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The work of White and Long (1) on the Kjeldahl digestion procedure in sealed tubes at 470° C. offers a major improvement in the reliability of this method for nitrogen analysis. The subsequent work of Grunbaum, Schaffer and Kirk (2) concerning the decomposition of ammonium sulfate at higher temperatures suggested that a rigorous set of conditions were required to avoid ammonia loss in the course of the digestion. This investigation also showed, as did the work of White and Long, that catalyst addition was unnecessary to affect decomposition of the organic material. Baker (1), in his investigation, confirmed the work of Grunbaum, et al. and stated that ammonia actually was lost above 420° C. when a mercuric sulfate-selenium catalyst was employed for the digestion.

The present investigation had several objectives. First, it was desired to identify the oxidation product or products of ammonia at elevated temperatures in sealed tubes. Also, it was intended to

establish a set of conditions for the digestion which would suppress the ammonia decomposition and would result in a less critical control of temperature. Finally it seemed important to compare results between the milligram and microgram scale analyses to determine whether or not there were surface or other effects which play a role in the digestion of microgram quantities of material which are not evident with milligram samples.

EXPERIMENTAL

The apparatus and procedure used for the analyses of microgram samples were identical with those described previously (2) and will not be discussed here, since the data of the previous investigation demonstrated the reliability of the method. The digestion tubes used on the milligram scale had the dimensions proposed by White and Long. The distillation apparatus employed was described by Kirk (3). The milligram method deviated from that described by White and Long in that the sulfur dioxide and carbon dioxide were driven off by heating the contents of the bomb to gentle boiling before transfer to the distillation apparatus. This eliminated the need for the addition of the base to the distillation apparatus ahead of the digestion mixture. Omission of the catalyst eliminated the need for the addition of sodium thiosulfate to the alkaline distillation mixture. The temperature range investigated for the digestion of both the milligram and microgram samples varied from about 150° C to 625° C. The error in reading

furnace temperature was less than 5° C.

RESULTS

Grunbaum et al. (2) showed that ammonia from organic, nitrogen-containing samples did not decompose as readily as ammonium sulfate during digestion and that the conditions established by White and Long, 0.5 hour digestion at 470° C., appeared to be optimum for the digestion of organic samples.

In this study similar experiments (2) concerning ammonium sulfate decomposition were repeated on both the milligram and microgram scales. Easily controlled conditions, i.e., sample size, temperature, quantity, and concentration of sulfuric acid, were investigated. The first three curves in Figure 1 illustrate the effect on recovery of varying temperature and sample size keeping the quantity of sulfuric acid constant. Curves A and B were obtained from measurements made on the microgram scale with sealed tubes having very nearly the same volume (approximately 0.8 ml.) as described previously (2). The data for Curve A were obtained using 10 μ l of concentrated sulfuric acid and approximately 25 μ g of ammonium sulfate in each tube. Curve B illustrates similar information for a series of determinations using 125 μ g of ammonium sulfate and 10 μ l of concentrated sulfuric acid. Curve C shows data obtained with both tubes which had a volume of approximately 25 ml. Each tube contained about 250 μ l of concentrated sulfuric acid and 6 mg. of ammonium sulfate. These volumes of acid afforded about the same overall gas pressures in the microgram and milligram determinations.

With 10 μ l of concentrated sulfuric acid in the microgram scale determination or 250 μ l of concentrated sulfuric acid in the milligram scale determination a liquid as well as a gas phase exists at temperatures much below 530° C. Above this temperature the sulfuric acid is completely vaporized. It is seen from the curves in Figure 1 that the ammonia loss depends upon the initial quantity of ammonium sulfate for a given volume of sulfuric acid.

Oxidation of Ammonia to Nitrogen Gas

Grunbaum et al. have suggested that the ammonia loss probably could be ascribed to oxidation of the ammonia to nitrogen gas. To confirm that this process was the one which was occurring, a 5.28 mg sample of ammonium sulfate and 250 μ l of concentrated sulfuric acid were sealed in a 25 ml bomb tube under vacuum (10^{-4} mm) and the contents were digested under conditions in which the ammonia was quantitatively lost (520° C. for 0.5 hr.). The composition of the gas phase was determined by use of the mass spectrograph. The sulfur trioxide and water were removed from the gas mixture by the proper control of temperature and the remaining gas showed 66.22 mole percent sulfur dioxide, 25.54 mole percent nitrogen and 8.24 mole percent oxygen. A calculation of the quantity of nitrogen gas from the pressure, volume and temperature values listed from the mass spectrographic analysis indicated that within experimental error the ammonia was oxidized quantitatively to nitrogen gas (gas recovery - 105%). The amount of sulfur dioxide formed in the course of the reaction was only in approximate agreement with the ratio from the equation for the net reaction, $SO_2/N_2 = 3$. Some of the sulfur dioxide is lost because it has an appreciable solubility in sulfuric acid at room temperature.

Additional loss may be incurred in the process of removing water vapor and traces of sulfur trioxide. The presence of oxygen is not due to contamination but rather to the decomposition of sulfur trioxide at the temperature of the digestion. In the temperature region where the dissociation of sulfur trioxide becomes appreciable, either sulfur trioxide or oxygen may serve to oxidize ammonia to nitrogen gas. For this reason, it is of interest to compare the decomposition of ammonia by oxygen with that by sulfuric acid. Curve D of Figure 1 shows the results of a series of determinations in which weighed quantities of ammonium chloride were sealed under one atmosphere of oxygen gas in 25 ml tubes. It is seen that the oxidation of ammonia occurs in approximately the same temperature region but at a slower rate in pure oxygen than in sulfuric acid. This effect may be due to greater concentration of oxidizing agent when sulfuric acid is present or it may result from a different mechanism in the oxidation process. The fact that oxidation of ammonia starts at approximately the same temperature in the presence of oxygen as when the sample is digested with sulfuric acid would seem to indicate that oxygen is responsible for the ammonia loss.

Curve A of Figure 2 shows the oxygen pressure due to the dissociation of sulfur trioxide which would exist in a 25 ml sealed tube if 250 μ l of concentrated sulfuric acid were completely vaporized at the temperatures listed. These calculations were carried out assuming that gaseous sulfuric acid does not exist as a major species. For these temperatures and pressures the validity of this assumption is uncertain. For the purposes of this comparison no corrections were made for gas imperfections since the calculations were intended only to demonstrate

the effect qualitatively. In addition, the uncertainty due to the species which are present does not warrant the lengthier and more exact treatment. In the oxidation of ammonium sulfate no large amount of sulfur dioxide is produced and as a consequence the oxygen pressure may approximate the amount calculated for Curve A, Fig. 2. Curve d was included to illustrate the reduced oxygen pressure that is obtained when appreciable quantities of sulfur trioxide are reduced in oxidizing the organic matter present. The curve represents the oxygen pressure when 10 mg of acetanilid and 250 μ l of concentrated sulfuric acid were sealed under vacuum and digested at the indicated temperatures. This lowering of the oxygen pressure due to the large amount of sulfur dioxide which is formed in the destruction of the organic matter can account for the increased stability which is observed when an organic nitrogen containing sample is digested.

The data discussed so far did not rule out the possibility that in this temperature region ammonia may also decompose to give nitrogen and hydrogen gas. Table I lists data which indicate that the reaction does not proceed according to this path. Ammonium sulfate and ammonium bisulfate samples were sealed in a bomb tube in the presence of air and heated for 0.5 hour at 550° C. These samples lost more ammonia than the stoichiometric amount permitted by the reduction of sulfur trioxide to sulfur dioxide, but this is explained by the presence of oxygen from the air contained in the sealed tube. When the same experiments were repeated in evacuated tubes and the samples were heated to 620° C the extent of oxidation was decreased. The reaction proceeded until the stoichiometrically available amount of sulfur trioxide was reduced to sulfur dioxide or slow by one-third of the

ammonia being lost in ammonium sulfate while two-thirds was lost with ammonium bisulfate. Within the certainty of the experiment, the reaction did not continue beyond the utilization of sulfur trioxide. It was concluded from these measurements that ammonia was not lost due to the reaction

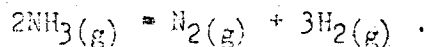


Table I

Oxidation of Ammonia in the Absence of Sulfuric Acid

<u>Sample</u>	<u>IN AIR</u>	
	<u>Furnace Temp.</u>	<u>% NH₃ Recovered</u>
(NH ₄) ₂ SO ₄	550° C	48.4
"	"	49.5
NH ₄ HSO ₄	"	20.9
<u>IN VACUUM</u>		
(NH ₄) ₂ SO ₄	620° C	66.4
NH ₄ HSO ₄	"	31.4
NH ₄ Cl	"	98.0

Effect of Concentrated Sulfuric Acid

The qualitative observation that ammonia was not decomposed as rapidly when an organic, nitrogen-containing sample was digested as when ammonium sulfate was heated under comparable conditions (2) led to an investigation of the effect of reactant and product concentrations in the digestion mixture. The variation in the amount of sulfuric acid added was investigated first. The results of these measurements which were carried out in 25 ml sealed tubes are shown in Figure 3.

The reproducibility of the values plotted is of the order of 10-20 percent. The minimum in this curve is associated with the quantity of sulfuric acid that produces a two phase system. Amounts of sulfuric acid less than about 500 μ l (approx. 20 μ l of concentrated sulfuric acid per milliliter of tube volume) result in a gaseous system in this temperature range. Amounts of sulfuric acid greater than about 500 μ l produce a two phase system. The distribution of the ammonia between the two phases diminishes the rate of oxidation of ammonia in the course of the digestion. The species responsible for the oxidation of ammonia (either sulfur trioxide or oxygen) will occur only in the gas phase and at the surface of the liquid. Figure 3 indicates that the least desirable quantity of sulfuric acid to use is that in which the vapor pressures have reached a maximum but no appreciable excess above this saturation amount is present.

The bulk of the work reported in this paper has been performed under conditions which permitted investigation of the gaseous process and which avoided the uncertainties introduced by a two phase system.

The starred point of Figure 3 represents the recovery of ammonia when 200 μ l of 30 percent fuming sulfuric acid were used instead of the regular, concentrated acid. As would be expected the loss of ammonia is greater in the presence of an increased amount of the oxidizing agent.

Effect of Digestion Time

It was proposed previously (2) that temperature played a more important role than time in the digestion process. At that time it was thought that after the one-half hour digestion period equilibrium

had been obtained. Work by Baker (1) with organic samples at 420° and 465° C corroborated this belief. Further work, however, in temperature ranges where the oxidation occurs at a more rapid rate, has shown that equilibrium for the oxidation is not obtained in such a short time interval even at temperatures where the reaction rate is relatively rapid. Table II shows two sets of data; one set in which the sample and acid were sealed in the presence of air and the second in which the tube was evacuated before sealing.

Table II
Variation in Ammonia Loss with Time

<u>IN AIR</u>			
<u>Composition of Digest</u>	<u>Temp.</u>	<u>Time of Digestion</u>	<u>% NH₃ Lost</u>
250 μ l H ₂ SO ₄	550° C	0.5 hr.	57, 69
" "	"	1.0 hr.	85
<u>IN VACUUM</u>			
100 μ l 1 M H ₂ SO ₄	620° C	0.5 hr.	63
" "	"	1.0 hr.	87

From these data it is seen that roughly the same fraction of ammonia is lost in the second half hour interval as in the first interval. This means that even at these considerably higher temperatures equilibrium conditions are not obtained in an hour's heating time and that at digestion temperatures approximating 470° C, establishment of equilibrium is sufficiently slow that a half hour heating period causes no serious loss.

this argument appears to be confirmed by the work of Baker on amino acetic acid at 165° C. The theoretical amount of ammonia is found in a half hour digestion period but after 3 and 4 hours at the same temperature the analysis shows an ammonia recovery of 98.7%. Further data with respect to loss with time will be given for tryptophan in a subsequent action.

Effect of Added Water

Initial experiments in which distilled water was added to the sulfuric acid digestion mixture indicated that the presence of water vapor suppressed the rate of ammonia loss. This is shown in Table III.

Table III

Effect of Water Above its Critical Temperature
on Ammonium Sulfate Digestions

0.5 hr. Digestion of Ammonium Sulfate at 620° C
on the Milligram Scale - in Vacuum

<u>Sample</u>	<u>Digestion Reagent Added</u>	<u>% NH₃ Recovered</u>
(NH ₄) ₂ SO ₄	None	66*
"	100 µl H ₂ O	74
"	100 µl, 1N H ₂ SO ₄	37
"	100 µl conc. H ₂ SO ₄	0

* The ammonia oxidized represents quantitative reduction of sulfur trioxide to sulfur dioxide.

The determinations show definitely that the presence of water suppresses the decomposition of ammonia.

Figure 4 illustrates the effect of added water on the re-oxidation of nitrogen in egg albumin. Curve A is included for comparison of the rate of oxidation of ammonium sulfate in 10 μ l of concentrated sulfuric acid as compared to that of egg albumin in a comparable amount of sulfuric acid. From these two curves it was concluded that the presence of sulfur dioxide does not suppress the decomposition of ammonia since the major difference in the two sets of experiments lies in the much larger quantity of sulfur dioxide formed in the decomposition of egg albumin than in the oxidation of ammonium sulfate. This observation is in agreement with those listed in Table II which showed that equilibrium is not attained in half-hour digestion periods. Since time of digestion and pressure of sulfur dioxide both indicate that an equilibrium state for the net reaction is not reached in a half-hour digestion period it is necessary to seek another explanation for the suppression of oxidation by the addition of water to the mixture as shown by curve C. A plausible reason for the decreased rate of oxidation in the presence of greater quantities of water vapor is associated with the quantity of oxidizing agent available to react with the ammonia species. A large increase in water pressure in the system will decrease the equilibrium pressure of sulfur trioxide as a result of the formation of sulfuric acid. Consequently, whether the oxidizing species is sulfur trioxide or oxygen, the pressure of either one should be lowered appreciably. This is confirmed by the observation on the milligram scale that a liquid phase is present when 250 μ l of concentrated sulfuric acid and 250 μ l of water are heated to temperatures as great as 540 $^{\circ}$ C in a 25 ml sealed tube. While the liquid phase was not actually observed on the microgram scale, the ratio of the volume of the work tube to the volume of concentrated sulfuric acid plus water implies that this also is true on the microgram scale.

Figure 5 shows the effect of digestion time on the decomposition of tryptophan at 550° C. Curve A indicates the rate of decomposition when 250 μ l of concentrated sulfuric acid were used to digest the sample and curve B illustrates the decomposition rate when 250 μ l of concentrated sulfuric acid plus 250 μ l of distilled water were used for the digestion. This temperature was selected because ammonia is rapidly oxidized by 250 μ l of concentrated sulfuric acid at 550° C and the effect of water can be shown using digestion periods of short duration. It is seen that the organic material was destroyed in less than 10 minutes in a furnace heated to 550° C in both cases but the rate of oxidation of ammonia was decreased considerably by the increased pressure of water. (Total pressure approximately 30 atm.). The circled points represent the amount of ammonia oxidized when ammonium sulfate was digested at this temperature. The reason for obtaining 100 percent ammonia recovery before appreciable loss occurs probably is due to the length of time that is required for the contents of the bomb to reach the temperature of the furnace. Since organic matter is decomposed by sulfuric acid at a rapid rate even at considerably lower temperatures than 400° C it is quite likely that by the time the sample has reached the temperature region where oxidation of ammonia occurs the organic portion of the sample has been completely oxidized.

Figure 6 shows that when tryptophan was digested at 530° C with 500 μ l of 9M sulfuric acid a digestion period of 10 - 30 minutes yielded a recovery of ammonia equal to or greater than 99 percent. Comparable experiments on the micro gram scale have shown that the digestion period may be shortened to a few minutes. This shorter period is due undoubtedly to the much smaller heat capacity of the smaller system which permits the

maximum temperature to be reached in a considerably shorter time interval.

As a result of this investigation on the sealed tube Kjeldahl digestion method it is recommended that the use of catalyst be avoided and if the temperature control can not be regulated to better than 10-20° C that a little water be added to reduce the rate of oxidation of ammonia. It should be pointed out that the critical temperature of water is exceeded and that the quantity added should be about 10 μ l water for each milliliter of gas space regardless of the quantity of concentrated sulfuric acid added.

SUMMARY

The investigation of conditions for the sealed tube Kjeldahl digestion demonstrated that ammonia loss in the digestion is due to oxidation of ammonia to nitrogen gas. It was shown that excellent agreement existed between the results on the milligram and microgram scales. Also it was found that oxidation of ammonia by sulfur trioxide or by oxygen (alone) occurs over the same temperature range. The difference in rate of the oxidation probably is due to differences in the partial pressure of the oxidizing species.

The data indicate that half-hour digestions are more than adequate for the destruction of the organic portion of a substance with compounds as refractory as tryptophane and that prolonged digestion can result in the oxidation of appreciable quantities of ammonia. The addition of water to the digestion mixture had a pronounced effect in lowering the rate of oxidation of ammonia. Consequently, digestion temperatures greater than 470° C may be safely used when small amounts of water are added without seriously altering the rate of destruction of the organic portion of the molecule and without increasing the ammonia loss.

A further study of the chemistry of the ammonia oxidation step is being conducted. It is hoped that such a study will lead to a better understanding of the factors which may be controlled in this oxidation.

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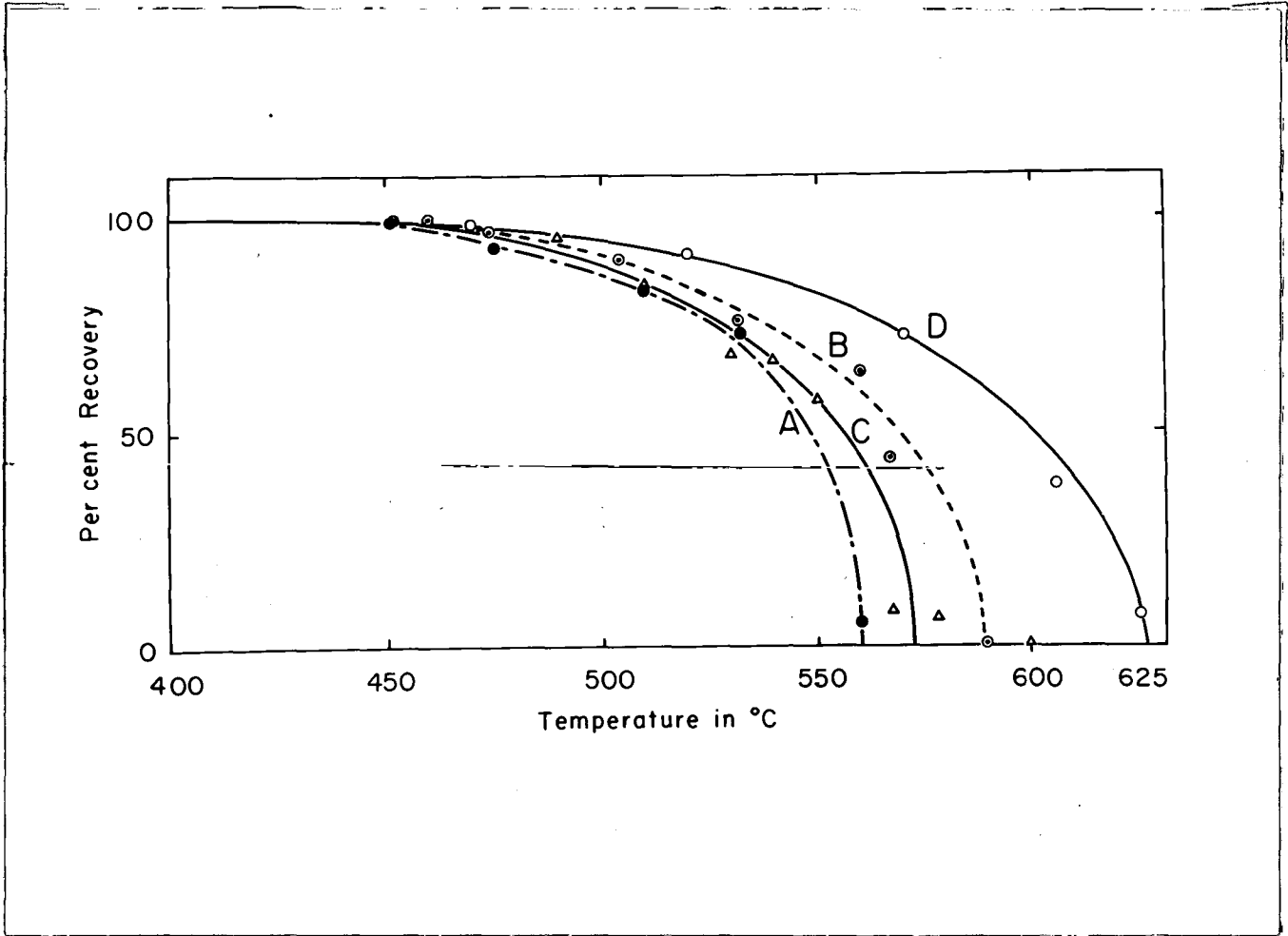


Figure 1

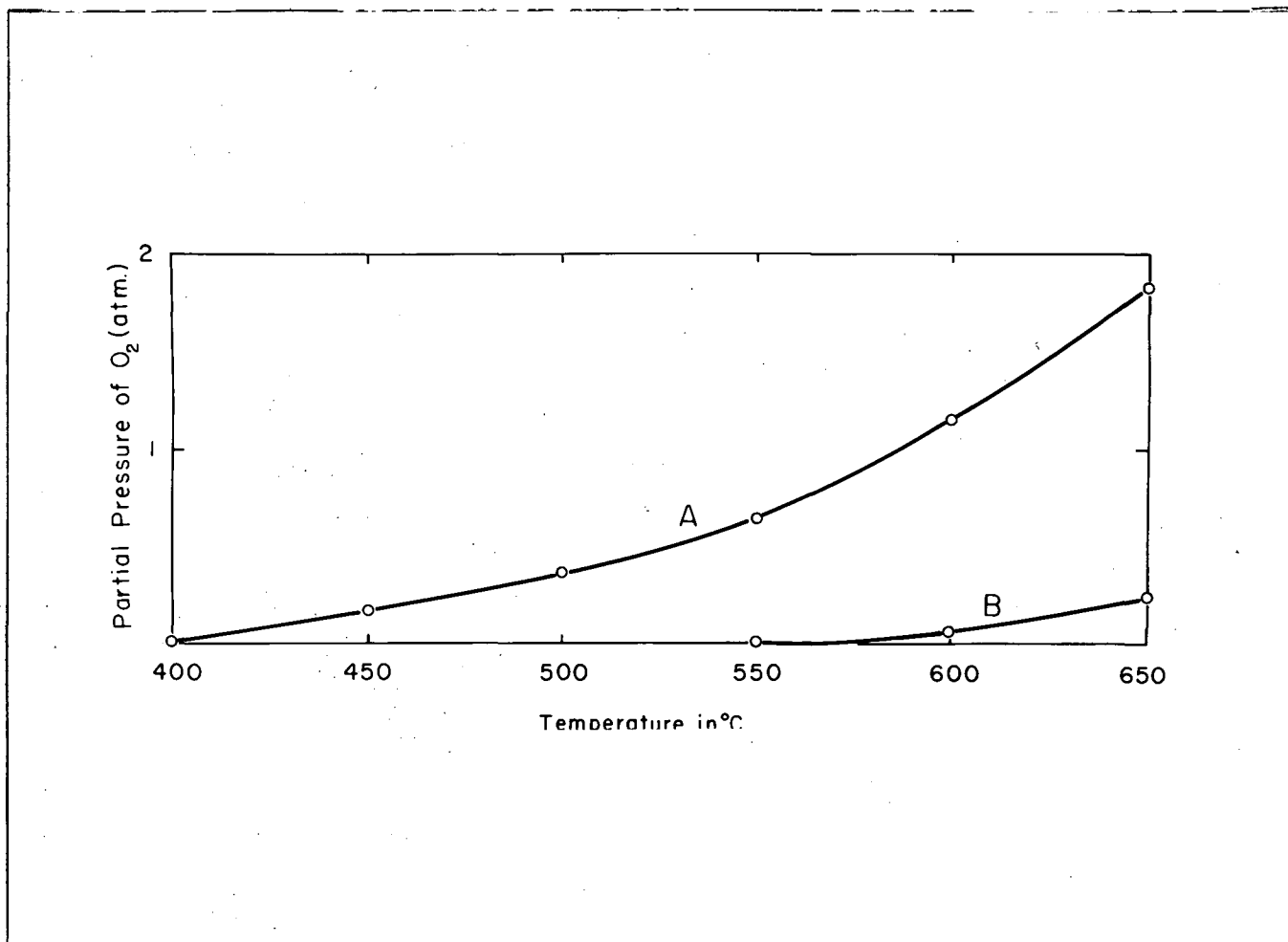


Figure 2

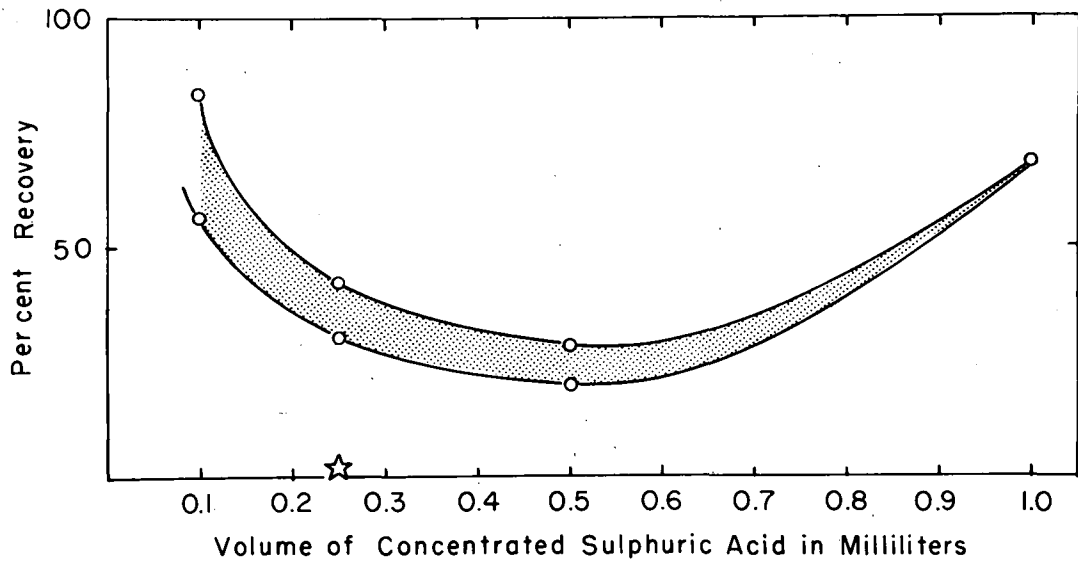


Figure 3

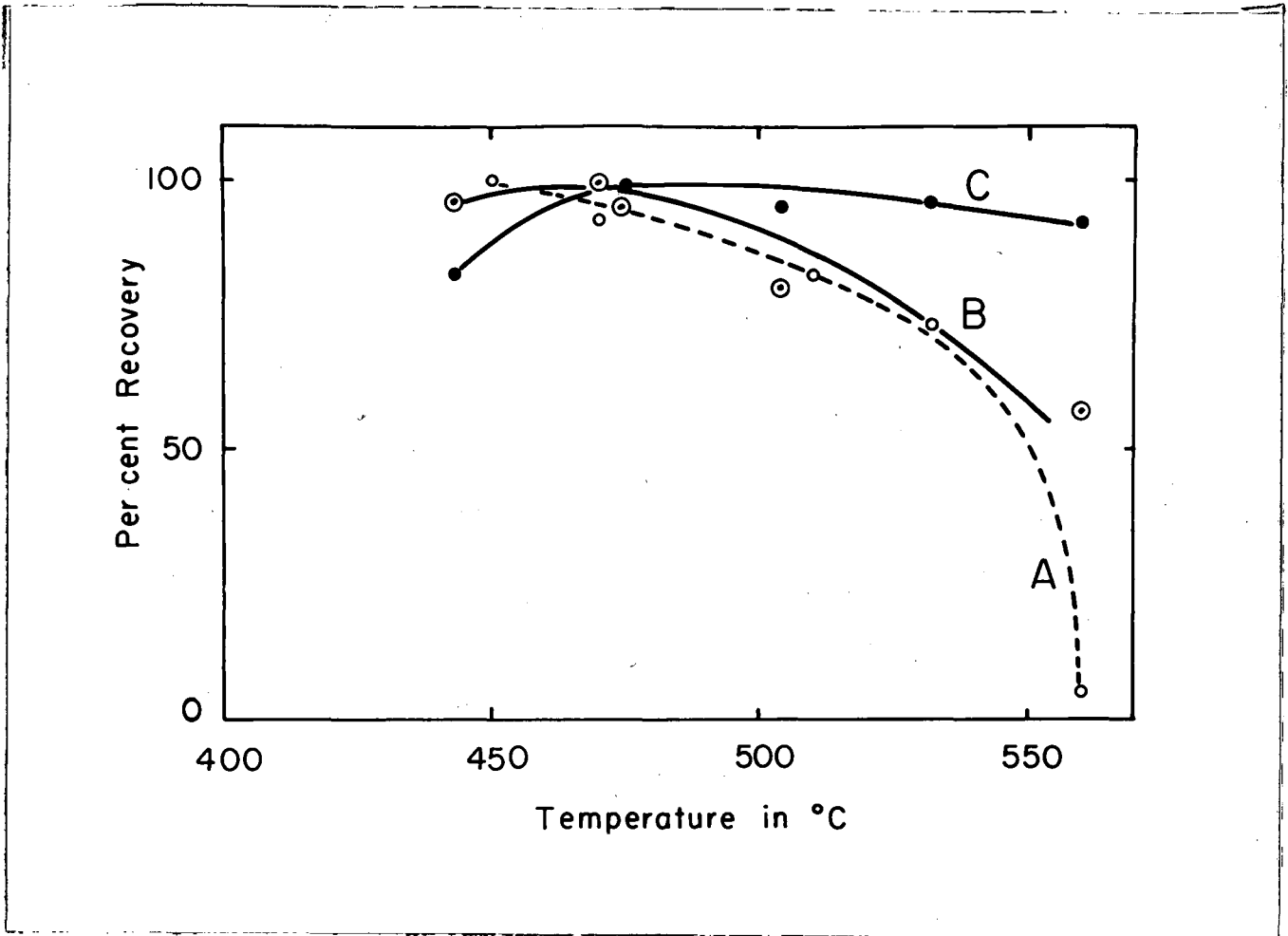


Figure 4

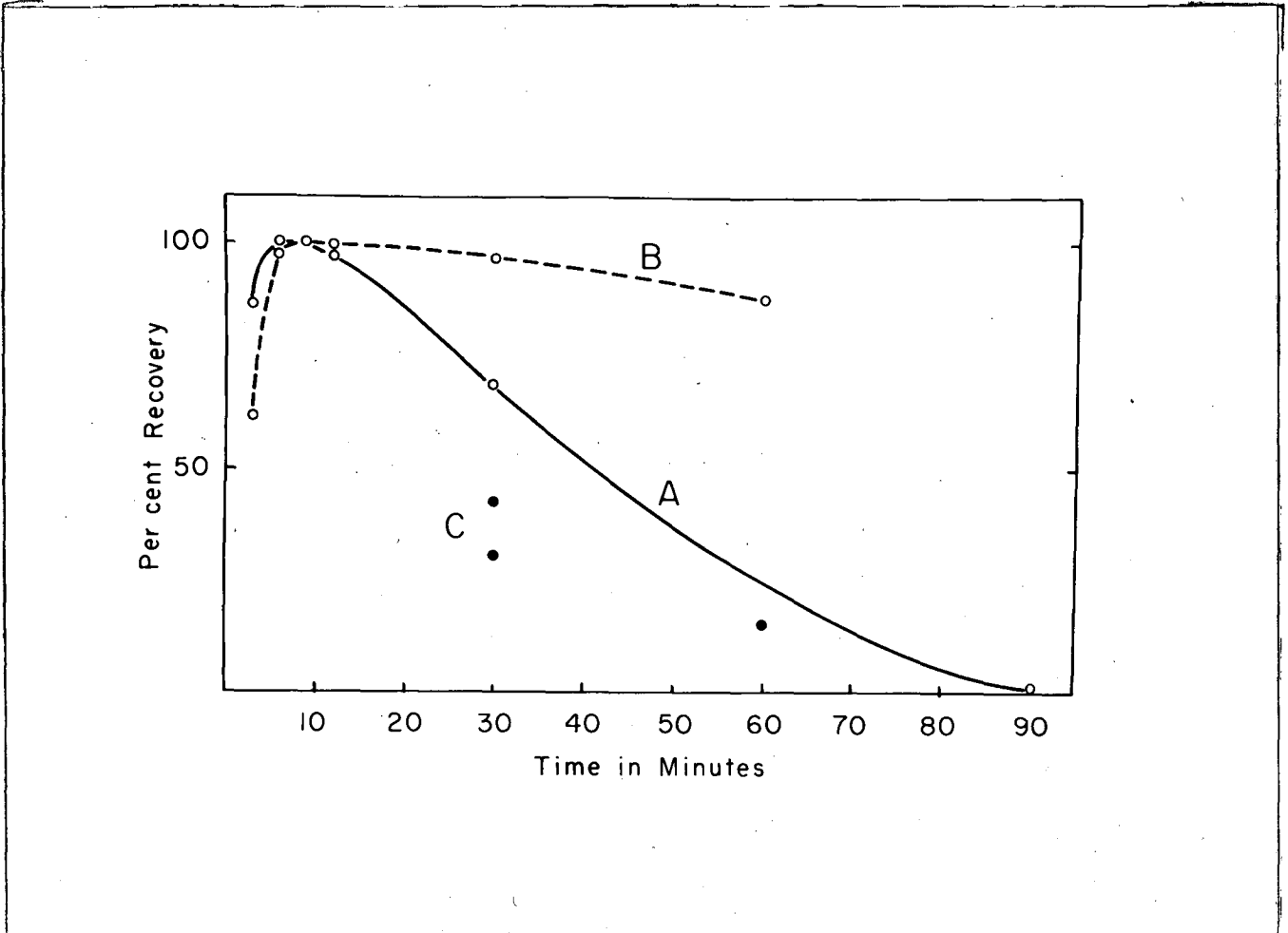


Figure 5

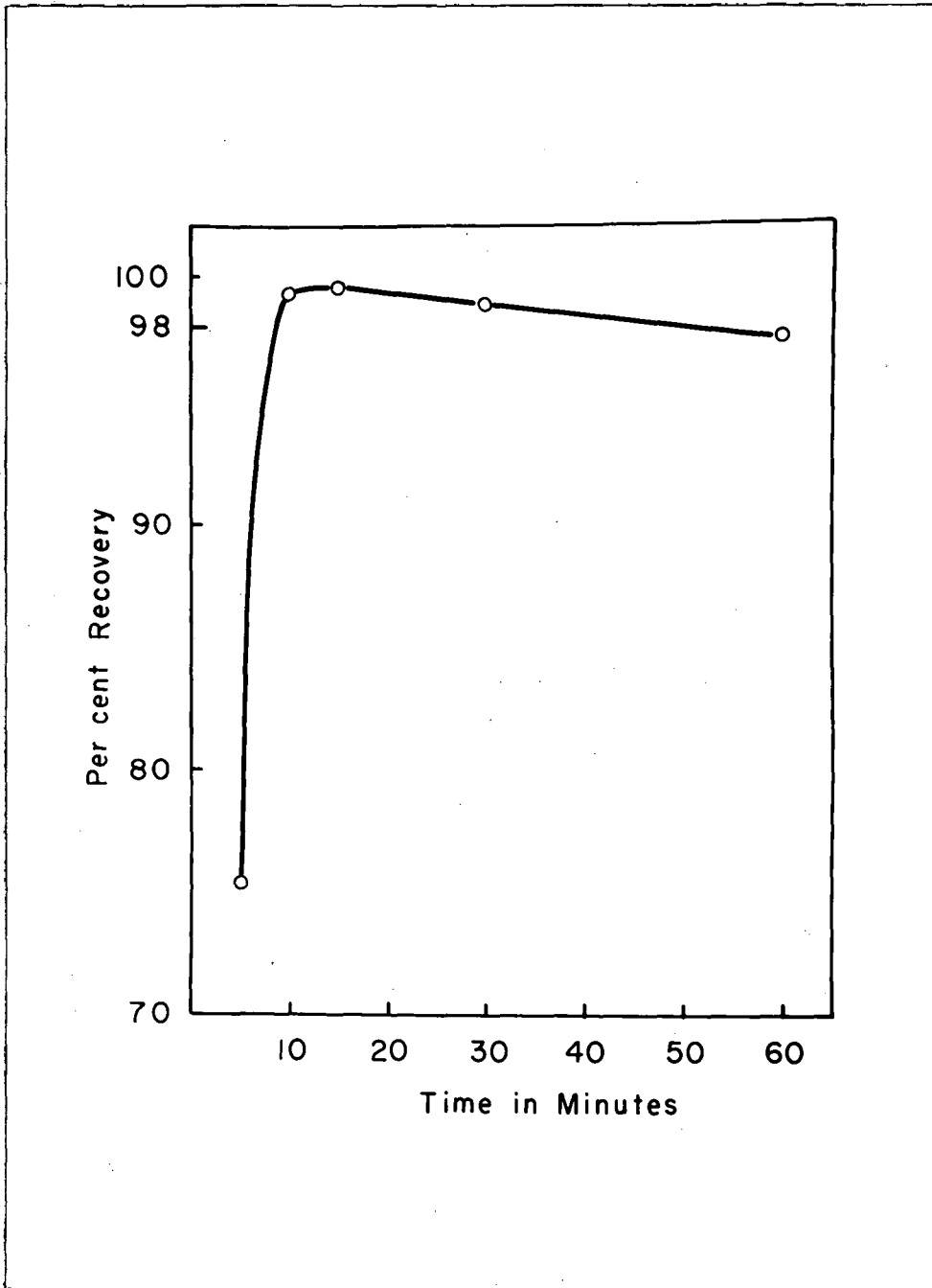


Figure 6