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Metal Complexes with Adenine 1-N-Oxide and Adenosine 1-N-Oxide

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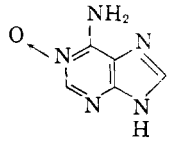
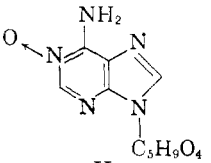
Stability constants have been obtained for complexes that adenine 1-N-oxide and adenosine 1-N-oxide form with manganese, iron, cobalt, nickel, copper and zinc. In the adenine-N-oxide complexes the binding of the metal involves the primary amino group and the nitrogen in position 7. In the adenosine-N-oxide complexes coördination of the metal is between the primary amino nitrogen and the oxygen of the N-oxide.

N-Oxides of adenine and certain of its derivatives have recently been shown to be of considerable biological interest, in that they may at times either act as antimetabolites or be metabolized to normal cellular purines.[1] They may also be significant in the metabolic roles of some co-enzymes in oxidation–reduction systems, as well as in the enzymatic hydroxylation of purines.[2] Because of the known metal-binding properties of adenine[3,4] and other purines,[5] a study has now been made of the avidity of adenine 1-N-oxide (I) and adenosine 1-N-oxide (II) for some divalent metal ions.



I



II

Both ligands belong to an interesting and apparently novel class in which coördination of a metal could possibly occur between the oxygen of the N-oxide and the nitrogen of the primary amino group. In (I), however, this would be opposed by the binding of the metal between this nitrogen and the nitrogen in position 7 (as in adenine complexes); the shape of the molecule prevents simultaneous attachment of all three groups to the same metal ion.

Experimental

Adenine-N-oxide and adenosine-N-oxide, generously provided by Drs. D. I. Magrath and G. B. Brown, were prepared by the method of Stevens, *et al.*[2] Manganese was added as chloride, iron and zinc as sulfates and the remaining cations as perchlorates prepared by ion-exchange on a column of Amberlite IR-120.

All potentiometric titrations were carried out at 20°, under nitrogen, using a Vibron Electrometer Model 33B (Electronic Instruments Ltd.) which was fitted with an internally shielded glass electrode and a saturated calomel electrode and which could be read directly to 0.001 *p*H unit. The output of the *p*H meter was also applied directly to a Recti-riter recording milliammeter (Texas Instruments Inc.) to facilitate assessment of *p*H constancy and of attainment of equilibria. The experimental procedure, which has been described previously,[6] consisted in the titration, adding 0.1 *M* KOH by micrometer syringe, of 50 ml. solutions containing known, low concentrations of metal salt, hydrochloric acid and purine-N-oxide. Titration curves were reproducible to better than 0.01 *p*H unit. The concentrations of free hydrogen ion used in calculations and constants were obtained from the measured *p*H values by using the limiting Debye–Huckel relation, $-\log f = 0.5\ I^{1/2}$. Stability constants of metal complexes were obtained from these titrations by Bjerrum's[7] method as developed by Flood and Lorås,[8] using the identity

$$K_1 = \frac{\bar{n}}{(1-\bar{n})[L^-]} - \beta_2 \frac{(2-\bar{n})[L^-]}{(1-\bar{n})}$$

where $\bar{n}$ is Bjerrum's "formation function" and K_1 and β_2 are the stability constants of the 1:1 and the 1:2 metal-ligand complexes. The plot of $\bar{n}/(1-\bar{n})[L^-]$ against $(2-\bar{n})[L^-]/(1-\bar{n})$ should be linear, with K_1 as intercept and β_2 as slope.

All absorption measurements were made with a Hilger Uvispek H700/305 spectrophotometer, following preliminary examinations of ultraviolet spectra using a Perkin-Elmer Spectracord Model 4000 spectrophotometer. The stability constants, K_1, of the 1:1 copper complexes were obtained from the optical densities, D, of a series of solutions of the N-oxides at constant *p*H and concentration but with progressively increased amounts of copper ion (copper in large excess) by plotting $(D - D_0)[H^+]/[Cu^{++}]$ against D, where D_0 refers to the copper-free solutions. This plot should be linear and of slope K_aK_1, where K_a is the dissociation constant of the ligand acid, provided that copper ion is not itself a light-absorbing species at the wave length chosen. This relation is derived:

Under the selected experimental conditions $[L^-] << [HL] << [Cu^{++}]$, so that

$$[HL] + [CuL^+] = [HL]_0$$

$$\epsilon_1[HL] + \epsilon_2[CuL^+] = D$$

Also, $[CuL^+] = K_1[Cu^{++}][L^-] = K_aK_1[Cu^{++}][HL]/[H^+]$ by definition. Elimination of $[CuL^+]$ and $[HL]$ from these three simultaneous equations gives directly

$(D - D_0)[H^+]/[Cu^{++}] = \epsilon_2K_aK_1[HL]_0 - DK_aK_1$, where $D_0 = \epsilon_1[HL]_0$, and, to a good approximation, $[Cu^{++}]$ may be taken as equal to the total copper concentration. If copper complex formation does not involve the displacement of a proton, the same relation, but without $[H^+]$ and K_a, is obtained.

Results and Discussion

Potentiometric titrations gave pK_a values at 20° of 2.69 and 8.845 for adenine-N-oxide and 2.25 for adenosine-N-oxide: earlier estimates from ultraviolet absorption spectra were 2.6, 9.0 and 2.14, respectively, at 25°.[2] Adenosine-N-oxide was found spectrophotometrically to have a further pK_a value of 12.86 (published pK_a = 12.5 at 25°[2]), taking 0.01 *M* KOH as *p*H 12.16. Although sugar groups are reported to have pK_a values around 13,[9] and a pK_a (= 12.5) of this type has been attributed to adenosine,[10] it is very unlikely that the spectro-

(1) (a) G. B. Brown, D. A. Clarke, J. J. Biesele, L. Kaplan and M. A. Stevens, *J. Biol. Chem.*, **233**, 1509 (1958); (b) D. Dunn, M. H. Maguire and G. B. Brown, *ibid.*, **234**, 620 (1959); (c) G. B. Brown, Proc. Fourth Internat. Congress of Biochemistry, Vol. XXIII, 1959, p. 111.

(2) M. A. Stevens, D. I. Magrath, H. W. Smith and G. B. Brown, THIS JOURNAL, **80**, 2755 (1958).

(3) T. R. Harkins and H. Freiser, *ibid.*, **80**, 1132 (1958).

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(5) A. Albert, *ibid.*, **54**, 646 (1953).

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(7) J. Bjerrum, "Metal Ammine Formation in Aqueous Solution," P. Haase and Son, Copenhagen, 1941.

(8) H. Flood and V. Lorås, *Tidsskr. Kjemi, Berg. og Metallurgi*, **5**, 83 (1945).

(9) R. Kuhn and H. Sobotka, *Z. Physik. Chem.*, **109**, 65 (1924).

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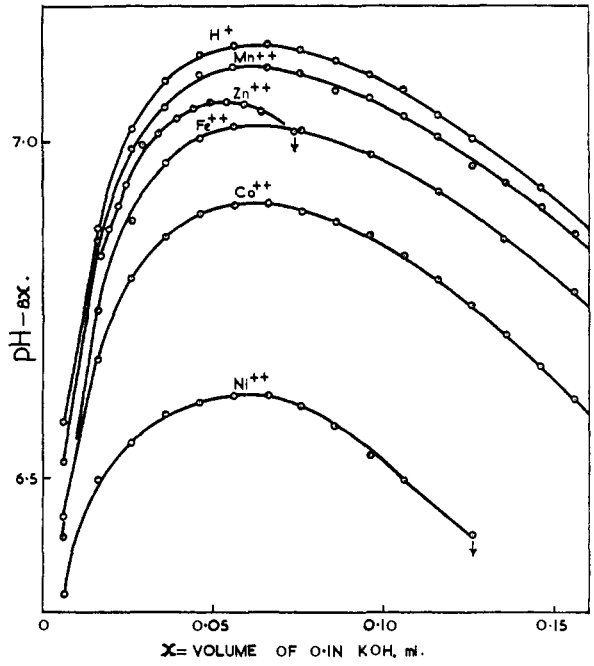
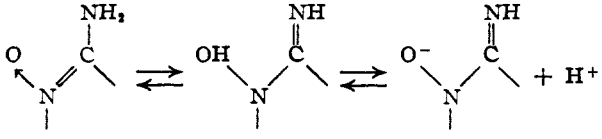


Fig. 1.—Titration curves of adenine-N-oxide (50 μmole) in water, alone and in the presence of some metal ions (all 25 μmole, except Zn^{++} 2.5 μmole). Arrows indicate onset of precipitation.

scopic pK_a = 12.86 of the N-oxide arises in this way. Thus, for wave lengths above 240 mμ, the ultraviolet absorption spectra of aqueous solutions of adenosine at *p*H 7 and in 0.5 *M* sodium hydroxide (*p*H ~ 13.9) are identical. In the N-oxide, however, there is a marked change in spectrum[2] which is much greater than is found in nucleosides when a sugar hydroxyl ionizes.[11] Stevens and Brown[12] have concluded that pK_a = 12.86 for adenosine-N-oxide is due in some way to the presence of the —NO group. It seems reasonable to attribute this *pK* to the loss of a proton from the tautomeric enol



The corresponding pK_a for adenine-N-oxide could not be measured accurately, but it is certainly much higher than "about 13," reported earlier[2]: present spectrophotometric results suggest that it is about 15.4 or even higher. The much weaker acidity of this group in adenine-N-oxide is to be expected because the proton must be removed from a mono-anion instead of from a neutral molecule.

Titration curves for adenine-N-oxide in the presence of a series of divalent metal ions are given in Figs. 1 and 2 and for adenosine-N-oxide in Fig. 3. In all cases complex formation leads to the displacement of protons by the metal ions. However, the accessible titration ranges are limited by the low solubility of the 1:2 copper complex with adenine-N-oxide (from results in Fig. 2 the solubility product is around $10^{-18.8}$) and the hydrolytic tendencies of the other cations studied, leading to precipitation. Onset of precipitation was, in all cases, clearly defined by the failure to reach equilibrium, *p*H meter readings drifting steadily to lower values. As titration proceeded, but before precipitation occurred, the increasing hydrolysis led to progres-

(11) J. J. Fox, L. F. Cavalieri and N. Chang, THIS JOURNAL, 75, 4315 (1953).

(12) M. A. Stevens and G. B. Brown, *ibid.*, 80, 2759 (1958).

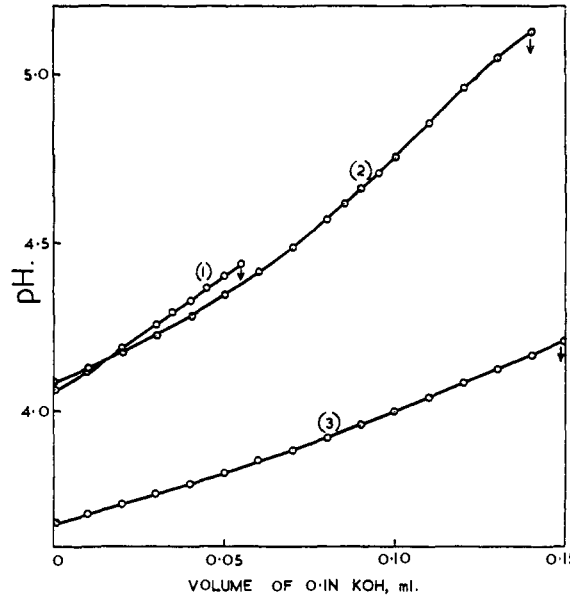


Fig. 2.—Titration curves of adenine-N-oxide (ANO) in water in the presence of copper perchlorate and hydrochloric acid: (1), (2) and (3), respectively, 25, 5 and 25 μmole ANO, 12.5, 12.5 and 50 μmole Cu^{++}, 2.7, 4.0, 9.4 μmole HCl.

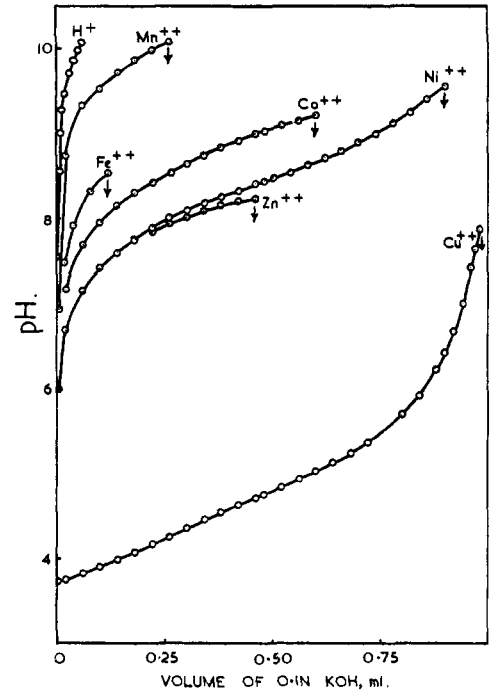


Fig. 3.—Titration curves of adenosine-N-oxide (100 μmole) in water, alone and in the presence of 50 μmole of some metal ions. Below 0.22 ml. KOH, points for Zn (not shown) differ from those for Ni by less than 0.03 *p*H unit.

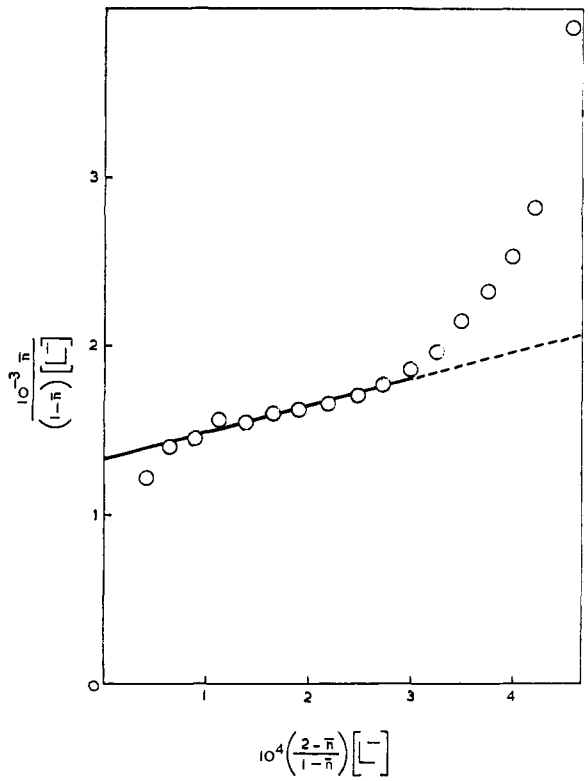


Fig. 4.—Evaluation of K_1 for the 1:1 adenine-N-oxide cobalt complex.

sively greater deviations from linearity of the plot of $\bar{n}/(1-\bar{n})[L^-]$ *vs.* $(2-\bar{n})[L^-]/(1-\bar{n})$, so that no reliable estimate of β_2 could be obtained. Only with adenosine-N-oxide and copper was there a good straight line over the entire range to $\bar{n} \sim 2$. Results for the system, cobalt adenine-N-oxide, shown in Fig. 4 are typical of all the other systems studied.

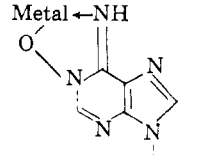
TABLE I

STABILITY CONSTANTS OF 1:1 METAL COMPLEXES WITH 1-N-OXIDES OF ADENINE AND ADENOSINE, AT 20°

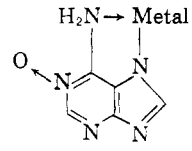
N-oxide conc., mole/l.	Metal ion,	Conc., moles/l.	log K_1	
(1) Adenine-N-oxide. pK_a = 2.69, 8.485 ± 0.01, ~15.4				
0.001	Mn^{++}	0.0005	2.13	
.001	Fe^{++}	.0005	2.80	
.001	Co^{++}	.0005	3.13	
.001	Ni^{++}	.0005	3.52	
.000094	Cu^{++}	.000236	7.16	Av. = 7.10
.000470	Cu^{++}	.000235	7.04	log β_2 ~14
.000500	Cu^{++}	.00100	7.09	
.001	Zn^{++}	.00005	3.47	
(2) Adenosine-N-oxide. pK_a = 2.25, 12.86				
0.002	Mn^{++}	0.001	5.37	
.002	Fe^{++}	.001	6.58	
.002	Co^{++}	.001	7.01	
.002	Ni^{++}	.001	7.52	
.002	Cu^{++}	.001	11.32	
		log β_2	22.18	
.002	Zn^{++}	.001	7.50	

No attempt was made to apply corrections for hydrolysis because, except for copper[6] (where hydrolysis was not significant), hydrolysis constants of the divalent ions studied are not known with sufficient accuracy. At the lower pH values, where hydrolysis was much less important, the experimental results were linear over a sufficient range for K_1 to be found by extrapolation. The values given in Table I were obtained in this way and have been calculated on the assumption that complex formation displaces one proton from each molecule of the neutral N-oxide.

These considerations suggest that 1:1 metal complexes with adenosine-N-oxide probably have the structure (III). On the other hand, if as seems reasonable, the pK_a = 8.485 of adenine-N-oxide corresponds to the pK_a = 9.7 of adenine, representing the loss of a proton from the imidazole ring,[12] the adenine-N-oxide complexes have structures similar to those found for adenine,[3] namely (IV).



III $C_5H_9O_4$



IV

The alternative structure for adenine-N-oxide complexes, corresponding to (III), does not fit the experimental results. Thus, if the ligand is a di-anion, as this would require, the data presented in Fig. 2 and Table I yield stability constants that vary directly with pH, whereas, if the ligand is a mono-anion as postulated, good constancy is obtained. The sparing and comparable solubilities of the copper complexes of adenine[3,4] and adenine-N-oxide are also consistent with this interpretation.

The spectral changes observed when copper(II) is added to adenine-N-oxide or adenosine-N-oxide provide an independent estimate of the stability constants of the complexes formed. The results summarized in Table II gave good straight lines

TABLE II

SPECTROPHOTOMETRIC EVALUATION OF LOG K_1 FOR COPPER COMPLEX OF ADENINE-N-OXIDE

(1) All solutions 200 μM in adenine-N-oxide, HCl added to adjust pH to 3.84

$[Cu^{++}]$, μM	1 cm. D_{2740}	$D - D_0$	$(D - D_0) \times a_{H^+}/[Cu^{++}]$
0	0.860		
483	0.944	0.084	0.03093
966	1.009	.149	.02940
1450	1.043	.183	.02698
1930	1.066	.206	.02502
2900	1.125	.265	.02192
3860	1.212	.352	.01995
4830	1.252	.392	.01818
9660	1.336	.476	.01356
19300	1.402	.542	.00909

Slope = 0.0464 gives log K_1 = 7.15

(2) All solutions 200 μM in adenine-N-oxide, range of copper conc. 5.10^{-4} to 1.10^{-2} M, pH 3.56 to 4.08, wave length 2800 Å. (near isosbestic point for cation and neutral ANO)

Slope = 0.0512, giving log K_1 = 7.19

(3) All solutions 40 μM in adenine-N-oxide, copper conc. 1.10^{-3} to 1.10^{-2} M, pH 4.50, wave length 2350 Å. (at this wave length Cu^{++} also absorbs and had to be corrected for)

Slope = 0.0455, giving log K_1 = 7.14

when $(D - D_0) \times a_{H^+}/[Cu^{++}]$ was plotted against D and led to values of log K_1 = 7.15, 7.19 and 7.14 for the 1:1 copper adenine-N-oxide complex, which agree with the constant obtained by potentiometric titration.

Any tendency for the neutral molecules to coordinate with metal ions must be very slight because at pH 4 a solution 0.4 μM in adenosine (which can coördinate in only this way) and 0.01 M in copper nitrate has an ultraviolet absorption spectrum close to that obtained by summing the spectra of the individual species. Similarly, Harkins and Freiser[3] found the titration curve of adenosine plus copper to be practically identical with the hydrolysis curve of copper(II), except that in sufficiently alkaline solution there was evidence of weak complex formation with the ribose group of adenosine.

Both series of complexes fit the Irving–Williams stability sequence, Mn < Fe < Co < Ni < Cu > Zn. Values of log K_1 for adenine-N-oxide complexes are less than for the corresponding adenine complexes, differences from reported[8] figures being about 1 log unit for nickel and cobalt and 0.2 unit for copper. This reduced stability is in line with the greater acidity of the N-oxide. In the same way, the greater stabilities by about 2–3 units of the metal complexes of adenosine-N-oxide relative to *o*-aminophenol[13] reflect the difference of 2.9 in their pK_a values.

Acknowledgments.—Dr. D. I. Magrath is thanked for suggesting this investigation and providing the adenine-N-oxide. The author is also indebted to Dr. G. B. Brown for a generous sample of adenosine-N-oxide and for his interest in this work.

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[CONTRIBUTION FROM THE INSTITUTE FOR ATOMIC RESEARCH AND DEPARTMENT OF CHEMISTRY, IOWA STATE UNIVERSITY, AMES, IOWA]

The Barium–Barium Hydride Phase System[1]

BY DAVID T. PETERSON AND M. INDIG

The Ba–BaH_2 phase diagram was established by thermal analysis and chemical analysis of equilibrated phases. The m.p. of barium is raised to a peritectic at 950° and 66 mole % BaH_2. A phase transformation in BaH_2 was found at 550°. The m.p. of BaH_2 obtained by extrapolation of the liquidus curve was 1200°. The solubility of BaH_2 in solid barium at 370° was 9.8 mole %.

Introduction

Barium hydride is a typical saline hydride, and the current interest in metal–salt equilibria and in metal–hydrogen systems made an investigation of the barium–barium hydride system seem worthwhile. Barium was chosen as the first of the alkaline earth group to be investigated because the metal undergoes no allotropic changes.[2] In addition, the Ba–$BaCl_2$ system shows larger solubilities than the other alkaline earth metal–halide systems,[3–5] and it was hoped that the Ba–BaH_2 system would also show easily measured solubilities. The equilibria were investigated by thermal analysis and chemical analysis of equilibrated phases rather than by measuring equilibrium hydrogen pressures because of the narrow temperature ranges over which this measurement can be made. Also, the interpretation of pressure–composition measurements can be difficult in the absence of any knowledge as to what phases are present in the system.

Experimental

Materials.—The Ba metal used for this investigation had been purified by double distillation under 10 mm. argon pressure. After distillation, the metal was handled in a glove box filled with argon to reduce contamination by reaction with the atmosphere. An analysis of a typical batch of this metal is given in Table I. Spectrographic analyses indicated that Al, Cu, Ca, Mg, Si and Sr were below 50 p.p.m. The m.p. was 729° and the thermal arrest was flat as expected for a pure material.

TABLE I

ANALYSIS OF BARIUM METAL

	Nitrogen, p.p.m.	Carbon, p.p.m.	Iron, p.p.m.	Manganese, p.p.m.
Crude barium	430	500–800	53	50
Distilled barium	73	35	23	80

Thermal Analysis.—The thermal analysis capsules were type 304 stainless steel cylinders 6.5 cm. long, 2.2 cm. diameter with 1.5 mm. walls and ends. A 5 mm. diameter with 1.5 mm. walls and ends. A 5 mm. diameter thermocouple well extended 8 mm. into the capsule from the bottom. A weighed charge of barium was placed in the capsule in the glove box. The loaded capsule was placed in a Vycor tube one end of which was closed and the other had a standard taper. A stopper with a stopcock was sealed to the standard taper with Apiezon W. The tube assembly was removed from the glove box and attached to the charging system. The sample tube was evacuated and a known volume of hydrogen introduced and allowed to react with the barium. The tube was heated to 250° to increase the rate of reaction. After the desired hydrogen concentration was reached, the tube assembly was returned to the glove box and the thermal analysis capsule closed by arc welding a cover plate in place.

The loss of hydrogen from the capsule by diffusion through the walls of the capsule during the thermal analysis was reduced by placing the capsule in a close-fitting quartz tube which was evacuated and closed. The amount of hydrogen which escaped was determined by measuring the pressure with a manometer. In all cases, the change in composition was negligible. A differential thermal analysis was used to give greater sensitivity in detecting small heat effects. The sample thermocouple was calibrated at the m.p. of an N.B.S. standard aluminum sample and a sample of

(1) (a) Contribution No. 855. Work was performed in the Ames Laboratory of the U. S. Atomic Energy Commission. (b) Based in part on the thesis submitted by M. Indig to Iowa State University in partial fulfillment of the requirements for the master of science degree.

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