions was determined to be 0.3 mM by optical absorption at room temperature in a 0.1-cm path length quartz cell with a Cary 14 spectrophotometer using an extinction coefficient of 34000 M^{-1} cm^{-1} at the absorption maximum of 312 nm. The wavelength scale of the Cary 14 in this range was calibrated with a holmium oxide filter and is accurate to ±0.3 nm.

Optical and ESR samples were prepared in a glovebag flushed with dry nitrogen. The ESR samples for room-temperature measurements were contained in 75 λ pipettes, and the ESR samples for 77 and 4 K experiments were prepared in 2-mm i.d. by 3-mm o.d. Suprasil quartz tubing. The samples were frozen in 2–3 s by plunging the sample tubes directly into liquid nitrogen. This is rapid enough that the water at a micellar surface retains a disordered, noncrystalline structure,[12] so it is assumed that micelle and vesicle shapes in the frozen solutions are similar to those in liquid solutions.

Photoirradiations were carried out with a 900-W high-pressure mercury lamp (Philips Model SP). A narrow band-pass filter (Corning no. 760) was used to pass light of 350 ± 30 nm. The total light flux was 1.0×10^2 W m^{-2} as measured by a radiometer. Typical irradiation times were 100 s at room temperature and 200 s at 7 K.

The ESR spectra were recorded at room temperature and at 77 K on a Varian-E4 ESR spectrometer. The electron spin echo data were recorded at 4.2 K on a home-built pulsed ESR spectrometer that has been described.[13]

Results

A. Optical Absorption. TMB dissolved in benzene has an optical absorption maximum at 312 ± 0.5 nm while in ethanol the maximum is at 306 ± 0.5 nm. The latter absorption peak in ethanol agrees witth the literature.[14] In DODAB, DODAC, and DHP vesicle solutions the optical absorption of dissolved TMB is at 313 ± 0.5 nm as shown in Figure 1 for DODAC vesicles. This absorption maximum contrasts with that for TMB dissolved in micellar solutions such as sodium dodecylsulfate (SDS) and dodecyltrimethylammonium chloride (DTAC) micelles where the absorption maximum is at 307 ± 0.5 nm; again see Figure 1. To the extent that the shift in the absorption maximum between ethanol and benzene solvents reflects a change in solvent polarity, it may be concluded that the environment of TMB in vesicles is less polar than the environment of TMB in micelles. It is also noteworthy that the micellar or vesicle surface charge does not affect the optical absorption maximum.

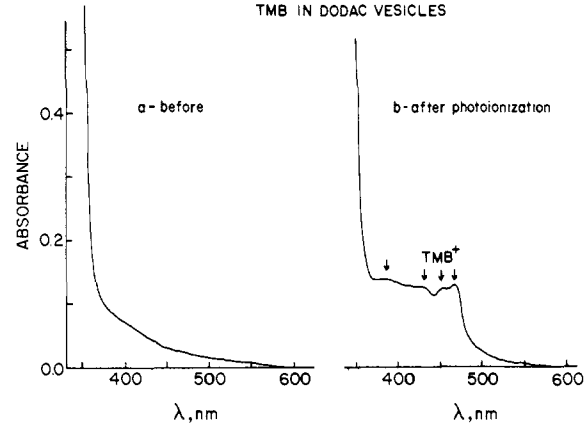

Figure 2. Optical absorption spectra of 3.7×10^{-4} M TMB in 0.01 M DODAC vesicles in a 1.0-cm path length cell (a) before and (b) after photoionization with 350-nm light for 100 s. New peaks appear after photoionization in the 425–475-nm region as shown by arrows that are assigned to TMB^+. Another peak near 385 nm (see arrow) is tentatively assigned to a charge-transfer complex between two TMB^+.

The above optical absorption results were obtained with solutions of pH 7 ± 1. Recent work by Beck and Brus discusses protonation equilibria of TMB in micellar solutions as a function of pH and micelle surface charge.[15] We have verified the optical spectral shifts they report as a function of pH for anionic and cationic micelles in which $TMBH^+$ and $TMBH_2^{2+}$ species are formed. In all of our results reported here the optical spectra indicate that we have an undetectable amount of protonated TMB species.

Photoirradiation of the vesicle solutions at 350 nm at room temperature causes the colorless solution to turn pale yellow. This color fades away in a couple of hours in both cationic and anionic vesicle solutions. Thus the yellow color after photoirradiatin denotes a relatively stable product, presumably an oxidation product of TMB. No such color changes were observed in the absence of TMB. The same color changes occur in micellar solutions where the oxidation product was confirmed by optical absorption and by electron spin resonance to be the TMB radical cation.[10] Figure 1 shows the optical absorption of the photoirradiated DODAC vesicles in comparison with the absorption before irradiation at room temperature. The TMB peak at 313 nm decreases about 15% after 100 s of irradiation, and a new broad peak extending from about 380 to 480 nm appears. Figure 2 shows this peak partially resolved when a longer path length cell is used. This absorption region is the same region in which the TMB radical cation absorbs as observed previously in micellar systems.[10] The TMB cation spectrum shows three clearly resolved peaks at approximately 435, 455, and 470 nm. The same three peaks are clearly observed in Figure 2. In addition there is an overlapping broad peak at shorter wavelength with an apparent maximum near 385 nm. At shorter photoirradiation times the 385-nm peak decreases relative to the 435, 455, and 470-nm peaks. The anionic DHP vesicle solutions also turned pale yellow when photoirradiated

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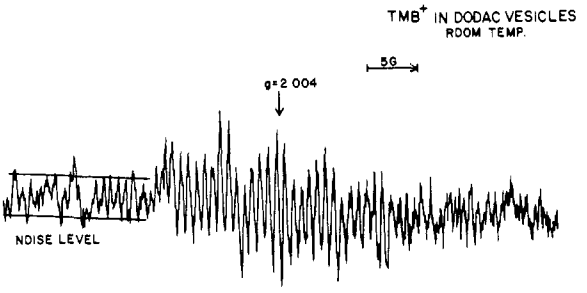

Figure 3. ESR spectrum at room temperature of photogenerated TMB^+ in DODAC vesicle solutions. The spectrum was taken during photolysis in the ESR cavity after 100 s of photolysis. The concentration of TMB and DODAC are $\sim 3 \times 10^{-4}$ and 0.01 M, respectively, and the vesicle concentration is $\sim 4.8 \times 10^{-7}$ M. The microwave power was ~ 5 mW, and the magnetic field modulation amplitude was 0.8 G.

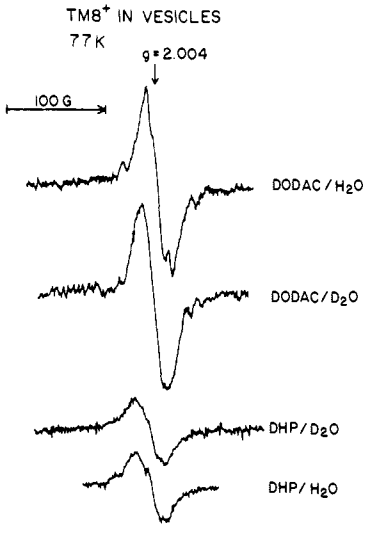

Figure 4. ESR spectra at 77 K of photogenerated TMB^+ in anionic DHP and cationic DODAC frozen vesicle solutions prepared in H_2O and D_2O. The concentrations of TMB, DHP, and DODAC are $\sim 3 \times 10^{-4}$, 0.07, and 0.01 M, respectively, and the vesicle concentrations are approximately 4.8×10^{-7} M for DODAC and 1.3×10^{-7} M for DHP. The microwave power was ~ 2 mW, and the magnetic field modulation amplitude was 5 G.

at 350 nm, and a similar new broad peak from about 380 to 480 nm is observed. However, it is only about 30% as intense as in the cationic DODAC vesicles when normalized for the difference in TMB concentration in the two different vesicle preparations.

A very crude estimate of the quantum yield of TMB^+ in the DODAC vesicle solutions gives ~ 0.01.

B. ESR and ESE. A weak, resolved ESR signal is observed at room temperature in the DODAC vesicle solutions containing TMB after photoirradiation as shown in Figure 3; no ESR is observed in the absence of TMB^+. Weak ESR is also observed in DHP vesicle solutions containing TMB. The ESR signal can be identified as TMB^+ by comparison to the ESR spectrum of TMB^+ in micellar solutions.[9] If we assign the weak optical band at 470 nm to the TMB cation and use the extinction coefficient for TMB^+ in acetonitrile ($\epsilon_{474} = 4 \times 10^4\ M^{-1}\ cm^{-1}$),[16] we obtain a TMB^+ concentration of 6×10^{-6} M. This is probably only accurate to a factor of 2, but it is compatible with our observation of only a very weak ESR spectrum.

If DHP or DODAC vesicle solutions containing TMB are rapidly frozen to 77 K, photoirradiation at 350 nm for 500 s produces an ESR signal that is shown in Figure 4. This spectrum is broad and difficult to unambiguously identify; however it is in width ($\Delta H_{pp} \approx 28$ G) and in magnetic field position ($g = 2.004$) identical with the well-characterized TMB cation observed in frozen micellar solutions.[9] Thus this signal is assigned to the TMB cation.

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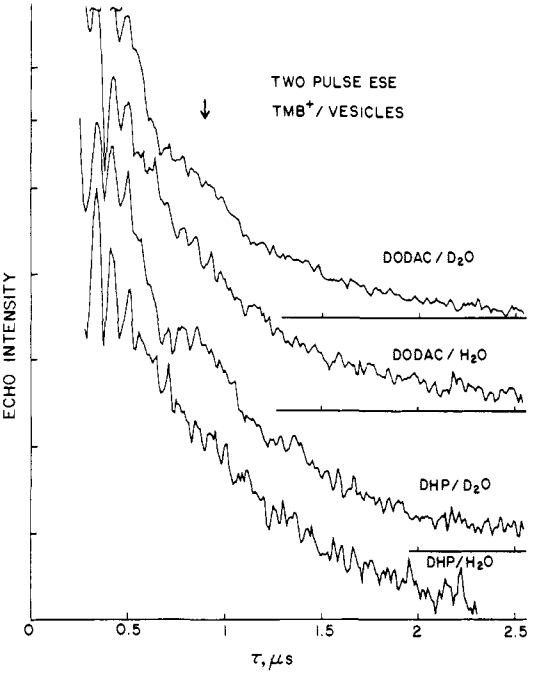


Figure 5. Two pulse ($\pi/2 - \pi$) electron spin echo decay envelopes at 4.2 K of photogeneated TMB^+ in cationic DODAC vesicles prepared in D_2O and H_2O and in anionic DHP vesicles prepared in D_2O and H_2O. The base lines have been offset vertically to avoid spectral overlap. The arrow shows the most prominent deuterium modulation period.

Immediately after photoirradiation of the frozen vesicle solutions green phosphorescence is seen, which lasts about 2 s, and the frozen matrix becomes yellow in color. These characteristics are the same as observed for frozen micellar solutions containing TMB. The frozen solutions are opaque, and no optical absorption measurements were made on them.

For the same irradiation time and the same TMB concentration it is found that the ESR signal height, whih is attributed to the TMB cation concentration in frozen solutions, is 2 times greater in the cationic DODAC vesicles than in the anionic DHP vesicles. This difference is somewhat comparable to the difference in the optical absorption attributed to TMB oxidation products in liquid solutions where a threefold greater yield was found in the cationic DODAC vs. the anionic DHP vesicle solutions.

Although the ESR signals in the frozen vesicle solutions are not too strong, it was possible to obtain electron spin echo spectra of these frozen solutions at 4.2 K. Figure 5 shows the results for DODAC vesicle solutions prepared in D_2O and H_2O and for DHP vesicle solutions prepared in D_2O and in H_2O. Although the spectra are quite noisy, a clear difference can be seen between the samples prepared in H_2O and D_2O. The prominent modulation in all samples corresponds to proton interactions, presumably mainly with protons on the surfactant molecules themselves since the modulation is still quite apparent in the samples prepared in D_2O. However, in the samples prepared in D_2O a very weak modulation with a characteristic time period of 0.5 μs corresponding to deuterium interactions is also observed. Such deuterium modulation clearly indicates that the TMB cations in frozen vesicle solutions are sufficiently close, namely, ≤ 0.6 nm, to interact with solvent water molecules but that the interaction is quite weak. The weakness of the interaction and the quality of the data does not permit a detailed quantitative analysis of this modulation data to be made. However, the deuterium modulation can be characterized for comparative purposes by its depth at the first modulation minimum at 0.68 μs. This depth is 9% in DODAC/D_2O and 12% in DHP/D_2O.

Discussion

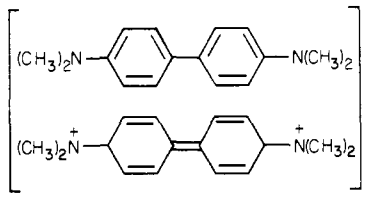
The observed optical absorption peaks of TMB of 306 nm in ethanol, 312 nm in benzene, 307 nm in micelles, and 313 nm in vesicles are of considerable interest. The difference between the

absorption peak in ethanol and benzene is probably due to a hydrogen-bonding solvent effect in ethanol in which the ground state is stabilized more than the excited state.[17] The shift in the optical absorption maximum between micelles and vesicles then implies that the environment of TMB is less good for hydrogen bonding in the vesicle systems than in the micelle systems. Furthermore, the independence of this shift on micellar or vesicle surface charge implies that the TMB is not located right in the Stern layer of the micelle where the surface charge is expected to have an effect.

Some difference in the average environment of TMB in micelle and vesicle systems is also suggested by electron spin echo modulation results on TMB^+ in frozen solutions. The location of TMB^+ within micellar structures appears to be the same as the TMB location based on nitroxide spin probe[18] and frozen solution[10] studies. The deuterium modulation depth associated with TMB^+ in micellar solutions prepared with D_2O at the first modulation minimum occurs at 0.68 μs in two-pulse echo spectra. This depth is 16% in anionic micelles formed with sodium dodecylsulfate[9] and is an average of 18% in cationic micelles formed with dodecyltrimethylammonium chloride and with hexadecyltrimethylammonium chloride.[10] In the anionic DHP and cationic DODAC vesicle systems prepared in D_2O, the deuterium modulation depth associated with TMB^+ at the first modulation minimum also occurs at 0.68 μs. In DHP anionic vesicles this modulation depth is 75% of the modulation depth in anionic micelles, and in DODAC cationic vesicles this modulation is 50% of the modulation depth in cationic micelles. The weaker deuterium modulation in the vesicle vs. the micelle systems means that the average interaction distance between TMB^+ and the nearest solvent water molecule is greater in the vesicle systems. This indicates that there is less water penetration into the vesicle surface than into the micellar surface. This, in turn, is consistent with the idea that TMB is in a worse environment for hydrogen bonding in the vesicle system compared to the micellar system.

A semiquantitative evaluation of the deuterium modulation effects between vesicles and micelles leads one to the following conclusions. Previous studies in micelles show that the TMB cation to solvent water molecule average interaction distance varies from about 0.4 to 0.5 nm depending on the surfactant molecule chain length.[10] On the basis of model simulations, the still weaker deuterium modulation in the vesicle systems indicates a TMB cation to solvent water interaction distance of 0.5–0.6 nm.

Photoionization of TMB in the vesicle solutions at room temperature generates a low concentration of the stable TMB cation radical based on the optical and ESR spectra. But, in addition, another species with $\lambda_{max} \approx 385$ nm appears to be formed. On the basis of the recent results of Josephy et al.,[19] we assign this other species to the nonparamagnetic diamine–diimine charge-transfer complex formed from two TMB cation radicals with the structure shown in **1**. Beck and Brus[15] have recently discussed a reaction of two TMB^+ in acidic solution (typically pH $\sim$1.8) to form TMB^{2+} and $TMBH_2^{2+}$. Our optical spectra do not match these products as expected since the local pH is not expected to be at all near to 1.8. Thus, the charge-transfer complex assignment seems best.



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The ESR spectra observed in frozen solutions also can be assigned to the TMB cation radical. This is additional evidence that photoionization does occur, even in frozen solution, as expected from previous evidence in homogeneous solution and in micellar solutions. The cation radical is stabilized to a greater extent in the frozen solution than in the liquid solution based on the relative intensities of the ESR spectra.

From the concentration of TMB in the vesicles and the vesicle concentration we calculate that there are approximately 770 TMB molecules/DODAC vesicle and about 1900 TMB molecules/DHP vesicle. This situation is in considerable contrast to that in the micellar solutions in which there is less than one TMB/micelle on the average. Because of the large number of TMB molecules/vesicle, it seems possible that two TMB cations may be formed in some vesicles and interact to form a nonparamagnetic diamine–diimine charge-transfer complex. This is supported by the decrease of the 385-nm peak relative to the TMB^+ peaks at shorter photoirradiation times. In other vesicles only one TMB cation is formed and so is stabilized as such. In frozen solutions TMB^+ is localized at its formation site and formation of the charge-transfer complex does not occur.

The initial yield of the TMB cation ESR spectrum observed in the frozen solutions can be used as a measure of efficiency of the photoionization process in the frozen vesicle solutions. It is interesting that the yield of the TMB cation is twice as great in the cationic vesicles involving DODAC and DODAB as it is in the anionic vesicle involving DHP. This apparent effect of surface vesicle charge is quantitatively identical with what has been previously observed in micellar systems.[10] For example, the photoionization efficiency of TMB is twice as great in dodecyltrimethylammonium chloride cationic micelles as it is in sodium dodecylsulfate anionic micelles. The exact nature of this surface charge effect is not understood quantitatively, but it has been qualitatively explained in terms of an asymmetric location for the TMB cation in the micelle structure. The same qualitative argument can be applied to vesicle systems. The weak deuterium modulations observed in the electron spin echo experiments also indicate that the TMB cation is located asymmetrically within the vesicle structure relatively close to one side.

Acknowledgment. This research was supported by the Department of Energy under Contract DE-AS05-80ER10745. We thank Dr. M. Narayana for operating the electron spin echo spectrometer to obtain the electron spin echo modulation results. We thank P. D. Josephy and R. Mason for a preprint of their results. We also thank Dr. R. Maldonado for checking TMB spectra as a function of pH.

Registry No. 1, 86584-38-5; *N,N,N',N'*-tetramethylbenzidine, 366-29-0; *N,N,N',N'*-tetramethylbenzidine radical cation, 21296-82-2; water, 7732-18-5; dihexadecyl phosphate, 2197-63-9; dioctadecyldimethylammonium chloride, 107-64-2.

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