tions. The characteristic constant, β, in the dialysis equation,[7] $\ln c/c_0 = -\beta t$, where c_0 is the substrate concentration at $t = 0$ and c is the concentration at time t, was determined to be 2.1×10^{-2} min^{-1} for a substrate solution containing 0.05 M Tris at pH 8.0 and 25°. To ensure that the disodium salt of the substrate was the diffusing species, 0.85 M NaCl was also present. When 0.85 M tetra-*n*-butylammonium bromide was substituted for the NaCl, the activity of the free alkaline phosphatase, in the absence of hollow fibers, was inhibited by a factor of 3.5, whereas the value of β decreased by a factor of seven to 0.3×10^{-2} min^{-1}, a value consistent with the increased bulkiness and decreased solubility in the membrane of the diffusing salt. We wish to emphasize that, when the hollow fiber reactor is operated in the diffusion-controlled regime, *a modest change either in the concentration or activity of the contained enzyme will have a negligible influence upon the measured value of* β.[7] The above values of β compare favorably with 3.9×10^{-2} min^{-1} for 4-nitrophenol dissolved in 0.05 M Tris at pH 8.0 and with the following data given in the Dow brochure on the b/HFD-1 beaker dialyzer: 12.5×10^{-2} min^{-1} for NaCl, 9.0×10^{-2} min^{-1} for creatinine, 4.8×10^{-2} min^{-1} for sucrose, 2.2×10^{-2} min^{-1} for raffinose, and 1.1×10^{-2} min^{-1} for vitamin B_{12}.

Experiments with chymotrypsin were similarly successful. Two milligrams of chymotrypsin (Worthington) were dissolved in 2 ml of 0.05 N HCl and placed in the interior of the hollow fibers of the same beaker dialyzer as used above. One hundred milliliters of a solution containing 0.07 M $CaCl_2$, 23.3% methanol, 0.035 M Tris at pH 7.80, and 5.0×10^{-4} M benzoyl-L-tyrosine ethyl ester (BTEE) was rapidly added to the dialyzer and the initial rate monitored at 256 nm and 25°. The measured value of β was 1.1×10^{-2} min^{-1}. When the amount of chymotrypsin was increased to *ca.* 20 mg, the value of β became 2.0×10^{-2} min^{-1}. As an upper limit for enzyme leakage, less than one part in 12,000 leaked out of the fibers during the course of 2 hr of experimentation with the more concentrated enzyme system. With hydrolytically stable substrates, such an enzyme leakage test is extremely sensitive. The activity of the chymotrypsin used in these experiments was, in the absence of the fibers, 31 μmol of substrate hydrolyzed per minute at 25° and the above solution conditions.

Less detailed exploratory experiments, which will not be discussed here, with urease and catalase also demonstrated the value of the hollow fiber reactor approach. The activity of the catalase was followed by winding 12–36 b/HFO-1 beaker osmolyzer strands containing the enzyme around a small magnetic stirrer present in a Yellow Springs Instrument Co. Model 53 biological oxygen monitor. The enzyme-containing sealed hollow fibers were also wound around a small stirring rod, which provided a convenient enzyme "tea bag" for conducting small batch reactions.

Our experiments clearly demonstrate that cellulose and cellulose acetate hollow fiber membranes can readily contain any individual enzyme, and presumably any mixture of enzymes as well, without the use of chemical reactions. *No enzyme leakage was detected with any of the four enzymes studied in this investigation.* The commercial and laboratory advantages of hollow fiber enzyme reactors are readily apparent. Contained enzymes can be re-used in different experimental runs and then recovered at the end of a series of runs by flushing the interior of the fiber bundles with deionized water. The ability to flush the fibers also permits the recirculation of an enzyme solution as well as the re-use of the fibers for different enzymes. Quantitation of such procedures for a variety of enzymes has yet to be performed, however.

Finally, we make no claims for enzyme stabilization within the hollow fibers; in fact, some enzymes may be destabilized owing to the large internal surface area. Enzyme stabilization must be treated as a topic that is separate from that of insolubilization or containment.[10]

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Measurement of Decay Rates of Triplet Excited States Using Time-Correlated Single Photon Counting. Benzophenone Triplets in Aromatic Solvents[1,2]

Sir:

The absolute measurement of excited state lifetimes in the submicrosecond time range was made possible recently by the development of the laser flash photolysis technique, which provides flash lifetimes of about 20 nsec.[3,4] Initial studies using the technique were directed toward determination of the absorption spectra and lifetimes of excited singlet states.[3,4] An extension of the method to determination of short triplet state lifetimes has been reported[5] in a case where the conventional microsecond flash technique was either inapplicable or gave results accompanied by large errors because the measured lifetimes were shorter than or similar to the flash decay times. Use of the nanosecond laser flash technique is, however, limited by the relatively high cost and complexity of the apparatus, and it is perhaps not surprising that the method is in use in a limited number of laboratories.

We have been engaged in studies designed to determine the factors responsible for the short lifetime (10 μsec) of benzophenone triplets in benzene solution, where simultaneously the quantum efficiency for disappearance of benzophenone is very small ($\sim 10^{-3}$).[6–9] Our initial approach involved determination of the

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(1) Photochemistry of Ketones in Solution. XXXVI. Part XXXV: D. I. Schuster and T. M. Weil, *Mol. Photochem.*, 4, 447 (1972).

(2) Presented at the 7th Middle Atlantic Regional Meeting of the American Chemical Society, Philadelphia, Pa., Feb 14, 1972, Abstracts, p 76, and the Central Regional Meeting, Pittsburgh, Pa., May 5, 1972.

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(8) J. Saltiel, H. C. Curtis, L. Metts, J. W. Miley, J. Winterle, and M. Wrighton, *ibid.*, 92, 410 (1970); J. Saltiel, H. C. Curtis, and B. Jones, *Mol. Photochem.*, 2, 331 (1970).

lifetime of benzophenone triplets in a variety of substituted benzenes as solvents. The lifetimes were too short to be measured using our high-energy microsecond flash apparatus (flash decay times of 15–40 μsec), so we thought to take advantage of the weak phosphorescence of benzophenone in fluid media at room temperature[7,8,10] by utilizing the time-correlated single photon counting apparatus which is capable of measuring lifetimes of faintly luminescent systems.[11] This communication reports what is apparently the first application of the single photon technique to the measurement of phosphorescence lifetimes, as an interesting and convenient alternative to the laser flash method for the study of short-lived triplet states.

The apparatus has characteristics similar to that of Halpern and Ware.[11] A deuterium-filled gated flashlamp operating at *ca.* 7 kHz was used for excitation, and radiation at 344 nm was isolated with a 0.25-m Jarrell-Ash monochromator. The phosphorescence from the benzophenone solutions (all 3×10^{-3} *M*) was viewed at right angles with respect to the excitation through a Corning 0-51 filter. Acquisitions were on the order of 60 min. Phosphorescence was observed to be exponential for *ca.* 1.5 orders of magnitude, although subsequent distortion resulted from a long-lived lamp tail which followed the main pulse. All of the aromatic solvents were purified by the Saltiel–Metts photochlorination method[12] and/or washing with concentrated sulfuric acid. As in our earlier study,[5] the solvent purification procedure of choice was that which resulted in the longest measured benzophenone triplet lifetime.

The results are summarized in Table I, along with data obtained earlier using the laser-flash technique.[5]

Table I. Decay Constants of Benzophenone Triplets in Aromatic Solvents at Room Temperature

Solvent	Ionization potential, V[a]	$1/\tau$, sec^{-1} Single photon counting[b]	$1/\tau$, sec^{-1} Laser flash photolysis[c]	k_q, l. mol^{-1} sec^{-1}
C_6H_6	9.245	1.07×10^5	1.0×10^5	9.6×10^3
C_6D_6		1.07×10^5	0.8×10^5	9.6×10^3
Pyridine	9.25	1.15×10^5		9.3×10^3
C_6H_5F	9.26	1.08×10^5		1.0×10^4
$C_6H_3F_3$	9.30	4.5×10^4		4.6×10^3
$C_6H_2F_4$	9.39[d]	5.8×10^5		6.2×10^4
$C_6H_4F_2$	9.40	2.3×10^5		2.2×10^4
C_6H_5Cl	9.41	1.6×10^5		1.6×10^4
C_6H_5Br	9.78	4.7×10^5		4.8×10^4
$C_6H_5CF_3$	9.81	4.9×10^4		7.2×10^3
C_6HF_5	9.92	6.8×10^5		7.5×10^4
C_6H_5CN	9.94	2.1×10^6		2.1×10^5
C_6F_6	10.02	1.6×10^6	1.4×10^6	2.0×10^5

[a] Taken from J. L. Franklin, J. G. Dillard, H. M. Rosenstock, J. T. Hesson, K. Draxl, and F. H. Field, "Ionization Potentials, Appearance Potentials and Heats of Formation of Gaseous Positive Ions," U.S. Department of Commerce, National Bureau of Standards, 1969. [b] This work. [c] Reference 5. [d] We have reason to doubt the reliability of this value.

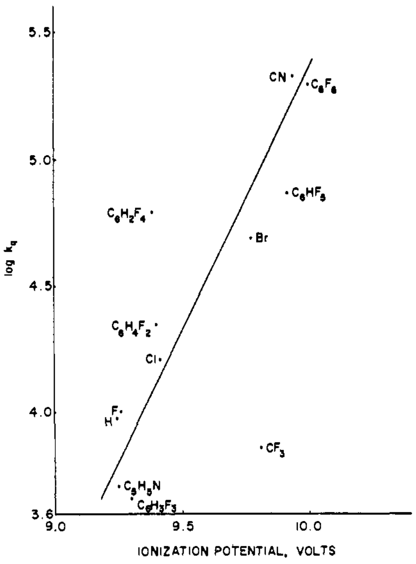
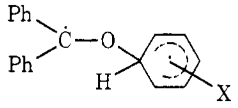


Figure 1. Interaction of benzophenone triplets with benzene, pyridine, and substituted benzenes. Plot of logarithm of absolute second-order rate constants *vs.* solvent ionization potentials.

Ionization potentials of the solvents studied are also given. Since in these systems, $1/\tau = k_q[\mathrm{Ar}]$, the lifetime data can be converted directly into the corresponding bimolecular quenching constants, k_q.

The following conclusions seem warranted on the basis of the data at hand. (1) In agreement with our earlier conclusion,[5] there is no solvent isotope effect on quenching of $^3Ph_2CO^*$ by benzene, indicating that reversible hydrogen transfer from benzene[6] is not an important mechanism for triplet deactivation.[13] (2) The dependence of quenching rates on solvent substituents suggests that some sort of complex or intermediate is involved in the quenching process.[14] While the data do not unequivocally rule out the formation of the diradical σ complex **1** proposed previously,[15,16] the most



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satisfactory correlation of the quenching data that we have found is illustrated in Figure 1, where log k_q is plotted *vs.* ionization potentials of the aromatics. For ten systems, the fit to a linear relationship is quite good. The only serious deviation from the line[17] is for $PhCF_3$, perhaps indicating a change in quenching mechanism in this case. Since the data show that k_q increases as

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(9) J. Dedinas, *J. Phys. Chem.*, **75**, 181 (1971); A. V. Buettner and J. Dedinas, *ibid.*, **75**, 187 (1971).

(10) C. A. Parker and T. A. Joyce, *Chem. Commun.*, 749 (1968); *Trans. Faraday Soc.*, **65**, 2823 (1969).

(11) See A. M. Halpern and W. R. Ware, *J. Chem. Phys.*, **54**, 1271 (1971); W. R. Ware in "Creation and Detection of the Excited State," A. Lamola, Ed., Marcel Dekker, New York, N. Y., 1971.

(12) J. Saltiel and L. Metts, unpublished results, private communication.

(13) The large isotope effects noted for formation of biphenyl in this system[8,9] imply that different interactions are involved in quenching and product-determining processes. Preliminary data show that the yields of benzpinacol in the various aromatic solvents decrease as the rate of quenching increases, in agreement with the conclusion that quenching and product formation are competitive processes.

(14) Reversible electron transfer without formation of a discrete complex is not rigorously exluded, although on the basis of the data and precedents, we do not prefer such an explanation.

(15) D. I. Schuster and D. F. Brizzolara, *J. Amer. Chem. Soc.*, **92**, 4357 (1970); E. J. Baum and R. O. C. Norman, *J. Chem. Soc. B*, 227 (1968).

(16) σ complexes analogous to **1** have been suggested in reactions of aryl radicals with fluoro- and hexafluorobenzene, and seem to be stabilized by the substituents: P. A. Claret, G. H. Williams, and J. Coulson, *J. Chem. Soc. C*, 341 (1968); P. Lewis and G. H. Williams, *J. Chem. Soc. B*, 120 (1969).

(17) For the case of $C_6H_2F_4$, see Table I, footnote *d*.

IP of the aromatic increases, an interaction involving at least partial charge transfer from the ketone triplet (as donor) toward the aromatic (as acceptor) is indicated. Interestingly, the reverse type of interaction involving charge transfer to $^3Ph_2CO^*$ from various aliphatic and electron-rich aromatic quenchers has recently been reported,[18] and seems well documented in other cases in quenching of ketone singlets as well as triplets.[19]

Further discussion of these data will be reserved for the full paper. We wish to emphasize, finally, that the time-correlated single photon technique deserves much more attention as a relatively simple and straightforward means of accurate measurement of short triplet lifetimes in solution.

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(18) J. B. Guttenplan and S. G. Cohen, *J. Amer. Chem. Soc.*, **94**, 4040 (1972).

(19) See, for example, P. J. Wagner and R. A. Leavitt, *ibid.*, **92**, 5806 (1970); I. E. Kochevar and P. J. Wagner, *ibid.*, **94**, 3859 (1972); N. C. Yang, M. H. Hui, and S. A. Bellard, *ibid.*, **93**, 4056 (1971); R. R. Hautala and N. J. Turro, *ibid.*, **93**, 5595 (1971).

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Ab Initio Molecular Orbital Studies of Cyclic Nitric Oxide Dimer

Sir:

Recently Williams and Murrell[1] published a nonempirical SCF–MO study of the nitric oxide dimer using a minimum basis of Slater atomic orbitals. They found that the trans conformation is the most stable in the gas phase with the cis conformation having an energy 3.5 kcal/mol higher. These findings are in disagreement with the vibrational spectra of pure solid NO,[2] of the matrix-isolated NO clusters,[2b,3] and of liquid[2a] and gaseous NO[4] which indicate that the most stable form of $(NO)_2$ is noncentrosymmetric. In this laboratory we have studied the NO dimer as a possible intermediate in nitrate ester combustion. The results of our minimum basis *ab initio* SCF calculations indicate that the cyclic isomer is the most stable.

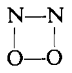
Our investigation of $(NO)_2$ was conducted using POLYATOM,[5] a Gaussian system of SCF programs kindly supplied by Professor Moskowitz. To obtain results of single ζ quality, we employed the 7,3 Gaussian atomic basis functions proposed by Whitman and Hornback.[6] These functions were contracted to two s and one p type function on each center. For the purposes of comparison we repeated the calculations of Williams and Murrell[1] using our basis set. The energy of the trans conformation was found to be −257.59643 au. However, the energy of the cis conformation suggested by these authors[1] was calculated to be −257.60110 au which is lower than the value for the trans isomer. These data are presented in Table I.

Table I. Calculated Geometries and Energies of $(NO)_2$[a]

Structure	R(NN)	∠NNO	R(NO)	Energy rel to trans
Cis	(1.57)	(110.2)	(1.23)	−2.9 (3.5)
Trans	(1.53)	(104.6)	(1.23)	0.0 (0.0)
Cyclic (I)	1.35	93.0	1.51	−8.8

[a] Bond length in ångströms, angles in degrees, and energies in kilocalories per mole. Values in parentheses are from ref 1.

We next considered the cyclic structures I and II for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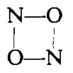
II

the NO dimer. Structure II was studied by restricting it to a planar-square configuration. A minimum energy of −257.51855 au was obtained at an ON bond length of 1.35 Å. Since this energy is somewhat above the lowest energy computed by Williams and Murrell,[1] structure II was not considered further. Structure I was also restricted to the square-planar conformation and the energy computed as a function of bond length. A minimum was obtained at a bond distance of 1.46 Å, the energy being −257.59595 au. This structure is quite competitive with the trans structure proposed in ref 1. To obtain a more realistic minimum, the "square" restriction was relaxed. A minimum energy of −257.61053 au was thus obtained. This energy corresponds to $R(NN) = 1.35$ Å and $R(NO) = R(OO) = 1.51$ Å. The energy minimization was carried out on a rather coarse grid of 0.05 Å so that the error in the above bond lengths should be plus or minus 0.05 Å. Structure I is thus about 8.8 kcal/mol more stable than the trans structure of Williams and Murrell[1] as can be seen in Table I.

Our calculations disagree with those of Williams and Murrell[1] on two points. We find that the cyclic isomer has the lowest energy and that the cis structure is 2.9 kcal/mol below, rather than 3.5 kcal/mol above, the trans. The first discrepancy may have occurred simply because Williams and Murrell[1] did not consider structures of type I. The second discrepancy is probably due to some loss of accuracy in the multicenter two-electron integrals. Guest, *et al.*,[7] have compared calculations of the type employed in ref 1 with more accurate calculations in the literature using the same basis set. Errors as large as 0.05 au in the case of ozone were found. These errors probably tend to cancel when similar geometries are compared. However, the cis and trans structures of ONNO seem to be sufficiently different so that the errors do not quite cancel and a discrepancy of several kilocalories per mole can occur.

The results of our calculations are in agreement with

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(1) J. E. Williams and J. N. Murrell, *J. Amer. Chem. Soc.*, **93**, 7149 (1971).

(2) (a) A. L. Smith, W. E. Keller, and H. L. Johnston, *J. Chem. Phys.*, **19**, 189 (1951); (b) W. G. Fateley, H. A. Bent, and B. Crawford, *ibid.*, **31**, 204 (1959).

(3) W. A. Guillory and C. E. Hunter, *ibid.*, **50**, 3516 (1969).

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