

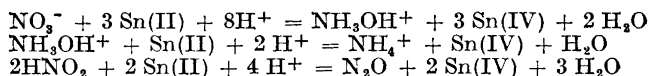
## The Mechanism of the Reduction of Nitrate

### I. Stoichiometry of Molybdate Catalyzed Reductions of Nitrate and Nitrite with Sn(II) in Hydrochloric and Sulfuric Acids

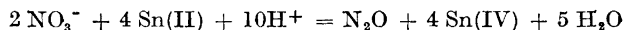
G. P. HAIGHT, Jr.\*, PATRICIA MOHILNER\*\* and ALFRED KATZ\*\*\*

*Contribution from Denmark's Technical University, Kem. Lab. A. Copenhagen, Denmark, and the Chemistry Departments of the University of Kansas and of Swarthmore College, U.S.A.*

The products of reduction of nitrate and nitrite with Sn(II) in the presence of molybdate catalyst in acid solution depend on the original oxidation state of nitrogen, the acid used, and the order of mixing reagents. In 3.0 M hydrochloric acid, when nitrate or nitrite is added to an excess of Sn(II) in the presence of molybdate, the following reactions have been observed.



In 3.0 M sulfuric acid, and 1.5 M sulfuric plus 1.5 M hydrochloric acids, nitrous acid gives the same reaction as given above but nitrate is reduced as follows.



The reduction of nitrate in hydrochloric acid is much faster than in sulfuric acid and proceeds in the two steps indicated. The reduction of hydroxylamine is much slower than the reduction of nitrate making the two steps quite distinct. In the reduction of nitrite some NO is observed if Sn(II) is added to nitrite in excess. Hydroxylamine is a very poor competitor with Sn(II) as a reducing agent for either nitrate or nitrite. It is proposed that in reactions involving excess Sn(II), the question of whether hydroxylamine or nitrous oxide is to be the product depends on the relative rates of dimerization and reduction of the active intermediate HNO.

During the past ten years considerable work has been done on the role of molybdate in the enzymatic reduction of nitrates in plants<sup>1-6</sup>. A number of studies of inorganic systems have shown molybdenum to be an

\* Chemistry Department, Swarthmore College (on leave to Kem. Lab. A 1960-1).

\*\* Chemistry Department, Louisiana State University, Baton Rouge.

\*\*\* University of Pennsylvania Medical School, Philadelphia.

effective catalyst in the reduction of oxyanions at the dropping mercury electrode<sup>7-9</sup>, with zinc metal<sup>10,11</sup> and with stannous chloride<sup>12</sup>. The present study was undertaken in the hope of providing a link in the two lines of research. Molybdate catalyzed reductions of nitrate to ammonia apparently play an important part in the nitrogen cycle of nature. Accordingly we have undertaken an investigation of the stoichiometry and kinetics of the reduction of nitrate in the presence of Sn(II) and molybdate catalyst. Both the stoichiometry and kinetics have proved to be very complex. Therefore we shall divide our findings, reporting the stoichiometry and kinetics, into separate communications. In the present paper we report conditions under which Sn(II) reduces nitrate to nitrous oxide, hydroxylamine, and to ammonia when molybdate is employed as a catalyst. In addition the reduction of nitrous acid to nitrous oxide both in the presence and absence of molybdate is discussed. Results are compared with previous noncatalytic studies of the reduction of nitrate and nitrite with stannous chloride<sup>13-17</sup>.

### EXPERIMENTAL

A very simple experimental procedure was employed. Standard solutions of nitrate or nitrite were added by means of a pipette to solutions containing a known excess of Sn(II) and approximately millimolar concentrations of sodium molybdate. Oxygen-free nitrogen was kept bubbling through the test solution at all times. From time to time samples were withdrawn and quenched in an excess of standard iodine-iodide solution which was then titrated with sodium thiosulfate solution. When successive determinations showed no further decrease of Sn(II) concentration with time the reaction was judged to be completed, and the total decrease in number of equivalents of Sn(II) was compared with the number of moles of nitrate or nitrite added. The analytical method was tested and found to be accurate in the presence of nitrate, nitrous oxide, hydroxylamine, and ammonium ions. Sn(II) reacts instantly with nitrous acid and nitrogen dioxide under the conditions of our experiments. NO if formed would be swept out by the nitrogen bubbling through the solution. It turned out that when reagents were mixed in the order indicated no evidence for reduction products other than nitrous oxide, hydroxylamine, and ammonium ions was ever detected.

Hydroxylamine was determined by the method of Bray, Simpson and Mackenzie<sup>18</sup> in which hydroxylamine is oxidized by ferric ions to nitrous oxide at 100°C, and the resulting ferrous ions are titrated with permanganate. If chloride was present, it was diluted one hundred fold before the titration with permanganate. Ammonium ions were determined by the Kjeldahl method after allowance for the hydroxylamine present. Once patterns were established, the number of equivalents of stannous ion consumed could be used to determine the proportions of the various nitrogen containing products at any time during a run.

Nitrous oxide was never determined quantitatively as such. In cases where it was suspected as a product, its odor and ability to support combustion served to indicate that it was a major product. In all such cases the number of equivalents of Sn(II) consumed corresponded to N(I) as the *only* oxidation state of nitrogen produced when neither hydroxylamine or ammonia could be detected.

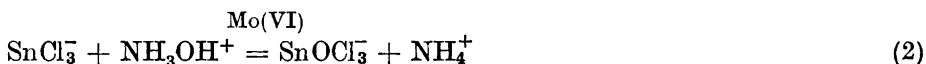
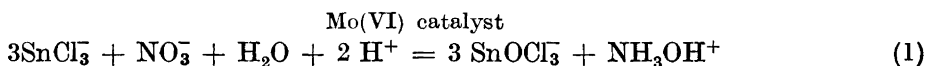
Reagent grade chemicals were used without further purification. Water purified by double distillation and by ion exchange gave identical results. Solutions of stannous chloride in 3.0 M hydrochloric acid were prepared by dissolving the reagent directly in acid and storing under nitrogen, and by passing solutions of cupric chloride in 3.0 M hydrochloric acid through a tin reductor. In the latter case reaction was complete in one pass. Stannous sulfate was prepared by shaking copper sulfate in 3.0 M sulfuric acid with metallic tin for over a week. The replacement of copper ions by tin proved to be extremely slow in the absence of chloride ions. Stannous ion stock solutions were standardized by titration with permanganate or iodometrically.

Nitric, hydrochloric, and sulfuric acids were standardized by sodium carbonate titrations using methyl orange indicator. Nitric acid was freed from oxides of nitrogen by bubbling oxygen free nitrogen through the solutions for one hour before use. Solutions of sodium nitrite were standardized by reaction with excess standard permanganate which was back titrated with standard ferrous ammonium sulfate solution. We found during these studies that we could also standardize nitrite solutions by reaction with excess Sn(II) and back titration with iodine. This method is not applicable if nitrate is present.

Stannous ions and nitrate ions were found not to react in the absence of molybdate over a two day period. Mixtures deteriorated however, if placed in flasks known to have once contained solutions of molybdate ions, regardless of how thoroughly they were cleaned.

## RESULTS

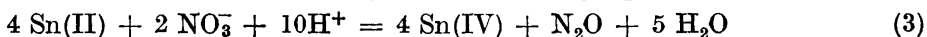
1. *The reduction of nitrate in 3.0 molar hydrochloric acid.* These conditions were selected because of the previous finding that with them hydroxylamine is most easily reduced to ammonia, and hydroxylamine must certainly be an intermediate in the reduction of nitrate to ammonia. Nitrate is in fact reduced to ammonia under these conditions in two distinctive steps.



The formula  $\text{SnCl}_3^-$  is used because it has been shown to be the most abundant and most reactive chlorotin(II) complex present in 3.0 M hydrochloric acid.<sup>19-21</sup> The formula  $\text{SnOCl}_3^-$  is purely speculative, but is a possible initial product if the reactions involve oxygen atom transfer to  $\text{SnCl}_3^-$ .

Fig. 1 shows the pattern of variation of Sn(II) concentration with time for runs containing excess Sn(II), nitrate, and sodium molybdate. A sharp break in the rate is always noted as soon as 3.0 moles of Sn(II) have been consumed for each mole of added nitrate. Table 1 shows how all the nitrogen present can be accounted for as hydroxylamine, ammonium ion, and/or unreacted nitrate at any time before completion of the run. (It is interesting to note that excess nitrate interfered very little with the determination of hydroxylamine.) Conclusive evidence that hydroxylamine is the primary product was found in our ability to calculate the points between  $([\text{Sn(II)}]_0 - [\text{Sn(II)}]) / [\text{NO}_3^-]_0 = 3$  and 4 from the previously established kinetics of the reduction of hydroxylamine<sup>19</sup>.

2. *The reduction of nitrate in sulfuric acid.* In the presence of excess stannous ion and sodium molybdate, nitrate is reduced to nitrous oxide in sulfuric acid of widely varying concentration and in equimolar sulfuric and hydrochloric acids at total ion normality of 3.0 (assuming  $\text{H}_2\text{SO}_4$  is a 1:1 electrolyte).



Here nothing can be said about the nature of stannous and stannic species present. In all cases  $\text{N}_2\text{O}$  was detected as a product and the number of equivalents of Sn(II) consumed corresponded almost exactly to reaction (3). This indicates

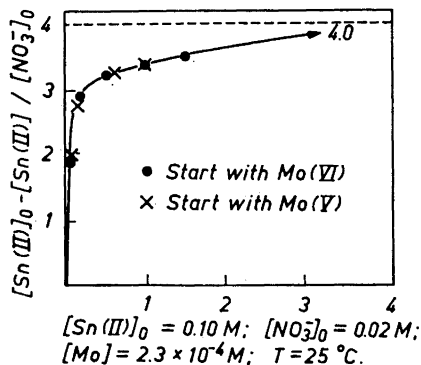


Fig. 1.

that nitrogen in the +1 oxidation state as nitrous oxide or hyponitrous acid ( $\text{H}_2\text{N}_2\text{O}_2$ ) is the only product of the reduction of nitrate under these conditions. This conclusion is strengthened by our inability to detect ammonium ions, hydroxylamine, or nitric oxide among the products. The reaction in 3.0 M sulfuric acid is extremely slow requiring several days time for completion at 25°C, even though one thousand fold more molybdate was used than in the study in hydrochloric acid. Qualitatively it was observed that up to two chloride ions per stannous ion decelerated the reaction somewhat. In equimolar sulfuric and hydrochloric acid the reaction rate was comparable with that in 3.0 M hydrochloric acid, though not quite so fast. The products in the mixed acids were the same as in sulfuric acid. Results obtained in solutions containing sulfuric acid are summarized in Table 2.

3. *The reduction of nitrous acid by Sn(II)*. All experiments in which sodium nitrite solutions were added to an excess of Sn(II) in either hydrochloric

Table 1. Nitrate reduction products in 3.0 M hydrochloric acid.

Time, min.	a Sn(II) <sub>0</sub> m/l	b NO <sub>3</sub> <sup>-</sup> <sub>0</sub> m/l	c Sn(II) m/l	d NH <sub>2</sub> OH m/l	e NH <sub>4</sub> <sup>+</sup> m/l	f NH <sub>4</sub> <sup>+</sup> m/l	g NO <sub>3</sub> <sup>-</sup> m/l
1	0.234	0.0492	0.1135	0.0383	0.00	0.0014	0.0092
15	0.234	0.0492	0.0770	0.0393	0.0094	0.0099	0.0000
1500	0.234	0.0492	0.0369	0.0000	0.0493	0.0492	0.0000
—	0.0423	0.150	0.0085	0.0113	0.000	0.0007	0.139

10<sup>-4</sup> M molybdate was present in each solution.

About 30 seconds elapsed between samples used to measure c, and samples used to measure d.

Columns a, b, c and d represent experimental measurements.

e = a - 3b - c if a > 3b

f = b - d, g = 3b - a + c if g > 0.0

The fourth row in Table 1 represents an experiment designed only to show qualitatively the presence of hydroxylamine in a run containing excess nitrate. The nitrate apparently did not interfere with hydroxylamine determination as was expected.

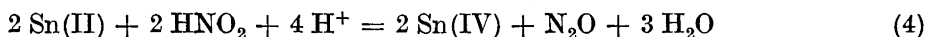
Table 2. Stoichiometry of the reduction of nitrate and nitrite by Sn(II) in sulfuric acid.

Mo m/l	a NO <sub>3</sub> <sup>-</sup> added millimoles	b NO <sub>2</sub> <sup>-</sup> added millimoles	c Sn(II) consumed millimoles	c/a	c/b	[H <sub>2</sub> SO <sub>4</sub> ] m/l
none	0.00	5.86	5.71	—	0.97	2
none	0.00	5.86	5.90	—	1.008	2
10 <sup>-3</sup>	0.00	5.86	5.81	—	0.99	2
none	23.5	5.86	6.70	—	1.15	2
none	11.75	5.86	6.46	—	1.10	2
none	22.97	0.00	0.10	0.005	—	2
10 <sup>-3</sup>	2.625	0.00	5.25*	2.00	—	2
10 <sup>-3</sup>	2.625	0.00	5.16*	1.98	—	2
10 <sup>-3</sup>	2.625	0.00	5.29*	2.01	—	3
10 <sup>-2</sup>	4.578	0.00	9.196 **	2.01	—	3.2
10 <sup>-2</sup>	4.578	0.00	9.224 **	2.01 <sub>5</sub>	—	9.0
0.005	0.150	0.00	0.187 (1 day)	2.05	—	3.0
			0.307 (2 days)			
0.005	0.150	0.00	0.285 (5 min.)	2.00	—	1.5
			0.300 (1 h.)			(+1.5 M HCl)

\* 35°C

\*\* 100°C, 10 min.

or sulfuric acids, with or without molybdate present, gave an instantaneous two equivalent reduction of nitrite according to the equation:



The same results were obtained employing back titration of excess Sn(II) with iodine, or by titrating Sn(II) to a potentiometric end point with the nitrite solution. (Raschig observed nitric oxide (NO) as a product when Sn(II) was added to nitrous acid in excess.) We have the paradoxical result that under conditions where nitrate is reduced to hydroxylamine and ammonia, nitrite is reduced only to nitrous oxide.

Table 3. Stoichiometry of the reduction of nitrite by Sn(II) in hydrochloric acid.

a NO <sub>2</sub> <sup>-a</sup> added millimoles	b Sn(II) consumed millimoles	Other N compounds present	b/a
0.2610	0.2610	none	1.000
4.058	3.996	none	0.985
4.058	4.240	none	1.04
7.250	7.333 <sup>a</sup>	none	1.01
1.480	1.475 <sup>a</sup>	none	0.995
6.270	6.210 <sup>a</sup>	none	0.99
0.478	0.457	0.018 M NH <sub>2</sub> OH	0.95
0.821	0.649	0.028 M NH <sub>2</sub> OH	0.79
0.1435	0.1207	0.009 M NH <sub>2</sub> OH	0.85

In all cases NaNO<sub>2</sub> solutions were added to other reagents in 3.0 M hydrochloric acid.

<sup>a</sup> Potentiometric titrations, all other results by iodometric analysis for excess Sn(II).

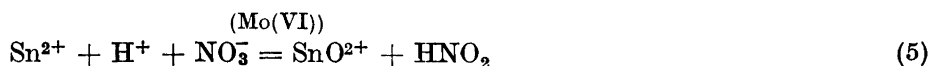
The fact that nitrate is not reduced when molybdate is absent, but nitrite is reduced quickly and quantitatively suggested that the non-catalyzed reaction could be used to determine nitrite in the presence of nitrate. Attempts to do this proved disappointing since the presence of nitrite evidently causes some reduction of nitrate to take place as is shown in two experiments recorded in Table 2. Similar synergistic effects were recorded by Klemenc and Klima<sup>14</sup> in 1929.

When nitrite solutions are added to solutions containing both hydroxylamine and Sn(II) in 3.0 M hydrochloric acid, somewhat less than one mole of stannous ion per mole of nitrous acid is consumed. The speed of reaction made rate and efficiency of mixing a large factor in the results and only qualitative conclusions can be drawn. It is clear however that hydroxylamine competes only slightly with SnCl<sub>2</sub>, and that the latter is a far more labile reducing agent in this case. Results of all experiments on the reduction of nitrite are summarized in Tables 2 and 3.

#### DISCUSSION

The patterns of products observed in molybdate catalyzed reductions of nitrate and in the reductions of nitrite with or without molybdate present are very similar to those observed at elevated temperatures without catalysis by Milligan<sup>13</sup>, Maumené<sup>15</sup> and Dumreicher<sup>16</sup>. This suggests that molybdate merely activates the robust nitrate ion, but does not further influence the course of reaction. The change in reaction product from hydroxylamine to nitrous oxide when sulfuric acid is substituted for hydrochloric acid is extremely interesting. It is not likely that the various species containing nitrogen in its various oxidation states are much affected by the change from hydrochloric to sulfuric acid at ion normality = 3.0. However, Sn(II) is principally SnCl<sub>2</sub> in 3.0 M hydrochloric acid. Since this is the most reactive of the chlorotin(II) complexes, the acceleration of reaction due to chloride is explainable, but not necessarily the change in reduction products of nitrate. The change in reduction products is believed to be explicable in terms of the kinetic properties of the active intermediate HNO.

Let us assume that stannous ion functions as a two-electron reducing agent (probably acting as an oxygen atom acceptor). Nitrate ions will then be reduced according to the following steps:



It is well known that HNO<sub>2</sub> in acid reacts with itself to form the anhydride N<sub>2</sub>O<sub>3</sub>. It seems reasonable that HNO, which would be a weaker acid, should form its anhydride even more readily. Thus:



is an alternative to reaction (7). The products of reactions (7) and (8) are the two products observed under different conditions. It now remains to explain how the various conditions employed favor one product completely over another.

Reaction (7) will be favored by low concentrations of HNO and by high reactivity of reducing agent. The rate of reaction (8) will increase as the square of the concentration of the active intermediate HNO, and is therefore favored when this concentration is high.

Comparing the reduction of nitrate in hydrochloric and sulfuric acids, we find evidence that reaction (7) is dominant when  $\text{SnCl}_3^-$  is the dominant form of the reducing agent present. This most reactive species of Sn(II) is able to make (7) the dominant reaction over (8). This is true, however, only when nitrate is the starting material. When nitrous acid is reduced, the initial reaction is very fast producing a large initial concentration of HNO which results in the formation of  $\text{N}_2\text{O}$  by reaction (8). It is hoped that future studies can be undertaken employing conditions where mixtures of products are obtained.

The great reactivity of the postulated species HNO can be used to account for the reduction of nitrate in the presence of nitrite, and for the production of NO when nitrite in excess reacts with solutions of stannous chloride<sup>17</sup>. HNO produced by the reduction of nitrite may give the following reactions as well as (7) and (8).



Labile  $\text{HNO}_2$  traps nearly all the HNO before it can dimerize while only a small, but significant, fraction of the robust nitrate ions are attacked.

The suggestion of Milligan<sup>13</sup>, that hydroxylamine is the primary reduction product of nitrate and is reoxidized to nitrous oxide by nitric or nitrous acids, is definitely refuted by our results. Nitrate does not oxidize hydroxylamine during the catalytic reductions, and hydroxylamine does not compete effectively with stannous ion as a reducing agent for nitrous acid. It is likely that the behavior of the very reactive HNO with respect to dimerization or reduction may be a key to the explanation of most observations on the reduction of nitrate which proceed beyond the oxidation states represented by  $\text{NO}_2^-$  and NO.

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