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A Spectroscopic Characterization of Metal Ion-Complexed NADH Models and Their Kinetic Behavior in Nitrosobenzene Reduction

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The structures of metal ion complexes of a series of NADH models were spectrophotometrically investigated. The electronic spectra were sensitive to variation of both the 1,4-dihydropyridines and the metals; particularly, 1-benzyl-3,5-bis(1-pyrrolidylcarbonyl)-1,4-dihydropyridine (BPDH) was recognized to produce two types of complexes with zinc and lead perchlorates, whereas 1-benzyl-1,4-dihydronicotinamides (BNAH) complexed metal ion only at the amide oxygen. In order to elucidate these structures, the effect of changing perchlorate ion concentrations was examined. In addition, reduction of nitrosobenzene in the presence of bivalent metal cations was kinetically investigated.

The coordination between zinc and NADH plays an important role in alcohol dehydrogenase enzymes.[1)] Thus, the role of metal ion is fundamental to our better understanding of NADH coenzymes. Then, there do exist a number of reports on NADH models. Ohno and other workers have demonstrated that metal ion coordination to NADH models such as BNAH and its optical active analogs exhibits a significant influence on rate acceleration[2a)] and stereodifferentiation[2b)] in carbonyl reductions. However, the systematic spectroscopic study on metal ion complexation has received less attention thus far.[3)] This study provides a spectroscopic analysis of zinc and lead complexes with NADH models, the results of which are discussed in this paper and also presents experimental data on the reduction of nitrosobenzenes by these complexes, since interest in a fundamental understanding of the relationship between the structure and the reactivity of NADH models continues to be an area of considerable experimental effort.

1,4-Dihydronicotinamides themselves have a disadvantage for their applications as coenzyme models owing to their relative instability toward electrophilic reagents. In order to overcome this drawback, we have recently synthesized symmetrically substituted 1,4-dihydropyridines and have used them for studies under acidic conditions. In this study we have employed such NADH models as a proper choice.

Experimental

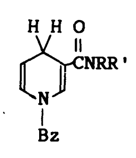
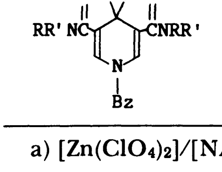
Materials. 1-Benzyl-3,5-bis(pyrrolidylcarbonyl)-1,4-dihydropyridine (BPDH, **1**) and its 1-(substituted benzyl) derivatives was prepared according to the method described in the previous paper.[4)] Special grade metal perchlorates as the hydrates from Kishida Chemicals Co. were used without further purification. Tetrabutylammonium perchlorate of special grade was purchased from Tokyo Kasei Kogyo Co. and dried in vacuo at 50 °C for one day. *p*-Substituted nitrosobenzenes were prepared according to the literature method.[5)] Acetonitrile was distilled from P_4O_{10}.

Kinetic Procedure. The kinetic data were determined at 25 °C in acetonitrile under anaerobic conditions, as described in our previous paper.[6)] UV-visible spectra and absorbance-time measurements were recorded on a Hitachi model-220 spectrophotometer equipped with constant temperature cell holders. The progress of the reaction was monitored by the disappearance of the absorbance at, for example, 355 nm due to **1**. Generally, the initial concentrations of **1** and nitrosobenzene were 0.1 mM and 1.0 mM, respectively, throughout this work (M=mol dm^{-3}).

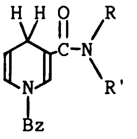
Results and Discussion

Spectral Changes of Dihydropyridine-Zinc(II) Complexes. The spectral changes in acetonitrile as solvent were monitored by UV-visible spectrometry in an appropriate region 300—400 nm. In Table 1 are summarized the spectroscopic data for a series of substituted 1-benzyl-1,4-dihydronicotinamides in the presence and absence of a 100-fold molar excess of zinc perchlorate. All these compounds have a strong absorption band somewhere ranging from 300 nm to 400 nm, depending on the metal ion and the substituents. It has been proposed that these absorption could be

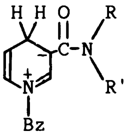
Table 1. Electronic Absorption Bands for NADH Model Compounds in the Presence and Absence of Zinc Perchlorate in Acetonitrile[a)]

Model compound[b)]	R, R′	λ/nm without Zn$(ClO_4)_2$	λ/nm with Zn$(ClO_4)_2$
	H, H	345	360
	H, Et	340	355
	$(CH_2)_5$	333	345
	Et, Et	330	338
	i-Pr, *i*-Pr	325	—
	$(CH_2)_4$	345	350
	H, Et	372	380
	Et, Et	355	368
	$(CH_2)_5$	340	370
	$(CH_2)_4$	355	320

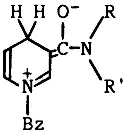
a) $[Zn(ClO_4)_2]/[NADH\ Model]=100$. b) Bz: $CH_2C_6H_5$.



(A)



(B)



(C)

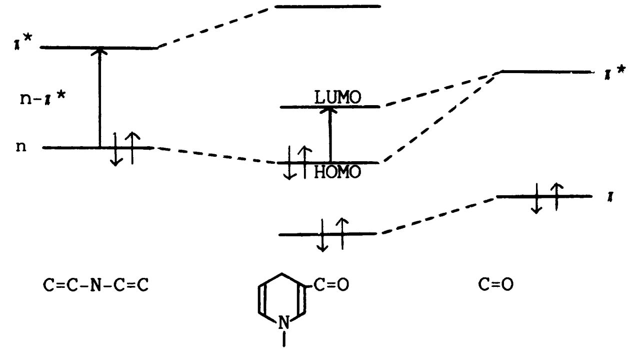


Fig. 1. Construction of the orbitals of 1,4-dihydronicotinamide. Only the major interactions are illustrated.

ascribed to the n-π^* transition.[7]

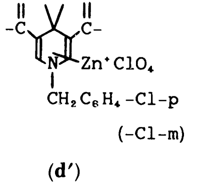
There are a few limiting resonance structures (**A**, **B**, and **C**) for 1,4-dihydronicotinamide in which the lone pair of electrons on N-1 is delocalized into the ring and conjugates further with the π-system of the carbonyl group. The delocalization will be maximized when the carbonyl group lies in the plane of the ring. Therefore, the twist of the bulky amide group out of the dihydropyridine ring destabilizes the LUMO more than destabilizes the HOMO, resulting in a blue shift of the electronic absorption band (Fig. 1). Indeed, the λ_{max} values shift toward a shorter wavelength in going from $R,R'=H,H$ (345 nm) to $R,R'=H,Et$ (340 nm) and to $R,R'=Et,Et$ (330 nm). Obviously, the steric repulsions diminish π-conjugation. Nevertheless, despite its bulkiness, $R,R'=(CH_2)_4$ substitution affords an electronic absorption band at an exceptionally rather longer wavelength.

Upon complexation with zinc ion, the dihydronicotinamides undergo spectral shifts toward long wavelengths without exception. The extent of the spectral shift for a less bulky amide is twice as large as that for a more bulky amide. This is because the relative extent of delocalization in nonplanar dihydropyridine molecules is originally small. Thus, two isopropyl groups in an amide may extremely destabilize the metal complex and actually the λ_{max} could not be defined.

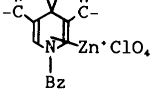
A similar situation is continued in 3,5-disubstituted 1,4-dihydropyridines; bulky carbamoyl groups may destabilize the dihydropyridines and causes a blue shift irrespective of with and without the metal ion. In Table 1 are collected observed λ_{max} values in the presence or absence of a 100-fold excess of zinc perchlorate. The most remarkable feature is the unusual behavior of the dihydropyridine having $R,R'=(CH_2)_4$, which shows a greater red shift and exceptional stability inspite of the bulkiness of the carbamoyl substituent. The exact reason for this behavior is not understood at present. This anomaly, however, may be understood if we accept the view that the hydrogens α to the nitrogen in a rigidly planar pyrrolidine ring fit well between the ring methylenes of the dihydropyridine without significant steric interference, making possible a nearly planar molecule with almost maximum conjugation.[7] Owing to this stability, only the dihydropyridines bearing 1-pyrrolidinylcarbonyl groups were used for further investigations.

The absorption peak of BPDH in acetonitrile was only at 355 nm (ε: 6070) in the absence of zinc perchlorate. An addition of a large excess of zinc perchlorate to that solution resulted in the displacement of the absorption maximum to 320 nm accompanied by a poor shoulder at a slightly longer wavelength than the original, i.e., at around 365 nm, which lies within the envelope of bands with λ_{max} 355 nm and 320 nm. In a previous paper,[6] the blue shift toward 320 nm (343 nm for $Mg(ClO_4)_2$) was attributed to complexation of the metal ion on the dihydropyridine nitrogen.[8] In contrast, the bathochromic shift toward 365 nm would be that generally expected for the complexation with the amide oxygen. The existence of the corresponding complex is not strictly recognized by the spectrum of the 1-benzyl or 1-(*p*-chlorobenzyl) derivative (Figs. 4 and 2a). However, the band of the 1-(*p*-methyl- or *p*-methoxybenzyl)-1,4-dihydropyridine derivative (**e′**) explicitly distinguishes itself as an independent band with a slightly smaller extinction coefficient in the presence of a 100-fold excess of zinc perchlorate relative to BDPH (Fig. 2b). The absorption belonging to the same category was also found with less Lewis acidic zinc nitrate (361, 5680) as well as perchlorates of manganese (λ_{max} 355 nm, ε 5590 M^{-1} cm^{-1}), cobalt (359, 5870), nickel (356, 5640), where no largely blue-shifted bands could be seen (Fig. 3).

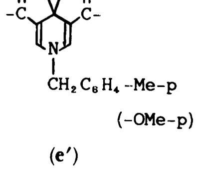
With the 1-benzyl or 1-(*p*-chlorobenzyl) derivative, the stronger absorption at 320 nm than at around 365 nm suggests that the complexation with the dihydropyridine nitrogen (**d′**) is dominant, while with the 1-(*p*-methylbenzyl) derivative, the amide oxygen is a major coordination site (**e′**).



(**d′**)



(**d**) and (**e**)



(**e′**)

These observations can be well-explained as follows: an electron-accepting group increases the contribution

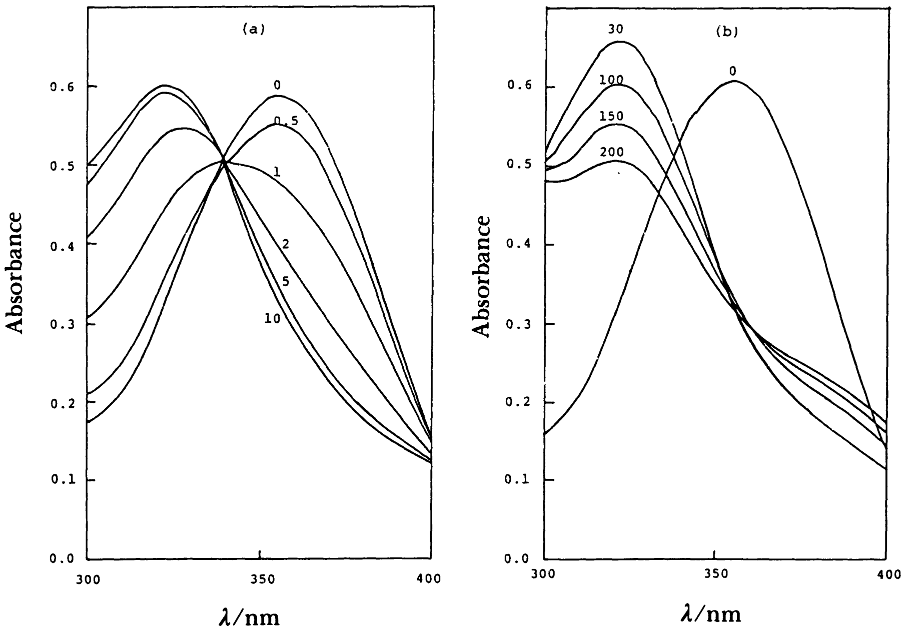


Fig. 2. Spectral changes of 1-(substituted benzyl)dihydropyridines (BPDH) in the presence of various amounts of zinc perchlorate in CH_3CN. [dihydropyridine]=0.1 mM. The numbers indicate the ratio $[Zn(ClO_4)_2]/[dihydropyridine]$. a) *p*-Cl, b) *p*-Me.

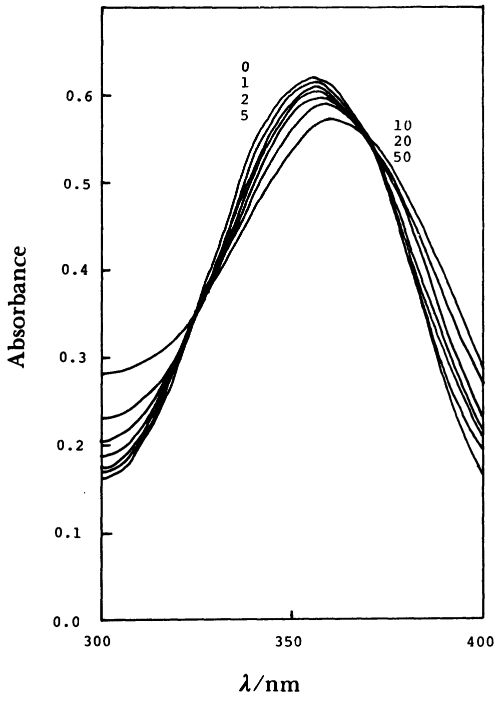


Fig. 3. Spectral changes of BPDH in the presence of $Zn(NO_3)_2$. The numbers indicate the ratio $[Zn(NO_3)_2]/[BPDH]$.

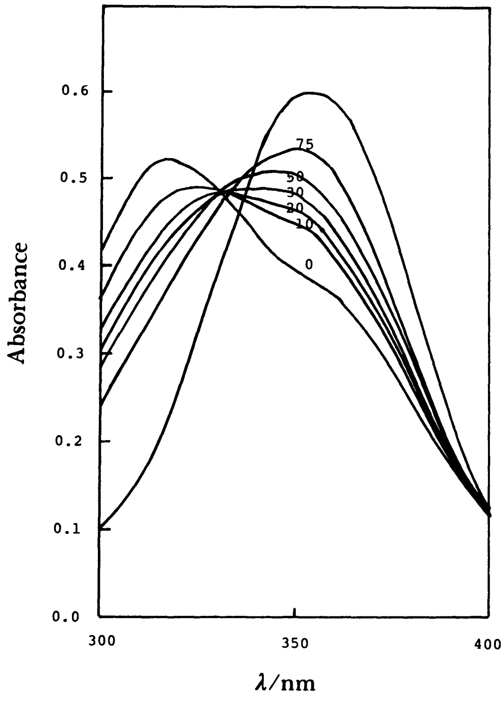
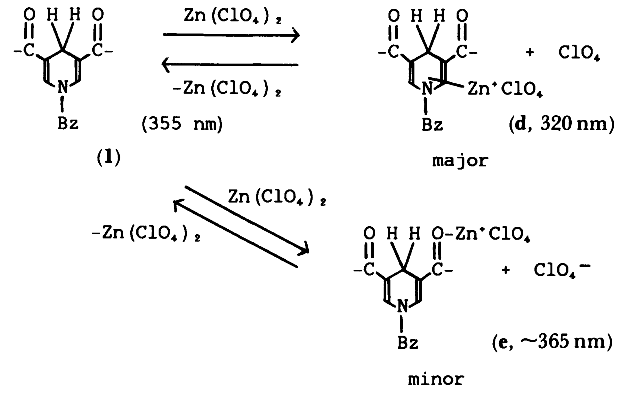


Fig. 4. Spectral changes of the BPDH–zinc ion complexes induced by addition of TBAP. [BPDH]=0.1 mM; $[Zn(ClO_4)]$=3 mM. The numbers indicate the ratio $[TBAP]/[Zn(ClO_4)_2]$.

of the resonance form **A** (shown in Fig. 1) and then the geometry around the ring nitrogen becomes more pyramidal to be suitable for accomodation of a metal cation, whereas an electron-donating group increases the contribution of the resonance form **C**, which makes the carbonyl oxygen more Lewis basic than the ring nitrogen.

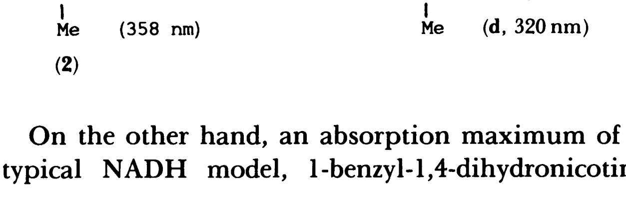
Figure 4 illustrates the spectral changes as a function of the concentration of tetrabutylammonium perchlorate (TBAP); as the 320 nm band diminishes, the original 355 nm band develops. These observations can only be consistent with the conclusion that the absorption at 320 and 365 nm should be ascribed to cationic complexes of zinc monoperchlorate with the dihydropyridine nitrogen and the amide oxygen, respectively, and these positively charged complexes would be readily decomposed by addition of perchlorate anion. In the meantime, these complexes are completely hydrolyzed by water in excess to regenerate the original BPDH. Thus, the following are the overall equilibria established for the complexation between zinc and BPDH in solution:

$$Zn(ClO_4)_2 \rightleftarrows Zn^{+}ClO_4 + ClO_4^-$$

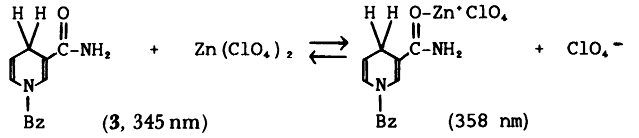


Scheme 1.

Meanwhile, the 1-methyl derivative (MPDH, **2**) behaves in spectroscopically a similar manner to the 1-benzyl derivative. In a series of the spectral changes an excellent isosbestic point can be seen at 342 nm on the original MPDH curve and no complex characterized by a red shift is produced in detectable concentration. Thus, metal coordination onto the dihydropyridine moiety rather than onto the amide carbonyl is dominating, since the ability of the 1-methyldihydropyridine moiety for coordination is superior to that of the 1-benzyldihydropyridine moiety owing to the less sterically hindering nature of the methyl vs. benzyl group rather than the electronic origin.



On the other hand, an absorption maximum of a typical NADH model, 1-benzyl-1,4-dihydronicotinamide (BNAH, **3**, λ_{max} 345 nm), was previously reported to shift toward a longer wavelength on complexation;[6] for example, 357 and 358 nm with magnesium and zinc perchlorates, respectively. It should be emphasized, however, that no spectral change was observed even by addition of a large excess (100-fold relative to BNAH) of TBAP probably because of the strong ability of the metal complexation of BNAH relative to the association of $Zn^{+}ClO_4$ with ClO_4^-.[6] Incidentally, the complexing ability (K_m) of BNAH has been estimated to be one order of magnitude greater than that of BPDH.[3,6]



Spectral Changes of Dihydropyridine-Lead(II) Complexes. Inspection of the spectra in Fig. 5a and 5b is quite instructive. Upon addition of lead perchlorate to a BPDH solution, two positively charged complexes may be inferred to be produced; a definite absorption band appears at 325 nm in the range of lead perchlorate concentrations 0—1 mM, which corresponds to a 1:1 complex of the dihydropyridine nitrogen and $Pb^{+}ClO_4$ (**g**). Further addition of the metal perchlorate gives, at the sacriface of the 325 nm band, another band at around 385 nm due probably to the complex between the dihydropyridine nitrogen and lead perchlorate (**h**). In order to verify this assignment, the effect of addition of TBAP on spectral changes was examined. The results are displayed in Fig. 6; as the band at 325 nm diminished, the corresponding broad band newly developed at around 385 nm and there was observed a single definite isosbestic point between these bands over the wide range of changing perchlorate concentrations. It is of particular interest to point out that the isosbestic point is not on the original BPDH curve, indicating that, in contrast to zinc complexes, the positively charged lead complexes do not furnish the free BPDH, even if large excesses of perchlorate anions are added to the solution. Instead, the proportion of the uncharged complex **h** is increased with increasing concentrations of TABP; namely, in the presence of excess TABP only the complex **h** can exist in a relatively stable form. This result allows the conclusion that bonding of BPDH to Pb^{2+} may be much stronger than that to Zn^{2+} because of the smaller hydration enthalpy and/or the larger ionic radii of Pb^{2+} than Zn^{2+} to be capable of accomodating two bulky perchlorate anions. Therefore, we may say that the ring nitrogen can be complexed not by lead perchlorate but by a lead monoperchlorate cation probably for its steric requirements.

In contrast to the characteristic feature of zinc com-

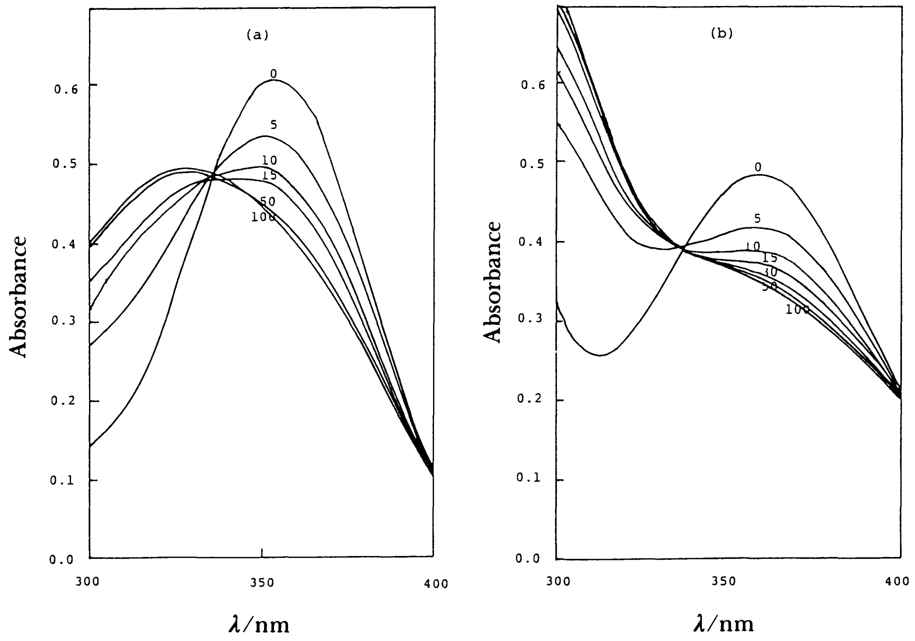


Fig. 5. Spectral changes of BPDH in the presence of various amounts of lead perchlorate: (a) 0—1.0 mM; (b) 3.0—20.0 mM. The numbers indicate the ratio $[Pb(ClO_4)_2]/[BPDH]$.

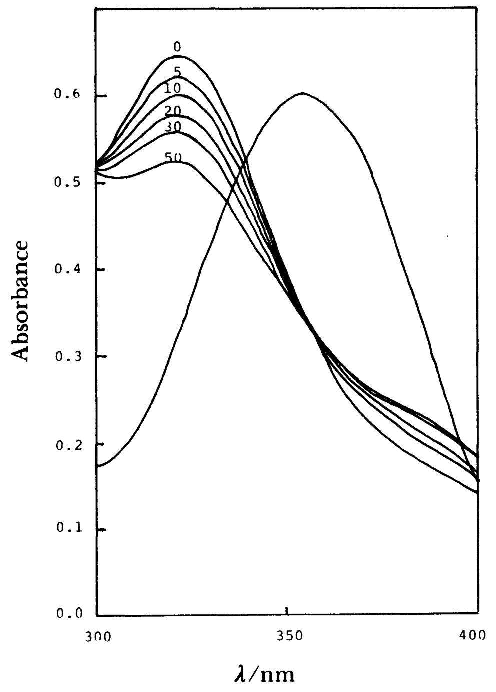
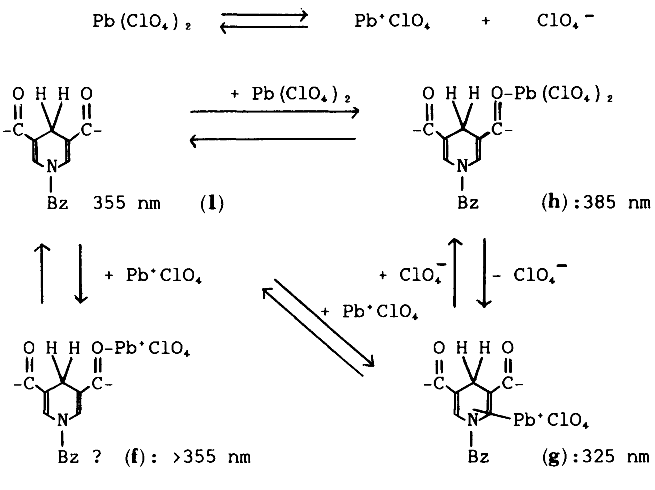


Fig. 6. Spectral changes of BPDH induced by addition of various amounts of TBAP in the presence of lead perchlorate. $[BPDH]=0.1$ mM; $[Pb(ClO_4)_2]=3.0$ mM. The numbers indicate the ratio $[TBAP]/[Pb(ClO_4)_2]$.

plexation, definite evidence for the presence of a complex **f** bearing a red-shifted band was not obtained. Even in any case examined here, the presence of a complex between the metal ion and the dihydropyridine with a stoichiometry of 1 : 2 or more was not evidenced under the present conditions, although such a higher metal complex of a dihydropyridine has been precedented with 3,5-diethoxy-2,6-dimethyl-1,4-dihydropyridine as was reported by Pandit.[9] From the above results, the complexation events can be summarized as in Scheme 2:



Scheme 2.

We shall discuss in the next section that the kinetic and spectroscopic features are closely interrelated.

Effect of Metal Cations on the Rate of Reduction. In the previous paper, we reported that bivalent metal ions catalyze the nitrosobenzene reduction with BPDH according to a Michaelis-Menthen type saturation kinetics and a linear relationship was found between

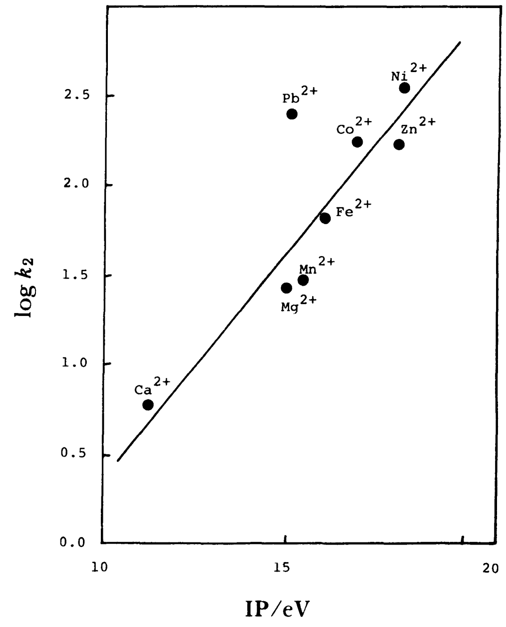


Fig. 7. Plot of log k_2 vs. ionization potential of the metal ion for the reduction of nitrosobenzene with BPDH in the presence of bivalent metal ions in CH_3CN under nitrogen at 25 °C. [metal perchlorates]=3.0 mM; [nitrosobenzene]=1.0 mM.

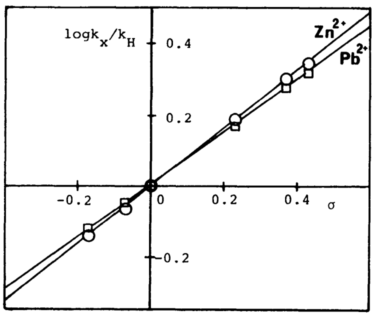


Fig. 8. Hammett plots for reduction of substituted nitrosobenzenes with BPDH in the presence of zinc and lead perchlorates in CH_3CN under nitrogen. [metal perchlorates]=3.0 mM; [nitrosobenzenes]=1.0 mM.

log k_2 and the ionization potentials of metal ions with a positive slope.[6)]

We have found here that the rate with lead perchlorate as catalyst is somewhat higher than the rate with zinc perchlorate and lead ion is one order of magnitude active than metal ions such as Mn^{2+} and Mg^{2+} having a similar ionization potential (Fig. 7). As for zinc and lead perchlorates, the effect of the substituents (X) in nitrosobenzenes was studied, the metal ion concentration being kept constant at 30×10^{-4} M. When σ parameters are used, the Hammett plots exhibit straight lines (ρ: 0.81 and 0.75 for zinc and lead salts, respectively). The similarlity in magnitude of the Hammett ρ values is indicative of both reductions as occurring by way of the same mechanism (Fig. 8).

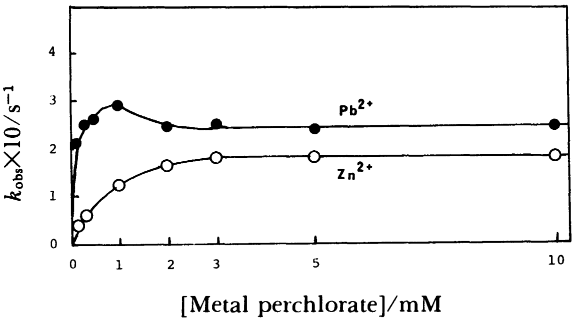


Fig. 9. Reduction of nitrosobenzene in the presence of changing amounts of bivalent metal perchlorates in CH_3CN at 25 °C under nitrogen. [BPDH]=0.1 mM; [nitrosobenzene]=1.0 mM.

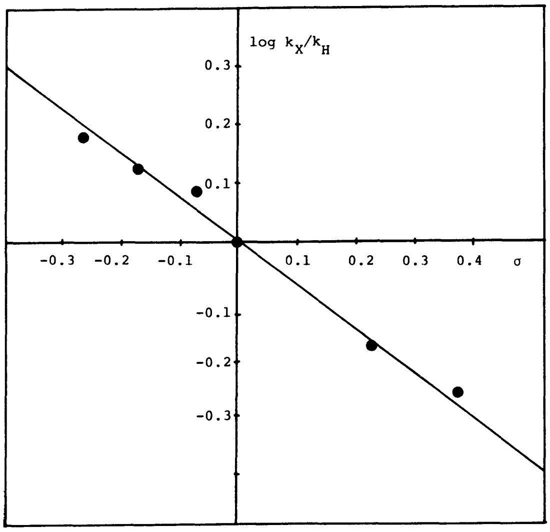


Fig. 10. Hammett plot for metal ion-catalyzed reduction of nitrosobenzene with 1-(substituted benzyl) BPDH in CH_3CN under nitrogen. [metal perchlorates]=5.0 mM. The reaction conditions are the same as described in Fig. 9, unless noted otherwise.

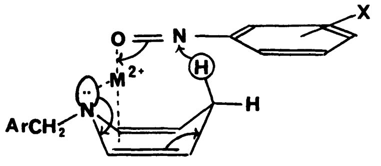
In Fig. 9 are depicted the plots of k_{obs} versus the concentrations of $Pb(ClO_4)_2$ and $Zn(ClO_4)_2$ for comparison. The clear saturation curve for zinc perchlorate might be explained by either or both of the following assumptions; the 320 and 360 nm zinc complexes (**d** and **e** types) are kinetically almost equivalent, or these complexes exist in the same ratio throughout changing concentrations of zinc perchlorate. In order to test the former assumption, an experiment was done, in which the reduction of nitrosobenzene with 1-(substituted benzyl)dihydropyridines was studied. The plotting of the logarithm of the observed rate constant against the Hammett σ constant affords a linear correlation within the experimental error (Fig. 10), which would presents a piece of evidence that, despite the overall differences in structure between complexes **d** and **e**, their kinetic behavior is inherently identical. Thus, we may safely say that zinc ion in any complex is displaced to the same position most suitable for the

Table 2. K_m and k_2 Values for Metal Ion-Catalyzed Nitrosobenzene Reduction

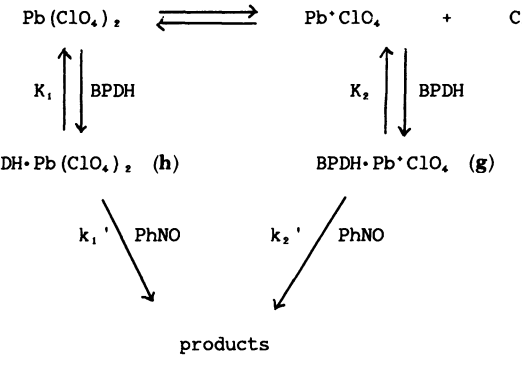
Metal ion	Ionization potential/eV	Ionic radius/Å	K_m/M^{-1}	$10^{-3}\,k_2$/M^{-1} s^{-1}
Zn^{2+}[a)]	17.96	0.74	1870	1.80
Zn^{2+}[b)]			1350	0.75
Pb^{2+}[a)]	15.03	1.24	18400	2.44

a) Counter anion: ClO_4^-. b) Counter anion: NO_3^-.

transition state of reduction, as illustrated below:[5)]



Unlike zinc perchlorate giving a usual saturation plot, lead perchlorate exhibits a significant feature in the rate-concentration plot; the maximum rate is observed at around 1 mM, suggesting that at least two kinetically important species are present in solution; namely, at its lower concentrations (<1 mM) added $Pb(ClO_4)_2$ would almost completely dissociate into Pb^+ClO_4 and ClO_4^- and the BPDH·Pb^+ClO_4 complex (**g**) thus formed could be the major species active for reduction, while at concentrations above 1 mM the BPDH·$Pb(ClO_4)_2$ complex (**h**) might also participate in the reaction. It is natural to conclude that the reaction with the former complex is faster than is the reaction with the latter and then the maximum rate has been observed. The contribution of the not identified complex **f** toward the overall rate, if any, is negligible, because of its only minor existence. Thus, the overall reaction proceeds by two competitive pathways involving totally different transition state, as illustrated below;



Scheme 3.

In Table 2 are compared the K_m and k_2 values calculated by assuming a Michaelis–Menthen equation, in which K_m is an association constant between BPDH and metal ion and k_2 is a second-order rate constant for the reaction of BPDH·metal complex and the substrate ArNO.[6)] In the case of lead perchlorate, the data points at lower metal ion concentrations (<1 mM) were only used for the calculation of K_m and k_2 values. The table shows that the apparent association

$$DH + M^{2+} \xrightleftharpoons{K_m} [DH\cdot M^{2+}] \xrightarrow{k_2[ArNO]} D^+ + PhNHO^- + M^{2+}$$

constant (K_m) for zinc complexes is tenfold smaller than that for lead complexes. This implies that the zinc complexes are relatively quite unstable as compared with the corresponding lead complexes. In order to verify this statement further, the effect of TBAP on the rate was also examined and the results thus obtained are depicted in Fig. 11; as for zinc perchlorate, the rate decreases steadily with increasing concentrations of added TBAP, indicating again all of the zinc complexes being unstable toward addition of perchlorate anions. On the other hand, as for the lead complexes, only a little rate depression resulted. This is in line with the conclusion which would be expected from the spectroscopic analysis and suggests that the uncharged BPDH·$Pb(ClO_4)_2$ (**h**) complex is slightly less reactive than the cationic BPDH·Pb^+ClO_4 complex (**g**).

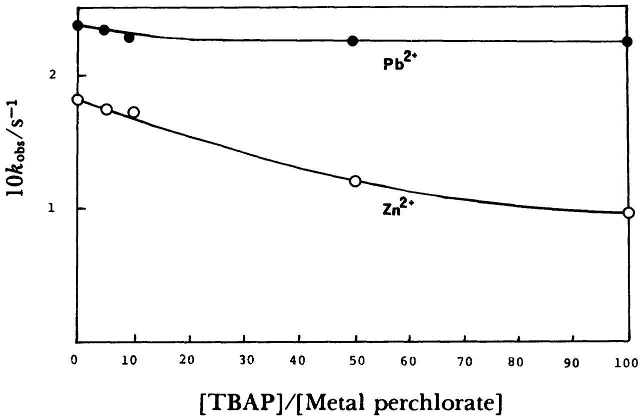


Fig. 11. Effect of addition of altering amounts of TBAP on rate of metal ion-catalyzed reduction of nitrosobenzene with BPDH in CH_3CN under nitrogen. [metal perchlorates]=3.0 mM.

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