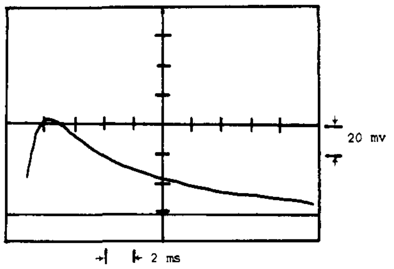

Figure 1. Oscilloscope trace of a solution containing $Eu(Phen)_3^{3+}$ and NPh_3 following flash photolysis. The reaction was monitored at λ_{max} for NPh_3^+ (650 nm) and the trace shows a decrease in transmittance followed by a slow regeneration step which follows equal-concentration, second-order kinetics. Horizontal sweep rate, 2 ms/div; vertical sensitivity 20 mV/division, base line is at 515 mV.

Eu^{3+} and some Eu(III) chelates has been studied,[7] but direct evidence for redox quenching was not obtained. We have investigated redox phenomena in acetonitrile solutions containing $Eu(phen)_3^{3+}$ and a series of potential organic reductants including diphenylamine, triphenyllamine, triethylamine, dimethylaniline, and triphenylphosphine using flash photolysis. The systems were studied by excitation of intraligand π-π* transitions in the uv because they have high absorptivities and intramolecular energy transfer to the emitting f-f states is facile and efficient.[6a] Filter solutions containing the added organic reductant were used; no transient phenomena were observed in the absence of the reductant.

With each organic reductant, quenching of the excited state was accompanied by the appearance of absorption bands attributable to one-electron oxidized radical cations[8] (Scheme II). The $Eu(phen)_3^{3+}$-NPh_3 system was studied in detail.

Scheme II

$$Eu(phen)_3^{3+} \xrightarrow{h\nu} Eu(phen)_3^{3+*} \qquad (4)$$

$$Eu(phen)_3^{3+*} + NPh_3 \xrightarrow{k_q} Eu(phen)_3^{2+} + NPh_3^+ \qquad (5)$$

$$Eu(phen)_3^{2+} + NPh_3^+ \xrightarrow{k_b} Eu(phen)_3^{3+} + NPh_3 \qquad (6)$$

The quenching step (eq 5) is sufficiently slow that it can be followed directly by conventional flash photolysis (Figure 1). The immediately observed product of the quenching is the one-electron oxidation product NPh_3^+ showing clearly that the quenching mechanism involves electron transfer. The rate constants for the quenching and back-reactions (Table I) were obtained by monitoring the appearance (pseudo-first-order kinetics) and subsequent disappearance (equal-concentration, second-order kinetics[10]) of the absorption due to NPh_3^+ (Figure 1). Both rate constants are well below the diffusion-controlled limit, but the origin of the relative slowness is unclear in the absence of redox potential[9] and detailed mechanistic information.

Redox quenching of the $\pi \rightarrow \pi^*$ excited states of metalloporphyrins is of importance because of possible analogies with photosynthesis, and because of the high near-uv-visible light absorption of the complexes. The $\pi \rightarrow \pi^*$ excited state of $Pd^{II}OEP$ (OEP is octaethylporphyrin) has a relatively long lifetime (Table I), and undergoes an efficient quenching process with paraquat in isobutyronitrile. Flash photolysis studies show that the initial rapid quenching leads to bleaching of the β band of the porphyrin at 544 nm, and to the appearance of reduced paraquat (λ_{max} 393 and 603 nm). The spectral changes observed are consistent with the formation of the ring oxidized porphyrin[11] (eq 8), followed by rapid, back-electron transfer (eq 9).

Scheme III

$$Pd^{II}OEP \xrightarrow{h\nu} Pd^{II}OEP^* \qquad (7)$$

$$Pd^{II}OEP^* + P^{2+} \xrightarrow{k_q} [Pd^{II}(OEP^+)]^+ + P^+ \qquad (8)$$

$$[Pd^{II}(OEP^+)]^+ + P^+ \xrightarrow{k_b} Pd^{II}OEP + P^{2+} \qquad (9)$$

The results described here show that rapid, bimolecular electron transfer processes can occur based on electron donation to or from the d-π* (CT), f-f*, and π-π* excited states of metal complexes. Such reactivity may be a common feature of long-lived excited states, providing that such processes are energetically favorable[12] and kinetic barriers are low.[13] Some of the reactions reported here share with the tris(2,2′-bipyridine)ruthenium(II) system the potentially useful properties of high chemical stability, high absorption in the visible, and the ability to undergo facile electron transfer, and may ultimately find application in energy conversion processes.[14]

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Isotope Effects on Gas Phase Reaction Processes. I. The Determination of Equilibrium Isotope Effects by Ion Cyclotron Resonance Spectroscopy

Sir:

Within the past decade ion cyclotron resonance (ICR) spectroscopy has developed into a formidable tool for the study of the thermodynamic stabilities of ions in the gas phase.[1,2] Of particular importance has been the determination by equilibrium ICR techniques of the acidities and basicities of a wide range of organic molecules.[3] Despite the considerable success which ion cyclotron resonance spectroscopy has entertained with regard to the elucidation of the thermodynamic properties of ions, its contributions to the general area of ion structure have been only slight. Thus, because the number of ions trapped within the ICR instrument is so small (10^5 to 10^6), normal absorption or emission spectroscopic techniques are not easily applied.[4] Some progress has been made, however, regarding the assignment of gross ion structure by indirect methods.[5]

One way in which information about the detailed structures of ions might be obtained from thermodynamic ICR measurements is by the use of isotope effects.[6] Although such measurements directly provide information only about molecular force fields, inferences may be made about geometrical structure, at least in the vicinity of the position of isotopic substitution.[6e] In particular, it should be possible to measure accurately the thermodynamics for a variety of proton, halide, or electron transfer reactions of the form:

$$A\text{-}H^+ + A^* \rightleftharpoons A + A^*\text{-}H^+ \qquad \text{proton transfer}$$
$$A^+ + A^*\text{-}X \rightleftharpoons A\text{-}X + A^{*+} \qquad \text{halide transfer}$$
$$A^+ + A^* \rightleftharpoons A + A^{*+} \qquad \text{electron transfer}$$

involving an equilibrium between some molecular species, A, and an isotopic variant, A*.[7] The experimental techniques required here are (qualitatively speaking) identical with those which have been used in numerous previous studies in order to establish the relative acidities and basicities of ions in the gas phase.

For the present, we shall be concerned with isotope effects on equilibrium constants. It should be noted, however, that rate constant isotope effects, which have received the widespread interest of the organic community,[6] may often be interpreted in terms of equilibrium constant isotope effects.[8] (Kinetic isotope effects may readily be measured directly using ICR spectroscopy; this will be the subject of a later study.) In particular, we shall concern ourselves with equilibrium constants for which sufficient spectroscopic data[9] are available to permit statistical mechanical calculation. Two such examples are the electron transfer equilibria involving the light and heavy isotopes of nitrogen and oxygen molecules

$${}^{15}N_2{\cdot}^+ + {}^{14}N_2 \rightleftharpoons {}^{15}N_2 + {}^{14}N_2{\cdot}^+$$
$${}^{18}O_2{\cdot}^+ + {}^{16}O_2 \rightleftharpoons {}^{18}O_2 + {}^{16}O_2{\cdot}^+$$

Our primary concern here will be to assess the accuracy of the ion cyclotron resonance spectroscopy experiment in determining the effects of isotopic substitution on equilibrium processes involving ions in the gas phase. Later reports will focus on connections between such equilibrium isotope effects and the structure of isolated ions.

For an isotopic exchange process

$$A + B^* \rightleftharpoons A^* + B$$

the equilibrium constant, K, may be expressed in the harmonic approximation in terms of the harmonic vibrational frequencies, ν, of the various species,[9]

$$K = \frac{\prod_i \dfrac{u_i(A^*)}{u_i(A)} \dfrac{1 - \exp[-u_i(A)]}{1 - \exp[-u_i(A^*)]} \exp[\tfrac{1}{2}\{u_i(A) - u_i(A^*)\}]}{\prod_j \dfrac{u_j(B^*)}{u_j(B)} \dfrac{1 - \exp[-u_j(B)]}{1 - \exp[-u_j(B^*)]} \exp[\tfrac{1}{2}\{u_j(B) - u_j(B^*)\}]} \tag{1}$$

Table I. Molecular Vibrational Frequencies (cm^{-1})[a]

Molecule	Vibrational frequency: Neutral	Vibrational frequency: Radical cation
$^{14}N_2$	2358	2207
$^{16}O_2$	1580	1905

[a] From: S. Bourcier, "Spectroscopic Data Relative to Diatomic Molecules," Vol. 17 of "Tables of Constants and Numerical Data," Pergamon, New York, N.Y., 1970.

Here $u = h\nu/kT$, and h, k, and T have their usual meanings. There are no symmetry number effects for the equilibria with which we are concerned. The main correction to eq 1 arises from anharmonicity;[10] this effect may easily be shown to be negligible here. $\Delta G°$ for the exchange process is related to the equilibrium constant in the usual manner,

$$\Delta G° = -RT \ln (K) \tag{2}$$

Because we are concerned here only with diatomic species, the product form of eq 1 reduces to a single term. Knowledge of only the harmonic frequency for one of each chemically different species is necessary, since

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k_e}{\mu}} \tag{3}$$

where k_e is the harmonic force constant and μ is the reduced mass. The experimental frequencies for $^{14}N_2$, $^{14}N_2^+$, $^{16}O_2$, and $^{16}O_2^+$ are presented in Table I. Using these data and eq 1, 2, and 3, we arrive at approximate statistical mechanical values for $\Delta G°$ for the two isotopic exchange reactions (at 298 K).

$${}^{15}N_2{\cdot}^+ + {}^{14}N_2 \rightleftharpoons {}^{15}N_2 + {}^{14}N_2{\cdot}^+ \quad \Delta G° \approx -7 \text{ cal/mol}, \quad K = 1.01 \tag{a}$$

$${}^{18}O_2{\cdot}^+ + {}^{16}O_2 \rightleftharpoons {}^{18}O_2 + {}^{16}O_2{\cdot}^+ \quad \Delta G° \approx 26 \text{ cal/mol}, \quad K = 0.96 \tag{b}$$

Ten determinations were made for $\Delta G°$ of reaction a,[11a] using pulsed ion cyclotron resonance techniques identical with those described previously,[2] at a variety of neutral pressure ratios ($P_{^{15}N_2}/P_{^{14}N_2}$) ranging from 0.4 to 2.1. A mean value of -10 ± 24 cal/mol results for the reaction as written (equilibrium constant equals 1.02 at 298 K), in good agreement with the statistical mechanical value. The range of individual $\Delta G°$ values was -59 to 17 cal/mol.

Six measurements were performed for reaction b[11b] at values of $P_{^{18}O_2}/P_{^{16}O_2}$ ranging from 0.6 to 1.4. Again the mean value of $\Delta G°$, 27 ± 15 cal/mol (equilibrium constant of 0.96 at 298 K), is in good accord with the statistical mechanical value. Here the individual $\Delta G°$'s ranged from 7 to 49 cal/mol.

The equilibrium constant for an isotopic exchange reaction, say, that involving the two oxygen isotopes,

$${}^{18}O_2{\cdot}^+ + {}^{16}O_2 \rightleftharpoons {}^{18}O_2 + {}^{16}O_2{\cdot}^+$$

may be simply expressed as

$$K = \frac{[{}^{18}O_2][{}^{16}O_2{\cdot}^+]}{[{}^{16}O_2][{}^{18}O_2{\cdot}^+]}$$

Thus, the determination of K by ICR techniques requires both the measurement of the ratio of the pressures of the isotopically related neutral molecules and of the relative abundances of the two ions. The measurement of ion ratios in this experiment may be carried out with high precision because the rates of ion loss (either diffusive or through chemical reaction) for the two isotopically related species are presumably very similar. We suspect that the measurement of the ratio of the pressures of the neutrals contains the major source of error. Indeed, drifts in total pressure of the neutrals during the course of a single determination (requiring on the order of 5–10 min) are large enough to account for the variation in measured $\Delta G°$'s. We believe that with presently available technology it is possible to measure the thermodynamics of isotopic exchange equilibria within a few tens of calories/mole.

Deviation from unity of the equilibrium constants for such processes as we have discussed here (i.e., involving heavy atom isotopic substitution) is expected and indeed observed to be very small (ΔG° are very close to zero). It is obvious, then, that the ICR technique is not really accurate enough to provide quantitatively useful information about such processes. *However, the errors in the technique should be no larger for reactions involving hydrogen-deuterium substitution where isotope effects are much larger. In these instances, the technique should be able to provide us with useful information.*

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Organic Synthesis Using Enzymes in Two-Phase Aqueous Ternary Polymer Systems

Sir:

The practicality of enzymes as catalysts in organic synthesis often depends on the efficiency with which they can be recovered from product mixtures and reused.[1,2] Two general approaches are presently available to the design of synthetic reactors based on enzymes: either the enzymes may be immobilized on (in) an insoluble support, or they may be used in solution and reisolated by ultrafiltration, adsorption, precipitation, or other methods.[2] When applicable, immobilization is usually the preferable approach: immobilized enzymes often enjoy protection against deactivation by adventitious proteases and are not exposed to the potentially deactivating conditions encountered during isolation from solution. Immobilized enzymes are, however, not applicable in reactions involving insoluble substrates, or in sequences requiring the enzymes to associate with or dissociate from other insoluble proteins or macromolecules during reaction.[3] Further, partial or complete deactivation often accompanies the immobilization of sensitive enzymes.

We wish to describe a new strategy for utilization of enzymes as catalysts in organic synthesis based on their partition in aqueous two-phase ternary polymer systems. Many aqueous polymer solutions show low mutual solubility, and two-phase ternary polymer systems have been extensively utilized for biochemical separations.[4,5] The partition of material between the two phases depends on the composition, pH, and ionic strength of the system,[4] and is also affected by the presence of polyelectrolytes[6] or specific ligands covalently bound to one of the polymers.[7] Characteristically, the partition coefficients, K,[8] of various proteins between the phases of the system formed from dextran and poly(ethylene glycol) (PEG) in water[9] are in the range $K = 0.1$ to 10.[4] Low molecular weight substances such as inorganic salts, amino acids, sugars, and nucleotides partition almost equally between the two phases (i.e., $K = 1$). We take advantage of the difference in K between substrate, product, and enzymatic catalyst to construct a two-phase biosynthetic reactor (TPBR). An efficient TPBR should consist of two, immiscible, aqueous polymer phases in which the enzyme is partitioned predominantly into one phase. If the partition coefficient for enzyme is far from unity, and that for substrates and products is close to unity, it is possible to separate the enzymatic catalyst from products efficiently by extraction. To assess the influence of the magnitude of K of an enzyme in a two-phase system on the operation of a TPBR, it is useful to analyze a simple model. We assume that the initial quantity of the enzyme added to the TPBR is A_0, the volume of the upper (enzyme poor) phase is V_1 and its enzyme concentration is C_1, and the volume of the lower (enzyme rich) phase is V_2 and its enzyme concentration is C_2. The loss of enzyme from the lower phase in each stage of separation is described by eq 3. For

$$K = C_1/C_2 \tag{1}$$

$$A_0 = A_1 + A_2 = C_1V_1 + C_2V_2 \tag{2}$$

$$A_1 = A_0(1 + V_2(KV_1)^{-1})^{-1} \tag{3}$$

an enzyme with $K = 0.001$ in a TPBR with $V_1/V_2 = 100$, the loss of enzyme from the lower phase to the upper in one stage would be 9.1%. Since for most enzymes values of $K < 0.001$ or > 1000 are unlikely,[3] countercurrent operation with multiple partition of the product mixture is necessary to minimize loss of the enzyme from a TPBR.

The operation of a single stage of this type of reactor has been demonstrated using a model system based on glucose 6-phosphate dehydrogenase (G-6-PDH, D-glucose 6-phosphate:NADP$^+$-oxidoreductase, EC 1.1.1.49) isolated from Torula yeast.[10] The partition coefficient of G-6-PDH in the commonly used dextran-PEG two-phase system is $K = 0.20$ (Table I); this value indicates that the protein partitions to the extent of 83% in the lower (dextran rich) phase at a phase volume ratio, $V_1/V_2 = 1$. Because this partition ratio