Abstract

DETERMINATION OF MERCURY BY FLAMELESS AAS

The procedure outlined describes an extremely sensitive and accurate method for the determination of mercury down to 50.0 ppb in solution. The procedure has been adapted to biological samples such as fish, brains and moldy wheat. The sample is taken into solution using wet digestion and an oxidizing agent. Mercury is then reduced to an elemental state and aerated from the solution in a reaction flask. The mercury vapor passes through an absorption cell of an atomic absorption spectrophotometer where its concentration is measured. The procedure is practically free from interferences except in extreme cases of organic vapors and the method shows a positive error.

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DETERMINATION OF MERCURY BY FLAMELESS AAS

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STATEMENT OF PROBLEM

The original problem was to apply a flameless atomic absorption spectroscopy (AAS) method for the determination of sub-microgram quantities of mercury in biological samples, specifically, lateral line muscle tissue from white bass captured within the state of Kansas, under contract for the Kansas Forestry, Fish and Game Commission. However, when the published analytical method of Hatch and Ott was applied, inconsistent results were recorded. The original problem was then changed to a two part problem, in order to include the development of a modified analytical procedure from the published method and application to biological samples. After revising the analytical method, parameters were needed to establish the desired degree of accuracy and consistency.

INTRODUCTION

In recent years findings of high levels of mercury in marine fish, such as the tuna and swordfish, have emphasized the influence of heavy metals on natural cycles and man's impact on those cycles. Man's use of mercury compounds has threatened bird populations in Sweden and contaminated some lakes and rivers in the United States to the point that fish are unsafe to eat.[1,2]

Mercury from human activities is a contributing factor to the contamination of the environment. Mercury is added to the environment through broken thermometers, burning of fossil fuels and ores, plastic wastes tainted with mercury catalysts, aqueous industrial wastes from chloro-alkali plants, and papermaking and grain seed dressings. Natural sources of mercury include the weathering of terresital rocks and soil. These releases of mercury by man each year probably constitute only a small fraction of the amount already in the seas and oceans.[1,2,3]

Poisoning due to the consumption of mercury is a poorly understood phenomenon. Recent experiments reveal that metallic mercury and mercuric and mercurous ion compounds usually attack the liver and kidney when ingested at toxic levels, while methyl mercury and other related alkyl mercurials attack the central nervous system and are retained in the body for long periods of time. Retention time studies have shown that methyl mercury in the human body has a half-life of seventy days; so even with low dosage, toxic levels may be reached.[1]

What actually constitutes a lethal concentration of methyl mercury in man is uncertain. Tolerance levels are observed to vary with the individual. The Food and Drug Administration set a weekly maximum consumption of four hundred and twenty grams of fish containing five hundred parts per billion (ppb) mercury for a full grown man and less for children. Persons who depend on fish for a major part of their diet could easily exceed this level.[1,6]

In 1964, Jack Halpern and J.P. Maher at the University of Chicago, had a demonstration showing that a model compound of Vitamin B_{12} could react with mercuric ion to give a methyl mercury compound. After a few years, J.M. Wood and co-workers had a demonstration showing that mono-methyl and di-methyl mercury compounds are capable of being synthesized in enzymatic-nonenzymatic biological systems. This confirms that biosysthesis could occur in anerobic and aerobic systems. The amount of product

(methyl mercury) is dependent on the concentration of mercuric ions and on the concentration of microorganisms. The concentration of microorganisms is, in turn, dependent on the concentrations of carbon, nitrogen, and phosphorus compounds in the water. The discovery that inorganic mercury could be alkylated in natural systems by microorganisms pointed out the present dimensions of mercury pollution of water and its toxicological implication as related to the resultant contamination of fish and, ultimately, of the human diet. Fish concentrate methyl mercury by means of their gills, so that their flesh contains concentrations several thousands times higher than the surrounding water.[4,7,10]

A variety of methods suitable for the determination of trace amounts of mercury in the ppb range are published. The need for these analytical techniques illustrates reports of environmental mercury pollution and the toxic levels in some areas. The most popular and sensitive of current analytical methods are based on spectroscopic instrumentation.[8,9,11]

The instrumental methods used extensively in this type of analysis work are either neutron activation analysis (NAA) or atomic absorption spectroscopy method. The NAA method uses an acid dissoluted sample, coupled with a chemical separation specific for mercury and an irradiation of the sample. The NAA method is extremely accurate but require sophisticated equipment, ususally an atomic reactor.[12]

The AAS method of determining mercury by a flameless technique is shown to be sensitive and accurate at the ppb range by the popular method of Hatch and Ott. The analytical procedure over the last several years is the most employed overall. In the standard method, the 253.7 nanometers (nm) radiation from a mercury line source (hollow cathode lamp) is focused onto a long path absorption cell containing the mercury vapor, and the absorption is registered automatically. The method utilizes a reducing reagent (stannous sulfate) and a carrier gas to sweep the mercury vapor through the absorption cell. The flameless AAS method is extremely sensitive and accurate but usually records a positive error where compared against recoverability of spiked samples.[11,13]

There are a few advantages of the cold mercury vapor technique as compared to other methods. The AAS instrument is relatively inexpensive, the sensitivity is extremely good, and simple attachments are available or can be constructed by glass blowers allowing any conventional AAS instruments to be used in the test. The AAS flameless method is also versatile, a large number of samples can be ran in a day, and the method is usually free of interference.[11,14,15,16]

The disadvantages of the cold mercury vapor technique are few. The AAS method is only quantitative not qualitative, there is a destruction of the sample, determination of more than one element not possible, and a limited number of elements are analyzed and some cation/anion interferences exist. The major interference is the spectral interference. The most severe of these is the nonspecific absorption that takes place when organic vapors are present with the mercury vapor within the absorption cell. Many common organics that are used frequently in the laboratory exhibit broadband absorption at the 253.7-nm line, and can lead to positive errors. The major way of overcoming the background absorption is to measure the interference and then subtract it from the total absorption, and the mercury content is found by the difference.[17,18]

THE HATCH AND OTT METHOD

Flameless AAS methods for the determination of mercury are sensitive and rapid due to the ready volatilization of mercury from an acid reducing solution at room temperature and the high absorbance of mercury vapor at the 253.7-nm line. The analytical procedure used in this research was based on the method of W.R. Hatch and W.L. Ott for the determination of sub-microgram quantities of mercury, which is reported to be extremely sensitive and accurate for concentrations down to 1.0 parts per billion.[13]

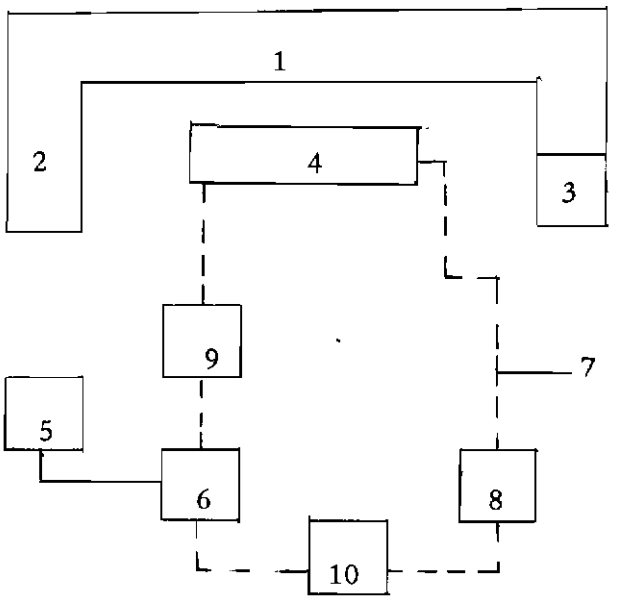
The method of producing volatile mercury from an acid solution using a stannous ion reagent is demonstrated in the published analytical method of Hatch and Ott. The acid digestion releases the mercuric, mercurous or some form of methylated mercury ions, if present. The acid digestion is followed by an oxidation step using a potassium permanganate solution. The reduction is accomplished by adding excess stannous ion to the oxidized sample mixture. The mercuric ion is reduced to elemental mercury or a volatile form of methyl mercury. The sample mixture is then aerated in a simple recirculating system and the absorbance is measured automatically at the 253.7-nm line and recorded. The method employs the simple principle of reduction of a volatile substance coupled with aeration using air as the carrier gas.[13]

The major components of the AAS apparatus of Hatch and Ott are: (Figure 1) 1) Atomic absorption Spectrophotometer–Perkin-Elmer 303 and 2) Mercury Hollow Cathode Tube–Westinghouse WL-22847, argon filled and 3) Recorder–Sargent Model SRL using a direct logarithmic mode for direct absorbance measurement and 4) Absorption Cell–constructed from borosilicate glass tubing (25 millimeter (mm) outside diameter (o.d.) by 15 centimeters (cm); quartz windows are cemented on each end (25mm diameter x 2mm thickness): gas inlets and outlets are placed 2 cm from each end as a source of placing the mercury vapor in the path of the cathode tube beam and 5) Variac–0 to 130 volts for controlling pump speed and 6) Neptune Dyna-Pump, Model 3–the pump is disassembled and sprayed with a clear plastic to resist corrosion and 7) Aeration Tubing–made from borasilicate glass of 5mm inside diameter (i.d.) and 8) Drier–suction filtration flask containing 19 grams of magnesium perchlorate for drying and 9) purging unit and 10) round bottom reactor flask.[13]

Tygon tubing is used to connect the pieces of the apparatus. The mercury vapors are generated in the reactor flask with the addition of the reductant and stirring. The carrier gas

SIMULATED DIAGRAM OF HATCH AND OTT'S RECIRCULATING SYSTEM FOR THE DETECTION OF COLD MERCURY VAPOR

FIGURE 1



1. Atomic Absorption Spectrophotometer.
2. Mercury Hollow Cathode Tube.
3. Recorder.
4. Absorption Cell.
5. Variac.
6. Neptune Dyna-Pump.
7. Aeration Tubing.
8. Drier
9. Purging Unit.
10. Reactor Flask.

enters the reaction flask and sweeps the vapors into the drier. The dry mercury vapors and carrier gas are then introduced into the absorption cell for detection and returned through the system over and over again. The recirculation of the mercury vapor is continued until a constant value is recorded automatically. A bleeding device is used to purge the vapors from the reaction system upon completion of analysis. After a complete cleaning, the system is ready for another sample.[13]

A trial model of the apparatus system of Hatch and Ott was set up in the laboratory. A few substitutions in equipment were needed but a recirculating system was assembled. Standards and reagents were prepared and eight attempts were made and inconsistent results were recorded (Table 1). The system was checked thoroughly and compacted to reduce air space within the system and inconsistent results were still recorded. After considering the published method and some of the techniques used in the method, the problem was changed from the application of the published analytical technique to devising a method that would be sensitive, consistent and accurate. The basic principle of reduction– aeration and wet digestion did not need adjusting; the problem was in the apparatus system.

RESULTS USING THE RECIRCULATING SYSTEM
OF HATCH AND OTT

TABLE 1

Nanogram Mercury Added	Absorbance 60 sec.	Absorbance 90 sec.	Absorbance 120 sec.	Absorbance 180 sec.
100.0	0.20	0.09	0.01	0.00
100.0	0.18	0.08	0.05	0.00
100.0	0.15	0.12	0.03	0.00
100.0	0.19	0.04	0.00	0.00
200.0	0.21	0.10	0.02	0.01
200.0	0.29	0.14	0.04	0.00
200.0	0.10	0.09	0.01	0.00
200.0	0.31	0.11	0.05	0.00

RESULTS AFTER COMPACTING RECIRCULATING
SYSTEM AND RESEALING PUMP

Nanogram Mercury Added	Absorbance 60 sec.	Absorbance 90 sec.	Absorbance 120 sec.	Absorbance 180 sec.
100.0	0.19	0.11	0.05	0.00
100.0	0.25	0.17	0.08	0.02
100.0	0.24	0.19	0.11	0.01
100.0	0.23	0.13	0.12	0.00
200.0	0.33	0.19	0.09	0.00
200.0	0.28	0.22	0.08	0.03
200.0	0.30	0.14	0.01	0.02
200.0	0.22	0.11	0.01	0.00

EXPERIMENTAL WORK WITH BASIC PRINCIPLES FROM HATCH AND OTT'S METHOD

The published analytical technique uses the simple principle of reduction-aeration of a volatile substance.[13] Further explanation of these principles and apparatus system will better define where the problem lies in the application of the cold mercury vapor technique. Examples to show how the principles relate to experimental work are provided.

A mercuric chloride standard was used to explain the chemistry of the reduction of mercuric ions to metallic mercury. The reactions followed this format:

1) $Sn^{++} + 2HgCl_2 + 4Cl^- \longrightarrow SnCl_6^{=} + Hg_2Cl_2$

2) Excess $Sn^{++} + Hg_2Cl_2 + 4Cl^- \longrightarrow SnCl_6^{=} + 2Hg^o$

As mercury (II) has a rather high standard reduction potential (0.854 Volt (V)), it was readily reduced to metal by excess stannous ion. In equation number one, the stannous ion was introduced slowly to the mercury (II) thus reducing this to mercury (I) and precipitating (ppt) a mercurous chloride product. With excess stannous ion introduced the mercury (I) further reduces to metallic mercury as seen in equation number two. The metallic mercury ppt. was aerated and swept by a carrier gas to the absorption cell for detection.[19]

Care was taken to assure complete volatization in the reactor flask. The method employed the other principle – aeration of a volatile substance. This method utilizes the fact that metallic mercury is the only element (other than inert gasses) that has an appreciable vapor pressure at room temperature, and whose vapor is practically wholly monoatomic. Metallic mercury is also characterized by a low affinity for oxygen and therefore a relatively high concentration of mercury atomic vapor can be maintained in the air at room temperature. Because of this relatively high vapor pressure exerted by mercury, no thermal energy from a flame (or other source) is required for vaporization and atomization of elemental mercury.

The high vapor pressure of metallic mercury was the reason for the principle of aeration. An inspection of chemical tables shows that the vapor pressure of metallic mercury is 0.0012 millimeters mercury at 20 degrees Centrigrade. Applying the ideal gas law, Pressure (P) x Volume (v)= number of moles (N) x gas constant (R) x temperature (T)

and quantitatively determining the micrograms of metallic mercury per liter, assuming the volume of the flask was two hundred fifty milliliters (ml), the conditions suggest a maximum of 1.289 micrograms are volatile. The quantitative determination serves as a rough check on complete vaporization since metallic mercury is not an ideal gas.[13,20,21]

The basic principles of reduction-aeration were applied to the research mercury standard solutions and the reactor flask to be studied. The next consideration was with the apparatus assembly, reagents and instrumentation. Sensitivity and accuracy are possible but both are dependent upon the conditions of the system. Data is to be presented and discussed to fully explain all parameters for the revised analytical technique.

EXPERIMENTAL DETAILS

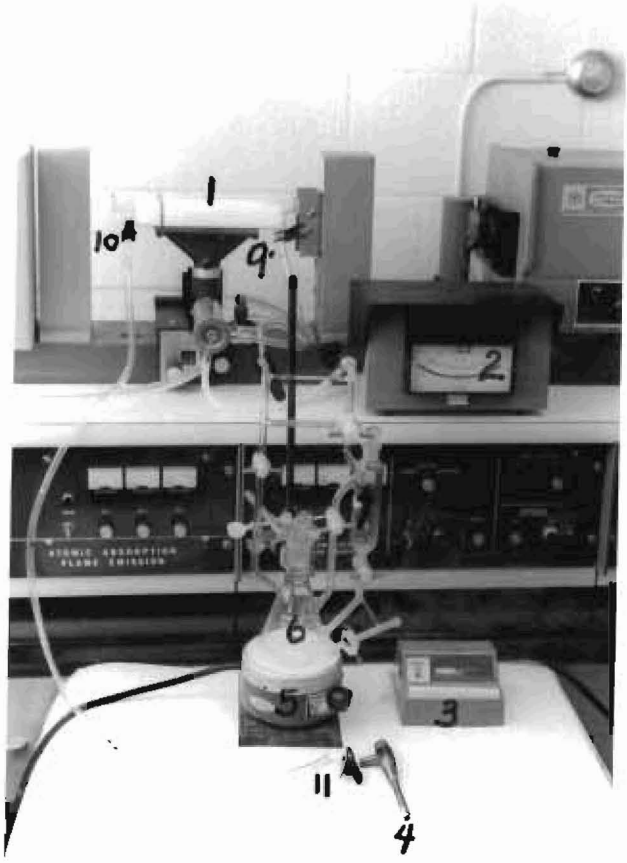
The research demonstrated the use of the open system opposed to the recirculatory system of Hatch and Ott's. The Hatch and Ott method recirculates the vapor through the absorption cell and reactor flask using a pump until a constant value is displayed automatically. After recording the absorption, the system is purged and another sample is analyzed.[13] Hatch and Ott's system was tried but values were inconsistent because the absorbance normally decreased rapidly within seconds of reaching a maximum. This loss was not traced but was probably caused by dilution of the vapor within the system or possibly a loss through the opening of the reactor flask for additions of sample and reductant. The equal dispersement is a basis for the Hatch and Ott method but results were inconsistent when applied in the laboratory (Figure 1). The equal dispersement of the vapor would certainly decrease the amount of mercury vapor in the absorption cell per unit space and unit time, such that, the dilution factor of the additional space in the apparatus reduced the absorption value beyond a detection limit or to almost a null point. To combat the large dilution factor, the tygon tubing used to connect the separate pieces of apparatus was shortened for the purpose of compacting the recirculating system of Hatch and Ott. Even though this system was compacted, consistent results were not recorded (Table 1). The later probable cause of dilution will be discussed later.

After finding that a recirculatory system did not work efficiently, an open system was devised and tried. In Figure 2 an apparatus set up of the open system was shown. The major components were: (1) the reactor flask with chamber, and (2) absorption cell mounted in the path of the hollow cathode tube, and (3) aspirator pump, and (4) the tygon tubing used to connect each piece of the apparatus system. The carrier gas entered at point 8 in Figure 2 and swept the cold vapor from the reactor flask into the inlet point 9 of the absorption cell. After the detection, the cold vapors were discharged through exit point 10 of the absorption cell and later through the aspirator terminal.

The open system (Figure 2) allowed the vapors to be swept through the cuvette by a carrier gas and immediately discharged. In this system, the vapor was pulled through as a "slug" and not allowed to disperse into the open space within the system. The slug size was dependent upon the flow rate of the carrier gas; such that, the slower the flow rate of the carrier gas, the density of vapor decreased and a lower percent absorption was recorded.

THE OPEN, NON-RECIRCULATING SYSTEM
FOR DETERMINING MERCURY VAPOR

FIGURE 2



1. Absorption cell with holder.
2. The meter.
3. Timer.
4. Aspiration pump.
5. Magnetic stirrer.
6. Reactor flask chamber A.
7. Reductant cylinder chamber B.
8. Entrance for carrier gas.
9. Entrance for carrier gas and mercury vapor into absorption cell.
10. Exit point of absorption cell.
11. Final discharge.

Thus the open system had consistent results with a negligible dilution factor to be accounted for.

In figure 2A an apparatus set up of the open system is shown again. The major components of this system were: (1) the reactor flask with chamber, and (2) the absorption cell mounted in the path of the hollow cathode tube and (3) the vibrator pump, and (4) the tygon tubing used to connect each piece of the apparatus system. The carrier gas was "pushed" instead of "pulled" through point 8 in figure 2A. Thus the only difference in figure 2 and 2A was the way the cold mercury vapors were transferred from the reactor flask to the absorption cell.

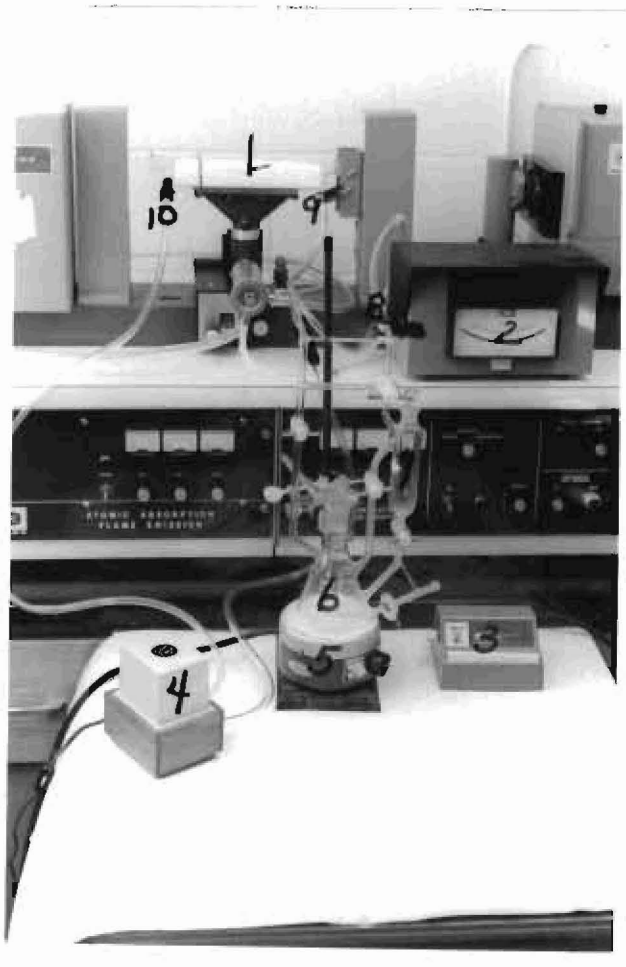
The use of an open, non-recirculating system eliminated the problem of obtaining a reliable, corrision proof pump. The pumps which were used either pushed (diaphragm pump) (Figure 2A) or pulled (aspirator pump) (Figure 2) the carrier gas through the absorption cell. A diaphragm pump was hooked up to entrance designated by point 8 Figure 2A. Neither pump was truely capable of recirculating the cold vapor reliably. The diaphragm pump could have recirculated the vapors, but corrision or possibly residual mercury could cause errors in analysis. Each pump gave dependable data with no maintanence in the open system.

The setup of Figures 2 and 2A was necessary to establish versatility of the method's pumping system. The versatility was demonstrated in that different pumping system's (aspirator or diaphragm pumps) could be used without disturbing the sensitivity and accuracy of the method. The figures also give a direct insight to the hookups of the systems. The two systems were both used in the project but Figure 2A was used consistently after the parameters involving different flow rates were completed.

The ability to handle a constant maximum amount of carrier gas in the range of twelve hundred fifty milliliters per minute (m1/min) was a pump requirement. The carrier gas capacity became an evident problem since not all pumps had this capacity. The capacity of the aspirator pump was dependent upon the amount of water pressure and the diaphragm pump was dependent on the pump size, number of vibrations per minute and efficiency of the pump. The aspirator pump was obtained from a laboratory stock and a trial and error process was ran on each to determine their capacity. The diaphragm pump was ordered directly because the capactiy was assured to be one thousand m1/min. Both pumps were chosen for their capacity and reliability. (Lower flow rates of carrier gas were obtained by muffling the output from each pump. Flow rates of six hundred fifty m1/min

A REVISED OPEN, NON-RECIRCULATING SYSTEM FOR THE DETERMINING OF MERCURY VAPOR BY USING A VIBRATOR PUMP

FIGURE 2A



1. Absorption cell with holder.
2. The meter.
3. Timer.
4. Vibrator pump.
5. Magnetic stirrer.
6. Reactor Flask chamber A.
7. Reductant cylinder chamber B.
8. Entrance for carrier gas.
9. Entrance for carrier gas and mercury vapor into absorption cell.
10. The exit point of absorption cell.

twelve hundred fifty ml/min were used to establish parameters for experimental work.) The aspirator pump was used more often because of the consistency, corrision resistent and the ability to flush the used sample from the reactor flask with a simple disconnect and hook up to the aspirator terminal of the reaction flask.

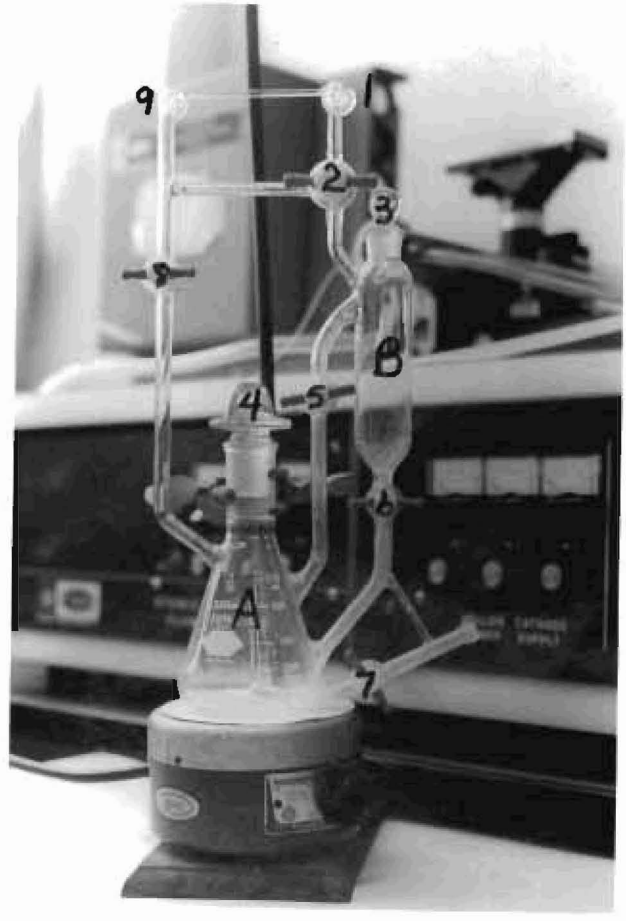
The flow rate of carrier gas was measured by using a buret, a soap solution and a timer graduated in hundreths of a minute. This apparatus was assembled in the following manner: (1) a ring stand was placed on a table and a clamp was attached to the stand, and (2) the one hundred milliliter buret was secured in a vertical position and (3) T-shaped nipple was attached to the lower end of the buret containing the soapy solution and (4) a piece of tygon tubing was attached to the horizontal end of the T-shaped nipple or the upper end of the buret, dependent upon the pump used. In the operation of this apparatus, the pump was started and a small amount of soap solution was injected into the stream of carrier gas. At the junction of the carrier gas and soap suds, a bubble was produced and the stream of carrier gas moved this bubble through the buret. One bubble was followed over a unit time and distance and the flow rate was figured in ml/min.

The open system used a different reactor apparatus. Pictured in Figure 3 is a complete schematic of the reactor flask. The reductant was retained in chamber B and the analysis sample in chamber A. All stopcocks were in a closed position, except for stopcock number 2 which diverts the carrier gas directly to the absorption cell until a unit time has elasped, in such case, the stopcock was turned 120 degrees, diverting the carrier gas through chamber B and chamber A. Stopcocks numbers 5 and 6 in this case were already opened for the addition of reductant. The stopcock number 8 was turned 90 degrees to allow the vapors to be swept toward the absorption cell. There were other ways to accomplish the transference of the carrier gas and mercury vapor. The stopcocks were all opened except for number 8. The aspirator pump was hooked up to pull the carrier gas directly through point number 1 and stopcocks numbers 5 and 6. Subsequently the only stopcock needed to be turned was number 8. In either case, the mercury vapors are transferred with minimum effort on the part of the operator. The fewer the operations needed, the more accurate a reaction time was arrived at continually.

In Hatch and Ott's work the experimental apparatus contains many separate pieces (Figure 1).[13] Their system used a round bottom flask containing sample in which the reductant is added to the flask from a pipette through an opening at the mouth of the flask.

REACTOR FLASK USED IN THE OPEN SYSTEM

FIGURE 3



1. Entrance for carrier gas.
2. Stopcock which diverts carrier gas toward or away from reactor chamber.
3. Ground glass stopper for chamber B.
4. Ground glass stopper for reactor chamber A.
5. Stopcock that regulates air flow and reductant addition.
6. Stopcock which regulates air flow and reductant addition.
7. Stopcock utilized in discharge of spent sample.
8. Stopcock regulating flow of carrier gas and mercury vapor toward absorption cell.
9. Exit point from reactor assembly.

At this stage in their technique, the sample mixture is exposed to the atmosphere, thus possibly allowing volatilization of some mercury. This loss may only be negligible but is significant at the sub-microgram level. A new reactor flask was designed to be a complete unit, in such case the reductant was contained in a separate chamber (B) and was directly attached to reactor chamber (A) (Figure 3). The direct attachment of these two vital chambers eliminated any loss of mercury vapor to the atmosphere, minimizing time of transit and dilution of vapor.

In the picture of Figure 4 a schematic of the absorption cell was pictured. The point number 1 was where the carrier gas and mercury vapor entered. The point number 2 was where the carrier gas and mercury was discharged. These two points could be interchanged if consistency was used in transferring the mercury vapors from reactor flask to absorption cell. Point number 3 designated the absorption cell glass tube.

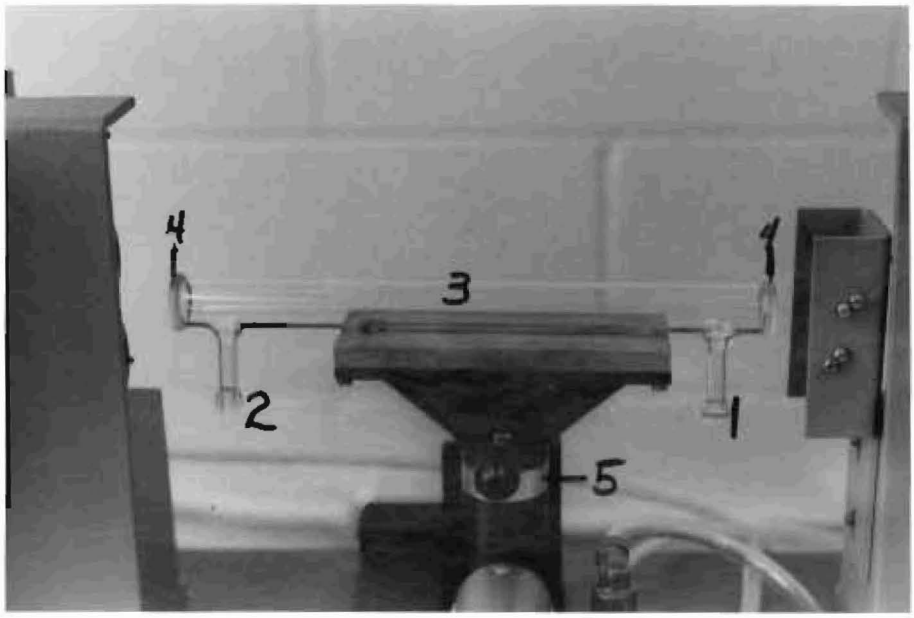
In the non-recirculating system the mercury vapor was generated in the reactor flask and was not exposed to any outside atmosphere. An aspirator pump was hooked up to pull the carrier gas through point 1 of Figure 3 and sweep the vapor into absorption cell through point 1 (Figure 4). The vapor was passed through the absorption cell for detection and then discharged through point 2. The system was simple to compact in comparison to the Hatch and Ott apparatus system.

Another consideration was the elimination of a drying agent for the system. The mercury vapor and carrier gas, as it is swept from the reactor flask, was not dried before entering the absorption cell. The Hatch and Ott recirculating system uses a drying agent (Figure1) but the open system eliminated this component (Figure 2). No substantially large interference was observed at the 253.7-nm line using just the chemical solutions in the procedures, but there were recorded values of the one per cent absorption in some cases. This minimal value might possibly be instrumental noise even though the suppression setting was number two. With this assumption taken into account, all sample values were not corrected by substracting the absorbance of 0.0044 from each value.

The experimental apparatus has been assembled and the open, nonrecirculatory system has been explained. Modifications to Hatch and Ott's analytical technique have been developed and these were: (1) use of nonrecirculatory system and (2) no drier and (3) different reactor flask assemble and (4) different aeration pumps. The results from these modifications were later applied to standard and spiked samples to ensure reproducibility,

ABSORPTION CELL

FIGURE 4



1. Entrance.
2. Exit.
3. Absorption tube.
4. Optical quartz windows sealed with epoxy glue.
5. Burner

accuracy , and consistency of the parameters. This was the first part of the problem -- revision of a published, popular analytical technique.

PROCEDURES OF MERCURY ANALYSIS

PROCEDURE OF SAMPLING

There were specific techniques in obtaining samples from fish and game birds. The sample of fish flesh from the white bass was taken by lancing the skin from the dorsal fin to the pectral fin to the ventral fin forming a triangle. A spatula was used to remove the lateral line muscle tissue from each fish and labels were attached to aluminium storage packages to designate location of capture for future reference after each sample of thirty grams was blended thoroughly with a mortor and pestal made from porcelain.

The technique of obtaining the brain from the pheasant, quail, or meadowlark was simple. In this technique either take a sharp dissecting knife or small dissecting sissors and cut the skull, removing all bone fragments that may contaminate the sample. A clean spatula was then used to dig out both lobes of the brain. These brain samples were not blended since the whole brain was used for the mercury analysis. Each brain sample was prepared the same day that the analysis was preformed.

The technique for obtaining a homongeneous sample of moldy wheat was difficult because the sample contained mold, dirt and sand. Since only a small amount of moldy wheat was salvaged from a grain elevator, two methods were used to obtain an analysis sample. In the first method the moldy wheat was dried at ninety degrees Farenheit and then ground to form a composite mixture. In the next method the moldy wheat was ground throughly without any drying before the analysis sample was taken. Each method was used repeatedly to ensure reproducibility and a practical approach to sampling. All samples were supplied by the Kansas Forestry Fish and Game Commission and geographical locations were logged in table 2 where capture was made.

GEOGRAPHIC LOCATIONS OF CAPTURED SAMPLES

TABLE 2

5	White Bass from Arkansas River at Arkansas City, Kansas.
2	White Bass from Milford Reservoir at Wakefield, Kansas.
7	Total (Collected 9/71).
2	Quail (male) from Pratt County.
2	Pheasants (male, female) from Pratt County.
4	Pheasants (male) from Pawnee County.
7	Pheasants (male) from Jewell County.
15	Total (Collected 11/71).
1	Moldy wheat sample from Lindsborg, Kansas.
1	Total (Collected 3/25/72).

WET DIGESTION

The destruction of the biological sample was carried out by wet digestion using concentrated sulfuric acid (1.84 specific gravity) at fifty-five degrees Centrigrade in a shaking water bath. The procedure for the dissolution of an analysis sample was completed as follows:

A weighed sample was placed in the bottom of a Kjeldahl flask, assuring that no sample was above the level of the five milliliters of sulfuric acid. The flask was incubated at fifty-five degrees Centrigrade until a clear, homogeneous solution was obtained. The color of the solution varied as to the type of material digested (fish--redish brown color, moldy wheat--dark brown to black, bird brain--light brown to brown). The complete dissolution of the sample was checked by using a one hundred fifty watt spot light. Upon a complete dissolution of the analysis sample, the Kjeldahl flask was removed from the shaking water bath, cooled and then fifteen milliliters of six percent potassium permanganate solution was added to the solution. The permanganated solution was allowed to stand for thirty minutes before actual analysis for mercury. The flask was then closed with polyethylene stoppers to assure no contamination or loss of mercury through evaporation. Samples at this stage can be kept for twenty-four hours with no apparent loss of mercury.[13,20]

Wet digestion technique variations were used in the digestion of the moldy wheat samples. Sample digestion of the dried ground wheat was accomplished by adding the same volume of sulfuric acid for digestion. The second method was accomplished by taking five milliliters of deionized water and two drops of sulfuric acid. Only the washings from both methods are used in the analysis since the moldy wheat contains some undissolved material, probably sand and dirt. The wet digestion technique was a versatile digestion technique in biological sample analysis because of the variation of proportions of acid and types of acids that could be used.[20]

The acid digestion technique caused some problems when potassium permanganate was added to the sulfuric acid in the digested solution. The first problem of the reaction was that it was very exothermic and could deteriorate the mercury content due to the possible loss through evaporation. Another observation was that there was much suspended material in a digested solution. This material usually dissolved to give a colorless or slightly opalescent solution in the last stages of the determination when the reductant was added. If the soltuion does not clear or show signs of completed dissolution, in this case, the wet digestion procedure needs to be repeated.[10]

LABORATORY TECHNIQUES INVOLVED USING REACTOR FLASK AND APPARATUS SYSTEM

After the digestion of the sample was completed using conc. sulfuric acid and the oxidation was completed using six percent potassium permanganate, the reactor flask and absorption cell was mounted on the atomic absorption spectrophotometer. The wavelength was adjusted to the 253.7-nm line and the slit setting was set at 100 X, damping set at the two and the absorption cell aligned for minimum absorbance. The reactor flask was clean with all stopcocks in the position shown (Figure 3). The carrier gas flow rate was adjusted to one thousand milliliter per minute and the magnetic stirrer was off. The instrument and apparatus was assembled and was ready for use in analysis.[20]

The digested oxidized sample contained in a Kjeldahl flask was transferred to the reactor flask A. A fifty milliliter aliquot of reductant was placed in a graduated cylinder. Two ten milliliter aliquots of reductant were used to completely rinse the Kjeldahl flask and these rinsings were added to the reactor flask A and stoppered quickly. The remaining reductant was added to funnel B. All stopcocks were replaced in the appropriate positions.[20]

After the sample was injected, the reactor flask was set up and the instrument was zeroed with the carrier gas flowing in the absorption cell. The taps number 5 and number 6 were opened to add the reductant to the reactor flask A, the stop watch was started while transferring the reductant and taps number 5 and number 6 were left opened. When sixty plus or minus two seconds elasped, tap number 8 was opened simultaneously with tap number 2 being turned one hundred twenty degrees clockwise opening the system so that the carrier gas entered through tap number 2. The varpors were swept from the reactor flask A by the carrier gas to the absorption cell for detection and then discharged. The meter reading was recorded automatically. The apparatus system was cleaned using diluted nitric acid, soap and water, and rinsed with deionized water. After this cleaning, the carrier gas was passed through the entire system to check for any residual mercury vapor within the system.[20]

The complete procedure for using the reactor flask was adapted from the work of a mercury determination method using wet digestion. Any variations which were used concerning the analytical technique are discussed in parameter section. This laboratory technique was used consistently throughout the research.

APPARATUS AND SOLUTIONS

Any conventional atomic absorption spectrophotometer could have been used in this research project which allowed for the mounting of an absorption cell in the path of the hollow cathode tube. Pictured in Figure 5 was the atomic absorption instrument used. The absorption cell was mounted directly in the path of the hollow cathode tube using the burner as a platform (Figure 4) and adjusting its height to align the center of the absorption cell with the cathode tube. The atmoic absorption spectrophotometer used was the Jarrell-Ash Model 82-500. The mercury cathode tube was manufactured by Westinghouse, and operating current of the cathode tube used was eight to ten amperes. The lower the operating current allowed for longer operational time of the cathode tube.

Another piece of the apparatus system was the flow-through absorption cell which was constructed of borosilicate glass tubing. The ends were closed off with optical quartz windows using an epoxy glue. The outside diameter was 2.4 centimeters and the overlength was 25.6 centimeters (Figure 4).

Another piece of the apparatus was the reactor flask. It was also constructed of borosilicate glass with polyethylene stoppers (Figure 3). Other pieces that were used: magnetic stirrer, an air pump that has a capability of moving one thousand milliliters of air per minute, an aspirator, air regulator, and one hundred milliliter buret, a stop watch graduated in hundreths of a minute, tygon tubing, ground glass fittings for assembly of apparatus system, Kjeldahl flasks (thirty milliliters), and fifty-five degree Centrigrade shaking water bath.

Reagents used in the analysis were an ACS analytical grade. These reagents included sodium chloride, hydroxylamine chloride, hydrated stannous chloride, mercuric chloride, concentrated (conc.) hydrochloric acid, conc. sulfuric acid (specific gravity 1.84) and potassium permanganate.

All solutions were prepared in advance of the experimental work. The reductant solution was prepared by mixing six hundred milliliters of water, one hundred milliliters of conc. hydrochloric acid, twenty grams of hydrated stannous chloride, thirty grams of hydroxylamine chloride and five grams of sodium chloride and then diluting to one liter with deionized water, in that order. The solution of six percent potassium permanganate was prepared by dissolving six grams potassium permanganate in ninety-four grams of

ATOMIC ABSORPTION INSTRUMENT
USED IN RESEARCH

FIGURE 5

1. Mounting platform for absorption cell or burner.
2. Meter.
3. Monochromator.
4. Mode selector switch.
5. Gain control switch.
6. Zero switch.
7. Damping switch.
8. On and off switch.
9. Hollow cathode tube.

water.[20]

The reductant was changed from stannous sulfate, hydroxylamine sulfate, sodium chloride and sulfuric acid to a stannous chloride, hydroxylamine, sodium chloride, and hydrochloric acid mixture. The stannous ion was the ion used in the reduction theory. This change was not significant since both solutions reduce mercury ions to mercury. Another chemical in the reductant was changed; the sulfuric acid was changed to hydrochloric acid. After this change, a report was found that linked hydrochloric acid to high absorbance values in similar analytical techniques. This possible problem was eliminated by pre-mixing the reducing reagent and hydrochloric acid. This procedure vaporized the mercury ions, if present. An analysis sample of each component was aerated in the reactor flask and the carrier gas was introduced to sweep the vapors through the absorption cell. The blank value for the reductant was one percent absorption. The one percent absorption value was substracted from each sample even though this value may only be instrumentation noise.[13,20]

PARAMETERS

With the first part of the problem completed and a theoretically sound method prepared, the next part of the problem was to establish parameters and then apply these to laboratory conditions. The conditions at which maximum response and accuracy were achieved will be discussed in the following sections. The major variables are flow rate of the carrier gas, volume of air within the reactor flask used for aeration of the sample, and the time of aeration of sample mixture before a carrier gas was introduced into the reactor flask for the vapor to be swept through the absorption cell for detection. All parameters were only applied to the AAS instrument, reactor flask, absorption cell and other related conditions set forth in the research techniques and procedures. For example, alterations in the length and diameter of the absorption cell may increase or decrease the sensitivity of the method (the longer the absorption cell and the smaller the diameter increases the absorption sensitivity).[9]

The first parameter established was that of absorbancevs. the flow rate of the carrier gas at two different concentrations. Thus the concentration of mercury vapor in the absorption cell was assumed dependent upon the flow rate of the carrier gas. To further explain this statement, data was taken using samples containing one hundred (C1) nanograms (ng) and three (C2) hundred ng per milliliter. The sample was aerated in the reactor chamber, after the reductant was added, for a time (t) with the flow rate of the carrier gas constant. The vapor and carrier gas was discharged into the absorption cell for detection and the results automatically recorded on the meter. The results presented in graph 3 and table 3 demonstrated the action of the cold mercury vapor. These results suggested that at lower flow rates of the carrier gas, the density of mercury vapor was less, such that the absorption was decreased. The higher the carrier gas flow rate introduced more mercury vapor into the absorption cell, thus increasing the absorption.

After demonstrating the dependency of the density of mercury vapor in the absorption cell upon the flow rate of the carrier gas at a constant aeration time, another parameter was established concerning the rate of reaction. The way the rate of reaction was determined was through a change in the time of aeration of the sample mixture. The dependency of the rate of reaction upon aeration time was shown in table 4 A, B and C graph 4 A, B and C. These results suggested that the rate of reaction was controllable and as the aeration time increased, the absorption increased. The same theory of "slug" movement

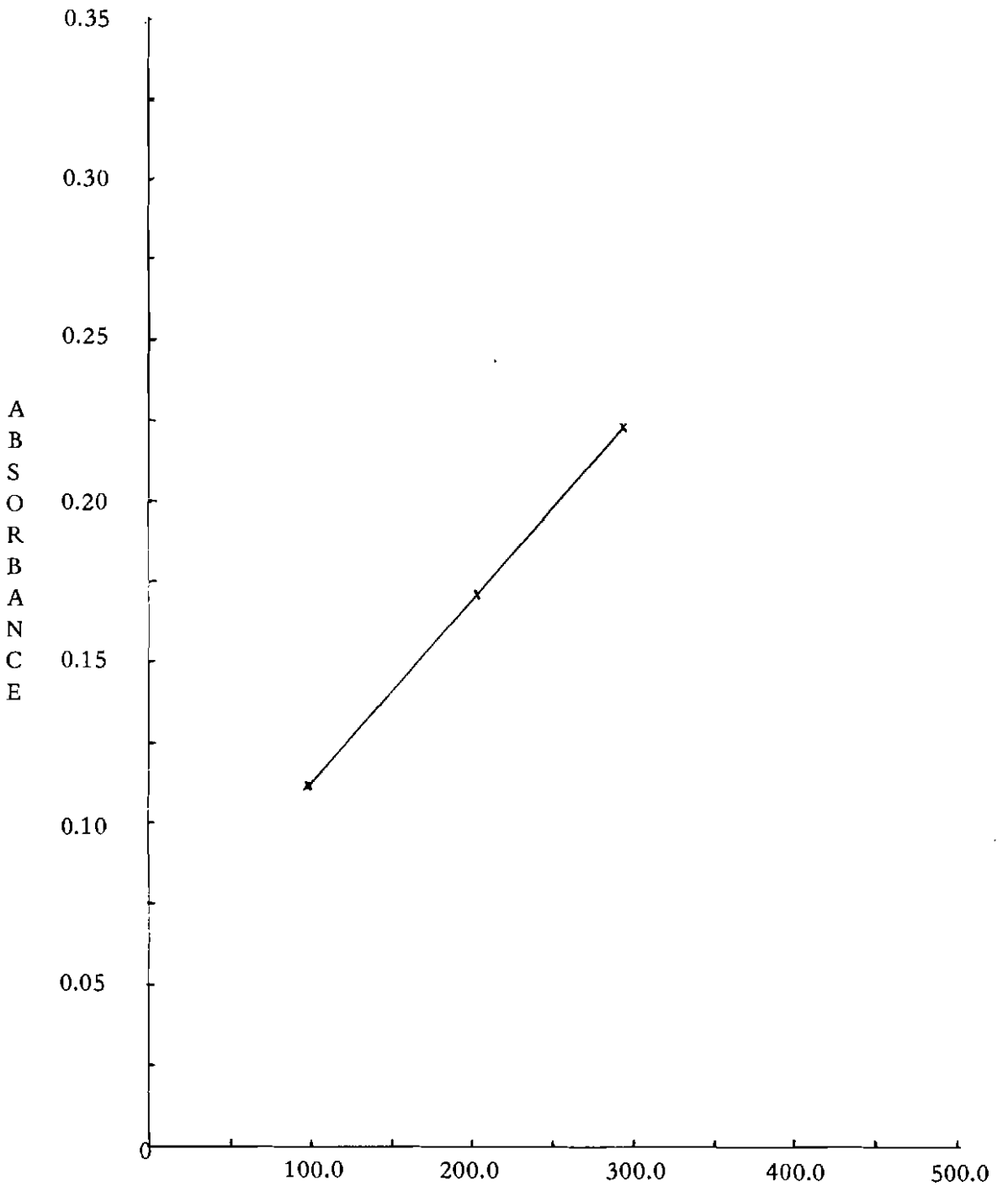
CONCENTRATION MERCURY VS. ABSORBANCE

STANDARD CALIBRATION CURVE DATA 1

Nanagrams of Mercury Added	Absorbance
100.0	0.1154
200.0	0.1700
300.0	0.2230

STANDARD CALIBRATION CURVE
CONCENTRATION MERCURY VS. ABSORBANCE

GRAPH 1



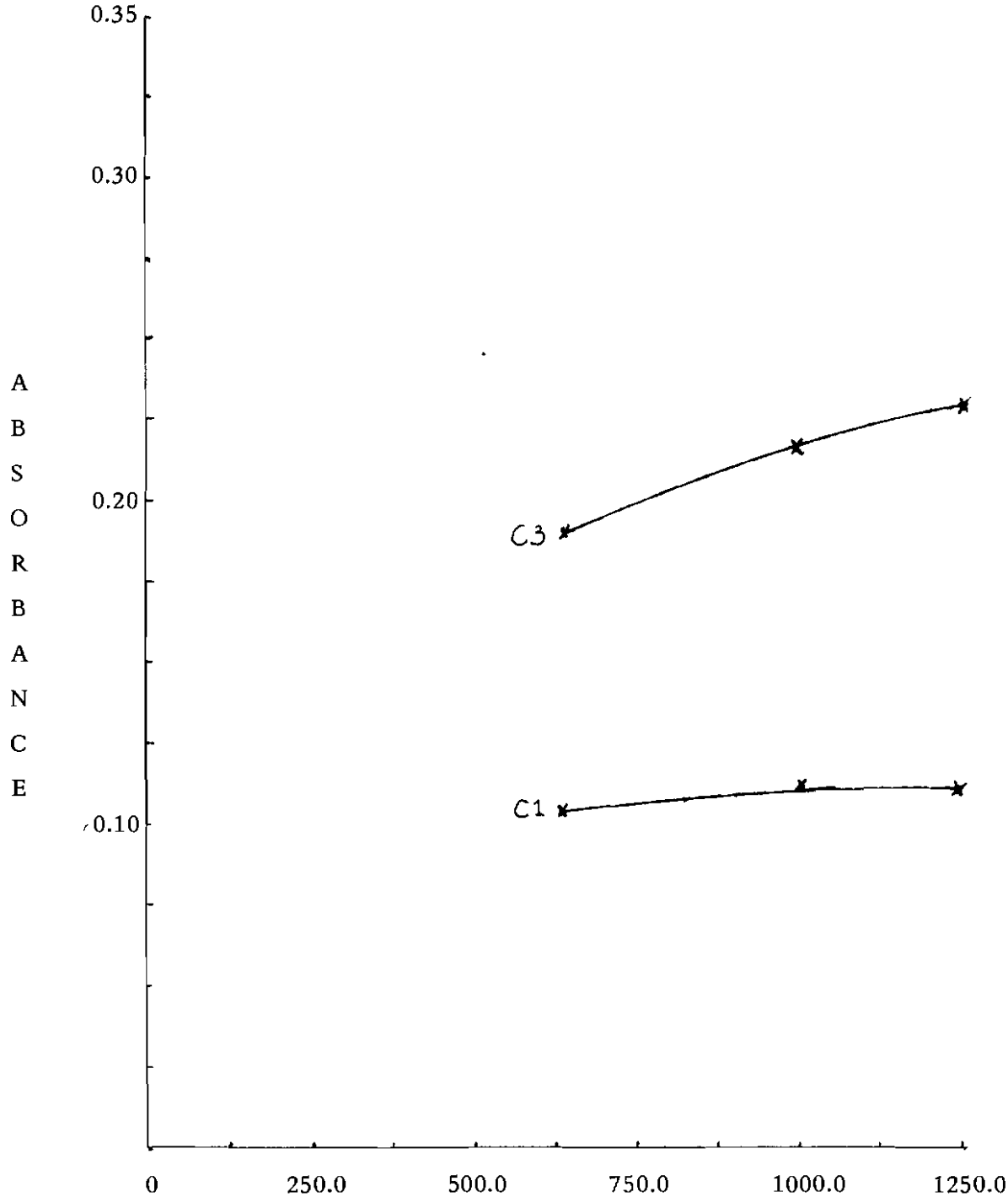
RESULTS OF ANALYSIS OF THE PARAMETER:
ABSORBANCE VS. FLOW RATE
MAINTAINING A CONSTANT CONCENTRATION AND A
CONSTANT AERATION TIME OF 60±2 SECONDS

TABLE 3

Nanograms Mercury Added	Aeration Time	Flow Rate	Absorbance
100.0	60±2 Sec.	1250.0	0.1144
100.0	"	1000.0	0.1144
100.0	"	625.0	0.1051
300.0	"	1250.0	0.2230
300.0	"	1000.0	0.2218
300.0	"	650.0	0.1926

ABSORBANCE VS. FLOW RATE
MAINTAINING A CONSTANT CONCENTRATION AND A
CONSTANT AERATION TIME OF 60±2 SECONDS

GRAPH 3



still applied, in that the density of the vapor per unit space within the absorption cell was dependent on the rate of vaporization or reaction.

Data and results have been established to show the dependence of the density of mercury vapor within the absorption cell on the rate of reaction and flow rate of the carrier gas. Graph 4 A, B and C and table 4 A, B and C demonstrated the effects of these parameters. The illustration demonstrated the reasoning for the choice of the flow rate of carrier gas and rate of reaction time for the analytical technique. The analytical technique used a rate of reaction time equivalent to sixty plus or minus two seconds and a carrier gas flow rate of one thousand two hundred fifty ml/min. The final decision as to the reaction rate and flow rate of the carrier gas were not made to take advantage of the greatest absorbance of the method but they allowed for shorter analysis times and also allowed for more samples to be analyzed the cheapest possible way without decreasing accuracy. The versatility of the method was also shown in graph 4 A, B and C. Thus more absorbance can be obtained with longer rates of reaction and higher flow rates of carrier gas (flow rate showed a limit was approached).

As the density of mercury vapor in the absorption cell was dependent on the rate of reaction and the carrier gas flow rate, the ideal gas laws also suggested the dependency of the density on the volume of air available for volatilization of mercury present. This parameter demonstrated the principle of whether or not a large amount of free air space was needed with the analysis sample mixture volume. The analysis sample mixture's volume was twenty milliliters and the reductant's volume was fifty milliliters. The reactor flask was two hundred fifty milliliters, so that left one hundred eighty milliliters of air space. As the sample mixture volume was increased through additions of water and reductant to keep a consistent concentration, the air space was deminished. The concentration of mercury was maintained through all dilutions. The flow rate was adjusted and an analysis was completed on each solution. The results showed that as air space was decreased, the absorbance also rapidly decreased (table 6 and graph 6). The vaporization dependency on the air space present within the reactor flask was one principle in reduction--aeration of cold mercury vapor.

ANALYSIS OF THE PARAMETER:

CONCENTRATION MERCURY VS. AERATION TIME
MAINTAINING A CONSTANT CARRIER GAS FLOW RATE

STANDARD CALIBRATION CURVE DATA 4

Nanagrams of Mercury Added	Absorbance
100.0	0.1154
200.0	0.1700
300.0	0.2230

RESULTS OF THE ANALYSIS OF THE PARAMETER:
ABSORBANCE VS. AERATION TIME
MAINTAINING A CONSTANT CARRIER GAS FLOW RATE

TABLE 4A

CASE I
Flow rate of carrier gas = 1250.0 m1/min.
Concentration C1 = 100.0 ng Hg added
Concentration C 2 = 300.0 ng Hg added

time in min.	0.5	1.0	1.8	3.0	5.0
C_1A*	0.0458	0.1135	0.1308	0.1549	0.1549
C_1A	0.0458	0.1192	0.1278	0.1549	0.1580
C_1A	0.0458	0.1135	0.1278	0.1518	0.1580
Ave_1	0.0458	0.1154	0.1288	0.1538	0.1569
C_2A	0.1249	0.2218	0.1366	0.2518	0.2518
C_2A	0.1278	0.2255	0.2328	0.2557	0.2557
C_2A	0.1249	0.2218	0.2328	0.2518	0.2557
Ave_2	0.1258	0.2230	o.2340	0.2531	0.2544

*A = Absorbance

RESULTS OF ANALYSIS OF THE PARAMETER:
ABSORBANCE VS. AERATION TIME
MAINTAINING A CONSTANT CARRIER GAS FLOW RATE

TABLE 4B

CASE II
Flow rate of carrier gas = 1000.0 ml/min.
Concentration C_1 = 100.0 ng Hg added
Concentration C_2 = 300.0 ng Hg added

time in min	0.5	1.0	1.8	3.0	5.0
C_1A	0.0458	0.1135	0.1278	0.1549	0.1549
C_1A	0.0458	0.1135	0.1308	0.1549	0.1580
C_1A	0.1410	0.1163	0.1308	0.1518	0.1549
Ave_1	0.0442	0.1144	0.1298	0.1538	0.1559
C_2A	0.1249	0.2218	0.2366	0.2518	0.2557
C_2A	0.1308	0.2218	0.2328	0.2518	0.2557
C_2A	0.1249	0.2218	0.2366	0.2557	0.2518
Ave_2	0.1268	0.2218	0.2353	0.2531	0.2544

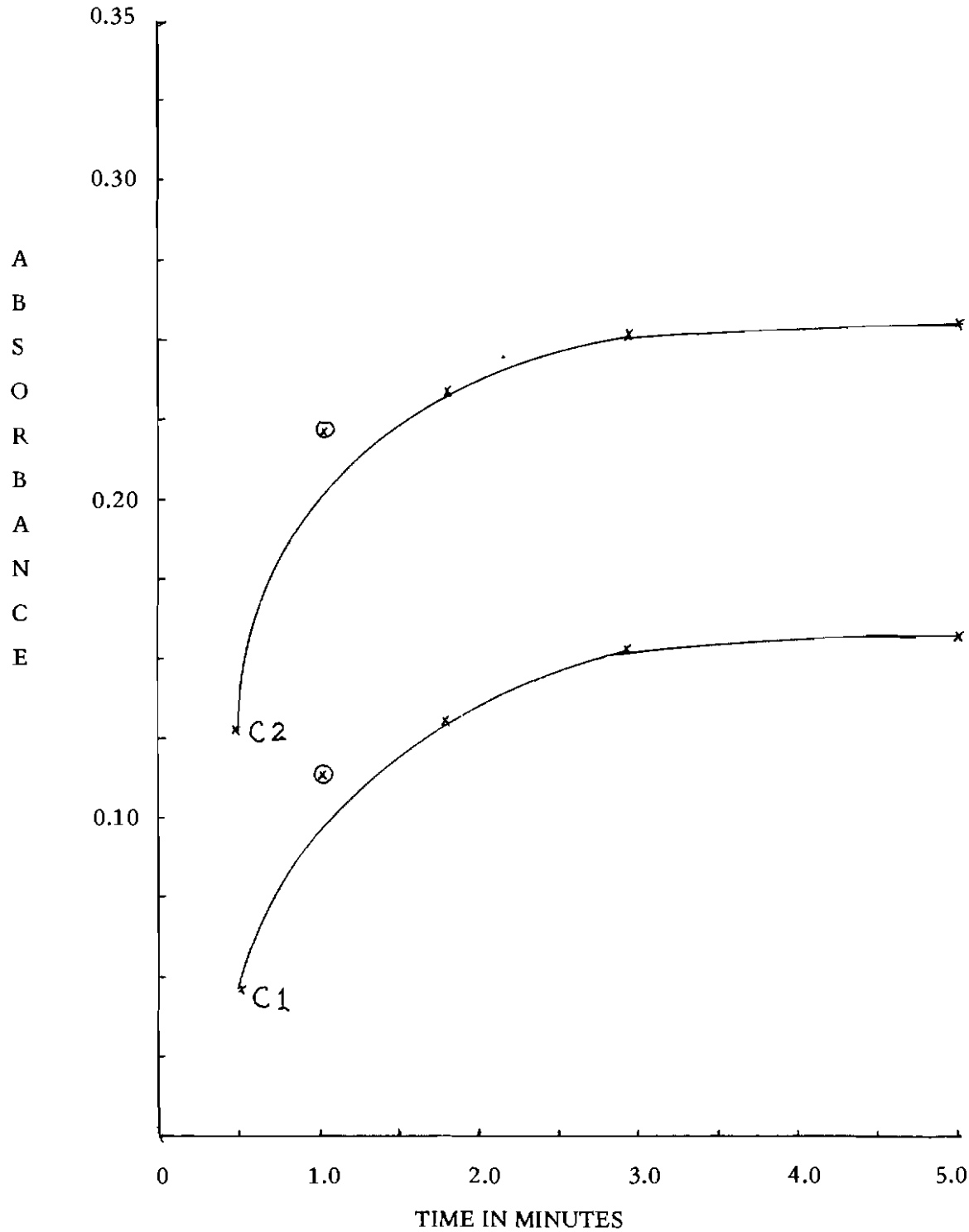
RESULTS OF ANALYSIS OF THE PARAMETER:
ABSORBANCE VS. AERATION TIME
MAINTAINING A CONSTANT CARRIER GAS
FLOW RATE AND VARYING CONCENTRATIONS

TABLE 4C

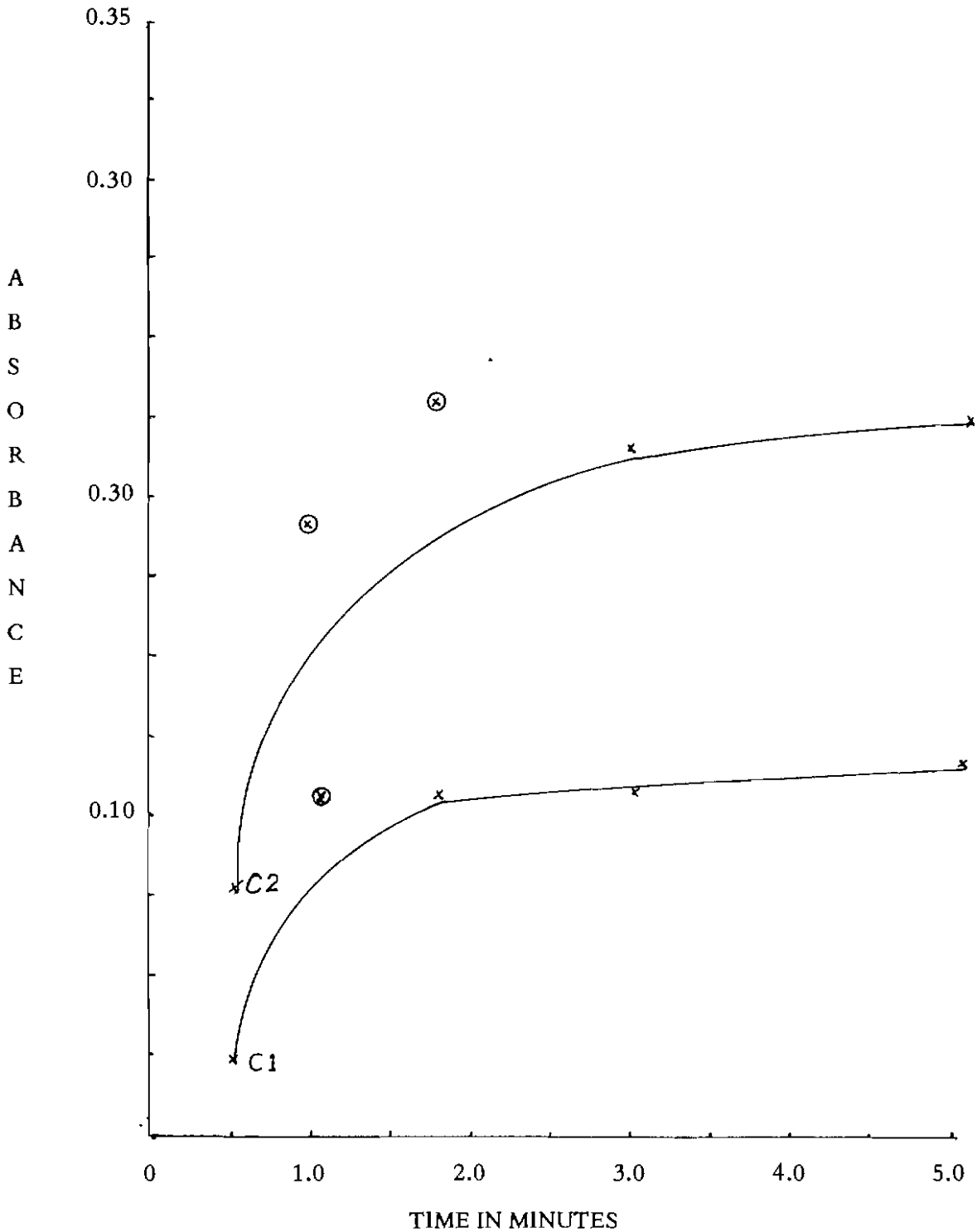
CASE III
Flow rate of Carrier Gas = 625.0 ml/min.
Concentration C_1 = 100.0 ng Hg added
Concentration C_2 = 300.0 ng Hg added

time in min	0.5	1.0	1.8	3.0	5.0
C_1A	0.0246	0.1051	0.1107	0.1051	0.1192
C_1A	0.0246	0.1024	0.1107	0.1079	0.1221
C_1A	0.0269	0.1051	0.1079	0.1051	0.1221
Ave_1	0.0254	0.1042	0.1079	0.1060	0.1221
C_2A	0.0757	0.1938	0.2366	0.2182	0.2255
C_2A	0.0757	0.1938	0.2366	0.2182	0.2218
C_2A	0.0783	0.1904	0.2366	0.2147	0.2255
Ave_2	0.0765	0.1926	0.2366	0.2170	0.2242

GRAPH 4A AND 4B
ABSORBANCE VS. AERATION TIME
MAINTAINING A CONSTANT CARRIER GAS FLOW RATE
OF 1250.0 m1/min. and 1000.0 m1/min.



GRAPH 4C
ABSORBANCE VS. AERATION TIME
MAINTAINING A CONSTANT CARRIER GAS FLOW RATE OF 625.0 m1/min.



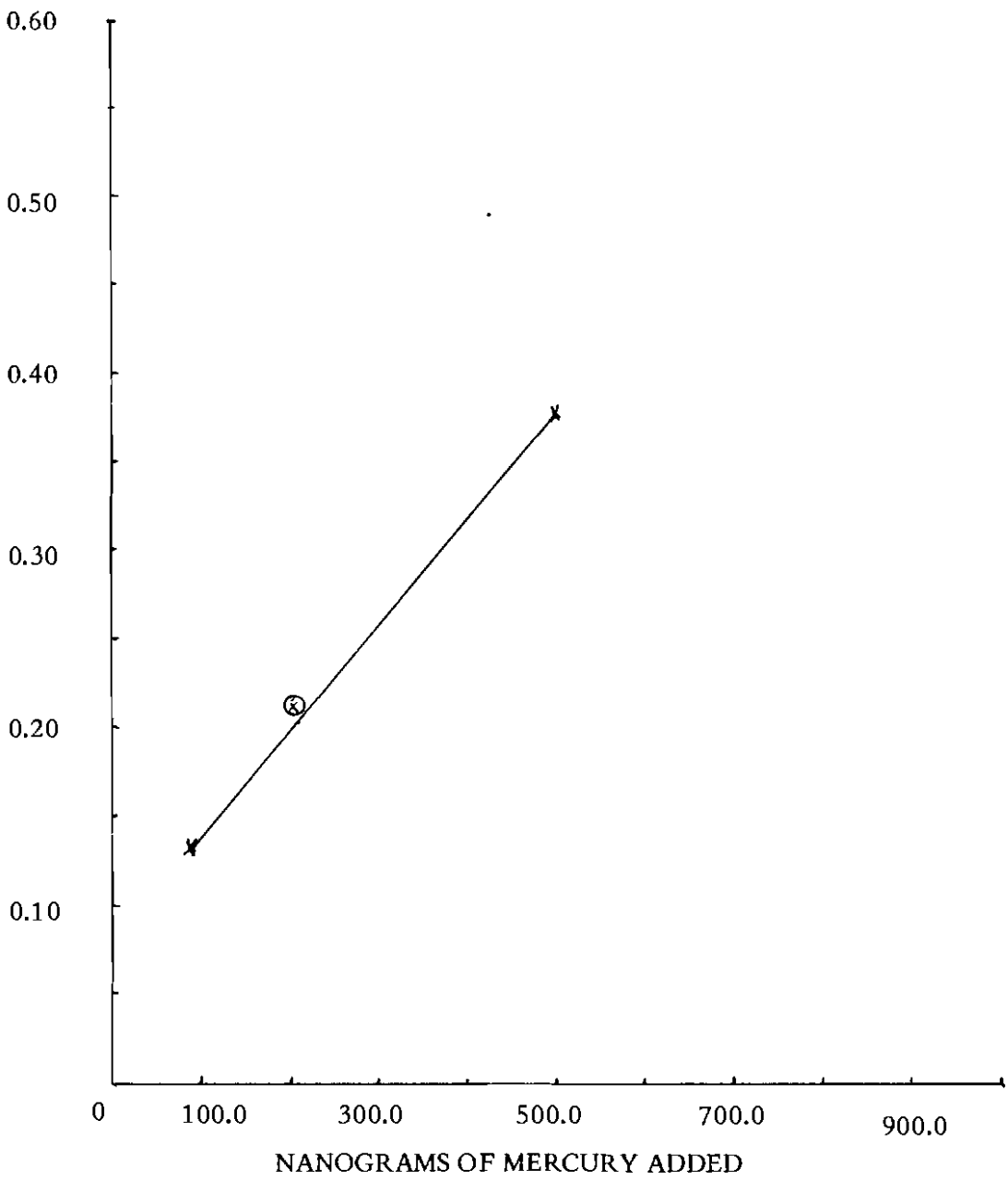
ABSORBANCE VS. TOTAL AIR SPACE

STANDARD CALIBRATION CURVE DATA 5

Nanograms Mercury Added	Absorbance
100.0	0.131
500.0	0.337
600.0	0.382

STANDARD CALIBRATION CURVE:
ABSORBANCE VS. NANOGRAMS MERCURY ADDED

GRAPH 5



RESULTS OF THE ANALYSIS OF THE PARAMETER:
ABSORBANCE VS. TOTAL AIR SPACE

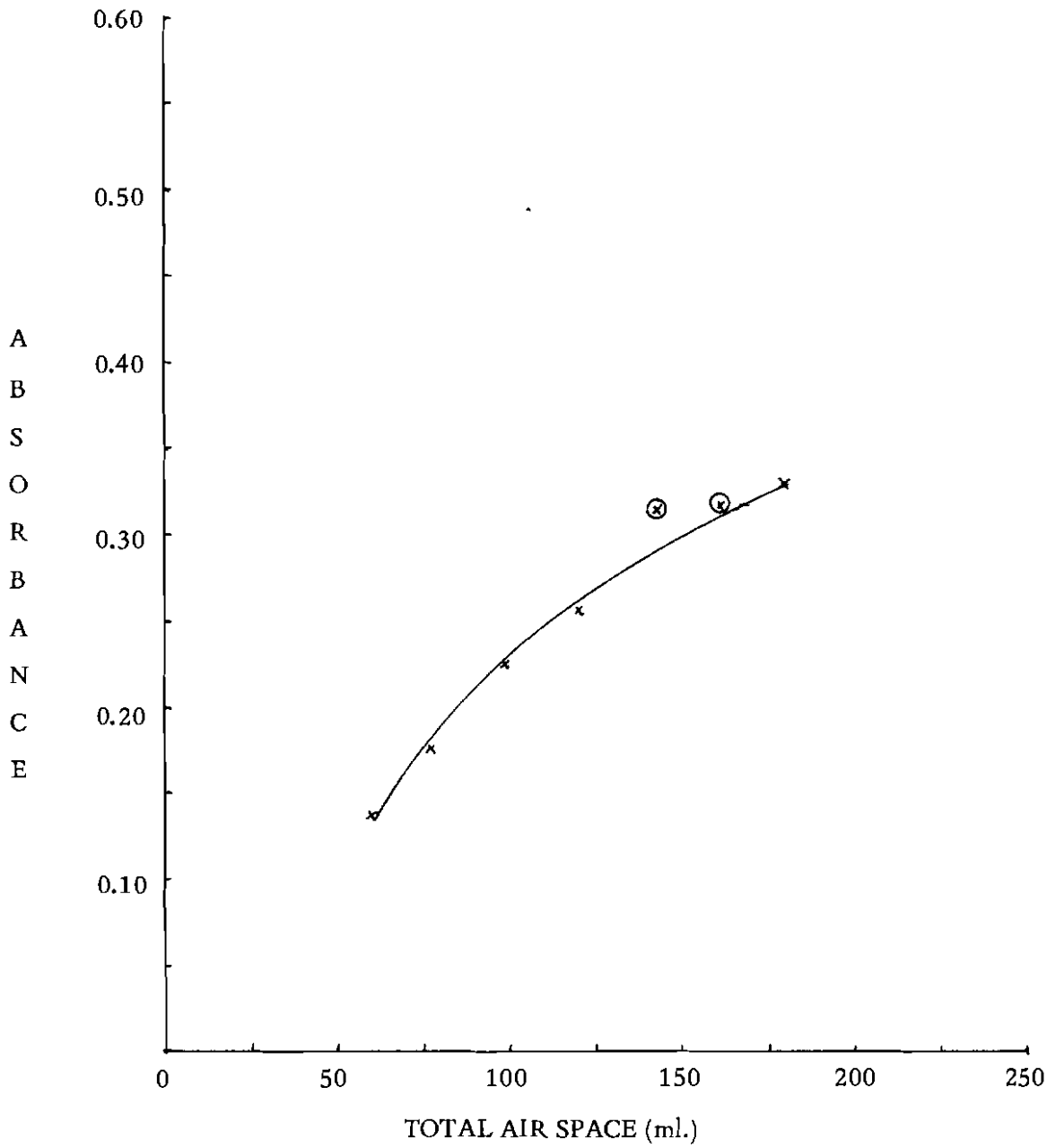
TABLE 6

Flow rate of carrier gas = 1000.0 ml/min
Concentration retained in reactor flask = 500.0 ng added
Constant aeration time = 60±2 seconds

Volume of Air (ml)	Nanograms of Mercury Added	Absorbance
60.0	500.0	0.1367
80.0	500.0	0.1739
100.0	500.0	0.2291
120.0	500.0	0.2676
140.0	500.0	0.3098
160.0	500.0	0.3233
180.0	500.0	0.3372

ABSORBANCE VS. TOTAL AIR SPACE

GRAPH 6



DATA AND RESULTS

The analytical technique has been revised from a popular, published method. A few modifications were used to adjust the technique for convenience and results. The parameters were established for the technique and standard curves were prepared to further demonstrate the reproducibility and linearity of the method. Each component of the apparatus has been prepared and tested. The last part of the problem was to use the technique under laboratory conditions. Fish flesh and assorted bird brains were gathered from the Kansas Forestry Fish and Game Commission for the purpose of applying the method. The flameless AAS method was used to calculate the relative amount of mercury in these biological samples.

After the apparatus system was completely assembled and all the chemicals were analyzed for impurities mainly mercury, a mercury standard was prepared by dissolving 0.1354 grams of dry mercuric chloride in one-hundred milliliters of deionized water. The mercury standard was then used to prepare dilutions (working standards) from one-hundred nanograms to six-hundred nanograms of mercury per milliliter. These working standards were used to establish data for a calibration curves (consult parameter section and table of graphs and standard calibration curve data) and these working standards were prepared daily for each calibration curve. Significant changes were not recorded on a day to day basis using the mercury standard. Some changes could be attributed to instrumentation, variance in flow rates, temperature and more notably the adherence of mercury ions to the glassware (in reference to the mercury standard). To combat excessive variance due to the housing of mercury standard for long periods of time, the mercury standard was prepared monthly or as needed.

The first application was to demonstrate the recovery properties of the revised analytical technique (Table 7). Fish samples were digested completely following the previously outlined procedures in the wet digestion section. The AAS instrument was readied and the samples were analyzed in triplicate. Each absorbance value was correlated against a daily standard calibration curve. The next set of samples were "spiked" (a known amount of mercury standard was added to each) and an analysis was preformed. The results were again correlated against the standard calibration curve. The "spiked" portion of the standard was substracted from each value. The difference was then correlated against the original sample and the difference was shown as a plus or minus value. The recovery of the

RECOVERY PROPERTIES OF THE REVISED ANALYTICAL METHOD OF HATCH AND OTT

TABLE 7

Initial Conc. Mercury (ng)	Mercury Added (ng)	Total Conc.	Difference	Average Difference	Absorbance	Recovery
100.0	0.0	100.0	0.0		0.131	100.0
	0.0	100.0	0.0	0.0	0.131	100.0
	0.0	100.0	0.0		0.131	100.0
100.0	100.0	200.0	+12.0		0.180	212.0
	100.0	200.0	+30.0	+24.7	0.188	230.0
	100.0	200.0	+32.0		0.1904	232.0
100.0	200.0	300.0	+25.0		0.240	325.0
	200.0	300.0	+25.0	+25.0	0.240	325.0
	200.0	300.0	+25.0		0.240	325.0
100.0	300.0	400.0	+05.0		0.264	405.0
	300.0	400.0	+05.0	+09.0	0.284	405.0
	300.0	400.0	+17.0		0.293	417.0
100.0	400.0	500.0	+00.0		0.337	500.0
		500.0	+00.0	+00.0	0.337	500.0
		500.0	+00.0		0.337	500.0

method was extremely good at the lower mercury concentrations. As the concentration of the spiked samples increased, so di the error; in other words, the method demonstrated a positive error in recovery.

Samples of white bass and pheasant were analyzed by the flameless AAS method. Each sample was captured by the Kansas Forestry Fish and Game Commission and locations are shown on Table 2. The sampling and digestion techniques were followed closely and each sample concentration was recorded along with other substantial data. Results of the analysis were shown along with a standard calibration curve data in the following sections (Results 1, 2, 3, 4, and 5).

It has been established that there is some mercury contamination in game birds, fish and other biological samples in Kansas. Since fish and game birds were not a regular diet item, there is little cause for concern---even if catches come from waters moderately contaminated with mercury wastes. No exact conclusions or patterns were drawn concerning the data since only a small number of samples were collected and the environmental conditions were unknown (terrestial rock and soil, fertilizers, accidental spillage of fungicides containing mercury compounds, etc...). The fish samples contained higher levels of mercury than the pheasant brain samples. None of the fish, quail, or pheasant samples approached or exceeded the United States Food and Drug Administration tolerance levels for mercury in edible products entered in commerce. As far as the moldy wheat sample containing excessive amounts of mercury, this was not any basis for concern since it was not a representative sample of the elevator.

ARKANSAS RIVER FISH SAMPLES AT ARKANSAS CITY, KANSAS

STANDARD CALIBRATION CURVE DATA: 6

Nanograms of Mercury Added	Absorbance
100.0	0.174
200.0	0.260
300.0	0.347
400.0	0.438
500.0	0.523

RESULTS OF ANALYSIS OF WHITE BASS LATERAL LINE MUSCLE, CAPTURED IN ARAKNSAS RIVER NEAR ARKANSAS CITY, KANSAS

RESULT 1

SIZE	WEIGHT GR.	ABSORBANCE	CONC.	CONC./GRAM
12.3"*	0.81322	0.2041	135.0	166.0
0.76 lb.	0.93521	0.2366	173.0	184.0
	0.87040	0.2147	148.0	170.0
14.6"	0.91505	0.1904	122.0	133.0
1.31 lb.	0.94002	0l1972	126.0	134.0
	0.96495	0.2041	135.0	140.0
13.0"	0.98105	0.2041	135.0	137.0
0.83 lb.	1.05012	0.2041	135.0	129.0
	0.97547	0.1997	130.0	133.0
15.3"	1.27022	0.2041	135.0	106.0
1.69 lb.	1.46751	0.2291	163.0	111.0
	1.62471	0.2557	198.0	121.0
10.3"	1.15451	0.2041	135.0	117.0
0.49 lb.	1.44107	0.2557	198.0	137.0
	1.30010	0.2291	163.0	125.0

*" = inch

JEWELL, PRATT AND PAWNEE COUNTIES

STANDARD CALIBRATION CURVE DATA: 7

Nanogram Mercury Added	Absorbance
25.0	0.086
50.0	0.149
100.0	0.200
200.0	0.260
500.0	0.420

RESULTS OF ANALYSIS OF BRAIN SAMPLES
AND MOLDY WHEAT SAMPLES
COMPOSITED FROM JEWELL, PRATT AND PAWNEE COUNTIES

RESULT 2

LOCATION	SPECIES	WEIGHT	A	CONC.	CONC./GRAM
Jewell	Pheasant	2.21553	0.1308	40.0	18.0
		2.73745	0.1308	40.0	14.6
		2.92165	0.0909	26.0	8.8
		2.21958	0.1024	30.0	13.0
		2.56025	0.1024	30.0	11.7
		2.84310	0.1107	33.0	11.6
		3.04499	0.0969	27.0	8.9
Pawnee	Pheasant	2.66869	0.0458	12.0	4.5
		2.87250	0.0605	19.0	6.6
		2.78468	0.0809	25.0	8.9
		1.9442	0.0506	14.0	7.2
Pratt	Quail	0.83850	0.0706	20.0	23.8
		0.20365	0.0506	14.0	47.6
	Moldy Wheat I	0.32677	0.3768	422.0	1291.4
	Moldy Wheat II	0.43570	0.4200	500.0	1147.5

FRIED FISH SAMPLES

CALIBRATION CURVE DATA: 8

Nanograms of Mercury Added	Absorbance
100.0	0.155
200.0	0.194
300.0	0.225
400.0	0.297

RESULTS OF ANALYSIS OF FISH SAMPLES
USED IN FRIED FISH SAMPLING
(WET WEIGHT ANALYSIS)

RESULT 3

LOCATION	SIZE	SAMPLE WEIGHT	ABSORBANCE	CONC.	CONC./GRAM
Arkansas River	1.69lb.	1.37565	0.1739	146.0	106.0
Arkansas City, Ks.	15.30"	1.71004	0.1938	200.0	116.0
Milford Res.	.78lb.	1.28365	0.2840	460.0	358.3
Near Wakefield, Ks.	11.90"	1.05578	0.2480	360.0	340.9
Milford Res.	12.40"	0.90500	0.1739	146.0	181.3
Near Wakefield, Ks.	.88lb.	1.10266	0.1938	200.0	181.3

RESULTS OF ANALYSIS OF FRIED FISH SAMPLES
(WET WEIGHT ANALYSIS)

RESULT 4

LOCATION	SIZE	CODE	WEIGHT	ABSORBANCE	CONC.	CONC./GRAM
Arkansas River	1.69 lb.	A*	1.37565	0.1739	146.0	106.1
	15.3"	A	1.71004	0.1938	200.0	116.9
		A	1.68825	0.1871	190.0	112.5
		B*	1.69875	0.1904	195.0	114.7
		B	1.17103	0.1675	130.0	111.0
		B	1.91585	0.2007	245.0	127.8
		C*	1.94490	0.2007	245.0	125.9
		C	1.92225	0.2041	233.0	121.2
		C	1.17655	0.1739	146.0	124.0

*A = raw fish packaged and cooked in aluminum foil.

*B = fish packaged and cooked in Crisco oil contained in aluminum foil.

*C = fish packaged and cooked in Crisco oil and flour contained in aluminum foil.

RESULTS OF ANALYSIS OF FRIED FISH SAMPLES
(WET WEIGHT ANALYSIS)

RESULT 5

LOCATION	SIZE	CODE	WEIGHT	ABSORBANCE	CONC.	CONC./GRAM
Milford Res.	12.4"	A	.73456	0.1739	146.0	198.7
	.88 lb.	A	1.05299	0.1938	200.0	190.4
		A	1.05331	0.1938	200.0	189.8
		B	1.20220	0.2007	245.0	203.8
		B	0.99979	0.1871	190.0	190.0
		B	0.74220	0.1739	146.0	196.7
		C	0.74431	0.1739	146.0	196.1
		C	0.98799	0.1904	195.0	197.3
		C	1.01990	0.1938	200.0	196.0
Milford Res.	11.9"	A	1.28292	0.2840	460.0	358.6
	.78 lb	A	1.06991	0.2480	355.0	331.8
		A	0.99927	0.2441	349.0	349.2
		B	1.07453	0.2480	355.0	330.3
		B	1.25278	0.2798	445.0	355.2
		B	1.082725	0.2518	370.0	340.3
		C	1.19119	0.2596	390.0	357.4
		C	0.99835	0.2441	349.0	351.2
		C	0.99372	0.2480	355.0	357.2

The question concerning the possibility of deminishing the mercury content in fish flesh by cooking. The hypothesis was examined and many fish samples were prepared to correlate a reasonable answer. The analysis went as follows: (1) the flesh was weighed and digested, and (2) a standard set of samples were analyzed and the results were correlated against a standard calibration curve, and (3) the other set of samples were fried over open flame and all remains were scraped off the aluminum foil and digested, and (4) the results of the fried fish were correlated against the same standard calibration curve and the results showed no substantial difference. Another set of samples were prepared using flour and fried in grease but no substantial difference was recorded again. The standard calibration curve data tabel 7 and results 3,4 and 5 as basis for statements. In conclusion, heat does not dislodge the mercury content of fish flesh.

In conclusion, it can be said from the results that no widespread epidemic of mercury contamination was underway in Kansas. However, intensive efforts should be maintained to reduce and regulate industrial mercury wastes, utilize less toxic agricultural fungicides and to pinpoint and control all other sources of mercury contamination of the environment.

The sensivitivy of the method depends of course on the relative volumes of sample plus reagents, air space in apparatus, volume and length of cuvette, rate of flow of the carrier gas, and the rate of reaction. The paper has only been concerned to get enough reduction and accuracy for an analysis down to fifty ppb. The revised analytical technique was successful in the application to biological samples and ease to operation.

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