arrive with the zenith angle mentioned above, requires a particle velocity of 70 m/sec or greater and an emission angle at or slightly below the horizontal.

It is true that particles entrained by LM exhaust would not initially follow a ballistic trajectory, but this is probably a good approximation away from the LM. The sharpness of the "permanent shadows" on the Surveyor camera shows that the incident particles were well collimated.

Thus, the discoloration pattern on the Surveyor 3 camera not only provides excellent evidence that the camera surface was whitened by the impact of particles blown from the lunar surface by the exhaust of the LM as it landed but also indicates the velocity and direction at which these particles were ejected. Many of the lunar particles moved at very low angles to the horizontal.

LEONARD D. JAFFE
Jet Propulsion Laboratory,
California Institute of Technology,
Pasadena 91103

References and Notes

1. P. Blair and G. R. Blair, *Summary Report on White Paint Development for Surveyor Spacecraft, Rep. TM-800* (Hughes Aircraft Corp., Culver City, Calif., 1964); W. Hagemeyer, *J. Spacecraft Rockets* **4**, 828 (1967); G. A. Zerlaut and J. E. Gilligan, *In Situ Degradation of Thermal Control Surfaces* (Illinois Institute of Technology Research Report U6061-17 to NASA Marshall Space Flight Center, Chicago, 7 March 1969); W. F. Carroll, personal communication.
2. B. G. Cour-Palais, in "News Release 70-23" (NASA, Washington, D.C., 1970).
3. J. J. Rennilson and R. M. Batson, in *Surveyor Program Results, Rep. SP-184* (NASA, Washington, D.C., 1969), pp. 38–42.
4. J. J. Rennilson, personal communication.
5. The sagging of the landing gear was noted by R. F. Scott and by R. Barlow (personal communication).
6. Army Map Service, *Lunar Map 1 : 500; Surveyor III Site* (Army Map Service, Washington, D.C., 1968).
7. R. M. Batson, in *Surveyor Program Results, Rep. SP-184* (NASA, Washington, D.C., 1969), pp. 45–54.
8. Mission Evaluation Team, "Apollo 12 Mission Report," *Rep. MSC-01855* (NASA Manned Spacecraft Center, Houston, 1970).
9. Lunar Sample Preliminary Examination Team, *Science* **167**, 1325 (1970).
10. I thank J. J. Rennilson for providing the transformation matrix relating Surveyor 3 camera coordinates to lunar coordinates and for other aid, and W. F. Carroll for helpful discussions. This report presents the results of one phase of research carried out at Jet Propulsion Laboratory under NASA contract NAS 7-100.

5 October 1970; revised 30 November 1970

Solvent Dependence of the Luminescence of N-Arylaminonaphthalenesulfonates

Abstract. *N-Methyl,N-phenyl-2-aminonaphthalene-6-sulfonate, a fluorescence probe, adsorbs to cycloheptaamylose with a stoichiometry of 1 : 1. The fluorescence of the complex is similar to that observed when the dye is dissolved in organic solvents. Similar fluorescence is observed with the dye in ice. The results are interpreted in terms of "solvent" relaxation during the excited state lifetime of the dye.*

Since the observation by Weber and Laurence (*1*) that *N*-arylaminonapthalenesulfonate dyes adsorb to bovine serum albumin with fluorescence enhancement, these dyes have been shown to adsorb to functionally important sites on several proteins (*2*). In addition they have been used in studies of nerves (*3*) and mitochondrial membranes (*4*). The fluorescence quantum yields of these compounds are low in polar solvents. When they are dissolved in nonpolar solvents or adsorbed to proteins, their quantum yields and average energies of fluorescence increase and the half-maximum bandwidths of emission decrease (*5–7*). Because the spectroscopic properties of these dyes adsorbed to proteins mimic those in nonpolar solvents, they have been used as probes for hydrophobic regions in protein molecules.

In homogeneous solutions, the fluorescence of these dyes reflect the polarity of the solvent (*5–7*). The question arises, however, to what extent the heterogeneous character of a protein or a membrane surface may be approximated by the homogeneous solvents in which the spectral characteristics of the chromophores have been investigated.

Cycloheptaamylose forms complexes with a variety of organic compounds (*8*). In the case of *N*-methyl,*N*-phenyl-2-aminonaphthalene-6-sulfonate, the interaction is indicated by a large increase in the quantum yield of fluorescence and a blue shift in the emission maximum. The fluorescence spectrum of the complex is shown in Fig. 1 and may be contrasted with the low fluorescence shown in water. The change of the fluorescence quantum yield was used to determine the stoichiometry and affinity between the dye and the sugar. A Scatchard (*9*) analysis indicated that one dye molecule bound to one sugar molecule with a Gibbs free energy of −6 kcal/mole.

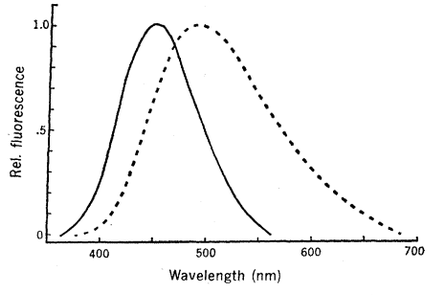

Fig. 1. Normalized fluorescence emission spectra of *N*-methyl,*N*-phenyl-2-aminonaphthalene-6-sulfonate in ice (solid curve) and adsorbed on cycloheptaamylose (dashed curve). The dashed curve was obtained from a solution containing $8.52 \times 10^{-6}M$ *N*-methyl,*N*-phenyl-2-aminonaphthalene-6-sulfonate and $1.50 \times 10^{-3}M$ cycloheptaamylose.

The structure of cycloheptaamylose is well known (*8*) and shows the presence of hydroxyl groups both in the cavity and on the outside of the cyclic sugar. Thus while the sugar surface may be less polar than water, it does not represent what a protein chemist would usually refer to as a hydrophobic region.

The fluorescence emission spectra of *N*-methyl,*N*-phenyl-2-aminonaphthalene-6-sulfonate in ice is also shown in Fig. 1. This spectrum was taken at a dye concentration where no concentration dependence of the emission energy was observed. In the case of ice the dye is certainly surrounded by polar molecules. The fluorescence properties of this dye in water, ice, and in the presence of cycloheptaamylose are summarized in Table 1.

We have observed that when dyes of this type are adsorbed to silica gel or when they are in a crystalline state, their fluorescence spectra are similar to those observed when the dyes are dissolved in nonpolar solvents. The fluorescence of a *hydrated* crystal of *N*-(*p*-tolyl)-2-aminonaphthalene-6-sulfonate has been reported (*10*) and showed an emission similar to that observed in fluid *polar* solvents.

We have recently investigated a series of fourteen 2,6-substituted aminonaphthalenesulfonates (*7, 11*). From the absorption and fluorescence spectra in various liquid solvents and the acid-base dependence of the spectra, we concluded that the lowest energy singlet-singlet transitions of these molecules contain significant intramolecular charge-transfer character of the type

Table 1. Comparison of fluorescence spectra of *N*-methyl,*N*-phenyl-2-aminonaphthalene-6-sulfonate in different chemical environments.

Addition to dye	Half-maximum bandwidth (nm)	Emission maximum (nm)	Quantum yield
H_2O (liquid, 300°K)	158	540	.006
H_2O (liquid, 300°K) + cycloheptaamylose	128	497	.2
H_2O (solid, 190°K)	~85	~455	~.5

$a_\pi \leftarrow l$ (*12*). Furthermore, we found a good correlation between the observed energies of the lower electronic states of these molecules and the relative energies predicted from theoretical considerations (*13*). Calculations of the dipole moment changes from experimental data for this series of molecules showed that the dipole moments of the first excited singlet states of the *N*-aryl-2-aminonaphthalene-6-sulfonates were all nearly four times larger than those of 2-aminonaphthalene-6-sulfonate. We also found that there was a good correlation between the dipole moments of the first excited singlet states of the *N*-arylaminonaphthalenesulfonates and their energies and quantum yields of fluorescence.

The shapes and energies of the fluorescence and phosphorescence spectra at 77°K in solid ethanol of all 14 derivatives suggest that the dipole moment of the lowest triplet state is intermediate between those of the first excited and ground singlet states. The work of Jackson and Porter (*14*) reinforces this conclusion. Because of the relative dipole moments of the lower electronic states it would be expected that the triplet state energy in fluid polar solvents would be nearly the same as that observed at 77°K for any of these molecules. On the basis of this fact it was shown that the fluorescence quantum yields of the *N*-arylaminonaphthalenesulfonates vary roughly as the square of the inverse of their first excited singlet-triplet energy interval. A reexamination of the fluorescence energies and quantum yields reported by Turner and Brand (*6*) for *N*-phenyl-1-aminonaphthalene-7-sulfonate in many different solvents showed an excellent correlation between the absolute quantum yield and the square of the inverse of the singlet-triplet interval. These results strongly suggest that the low quantum yields of the *N*-arylaminonaphthalenesulfonates in fluid polar solvents are a result of an enhanced intersystem-crossing to the triplet state under these conditions.

Further evidence for this suggestion was obtained from fluorescence studies in glycerol. The molecular relaxation time of glycerol as a function of temperature has been reported (*15*). It was found that for *N*-methyl,*N*-phenyl-2-aminonaphthalene-6-sulfonate, the energy and quantum yield of fluorescence increased when, due to a change in temperature, solvent relaxation occurred on a time scale longer than the fluorescence lifetime of the dye molecule.

It thus appears that solvent relaxation during the lifetime of the first excited singlet state is a key feature involved in the spectral shifts and quantum yield changes observed with these molecules. In homogeneous *fluid* solvents the solvent dipole moment is the main factor influencing the fluorescence. However, it is important to reemphasize that under certain conditions—restricted solvent relaxation in a polar medium—the fluorescence of these dyes does not indicate the hydrophobicity (dipole moment) of the solvent as has commonly been assumed.

The present state of our understanding of the excited state processes of the *N*-arylaminonaphthalenesulfonates is described in Fig. 2. Enclosed in the boxed area are the variables that are important in influencing the fluorescence of these molecules. We believe that solvent molecule relaxation is a key factor in the excited processes. Solvent polarity, viscosity, and temperature influence the fluorescence mainly by their effects on solvent reorientation. However, there may also be other important factors which have not yet been found. Our results should be taken into account in the interpretation of the fluorescence observed when these and similar dyes are adsorbed to proteins.

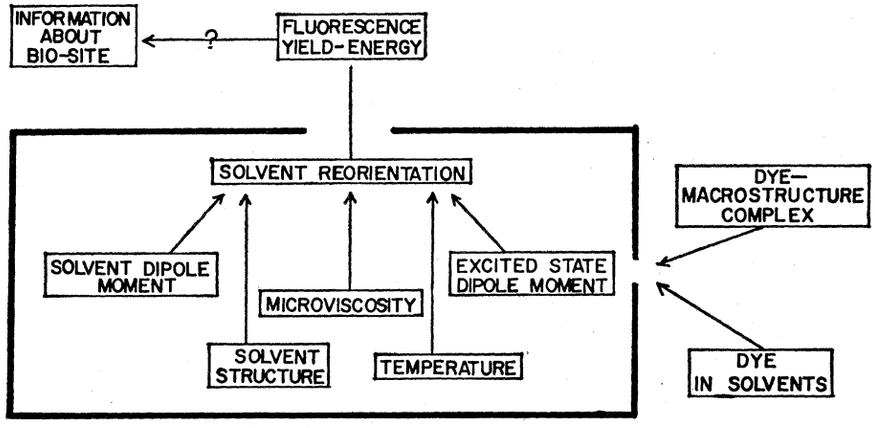

Fig. 2. The interplay of environmental factors effecting the fluorescence characteristics of *N*-arylaminonaphthalenesulfonates.

CARL J. SELISKAR
LUDWIG BRAND
McCollum-Pratt Institute,
Johns Hopkins University,
Baltimore, Maryland 21209

References and Notes

1. G. Weber and D. J. R. Laurence, *Biochem. J.* **56**, 31P (1954).
2. L. Stryer, *J. Mol. Biol.* **13**, 482 (1965); L. Brand, J. R. Gohlke, D. S. Rao, *Biochemistry* **6**, 3510 1967); W. O. McClure and G. M. Edelman, *ibid.*, p. 559; M. DeLuca, *ibid.* **8**, 160 (1969).
3. I. Tasaki, R. Watanabi, R. Saudlin, L. Carnay, *Proc. Nat. Acad. Sci. U.S.* **61**, 883 (1968).
4. A. Azzi, B. Chance, G. K. Radda, C. P. Lee, *ibid.* **62**, 612 (1969).
5. W. O. McClure and G. M. Edelman, *Biochemistry* **5**, 1908 (1966).
6. D. C. Turner and L. Brand, *ibid.* **7**, 3381 (1968).
7. C. J. Seliskar, D. C. Turner, J. R. Gohlke, L. Brand, in *Molecular Luminescence*, E. C. Lim, Ed. (Benjamin, New York, 1969), p. 677.
8. D. French, *Advan. Carbohydrate Chem.* **12**, 189 (1957).
9. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
10. A. Camerman and L. H. Jensen, *Science* **165**, 493 (1969).
11. C. J. Seliskar and L. Brand, in preparation.
12. M. Kasha, in *Light and Life*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1961), p. 35.
13. J. N. Murrell, *Proc. Phys. Soc. Ser. A* **68**, 969 (1955).
14. G. Jackson and G. Porter, *Proc. Roy. Soc. London Ser. A* 260, 13 (1961).
15. J. G. Powles, in *Molecular Relaxation Processes* (Academic Press, New York, 1966), p. 127.
16. Supported by NIH grant GM 11632. Contribution No. 609 from the McCollum-Pratt Institute.

24 August 1970; revised 14 October 1970