pointing away from the double bond (i.e., with $\alpha > 180°$, cf. Figure 1) as advocated by Yamaguchi et al.[60] is an interesting proposition. Subsequently a rotation around the newly formed CO bond gives dioxetane in a process which involves a totally asymmetrical transition state.

The conclusion of this paper is, then, that the dioxetane pathway has somewhat higher barrier than the peroxirane pathway, provided that the conversion of peroxirane to dioxetane is not the rate-determining step. The present study is noncommital to the totally unsymmetrical reaction paths.

Acknowledgment. Many encouraging discussions with Professor P. Pyykkö are gratefully acknowledged. M. H. wishes to thank The Academy of Finland for financial support. Besides the Finnish Universities' UNIVAC 1108, computer resources have been made available to us by the Computing Centre at University of Oulu and the Computer Centre in Lund.

Registry No. Oxygen, 7782-44-7; ethylene, 74-85-1.

Clustering of Hydrophobic Ions in the Presence and Absence of the Polysoap Poly(vinylbenzo-18-crown-6)

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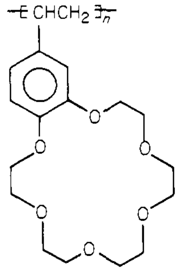
Contribution from the Chemistry Department, College of Environmental Science and Forestry, State University of New York, Syracuse, New York 13210. *Received January 19, 1983*

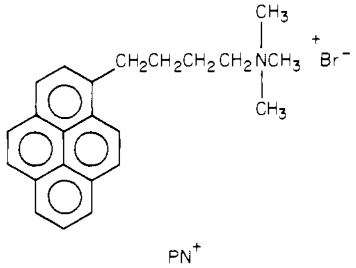
Abstract: Measurements of optical absorption and fluorescence spectra and of turbidity in dilute aqueous solutions of 4-((1-pyrenyl)butyl)trimethylammonium bromide (PN^+) and sodium tetraphenylborate (BPh_4^-) point to the formation of stable aggregates with the preferred stoichiometry $(PN^+)_2(BPh_4^-)_3$. The complex only shows the pyrene excimer emission, which disappears, together with the coacervate phase, on adding an excess of the polysoap poly(vinylbenzo-18-crown-6) (P18C6). The presence of BPh_4^- ions greatly enhances the binding of PN^+ to P18C6, as the binding capacity of the polymer is increased from one PN^+ molecule per 26 monomer base units in the absence of BPh_4^- to about one PN^+ per 2.5 monomer units in the presence of BPh_4^-. It is speculated that under these saturation conditions the pyrene moieties of the $(PN^+)_2(BPh_4^-)_3$ complex are inserted in between adjacent benzocrown ether ligands with the $-N^+(CH_3)_3$ groups protruding into the aqueous phase and paired to BPh_4^- ions.

Formation of 1:1 ion-pair complexes in dilute aqueous solutions of hydrophobic solutes of opposite charge and the subsequent appearance of a complex coacervate phase have been well documented.[1,2] In a recent communication, Atik and Singer[3] reported the formation of premicellar aggregates between the fluorophor sodium 5-(1-pyrenyl)pentanoate (PP^-) and cationic surfactants (S^+) such as cetyltrimethylammonium chloride (CTAC). They deduced from ESR studies with a surfactant nitroxyl radical that the predominant aggregate structure was $(PP^-)_2(S^+)_3$. The latter complex, with two pyrene moieties in close proximity, exhibited exclusively the characteristic pyrene excimer emission. An earlier report by Davis[4] on the fluorescence of potassium 4-(1-pyrenyl)butanoate (PB^-) in the presence of cetyltrimethylammonium bromide (CTAB) showed complete conversion of monomer into excimer emission when the ratio $CTAB/PB^-$ reached the value 1.5 for $[PB^-] = 8.8 \times 10^{-5}$ M, although no conclusion was drawn as to the possible significance of this observation in terms of a specific aggregate stoichiometry.

In recent years we studied in detail the polysoap-type properties of poly(vinylbenzo-18-crown-6) (P18C6), a polymer which binds a variety of neutral and ionic organic solutes.[5-9] While investigating the interaction of P18C6 with cationic and anionic pyrene derivatives[10] we observed that the binding of 4-((1-pyrenyl)butyl)trimethylammonium bromide (PN^+) to P18C6 in water can be dramatically enhanced by adding minute amounts of sodium tetraphenylborate. Subsequent studies on dilute solutions of PN^+ and BPh_4^- in the presence and absence of P18C6 indicate the presence of complexes of the type $(PN^+)_2(BPh_4^-)_3$, similar to the premicellar aggregates found by Atik and Singer for the anionic pyrene derivative and the cationic surfactant molecule. Apparently, such complexes between hydrophobic ions may be more common, and their formation profoundly affects their binding to polysoap-type macromolecules like P18C6.



P18C6



PN^+

Experimental Section

Materials. 4-((1-Pyrenyl)butyl)trimethylammonium bromide was obtained for Molecular Probes, Inc., while sodium tetraphenylborate of 99.5% purity was a Fisher product. The synthesis of poly(vinylbenzo-18-crown-6) was reported earlier.[11] The number average molecular weight of the polymer as measured by osmometry was 110 000 (DP_n = 320).

Measurements. Optical spectra were recorded on a Beckman Acta M VI spectrophotometer, and fluorescence spectra were obtained by means of a Perkin-Elmer 650-10S spectrofluorimeter. Turbidities of aqueous mixtures of PN^+Br^- and $NaBPh_4$ were measured with a DRT-100 tur-

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(1) Mukhayer, G. I.; Davis, S. S. *J. Colloid Interface Sci.* **1975**, *53*, 224.
(2) Tomlinson, E.; Davis, S. S. *J. Colloid Interface Sci.* **1980**, *74*, 349.
(3) Atik, S. S.; Singer, L. A. *J. Am. Chem. Soc.* **1979**, *101*, 6759.
(4) Davis, G. A. *J. Chem. Soc., Chem. Commun.* **1973**, 728.
(5) Wong, L.; Smid, J. *J. Am. Chem. Soc.* **1977**, *99*, 5637.
(6) Shah, S. C.; Smid, J. *J. Am. Chem. Soc.* **1978**, *100*, 1426.
(7) Kimura, K., Smid, J. *Macromolecules* **1982**, *15*, 966.
(8) Smid, J. *Pure Appl. Chem.* **1982**, *54*, 2129.
(9) Wong, K. H.; Kimura, K.; Smid, J. *J. Polym. Sci., Polym. Chem. Ed.* **1983**, *21*, 579.
(10) Roland, B.; Smid, J.; to be published.
(11) Kopolow, S.; Hogen Esch, T. E.; Smid, J. *Macromolecules* **1973**, *6*, 133.

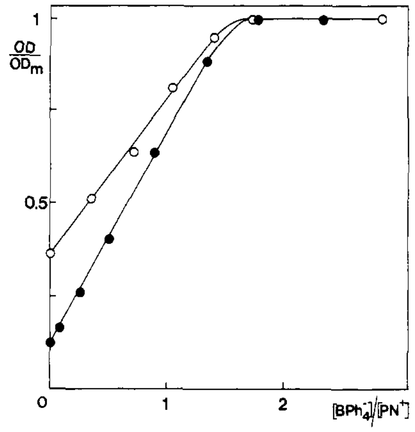


Figure 1. Plot of the ratio of optical density to maximum optical density of PN^+ absorption as a function of the ratio BPh_4^-/PN^+ in the presence and absence of P18C6: (●) no P18C6, $[PN^+] = 2.1 \times 10^{-5}$ M, optical density mesured at 352 nm; (○), $[P18C6] = 5 \times 10^{-5}$ M, $[PN^+] = 2.1 \times 10^{-5}$ M, optical density measured at 348 nm.

bidimeter, and expressed in terms of nephelometric turbidity units, NTU. All spectral and turbidity studies were carried out in twice-distilled water at 25 °C.

Results and Discussion

The optical absorption spectrum of the pyrene compound PN^+ in water (λ_m 326 and 342 nm) is changed on adding small amounts of the P18C6 polymer, and the appearance of isosbestic points confirms the presence of P18C6-bound PN^+ (λ_m 332 and 348 nm). Studies to be reported elsewhere[10] demonstrate that the binding follows Langmuir adsorption behavior, and linear Klotz plots yield a value $K = 9 \times 10^4$ M^{-1} for the intrinsic binding constant of PN^+ to P18C6. The minimum number of crown monomer base units per bound PN^+ molecule is 26, i.e., a P18C6 chain of DP = 320 will bind a maximum number of 12 PN^+ molecules.

The tetraphenylborate anion also has a high binding constant to P18C6.[5] It easily replaces anionic dyes like picrate, methyl orange, or pyrene carboxylate bound to the polymer domain. However, it might be anticipated that in mixtures of PN^+ and BPh_4^- the binding of both species to P18C6 could be enhanced as a result of electrostatic interaction between bound PN^+ and bound BPh_4^-, possibly leading to ion pairing in the polymer domain. Indeed, small amounts of $NaBPh_4$ dramatically increase the binding of PN^+ to P18C6. Figure 1 shows that the optical density of the 348-nm absorption maximum of the P18C6-bound PN^+ increases as a function of the ratio BPh_4^-/PN^+ at constant PN^+ concentration. Taking the point of intersection of the two linear sections of the plot, a maximum value is reached at a ratio $BPh_4^-/PN^+ = 1.5$, where all PN^+ is bound to P18C6. The value of 1.5 is obtained irrespective of the PN^+ concentration as long as $[PN^+] > 4 \times 10^{-6}$ M.

A shift in the PN^+ spectrum is also observed when $NaBPh_4$ is added to the pyrene compound in the *absence* of P18C6. The new absorption maximum is at 352 nm, and even at 10^{-5} M concentration of the two reagents a coacervate phase can be detected. Figure 1 shows that the conversion into the new species is complete when the ratio BPh_4^-/PN^+ equals 1.5, the same value as found for the reaction in the presence of P18C6. The turbidity in the PN^+–BPh_4^- solution disappears on adding P18C6. At the same time the 352-nm peak of the $PN^+ \cdots BPh_4^-$ species shifts back to 348 nm, the characteristic maximum of P18C6-bound PN^+. The change is complete and the turbidity has disappeared when the ratio P18C6/PN^+ exceeds the value 2.5.

Apparently, the interaction between PN^+ and BPh_4^- does not simply yield 1:1 ion-pair complexes but higher aggregates in which the ratio BPh_4^-/PN^+ is 1.5. This value is further confirmed from turbidity measurements in mixtures of PN^+ and BPh_4^-. Turbidities were measured at constant $[PN^+]$ for different ratios of BPh_4^-/PN^+. This was repeated for solutions with different initial concentrations of PN^+. The results, graphically depicted in Figure 2, demonstrate that above $[PN^+] = 5 \times 10^{-6}$ M the turbidity reaches rather abruptly its maximum value at $BPh_4^-/PN^+ = 1.5$. At very low PN^+ concentrations ($\approx 10^{-6}$ M) the equilibrium concentrations of free BPh_4^- and PN^+ constitute a considerable fraction of the total concentrations of added reagents, and no sharp transition is found at the ratio 1.5. The maximum turbidity is proportional to the initial PN^+ concentration, the only significant deviation being at the highest concentration of PN^+.

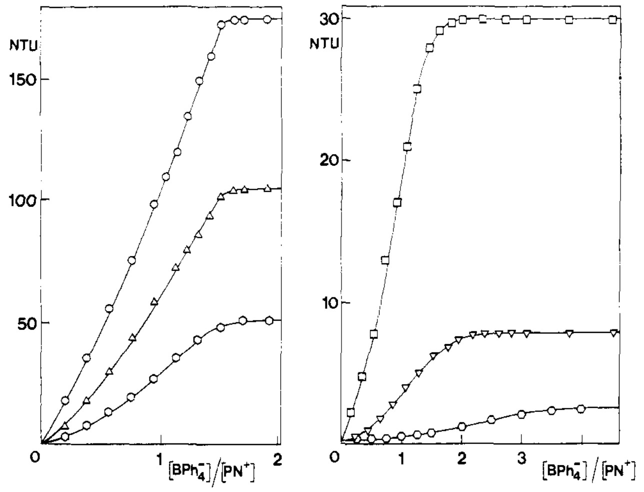


Figure 2. Plot of turbidity (in nephelometric turbidity units, NTU) vs. the ratio BPh_4^-/PN^+ in mixtures of PN^+ and BPh_4^- at 25 °C. $[PN^+] = 4.15 \times 10^{-5}$ M (○); 2.12×10^{-5} M (△); 1.05×10^{-5} M (○); 5.55×10^{-6} M (□); 1.83×10^{-6} M (▽); 8.0×10^{-7} M (○).

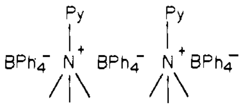
An important observation is also that the characteristic fluorescence spectrum of the PN^+ molecule decreases on adding BPh_4^- and is replaced by the broad excimer emission peak at λ_m 480 nm. This resembles the behavior of pyrene–carboxylate mixtures with cationic surfactants as reported by Atik and Singers and by Davis. At $[PN^+] = 10^{-5}$ M the conversion into the excimer spectrum is complete when $BPh_4^-/PN^+ = 1.5$.

The combined observations on the PN^+–BPh_4^- system in the absence of P18C6 point to the formation of stable complexes of the type $(PN^+)_m(BPh_4^-)_n$ with $n/m = 1.5$. This aggregate exhibits exclusively an excimer emission, implying that it contains at least two PN^+ molecules in close proximity. As in the case of Atik and Singer, the preferred aggregate structure in our case also appears to be a 2:3 complex, $(PN^+)_2(BPh_4^-)_3$. This structure is apparently maintained as increased clustering results in the formation of a coacervate phase.

The observation that the turbidity of aqueous mixtures of PN^+ and BPh_4^- disappears on adding P18C6 and that the binding of PN^+ to P18C6 in the presence of BPh_4^- at a constant ratio of P18C6/PN^+ reaches its maximum value for $BPh_4^-/PN^+ = 1.5$ (Figure 1) suggests that the $(PN^+)_2(BPh_4^-)_3$ aggregate itself binds to P18C6, and that its formation in water is the cause for the strong increase in PN^+ binding to P18C6 when tetraphenylborate is added. In the absence of BPh_4^- a minimum average number of 26 crown monomer base units are needed to accommodate one PN^+ molecule in the P18C6 domain, and the pyrene compound is most likely located in the hydrophobic interior of the polymer coil. With BPh_4^- present the capacity of P18C6 to bind PN^+ under saturation conditions increases to one PN^+ molecule per 2.5 crown monomer base units. The bound species in the complex-saturated polymer has an absorption maximum in between 348 (P18C6-bound PN^+) and 352 nm (complex in water). Also, its fluorescence spectrum depicts a strong excimer emission, contrary to the normal monomer emission spectrum of PN^+ bound to P18C6 in the absence of BPh_4^-. As the concentration of P18C6 is increased, the P18C6-bound $(PN^+)_2(BPh_4^-)_3$ complex gradually breaks up as PN^+ and BPh_4^- molecules are dispersed over the newly added polymer coils. This is accompanied by a change in λ_m to 348 nm and an increase in the monomer PN^+ emission spectrum at the expense of the excimer emission intensity. From intensity ratios of monomer and excimer emission peaks it is estimated that in mixtures of 2×10^{-5} M PN^+ and 3.2×10^{-5} M BPh_4^- the respective monomer to excimer ratios are approximately 0.13, 0.33, 0.55, and 0.9 for P18C6/PN$^+$ = 1.0, 3.0, 7.5, and 30 (all complex is polymer bound when P18C6/PN$^+$ > 2.5). Even at high P18C6/PN$^+$ ratios, when the excimer emission of bound PN$^+$ disappears, the binding of PN$^+$ is still strongly enhanced by the presence of BPh$_4^-$. In that case, the bound PN$^+$ may be in the form of an ion pair PN$^+$BPh$_4^-$ which is expected to have a higher binding constant to P18C6 than the free PN$^+$ ion.

It may be argued that the strong binding of (PN$^+$)$_2$(BPh$_4^-$)$_3$ to P18C6 is caused by Na$^+$ binding to P18C6. This would convert P18C6 into a polycation which then interacts electrostatically with the negatively charged complex. However, the binding constant of Na$^+$ to P18C6 in water is only 2.4 M^{-1},[12] and under our conditions ([NaBPh$_4$] < 5 × 10^{-5} M) binding of Na$^+$ to P18C6 would be negligibly small. On the other hand, Na$^+$ binding to P18C6 may be enhanced as a result of the strong binding of the anionic complex to P18C6, which especially under saturation conditions converts P18C6 into a polyanion of rather high charge density.

The large number of PN$^+$ molecules that can be adsorbed onto P18C6 in the presence of BPh$_4^-$ suggests that under these conditions the pyrene molecules and, thus, the complexes are now located close to the periphery of the coiled P18C6 chain. It could be speculated that the ability to accommodate so many PN$^+$ molecules is accomplished by insertion of the planar pyrene ring in between adjacent benzocrown ether ligands with the trimethylammonium cationic ends protruding into the aqueous phase and paired to BPh$_4^-$ ions. The stability of the (PN$^+$)$_2$(BPh$_4^-$)$_3$ aggregate (or a multiple of this complex) in water may in part derive from ground-state interactions between the two pyrene moieties as suggested in the drawing below, causing the optical absorption of pyrene to shift from 342 to 352 nm and the monomer emission spectrum to change to an excimer spectrum. Apparently, this structure is maintained to some degree when the complex binds to P18C6, at least when the ratio P18C6/PN$^+$ is not too large. More information on the location of P18C6-bound PN$^+$ in the presence and absence of BPh$_4^-$ could probably be obtained by addition of pyrene quenchers, and such studies are being planned.



Preliminary studies have revealed that PN$^+$ binding to P18C6 is also enhanced by anions like SCN$^-$ or I$^-$ even when added in the form of their potassium or cesium salts. These cations bind to P18C6, and their presence should actually decrease PN$^+$ binding to P18C6 as was also demonstrated in the binding of the cationic fluorophor auramine 0.[9] In comparison to BPh$_4^-$, much higher concentrations ($\approx 10^{-2}$ M) of SCN$^-$ or I$^-$ are needed to enhance PN$^+$ binding to P18C6, and it is possible that under these conditions ion pairing with PN$^+$ occurs which in turn causes the increased binding to P18C6.

In conclusion, our studies demonstrate that even at very low concentrations hydrophobic ions of opposite charge aggregate into complexes of well-defined stoichiometry (in our system 2:3) which then cluster into higher aggregates under formation of a coacervate phase. The aggregation profoundly affects the binding of these solutes to neutral polysoap-type molecules like poly(vinylbenzo-18-crown-6), and probably also to micelles or to macromolecules with hydrophobic regions such as proteins. It especially increases the total number of solute molecules that can be bound to the poly(crown ether). The aggregation also causes changes in the optical and fluorescence spectra of the solutes e.g., the formation of excimers. Since these solutes are frequently used to probe the structures of micelles and biological macromolecules, the observed phenomena may have important implications in these fields of study.

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Registry No. PN$^+$, 81341-11-9; BPh$_4^-$, 4358-26-3; P18C6, 31943-71-2.

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[12] Wong, L.; Smid, J. *J. Polym.* **1980**, *21*, 195.

Electrochemical Behavior of a Dopamine Polymer. Dopamine Release as a Primitive Analogue of a Synapse

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Abstract: A modified polystyrene was synthesized and characterized which held *N*-(2-(3,4-dihydroxyphenyl)ethyl)isonicotinamide units. This polymer was dissolved in DMF, syringed onto glassy carbon electrodes, and dried; the electrode was then used in aqueous electrolyte solutions. In pH 7 solution, at potentials more negative than −0.9 V (SCE), cathodic current caused cleavage of the amide linkage and release of the neurotransmitter dopamine. The released dopamine was detected at a second electrode by its oxidation or by HPLC. Voltammetric studies of the reduction process as well as the oxidation of the hydroquinone units in the polymer layer were performed. It was shown that only a few equivalent monolayers of polymer units could be oxidized or reduced even if the layer contained many more such units. The oxidation of solution-phase NADH on these electrodes was studied voltammetrically. It was shown that the polymer acted as an electrocatalyst. Quinone units were formed on the polymer and they in turn oxidized NADH molecules. Maximum catalytic efficiency was obtained with a layer holding approximately one equivalent monolayer of dopamine units.

The conduction of electrical signals in vivo involves chemical communication between neutrons.[1] At a synapse, transmitter substances are released from the presynaptic terminal in response to a change in the neuron's potential. These neurotransmitters diffuse across a small volume of solution, the synaptic cleft, and then are detected at receptor sites on the postsynaptic membrane. We have recently reported that a solid electrode, modified with a thin layer of a suitable polymer, will similarly respond to a change in potential to release the neurotransmitter, dopamine.[2]

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[1] Kuffler, S. W.; Nicholls, J. G. "From Neuron to Brain"; Sinauer Associates: Sunderland, MA, 1976; pp 132-176 and references therein.

[2] (a) Miller, L. L.; Lau, A. N. K.; Miller, E. K. *J. Am. Chem. Soc.* **1982**, *104*, 5242. (b) Lau, A. N. K.; Zinger, E.; Miller, L. L. *Neurosci. Lett.* **1983**, *35*, 101.

0002-7863/83/1505-5271$01.50/0