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RESEARCH AND DEVELOPMENT DIVISION
ORGANIC CHEMICALS DEPARTMENT
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IMPROVEMENT IN THE DETERMINATION OF TRACE LEAD IN BODY FLUIDS AND TISSUES PART IV - APPLICATION OF ANODIC STRIPPING VOLTAMMETRY

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ABSTRACT

Anodic stripping voltammetry determination for sub-microgram amounts of Pb has been developed to a practical technique. Two problems with the electrodes have been solved. These are (1) decreasing sensitivity for Pb with the life of the electrode and, (2) reproducible production of electrodes with good separation of the Pb peak from the hydrogen reduction wave.

Digestion procedures compatible with the anodic stripping determination of Pb have been devised for bones (2 ppm), air filters (< 1 to 100 μg), urine (0.03 ppm) and milk (0.007 ppm). The conditions for the oxidation of blood for quantitative determination of Pb (~0.2 μg) are still under investigation.

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OBJECTIVE

To develop a method for determining lead in biological samples which is more sensitive, accurate and precise than existing procedures.

BACKGROUND

The necessity for a more sensitive and accurate method for determining lead in biological samples had been stated in a previous report (Ref. 1). Anodic stripping voltammetry (ASV) had been shown to have adequate sensitivity to meet the objective (Ref. 1). However, the routine use of the technique for analysis of a large number of samples was hindered by the difficulty of producing and maintaining the working electrode surface which gave a separation of the Pb dissolution wave from the H^+ reduction wave (Ref. 2).

CONCLUSIONS

1. The anodic stripping voltammetry technique has been developed into a practical method for routine determination of trace lead. Three procedure changes were made: use of a polymethylmethacrylate-filled graphite for the working electrode; plating larger quantities of mercury onto the electrode, and maintaining the mercury in the reduced state on the electrode from determination to determination.

2. Wet chemical digestion procedures were developed for processing bones, air filters, urine and milk. These procedures are compatible with anodic stripping determination of lead in the sample solution.

PATENT SITUATION

The patent status of this work will not be investigated because of the nature of the work.

SAFETY

Wet ashing of organic samples with perchloric acid must be carried out by a procedure shown to proceed without explosive oxidation. A fume scrubber system should be provided so that perchloric acid will not condense and collect, possibly mixed with dust or other organics, in the duct work of the laboratory hood system.

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WASTE DISPOSAL

Only laboratory quantities of chemicals are used. Regular solvent disposal provided by the laboratory is used for organic materials.

PUBLICATION STATUS

Publication of this work is not planned at this time.

FUTURE WORK

1. Develop a method to digest 1.0 to 2.0 g of blood (0.2 - 0.4 μg Pb) to give 100% recovery of lead with a precision of ± 5%.

2. Extend the analysis to 0.1 g of blood.

3. Determine if solvent extraction and atomic absorption can be used to determine Pb in urine in order to provide a more rapid method of analysis.

4. Conduct an interlaboratory check on methods for determining Pb in urine and blood with Chambers Works Medical Laboratory.

5. Determine the loss of Pb from ashing bones at 550°C.

6. Determine optimum polymerization conditions for producing a bubble-free and crack-free polymer electrode coating.

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I. DISCUSSION

A. Introduction

The need for a more sensitive method for determining Pb in biological samples has been shown previously (Ref. 1). The goal is to determine routinely 0.01 to 0.1 ppm ± 0.005 ppm Pb in urine and 0.2 ± 0.02 ppm Pb in 1.0 g of blood and tissues. Anodic stripping voltammetry (ASV) has been developed to almost routine use. The techniques of preparing several types of samples for determination of Pb by ASV are described, as well as the technique of ASV. Attention to small details is essential in utilizing the ASV technique and for controlling contamination at the level of Pb being determined. Many details which may seem inconsequential to the reader have been included in this report in order to provide usable procedures.

B. Anodic Stripping Voltammetry

1. Electrodes - Existing Conditions

Anodic stripping voltammetry (ASV) consists of concentrating metals onto an electrode surface by electrolytic reduction from a solution. After a period of concentration, the applied voltage to the working electrode is increased in the positive direction linearly with time while the current through the electrode is measured. A current peak occurs in the current-voltage curve at the oxidation voltage for each metal on the electrode. Figure 1 shows a typical current-voltage scan. This scan was made under the conditions described in the Experimental Section II-A.

Previous work with paraffin-coated, vitreous carbon electrodes showed that the dissolution peak for Pb in 1 N HCl is well separated from all metals expected to be present from blood, tissue or air filter samples. The use of this concentration of acid caused the H^+ reduction wave to tail into the Pb peak with some of these electrodes and not with others. Those electrodes which at first did not show this tailing deteriorated with use. A good electrode lasted one or two days before the H^+ reduction wave interfered with the Pb oxidation peak.

In addition, as the electrodes were used more frequently, the sensitivity gradually decreased. This latter difficulty was thought to have been solved (Ref. 2), but the solution proved to be ineffective on long term use.

2. Electrodes - Present Work

The loss in sensitivity with use was corrected by halting the scan voltage during anodic stripping at -0.2 V versus the Ag/AgCl reference electrode. This change provided a long term solution to the problem. The mercury is maintained in the reduced or metallic state. The oxidation of Hg probably produced a layer of Hg_2Cl_2 precipitate on the electrode which inhibited the diffusion of Pb^{++} to the reduced mercury surface.

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In an effort to correct the H^+ reduction wave tail problem an acetic acid buffer (pH 5.0) was tried in order to increase the separation of the reduction wave from the Pb dissolution peak. The Pb peak is well separated from other metals, with the exception of thallium, in this medium (Ref. 6). In the presence of Fe in the amount normally present in a 1 g sample of blood, the amount of Pb found by ASV in acetate buffer was only 75 to 85% of the added Pb. Evidently Pb was coprecipitated with $Fe(OH)_3$ which was visible in the acetate buffer. To avoid coprecipitation, Pb was plated from 1 N HCl and stripped in a separate acetate buffer. This also produced low recovery. This is apparently due to the open circuit present when the solutions are changed. Table I shows the effect of the duration of an open circuit on the amount of Pb determined.

The performance of the vitreous carbon, paraffin-coated electrode was improved (greater separation between H^+ reduction tail and Pb dissolution peak) when plated for 20 minutes with a solution containing 50 mg Hg^{++} as compared with 0.8 mg Hg^{++}. The sensitivity for Pb was not changed over this range of Hg^{++} concentration in the plating solution.

Graphite rods impregnated with paraffin gave initially better and more reproducible electrodes than vitreous carbon, but they lasted only about one-half day. It was thought that a harder filling in graphite would increase the useful life. Therefore polymethylmethacrylate-filled graphite rods were prepared (see Experimental Section II A-2). The advantages of this electrode are characterized by:

1. Good separation of the H^+ reduction tail from the Pb peak - when properly polished.

2. Useful life usually two weeks.

3. Good performance can be restored by polishing with jewelers' rouge.

In order to restore good performance of the vitreous carbon electrode one must polish and recoat with paraffin.

The polymerization conditions need to be improved to yield bubble-free and crack-free polymer coating. At present, care must be taken in polishing to be sure that no bubble or crack breaks the polished surface.

3. System Performance

The evaluation of the method was carried out using the new methacrylate-graphite electrode and the 3-electrode system previously described (Ref. 2). The response of the system (as measured by $\mu a/\mu g$ Pb in 50 ml) is non-linear over the full range of the recorder and the non-linearity increases with peak height. However, the response is linear within the precision of the method up to a peak height equivalent to about 60% of the recorder span. Figure 2 shows a plot of method response ($\mu a/\mu g$ Pb in 50 ml) versus peak height for two greatly different lead concentrations run at different recorder attenuations. The decrease in

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response with peak height is 2-3% from 25% to 65% of the recorder span, above which there is a rapid decrease. Because of this, samples are run with an attenuation which will give a peak height nearly the same as the standard. Samples must, therefore, be replated and rescanned at different attenuation if the peak is too large.

The agitation of the plating solution affects the rate at which Pb plates as would be expected due to the change in the thickness of the concentration gradient layer at the electrode surface - thinner at higher agitation speeds (Figure 3).

Several plating runs were made with different clearance between the working electrode and the agitator and the working electrode and the vessel walls. The plating solution was not changed during these runs. The results (Table II) show that the configuration of the electrodes, agitator and vessel are critical for precise determinations. A jig was therefore introduced to center the vessel on the magnetic stirrer and to reduce variation in the working electrode-vessel wall distance.

Table III shows the precision obtained by making several runs without disturbing the system. There is little difference in precision depending upon whether one uses a rapid or slow agitation rate or whether the plating solution is allowed to approach rest conditions for 30 or 60 seconds between cessation of agitation and the start of the voltage scan. Because the precision was the same at slow and rapid agitation, a rapid agitation was selected (4.5 setting 930 rpm) because it gave greater sensitivity.

The agitation setting that was selected is the greatest possible without causing the magnetic bar to become decoupled from the drive magnet. Decoupling causes the bar to move erratically in the vessel striking the electrode surface. If this occurs, the Pb dissolution peak becomes unsymmetrical and sometimes double. The electrode surface must then be cleaned and replated with Hg before accurate determinations can be made (see Experimental Section II-A-2 and II-A-6).

The optimum conditions derived from the above studies are given in the procedure as described in the Experimental Section, II A.

Possible interferences from variations in the amount of HCl, the presence of H_2SO_4, Na_2SO_4 or $Ca(H_2PO_4)_2$ are shown in Tables IV, V and VI. The effects of these substances on the sensitivity for Pb are small, but as in the case of polarography the standard solution should match the sample solutions closely enough to avoid errors due to different rates of diffusion of Pb ions in the different solutions.

C. Samples

The earlier work covered by this report used 50-ml Erlenmeyer flasks for the digestions of bones and filters. The subsequent use of 30-ml Kjeldahl flasks proved superior. The oxidations of bone and filter papers are more rapid in

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Kjeldahl flasks than in Erlenmeyers. The reagent blank values are less and more consistent with the Kjeldahl flask. Generally the blanks are < 0.1 μg Pb and two blanks with each set of samples usually agree within 0.02 μg Pb. The improvement of digestion in Kjeldahl flasks is because the HNO_3 used is evaporated more slowly from the Kjeldahl giving more time for the acid to oxidize the sample. Less HNO_3 is used giving lower blanks and the Kjeldahl flask is covered by its fume duct so as to keep out contamination.

1. Bones

A wide range of temperatures (450 to 750°C) has been used to dry ash bone samples (Refs. 7, 8, 9, 10). Of these authors only one (Ref. 7) reported a possible loss of Pb at temperatures greater than 600°C, but no data were given. Burnham, et al, (Ref. 12) had reported 11 to 13% loss of Pb from air filter samples when ashed at 500°C. Wet-ashing with HNO_3 followed by ASV determination is being used for Pb in bone samples in this laboratory.

It was not until this work was well under way that the work of Martin and Blanchard (Ref. 11) was known. Their data show that Pb^{210} is not appreciably volatilized from caribou bone when dry-ashed at temperatures up to 600°C with a 20% loss at 700°C ashing. Validation of dry-ashing bones without Pb loss has not yet been done in our laboratory.

In order to determine Pb by ASV in the digest from bones, it was necessary to determine the effect of calcium phosphate on the plating rate of Pb. Table IV shows the sensitivities for a series of Pb determinations with and without the addition of $Ca(H_2PO_4)_2$. A small negative bias is seen in the presence of calcium phosphate.

It was shown by ASV determination that less than 0.01 ppm Pb was extracted from bone by benzene-ethanol extraction in the preparation of dry, fat-free bone before determination of Pb in the bone. This bone contained 2 ppm Pb.

We reported (Ref. 3) that ASV agreed with atomic absorption in the determination of Pb in bone.

2. Filters

A wet oxidation (H_2SO_4 + HNO_3) of the air filter samples has been used in preference to a dry oxidation which had been reported to give low recovery of Pb (Ref. 12). Recovery of added Pb carried through the wet oxidation was 111 μg from 112 μg added and 4.6 and 5.1 from 5.15 μg added (ORNB 256-174). The current procedure is described in the Experimental Section.

3. Urine

The current method for urine is described in the Experimental Section. A more rapid procedure will be investigated based on extraction and determination of

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Pb by atomic absorption. In developing the current procedure several difficulties need to be considered.

Upon standing, calcium phosphate precipitates from urine, some of which adheres to the walls of the container. This precipitate contains Pb. Lead is recovered from the walls by removing the sample from the bottle, washing the walls with acid and combining the acid wash and the sample (Ref. 14).

A wet digestion of urine with H_2SO_4 proved inadvisable due to the difficulty in dissolving the precipitate formed during the digestion, presumably $CaSO_4$. Normal urine ranges from 30 to 400 ppm Ca (Ref. 13).

Wet digestions with only HNO_3 in Vycor Kjeldahl flasks gave low results as indicated by incomplete recovery of added Pb (ORNB 256-133). Wet digestions of urine with HNO_3 and 200 mg KNO_3 yielded better recovery of Pb. Six determinations were made with 0.19, 0.48 and 0.96 μg of Pb added to reagent blanks of HNO_3 + KNO_3. A normal urine sample (0.03 ppm) contains 0.45 μg Pb. A recovery of 95.5% with a relative standard deviation of 3.0% was obtained (ORNB 256-138).

4. Blood

The proper conditions for oxidizing the organic matter in blood have not been defined. Dry-ashing at 500-550°C is possible. Wet-ashing with H_2SO_4 and HNO_3 alone is not complete. Addition of either H_2O_2 (30 %) or $HClO_4$ to the digest causes complete oxidation. However, wet digestion with either oxidant is slow. Lots of reagent H_2O_2 analyze between 0.25 and 1.8 ppm Pb and cannot be used for 1.0 g blood samples which contain ~0.2 μg of Pb. $HClO_4$ analyzes 0.009 ppm Pb and can be used since only 0.1 ml of the acid was used in each digestion. Runs for determining recovery of Pb have not yet been made. The reproducibility of 0.3 μg Pb standards diluted to 40 ml volume as would a digested sample is shown in Table V to be within 5% relative. Work must be continued on blood samples to provide a workable procedure for 1-gram samples.

5. Milk

Milk samples were received from Ethyl Corporation for determination of Pb. These samples could not be digested by H_2SO_4 + HNO_3 due to the residue $CaSO_4$ which would not dissolve. HNO_3 alone caused a violent oxidation spilling the sample. A modified dry ashing procedure was used successfully. The procedure and our results are reported in Ref. 4. A comparison of our results with other laboratories is in Ref. 5. These two references are included in the Appendix. The results show that the procedure used in this laboratory gives good results at the 0.01 ppm level. No further work is planned on milk samples.

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II. EXPERIMENTAL

A. Anodic Stripping Voltammetry

1. Apparatus

A Sargent Polarograph and compensator were used to provide three-electrode operation for recording the current-voltage curves of the anodic stripping process. These items were described previously (Ref. 1, 2). A schematic diagram showing the electrical circuit as it is presently used is in the Appendix (Figures 4, 5). The quartz plating cell, agitator and electrodes (reference and auxiliary) are also described in References 1 and 2. The working electrode is described in the next section. Photographs of the cell and electrodes are shown in Figure 6. The magnetic stirring bar is driven by an A. H. Thomas Model 15 magnetic stirrer with an aluminum speed-reducing cap (Cat. No. 9235 B20). The agitation speed was maintained reasonably constant by setting the speed adjustment to a selected position and operating the stirrer by a separate on-off switch.

2. Working Electrode

The working electrode was prepared by impregnating a graphite rod with polymethylmethacrylate as follows:

a. Evacuate a tube (30 mm x 180 mm) containing 4" x 1/4" spectrographic graphite rods (Fisher Scientific, Cat. No. 4-676-5) to a pressure <0.1 Torr. Refer to Figure 7.

b. Fill the system to atmospheric pressure with N_2. Repeat step a and b again.

c. Re-evacuate to 0.1 Torr and isolate the system from the vacuum pump.

d. Introduce methyl methacrylate into the system to cover the rods with the monomer.

   The methyl methacrylate used in this work was obtained from the Experimental Station storeroom; it is inhibited with 22-28 ppm hydroquinone, Vazo® initiator, 0.1% (w/w), was added just before adding monomer to the system.

e. Introduce N_2 into the system to increase the pressure to atmospheric.

f. Transfer the rods individually to 10 mm diameter glass tubes and add monomer to the tubes to cover the graphite. Stopper the tubes to prevent evaporation.

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g. Place the tubes in a water bath at 40°C with the water level about 2 cm below the top of the graphite rods. Leave in the bath for 3-4 days until hard.

h. Remove from the bath and place in an oven at 100°C for one hour to cure.

i. Break the glass away from the plastic coated rods gently.

j. Polish one end of the rod (a cross section only) with successively finer paper until 3/0 emery paper is used. There will be some bubbles in the polymer. Finish the polishing at a point which has no bubble touching the surface. Polish with jewelers' rouge on filter paper (Whatman 41 or 42) to remove all scratches and depressions.

k. Wipe the electrode free of the major portion of rouge with filter paper. Soak a few minutes in 1:1 HCl and rinse with water.

Never touch the active surface with the fingers.

Once a day the working electrode is coated with fresh mercury as follows:

a. Add 5 ml of $HgCl_2$ solution (10 mg Hg/ml) to the quartz plating vessel containing a Kel-F agitator (7/8" length).

b. Add 1.0 ml of HCl (conc.) and dilute to about 15 ml with H_2O.

c. Place the plating vessel up around the electrodes, immersing them into the solution. Lower the assembly onto the magnetic drive unit.

d. Start the agitator.

e. Switch the operation switch from "stripping" to "plating". Adjust the voltage on the graphite electrode to -0.4 volts versus the Ag/AgCl reference electrode.

f. Plate for 20-25 minutes. Turn off the agitator.

g. Remove the plating vessel and rinse the electrodes and the vessel well with water. The electrode is now ready for standardization.

3. Reagents and Standard Solutions

All water referred to in this report is distilled water normally supplied to the laboratory, which has been redistilled in the laboratory from an all quartz still and stored in polyethylene before use. The acids were reagent grade and used without purification.

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Standard solutions of Pb were prepared by dissolving individually reagent grade $PbCl_2$ (1.3430 g) and $Pb(NO_3)_2$ (1.5996 g) in water containing 1.0 ml HCl and diluting to 1.000 l. The salts were dried at 80°C and 130 Torr for an hour before use. Working standards were prepared by diluting the stock solutions by weight in FEP Teflon® bottles with water and HCl to make 0.01 N in acid. The dilute standards (0.1 to 50 μg Pb/ml) were stored in Teflon® bottles and were stable for up to six months. No difference from the calculated values were noted (±5% relative) between those standards made from $PbCl_2$ and $Pb(NO_3)_2$.

4. Standardization

Determination of the sensitivity of the system for Pb should be made every fifth determination, as follows:

a. Pipet an aliquot of a standard solution of Pb into a clean, deleaded 50-ml volumetric flask. The amount of Pb should be the same order of magnitude as that expected in the samples.

b. Add 2.0 ml HCl (conc.) and one drop of $HgCl_2$ solution (10 mg Hg^{++}/ml).

c. Dilute to 50.0 ml with H_2O and follow section on determination of Pb.

d. Calculate the sensitivity factor:

$$F = \frac{H \times S}{W} = \mu a/\mu g - 50 \text{ ml}$$

where: H = peak height in mm.

S = sensitivity, μa/mm.

W = μg Pb in 50 ml of solution, step a.

5. Determination of Pb

a. Set the dials and switches on the Sargent Polarograph Model XXI as follows:

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Span EMF:	2.12 V (Scan rate then 19.5 mV/sec)		
Initial EMF:	1.00 V	A.C.:	On position so that green light is "on" showing proper polarity
DC EMF:	3.0 V		
Initial EMF:	Opposed		
		Downscale:	0.0
Damping:	Off		
		Upscale:	To be selected
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Sensitivity: (according to the concentration of Pb):

0.06 μa/mm	0 to 2.0 μg Pb/50 ml
0.10	1.5 to 3.0
0.20	3.0 to 6.0
0.40	6.0 to 12.0
0.80	12.0 to 24.0
1.50	22.0 to 45.0

b. Provide a N_2 sweep of about 2 l/min. to the plating vessel directed over the liquid surface.

c. Add HCl (conc.) to make 2 ml per 50 ml to which the sample will be diluted (step d). Add 1 drop of $HgCl_2$ solution (10 mg Hg/ml) for each 50 ml of diluted sample.

d. Dilute the sample to a known volume with H_2O.

e. Lift the electrodes out of the plating vessel.

f. Rinse the electrodes well with water.
A 400-ml plastic beaker is handy to catch the rinse water and for use in step i.

g. Rinse the plating vessel three times with water.
Hold the stirring bar in the vessel with another magnet on the outside of the vessel.

Use a vigorous stream of water from a squeeze bottle directed all around the side walls. Empty the vessel each time at a different portion of the lip or circumference. Wiggle the magnetic stirrer vigorously when the water is in the vessel during the last rinse.

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h. Pour enough sample into the plating vessel to cover the stirring bar. Wiggle the bar. Rotate the vessel to wet all the sides with the solution. Empty out the solution and repeat this step for a total of two rinses with the sample solution.

i. Add sample solution to the vessel to fill to the 15-ml mark ± 2 ml.

j. Tip off drops hanging onto the bottom of the electrodes by touching them with the lip of the plastic beaker.

DO NOT touch the active surface of the carbon electrode.

k. Place the plating vessel up around the electrodes, immersing them into the solution. Lower the assembly onto the agitator.

l. Start the agitator. Start a timer to ring about 5.5 minutes.

m. Switch the operation switch from "stripping" to "plating" and at the same time start a stop watch.

The voltage setting on the plating box must be at 1.0 to 1.1 volts before switching to "plating."

n. Check that the % Span is at 10% and the Initial and Span voltages are at 1.00 and 2.12 volts, respectively.

o. Set the EMF switch to Constant when the timer rings.

p. At 6 Min., 0 Sec. (± 0.5 Sec.):

Switch the operation switch to "stripping" and turn off the agitator.

q. Adjust the Upscale dial to place the pen at the right side of the chart. (0 to 20 mm from the side).

r. At 6 Min., 30 Sec. (± 0.5 Sec.):

Switch the Constant EMF to EMF Increasing and turn on the chart drive.

s. Scan to -0.20 V (38% span). Turn off the chart drive and the EMF scan motor.

The equipment is ready for the next sample which should be started immediatel (Step a, this section).

t. Measure the peak height of the Pb dissolution peak (Figure 1).

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Calculation:

$$\mu g\ Pb = \frac{H \times S}{F} \times \frac{V}{50}$$

where: H = peak height for Pb in mm.

S = sensitivity in μa/mm.

F = calibration factor

V = volume in ml (step d)

50 = volume of the standardizing solution

6. Cleaning the Electrode

Cleaning of the electrode is necessary when the Pb peaks become broad or are double peaked or when the plating rate (sensitivity) decreases to a small value. If the following procedure for cleaning the electrode does not improve the operation of the electrode, then one must repolish the active surface with rouge (section II-2).

Rinse the electrodes and the plating vessel well with H_2O to remove all chloride. Fill the vessel with 0.5 M $HClO_4$ and immerse the electrodes in it. Start the agitator. Switch the electrodes to stripping. Scan the voltage from -0.8 V to +0.4 V (voltage settings as in section II-5 and scan from 10% to 70% span). Rinse the electrodes well with H_2O. The system is now ready for a calibration run, section 4.

B. Samples

1. Bones

A procedure for determination of Pb in bones has been given in Reference 3. That procedure has been changed in the digestion of the sample to be compatible with anodic stripping determination. The procedure as now used is below.

a. Weigh (±0.001 g) 0.3 to 0.5 g of dry, fat-free bone into a deleaded 30-ml Vycor Kjeldahl flask.

   The nominal 30 ml flask must previously have been calibrated at the 40 ml volume.

b. Add 2 ml (dropper) of HNO_3, concentrated. Heat on a Kjeldahl digestion rack to dryness and heat strongly.

c. Repeat step b until there is no further darkening of the residue when dried.

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d. Cool and add 1 ml (dropper) of HCl, concentrated, and evaporate to almost dryness. Repeat twice.

e. Cool and add 2 ml (dropper) of HCl, concentrated, and ~2 ml of water. Boil gently for 5-10 minutes.

f. Cool. Stopper and store for determination by anodic stripping voltammetry.

2. Filters

Two sizes of cellulose Millipore filters have been analyzed. The procedure for the 3-cm personal sampler filter is given below. The 4-inch filter is ashed slightly differently to prevent a foam from forming in the Kjeldahl flask and overflowing in the worst cases. After placing the 4-inch filter into the flask, add 1.0 ml of H_2SO_4 and heat to completely char the sample. HNO_3, 1 ml, may now be added and heating continued. More than one addition of HNO_3 may be required. to completely oxidize it. The digestion is continued from step d as in the case with the small filters.

Digestion of Cellulose Filters - 3 cm Diameter

a. Place the filter into a deleaded 30-ml Vycor Kjeldahl flask.

Handle the filter in such a manner that no sample is lost from the filter surface.

b. Add 0.5 ml of H_2SO_4 and 1 ml of HNO_3.

c. Place on the digestion rack and heat at a low rate at first and finally heat strongly to fumes of H_2SO_4.

d. Cool and add ~2 ml of H_2O. Heat to fumes of H_2SO_4.

e. Cool and add ~1 ml of HCl. Heat to fumes of H_2SO_4.

f. Cool and add 2 ml of HCl and 2 ml of H_2O. Boil gently for 5 minutes.

g. Cool, stopper and set aside for determination by anodic stripping voltammetry.

3. Urine

a. Transfer the sample to a clean plastic graduated cylinder. Record the volume.

b. Pipet 10 ml of HNO_3 to the sample bottle. Rinse the walls of the sample bottle with the acid to dissolve any precipitate from the side walls.

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c. Pour all the sample back into the sample bottle. Mix well.

d. Pipet 15.0 ml of sample and acid solution into a deleaded 30-ml Vycor Kjeldahl flask.

e. Add 1.0 ml of 0.2 mg KNO_3/ml solution.

f. Boil gently to dryness on a Kjeldahl digestion rack.

g. Cool and add 1 ml of HNO_3. Heat to dryness.

h. Cool and add 1 ml of HCl and 1 ml of H_2O. Heat to dryness. Repeat once.

i. Add 2 ml of HCl and 2 ml of H_2O and boil gently for 5 minutes. Cool, stopper and set aside for determination of Pb by anodic stripping voltammetry.

In calculating the concentration of Pb in the urine, the sample volume used is not 15 ml; but $15 \times \frac{V}{V + 10}$, where V = the volume of urine measured in step a.

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APPENDIX

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FIGURE 1

ANODIC STRIPPING SCAN FOR LEAD

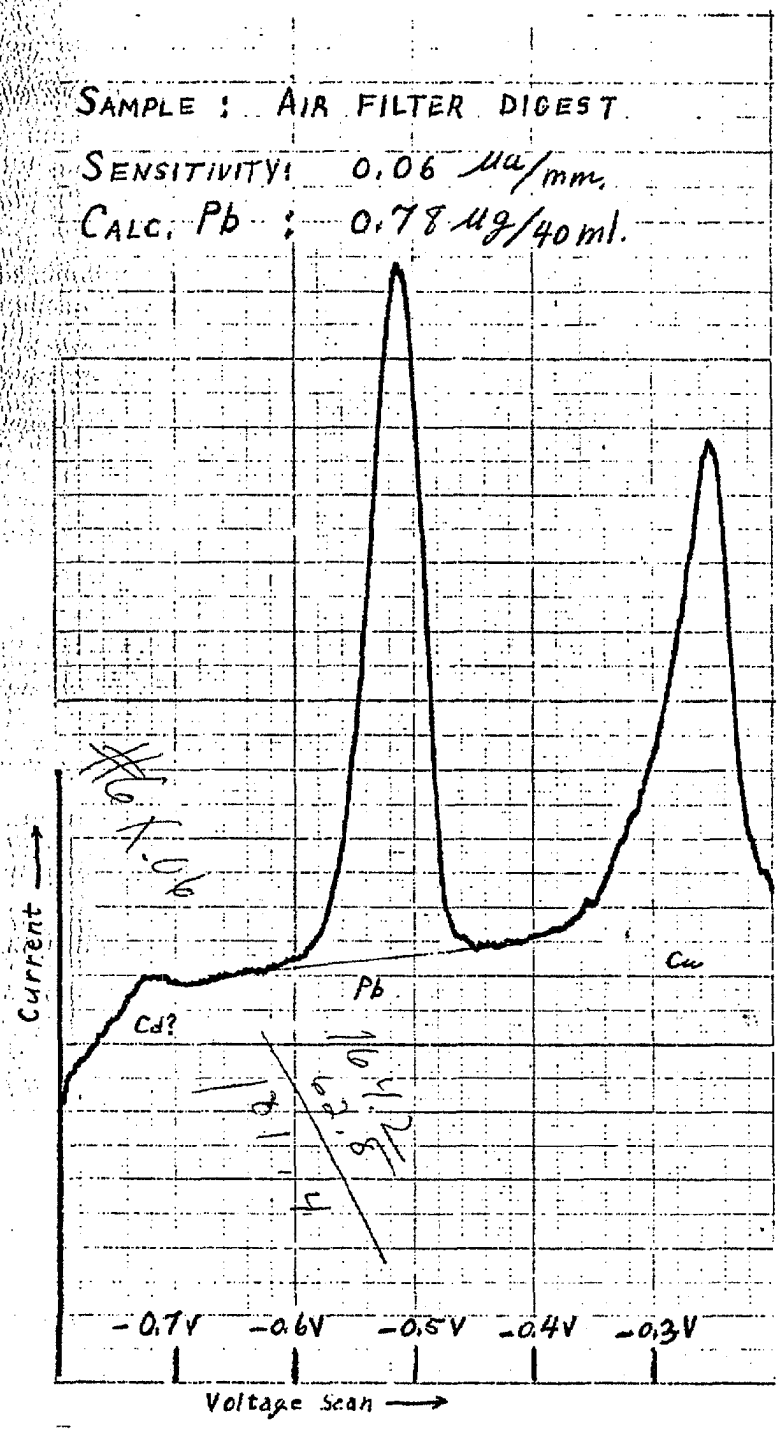

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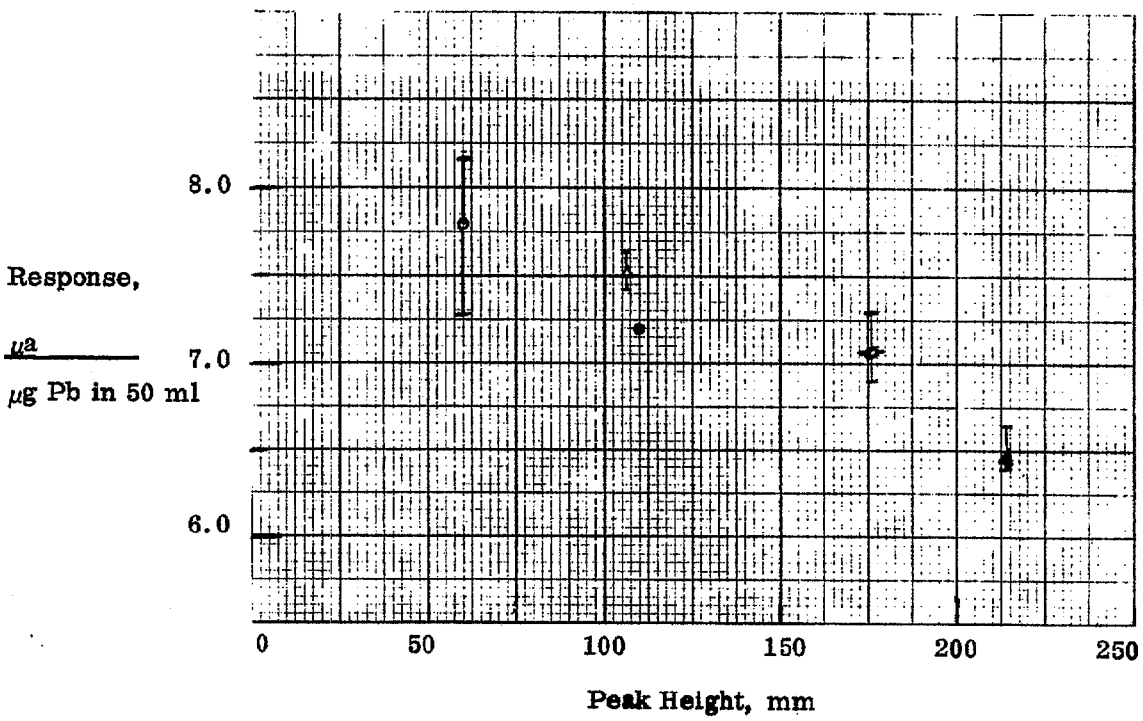
FIGURE 2

METHOD RESPONSE vs. PEAK HEIGHT

OCNB 65-171



o 1.5 μg Pb per 50 ml at 0.2 μa/mm
● 1.5 μg Pb per 50 ml at 0.1
-o- 1.5 μg Pb per 50 ml at 0.06
△ 21 μg Pb per 50 ml at 1.5
▲ 21 μg Pb per 50 ml at 0.6

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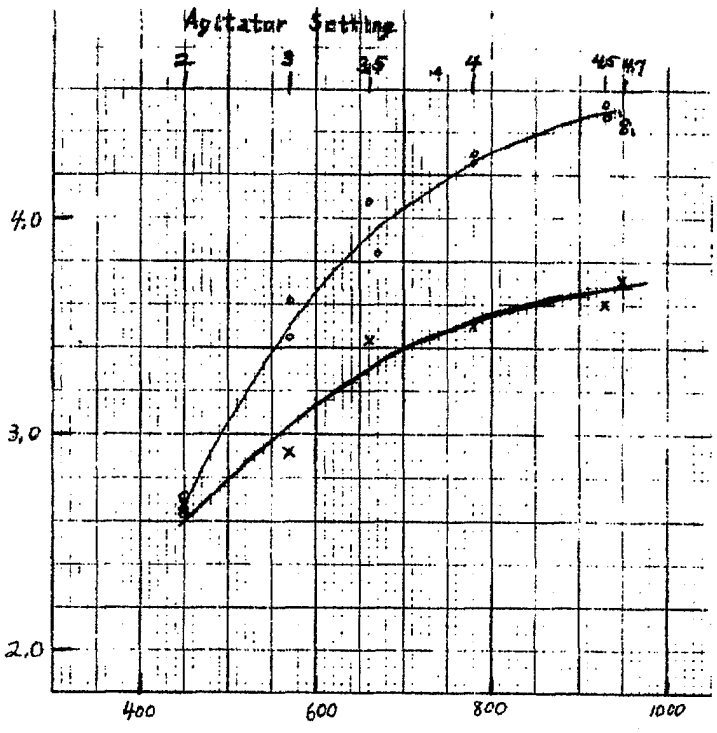
FIGURE 3

SENSITIVITY vs. AGITATOR SPEED

Reference: ORNB 256-144



Sensitivity, $\frac{\mu a}{\mu g\ Pb/50\ ml}$

Agitation, RPM

o = Agitator-electrode surface distance of 1 mm

x = Agitator-electrode surface distance of 13 mm

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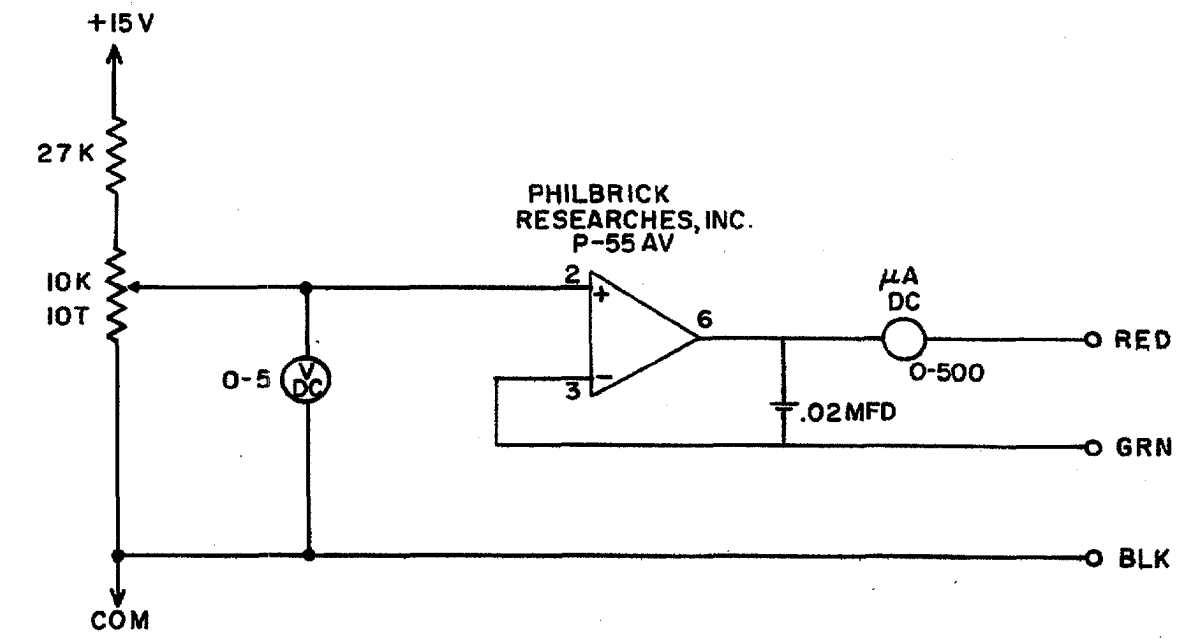
FIGURE 4

SCHEMATIC FOR ANODIC STRIPPING

SIMPLIFIED PLATING UNIT



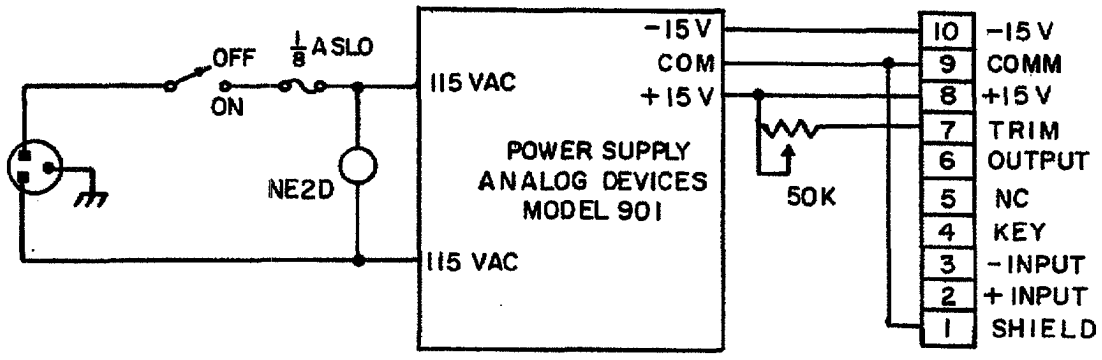


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FIGURE 5

SCHEMATIC FOR ANODIC STRIPPING POLAROGRAPHIC COMPENSATOR

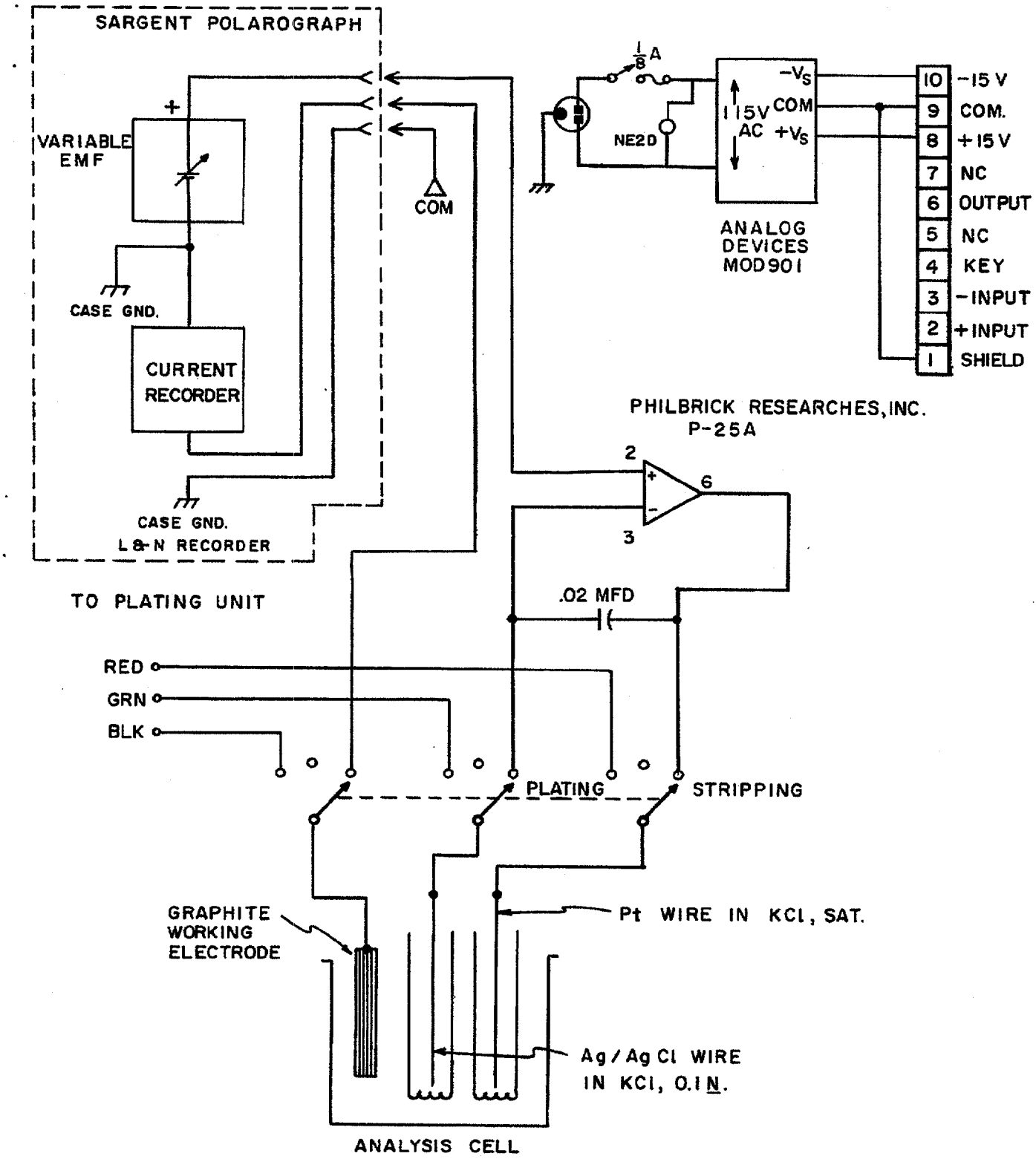

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FIGURE 6

ANODIC STRIPPING VOLTAMMETRY
VESSEL AND ELECTRODES

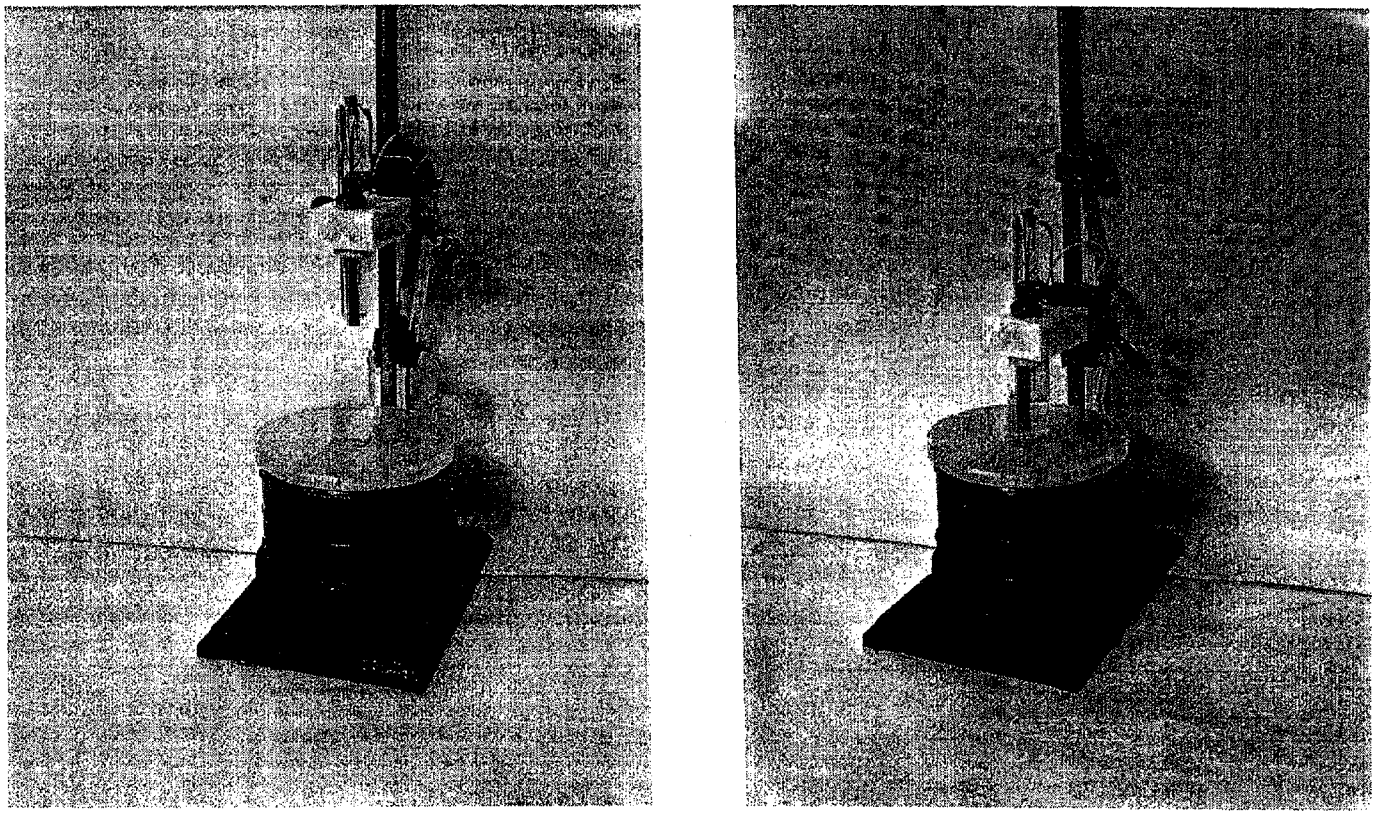

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FIGURE 7

VACUUM SYSTEM FOR PREPARATION OF ELECTRODES

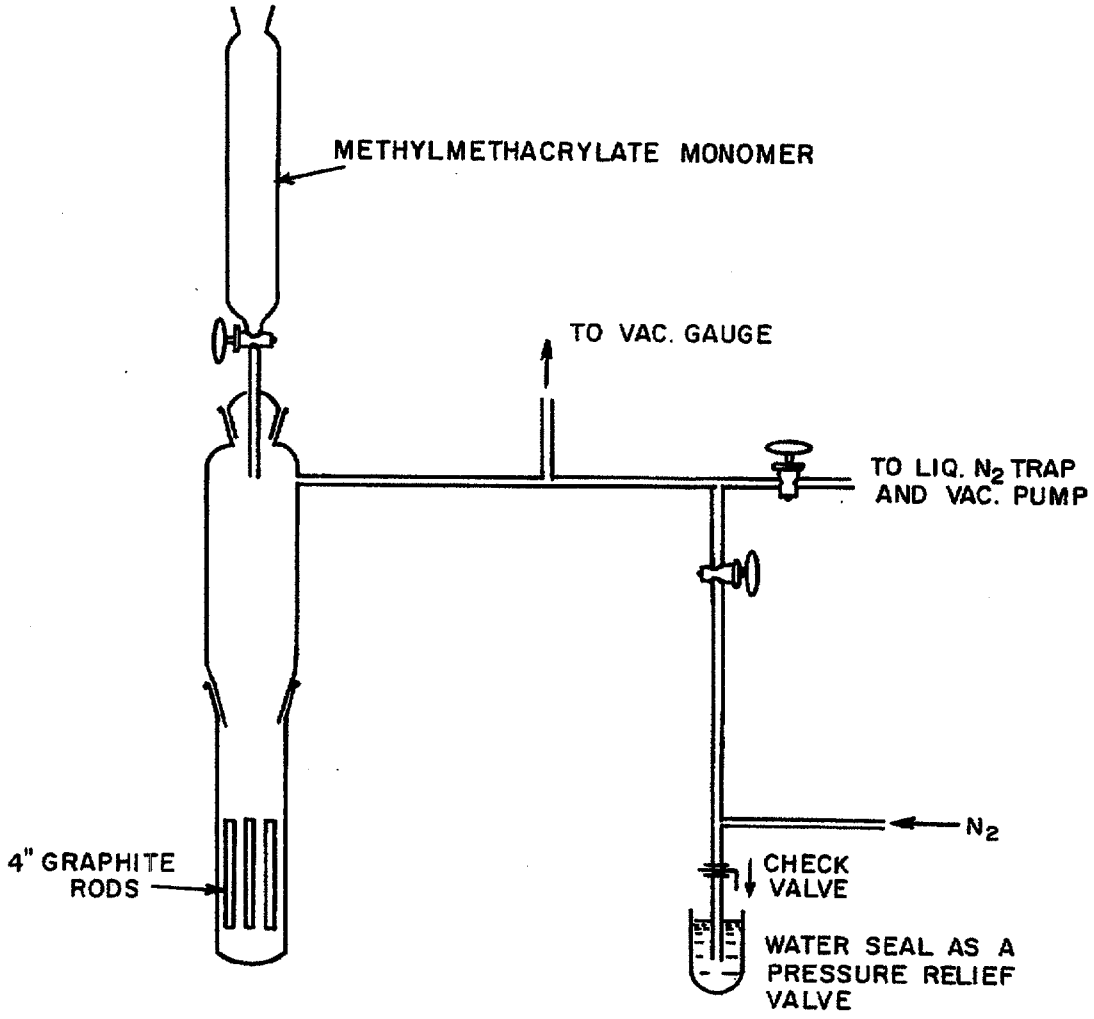

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TABLE I

EFFECT OF OPEN CIRCUIT DURATION ON Pb PEAK CURRENT

OCNB 65-163

0.29 μg Pb/15 ml; Plated 5.0 Min. in 0.8 N HCl; Sens. 0.08 μa/mm

Open Circuit Duration, Sec. (in order run)	Lead Peak Height, mm
0.1 estimated	89.6
0.2 "	70.3
<0.1 "	95.8
<0.1 "	93.0
1 "	9.6
<0.1 "	95.2

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TABLE II

SENSITIVITY vs. ELECTRODE PLACEMENT

Reference: ORNB 256-145

Common Conditions:

Agitator Setting:	4.5
Agitation Time:	180 Sec.
Quieting Time:	60 Sec.

	A	B	C	D
Agitator-Electrode Distance, mm	2	2	13	13
Vessel Wall-Electrode Distance, mm	1	3	1	3
Sensitivity, $\mu a/\mu g$ Pb-50 ml	3.33	2.94	3.05	2.75
	3.42	3.08	3.01	2.89
	3.45	2.91	3.05	2.55
	3.36	2.96	3.29	2.81
Mean	3.39	2.97	3.10	2.72

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TABLE III

EFFECT OF AGITATION ON PRECISION

(ORNB 256-144)

	A	B	C	D
Electrode-Agitator Distance, mm	1	1	1	1
Agitator Setting	2.0 (450 rpm)	4.7 (950 rpm)	2.0	4.7
Agitation Time, Sec.	180	180	210	210
Solution Quieting Time, Sec.	60	60	30	30
No. of Determinations	10	5	5	5
Sensitivity Range ($\frac{\mu a}{\mu g/50\ ml}$)	2.27-2.14	3.73-3.88	3.04-3.19	4.21-4.47
Mean Sensitivity	2.33	3.80	3.11	4.33
Standard Deviation	0.063	0.055	0.065	0.105
Relative Std. Deviation	0.027	0.014	0.021	0.024

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TABLE IV

EFFECT OF CALCIUM PHOSPHATE ON THE DETERMINATION OF Pb BY ASV

(ORNB 65-172)

μg Pb Added	Total μg Pb Present	g $Ca(H_2PO_4)_2 \cdot H_2O$	Sensitivity, μa/μg-50 ml With Ca	Sensitivity, μa/μg-50 ml Without Ca
1.03	1.03	0	-	7.91
1.03	1.03	0	-	7.40
1.03	1.84	0.46	6.98	
1.03	1.84	0.46	7.21	
21.0	21.0	0		7.10
21.0	21.0	0		7.01
21.0	21.8	0.46	6.55	
21.0	21.8	0.46	6.70	
31.5	31.5	0		6.25
31.5	31.5	0		6.42
31.5	32.3	0.46	6.09	

All samples contained 3 ml of HCl and were diluted to 50 ml. There was a gradual drift in sensitivity with time when this work was done.

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TABLE V

ANODIC STRIPPING PEAK HEIGHT
FOR Pb IN THE PRESENCE OF HCl AND H_2SO_4

(ORNB 65-164)

Acid Added/15 ml	Pb Added/15 ml	
	0.29 μg	0 μg
1.0 ml HCl	95.8) mm	0.0 mm
	93.0)	
	95.2)	
	96.8)	
2.0 ml HCl	94.6)	
	102.5)	
1.0 ml HCl + 0.5 ml H_2SO_4	95.8)	2.5
	91.0)	

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TABLE VI

EFFECT OF Na_2SO_4 ON Pb BY ANODIC STRIPPING

(ORNB 984-9)

Present in 40 ml Volume	Peak Height, mm at 0.04 μa/mm
0.00 μg Pb)	5.1
2.0 ml HCl)	7.9
0.5 ml H_2SO_4)	5.1
0.12 g Na_2SO_4)	5.7
0.583 μg Pb)	135.9
2.0 ml HCl)	129.2
0.5 ml H_2SO_4)	133.3
0.00 g Na_2SO_4)	
0.583 μg Pb)	129.2
2.0 ml HCl)	132.1
0.5 ml H_2SO_4)	135.0
0.00 g Na_2SO_4)	

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TABLE VII

REPEATABILITY BY ANODIC STRIPPING VOLTAMMETRY

(ORNB 256-149)

Sample: 0.194 μg Pb Diluted to 40 ml

Agitation-Plating Time	6.0 Min.	10.0 Min.	
Peak Heights	35.5 mm X 0.06 μa/mm	51.8 mm X 0.06 μa/mm	
	31.5	50.3	
	32.6	60.2	
	31.5	52.0	
	33.8	50.5	
	34.2	52.4	Excluding 60.2
Mean, mm	33.0	52.9	51.4
Std. Dev.	1.6	3.7	0.9
Rel. Std. Dev.	4.8%	7.0%	1.8%

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OR-6828-E REV. 5-61

APPENDIX

bcc: W. H. Linton, J. L.
J. J. Mikita/D. R. Diggs
A. J. Pahnke/E. N. Cantw
L. A. Williams, Exp. Sta.

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
WILMINGTON, DELAWARE 19898

ORGANIC CHEMICALS DEPARTMENT
RESEARCH AND DEVELOPMENT DIVISION
PETROLEUM LABORATORY

December 4, 1969

Dr. M. Brandt
Ethyl Corporation
Research Laboratories
1600 West Eight Mile Road
Ferndale, Michigan 48220

Dear Dr. Brandt:

Our L. A. Williams has determined the lead concentration in the two samples of milk (10969-A, -B) supplied (your letter of October 16, 1969) with the results tabulated below:

Sample No.	Pb (μg/l)	Avg.
10969A	6.5, 6.6, 8.4	7.2
10969B	58, 61, 63	61
10969A + 5.6μg Pb/l	10.0, 10.8	10.4
Reagents blanks (Calcd. on 50-ml sample basis)	2.9, 3.3	3.1

We understand that the samples were identical but sample 10969-B was fortified with 59 micrograms of Pb per liter as lead nitrate. Mr. Williams was not informed of the level of lead spiking until his analysis was complete.

The lead determination was made by anodic stripping voltammetry after the sample had been ashed by the procedure outlined below. The milk samples did not ash easily under normal procedures.

1. Add 50 ml. (polypropylene graduate) of milk to 250 ml. Vycor beaker. Cover with watch glass.

2. Add 1 ml. of HNO_3, concentrated.

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3. Boil to reduce the sample volume on a low-temperature hot plate until the sample begins to bump.

4. Place the covered beaker into an oven, set at 110°C, to dry overnight.

5. Place the uncovered beaker in a muffle furnace at 525°C for four hours. Cool, add 0.3 ml. of 1% NH_4NO_3 solution and remuffle for one hour.

6. Dissolve residue in four ml. of 1:1 HCl.

7. Transfer and dilute to volume with water in a 50 ml. volumetric flask.

8. Determine Pb by anodic stripping voltammetry. Standardize by comparison with standard Pb solutions run at time samples are run.

We appreciate the opportunity to analyze these samples. We would be interested in the experience of your laboratory and others in analyzing these samples.

Sincerely yours,

G. H. Patterson

G. H. Patterson, Head
Automotive Emission
Studies Division

GHP/jel

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ETHYL CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT
RESEARCH LABORATORIES • 1600 WEST EIGHT MILE ROAD
FERNDALE, MICHIGAN 48220

January 7, 1970

Dr. G. H. Patterson
Petroleum Laboratory
E. I. DuPont de Nemour Co.
Wilmington, Delaware 19898

Dear Dr. Patterson:

You will recall that some time ago we shipped milk samples to you for lead determination. This was prompted by our own difficulties in carrying out the analysis. It had been anticipated that this would be a difficult analysis because of the high protein content and the low concentration of lead. Data in the literature indicated an average of about 50 µg of lead per liter.

Our initial trials verified that the analysis was indeed a formidable one. In these analyses we found about one-tenth of the reported average and concluded that lead was being lost during the course of the analysis. Various kinds of sample treatment were applied but in all cases the amount of lead was found to be at a low level.

A quantity of dry milk solids was obtained and a portion reconstituted. A known amount of lead was added to a part of the liquid milk. This material, the untreated reconstituted milk and the dry milk solids were analyzed (Tables 1 and 2). At the same time, the assistance of several laboratories was solicited. These were either experienced in determining lead in milk or were known to be competent in the determination of trace amounts of lead in organic matrices.

Samples of the reconstituted milks were sent to these laboratories for analysis by their methods. In some cases, a portion of the dry milk solids was also forwarded. Laboratories A, B, C, D and E received milk from dry solids Lot 10J25E. Laboratories B and C received samples from the same batch of liquid milk prepared from this lot. Laboratories F, G and the A (second sample) received samples from Lot 2A15E. Our analyses of the two lots of dry solids shows the lead content to be essentially the same (Table 2).

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Results of the analyses by the participating laboratories are shown in the attached table (Table 3). Your laboratory is designated as G .

Table 4 lists data obtained on a composite sample of market whole milks supplied by Laboratory C.

In each case, those laboratories which were inexperienced in the analysis, expressed varying degrees of dissatisfaction with their results.

Inasmuch as particulars are not known on the various procedures used, it is not possible to show specific sources of error. However, there are indications that the following are major contributors:

1. Inadvertent contamination during sample preparation.
2. Small sample size with consequent large multiplication of error.
3. Inability of the final measurement technique to adequately discriminate.

This exercise has demonstrated that the determination of lead in milk is considerably more challenging than previously suspected and the likelihood is great that the lead content of milk is substantially lower than previously reported.

Very truly yours,

Manuel Brandt

MB:cjd

DUP050312288

Table 1

LEAD IN DRIED MILK

	Laboratory	Sample Wt, g	Po, µg	Equivalence in µg Pb/l
Lot 10J25E	Ethyl	10 (equiv. to 100 ml)	0.40	4.0
			0.30	3.0
		50 (equiv. to 500 ml)	0.84	1.7
			0.46	0.9
	Laboratory E	24*		11.6
Lot 2A15E	Ethyl	50	1.80	3.6
			2.22	4.3
	Laboratory F	5		AAS 38
		5		Dz 26

*Pb determined on 2/5 of original sample.

MB:cjd
1/7/70

DUP050312289

Table 2

Pb IN RECONSTITUTED MILK
(200-ml samples)

	Pb, µg/l		
		Treated	
Lot 10J25E	Untreated	59 µg/l	5.9 µg/l
	6.7(1)	63.9(1)	
	6.2(1)	59.8(1)	
	9.2(2)	66.4(2)	
	7.7(2)	62.9(2)	
	6.9		14.7
	7.0		13.2
	6.8		
	7.0		
Lot 2A15E	6.1(3)	58.8(3)	
	7.2(3)	62.1(3)	

(1) Portions of batches sent to Laboratories B and C. Analyzed about two weeks after shipment.

(2) Same samples as (1); analyzed about four weeks after shipment.

(3) Inadvertently ashed at 580°C for 2 hours.

MB:cjd
1/7/70

DUP050312290

Table 3

LEAD IN MILK

	Sample, ml	Pb, µg/l Untreated	Pb, µg/l Treated (59 µg/l)
A(3,4)	50	110(1)	110(1)
	?	150(2)	192(1)
	?	56(2)	100(2)
B	?	37	89
C	1000	9(2)	30(2)
		7(2)	65(2)
D	50	24(5)	79(5)
		29.6	54.8
E	250(6)	12	65
		AAS Dz	AAS Dz
F	100	27 18	- -
	50	- -	85 62
G DuPont	50(7)	7.2	61
Ethyl	200	7	62

(1) Second analysis on original samples.
(2) Second samples.
(3) Labs A, B, C, D, E from Lot 10J25E; B and C received same batch of reconstituted milk.
(4) A (second sample), F and G from Lot 2A15E.
(5) Tentative data obtained on initial attempt.
(6) Used 2/5 aliquot for Pb measurement.
(7) Used undisclosed aliquot for Pb measurement by ASV.

MB:cjd
1/7/70

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Table 4

LEAD IN MARKET WHOLE MILK (COMPOSITE SAMPLE)

Pb, µg/l	
Ethyl	Laboratory C
5.1	≤ 3
4.9	≤ 3
4.4	
5.4	

MB:cjd
1/7/70

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