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ABSTRACT

Title of Thesis: Determination of Trace Quantities of Selenium and Arsenic in Canned Tuna Fish Using Electroanalytical Techniques.

Ann Marie Higham, Master of Science, 1986

Thesis Directed By: Dr. Reginald P.T. Tomkins

Toxic metals are present in the environment and are known to be present in food. Selenium, which is considered an essential micronutrient, is recognized as a toxic metal at slightly higher concentrations. Arsenic is also recognized as a toxic metal. Selenium and Arsenic tend to accumulate in food, specifically fish and seafood.

Fifteen brands and types of tuna fish were analyzed for selenium using differential pulse cathodic stripping voltammetry. The cans with the high and low concentrations of selenium were analyzed for arsenic using differential pulse polarography. Three samples with three percent recoveries and a blank were analyzed for each can of tuna fish. Four sample digestion methods with several variations of each were tested to determine the most reliable technique. An acid digestion procedure using HNO_3 and $Mg(NO_3)_2.6H_2O$ with an 18 hour predigestion step gave the best results with an average recovery of 98.2 percent.

The selenium concentration of the cans analyzed ranged from 0.034 to 1.20 μg/g with an average concentration of 0.68±0.268 μg/g. The arsenic concentrations of the two cans analyzed were 1.62 μg/g and 2.41 μg/g in the low and high selenium cans respectively.

The selenium concentrations found in the tuna fish are not excessively high and do not seem to pose a problem. The arsenic concentration of 2.41 μg/g does however approach the maximum allowable level set by the FDA at 2.6 ppm.

DETERMINATION OF TRACE QUANTITIES OF SELENIUM AND ARSENIC IN CANNED TUNA FISH USING ELECTROANALYTICAL TECHNIQUES

by
Ann Marie Higham

Thesis submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of
Master of Science
1986

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I. TOXIC METALS IN FOOD

Heavy metals are found in trace quantities in plants and in animal tissues and therefore also in food. Although toxic metals are naturally present in the environment, industrial processes have resulted in an increased concentration of heavy metals in air, water and soil. Subsequently these metals are taken up by plants and animals and they make their way into the food chain.

Traces of many heavy metals in food are a health hazard, but the determination of the levels when various materials become dangerous is not so simple. Several elements are known to be essential at low concentrations, but at higher levels they are toxic. This is complicated even further if there is a very narrow range between the concentration at which the metal is considered essential and the concentration at which it is considered toxic. This presents a problem particularly in the case of selenium.

Some of the more common metals that pose problems in food are cadmium, lead, mercury, arsenic and selenium. Below is a brief description of the toxic effects of each of these metals along with the problems that they pose in food.

Cadmium

Cadmium is a non-essential and highly toxic metal. Although its concentration in foods is usually less than 0.1 μg/g wet weight, and it is only partially absorbed by the body, ranging from 0.5 to 12 percent in various species of animals (Doull et al., 1980), it does accumulate in body tissues, particularly in the kidneys. Its tendency to accumulate in the body even over a period of decades due to its slow excretion rate has

caused much concern over the levels at which man is exposed to Cd. Acute Cd poisoning is generally not of concern when dealing with Cd in food. Chronic toxicity is much more of a problem when dealing with a relatively low daily dose over a period of years or decades.

Chronic Cd poisoning has been shown to cause hypertension, cardiovascular problems, osteomalacia with spontaneous multiple bone fractures, renal damage, depression of growth with reduced fat and protein digestion, irreversible binding in the replacement of zinc, hyperglycemia and glucose intolerance. Cd usually accumulates in the kidney, liver and reproductive organs where it exhibits its most toxic effects.

The kidney accumulates much higher concentrations of Cd than the other organs, so it is the primary site to exhibit Cd toxicity. Tubular and glomerular damage will result in increased excretion of both high and low molecular weight proteins. Proteinuria, glycosuria and aminoaciduria are all results of Cd toxicity on the kidney.

Cd taken through the diet inhibits enzymes associated with zinc by competing with and displacing the zinc from the enzymes. Cd inhibits the uptake of zinc and copper and increases the urinary excretion of copper which can lead to bone malformations. Cd also inhibits iron absorption and hemoglobin formation which can lead to anemia, growth retardation and poor bone mineralization.

Cd affects the reproductive organs and has caused gonadal tissue damage and sterility in both sexes of experimental animals. Cd has been shown to cross the placental barrier in experimental animals and cause skeletal, kidney and heart abnormalities in fetuses (Doull et al., 1980). Ingested Cd however is not carcinogenic and will not exhibit effects on the lungs which occurs with inhalation (Sunderman,1972).

Cd poisoning is caused primarily by environmental contamination. Commercial phosphate fertilizers and cow manure, extensive food processing and refining, industrial air emissions and industrial wastewaters have all been shown to somehow raise the Cd content of various foods. Both air and waterborne Cd, as well as commercial fertilizers, cow manure and sewage sludge used as a fertilizer, can cause an increase in the Cd concentration of the soil. Cd concentrations are usually less than 1 μg/g in uncontaminated areas, but it has reached as high as 69 μg/g in contaminated soil in Japanese rice fields (Fribergh et al., 1974). Rice, wheat, radish and lettuce are able to take up considerable quantities of Cd from the soil which results in an increased Cd content in grains of cereals or in the edible portions of vegetables.

The Cd content of fish has also been examined. An average Cd concentration of 0.094 μg/g wet weight was reported for 19 fish from the Great Lakes (Fribergh et al., 1974). Oregon groundfish were found to have a Cd content of less than 0.10 ppm except for the lingcod which had an average concentration of 0.224 ppm (Childs and Gaffke, 1974). In the analysis of fish and fish products from Great Britain, Cd concentrations in 48 samples of tuna fish were all below 0.2 μg/g, but some salmon meat was reported as having more than 3 μg/g (Fribergh et al., 1974).

Shellfish tend to accumulate Cd to a much greater extent (Fribergh et al., 1974). Values of 110 to 420 μg/g wet weight were reported in the livers of shellfish from polluted waters. Oysters from the East Coast of the U.S. were found to have Cd concentrations of up to 7.8 μg/g wet weight, while oysters from the West Coast had concentrations of up to 2.1 μg/g wet weight. Table 1.1 shows the Cd content that has been reported for many types of Oregon groundfish. Table 1.2 shows the Cd concentration that has been reported for many other types of food.

TABLE 1.1. Cd Content of Oregon Groundfish

Species	Southern Coast Cd (μg/g wet weight)	Northern Coast Cd (μg/g wet weight)
Rex sole	0.012±0.001	0.026±0.007
English sole	0.013±0.001	0.007±0.001
Dover sole	0.014±0.003	0.011±0.001
Petrale sole	0.012±0.002	0.008±0.001
Orange rockfish	0.014±0.003	0.088±0.015
Lingcod	0.009±0.002	0.224±0.012
Hake	0.014±0.002	
Starry flounder	0.018±0.002	
Sand sole	0.014±0.003	

Source: Childs and Gaffke, 1974.

TABLE 1.2. Maximum Cd Content Reported for Foods in the U.S.

Type of Food	Cd (μg/g)	Type of Food	Cd (μg/g)
Dairy products	0.01	Root vegetables	0.08
Grain and cereals	0.06	Garden fruits	0.07
Meat, fish and poultry	0.03	Sugar and adjuncts	0.04
Leafy vegetables	0.14	Oils and fats	0.04
Legume vegetables	0.04	Potatoes	0.08

Source: Fribergh et al., 1974.

In uncontaminated areas most foods will contain less than 0.1 μg/g of Cd. This does not pose a problem. The World Health Organization proposed a tolerable weekly intake of 400 to 500 ug which is about 70 μg/day.

The only foods that usually accumulate excessively high concentrations of Cd are shellfish, liver and kidney. With these foods the concentration often exceeds 10 μg/g (Doull et al. 1980). This can pose a problem if sufficient quantities are ingested. The waters from which shellfish are harvested should be closely monitored to insure that industrial pollutants containing Cd are limited.

In contaminated soils the Cd concentration may increase considerably which can cause a severe problem. In Japan the itai-itai disease, which produced osteomalacia and renal damage, was caused by the consumption of Cd contaminated rice. The Cd concentration of high accumulator foods and foods grown in areas of suspected Cd contamination should be closely monitored to insure that the level in the foods to be consumed is not toxic.

Lead

Lead is a highly toxic non-essential metal that is widely distributed throughout all parts of the environment. Pb has been used in the manufacturing industry, pottery industry, as pigments in paints and varnishes, in automobile engines as well as in many other areas. The widespread use of Pb, along with its natural occurence, has lead to the presence of Pb in a wide variety of foods averaging about 0.1 μg/g (Mair and Cresser, 1962). Up to two thirds of the daily intake of Pb was found to be of dietary origin (Rabinowitz et al., 1973).

Pb accumulates in the body to varying degrees depending on the environmental load and the nature of the Pb compound. The absorption of Pb through the gastrointestinal tract is approximately 8 percent but it

depends on the calcium, iron, fat and protein content along with age. In children the absorption of dietary Pb is approximately 40 percent (Doull et al., 1980). Pb has been shown to concentrate in the bones and soft tissues, especially in the brain where it causes reduced function. It also inhibits the biosynthesis of heme, producing anemia, causes damage to the kidneys and liver, and it produces hyperactivity.

Chronic exposure to high doses of inorganic Pb often results in a severe and fatal condition of Pb encephalopathy. Damage occurs to the cerebellum, spinal cord and motor and sensory nerves. Peripheral neuropathy is seen after prolonged exposure in which demyelination and axonal degeneration is observed. Lower levels of Pb exposure that do not cause encephalopathy have been shown to still cause psychological problems in children (Doull et al., 1980).

Pb also damages the proximal tubules of the kidney depressing the reabsorption of glucose, amino acids and phosphate. Prolonged high Pb exposure causes a progressive disease in which death due to renal failure usually ensues.

One of the early manifestations of Pb poisoning is anemia. Pb impairs the synthesis of hemoglobin at even low levels of exposure. Pb causes extensive excretion of the precursors of porphyrin and hemoglobin in urine, interferes with the incorporation of iron into protoporphyrin and inactivates aminolevulinic acid dehydratase and porphyrinogen decarboxylase, two enzymes necessary for hemoglobin synthesis.

The average daily intake of Pb is about 0.45 mg with 0.22 to 0.40 mg attributed to food (Venugopal and Luckey, 1978). Extensive tests and analysis have been done on the lead content in various foods.

One of the major sources of Pb in the food supply is from Pb-soldered cans. The Pb leaches from the solder used in the can manufacture causing

the Pb concentration in the food to increase with time. The Pb concentration of various foods canned in vinegar were reported to have more than 20 times the levels of Pb contained in similar fresh and jarred products (Mair and Cresser, 1962). The mean Pb level in products canned in vinegar was 6.6 ppm while the mean Pb level of other canned foods is reported as 0.20 ppm (Boyer and Johnson, 1982). Table 1.3 shows the Pb content of various foods canned with vinegar. Table 1.4 shows the Pb content of other canned foods.

TABLE 1.3. Pb Content of Products Canned in Vinegar

Product	Can#	Pb (ppm)	Product	Can#	Pb(ppm)
Cucumber	1	10.0±0.20	Leeks	1	6.6±0.15
	2	8.2±0.16		2	6.8±0.14
	3	9.6±0.18		3	6.4±0.13
Mustard greens	1	6.8±0.14	Ginger and	1	6.3±0.13
	2	6.6±0.14	mixed vegetables	2	6.5±0.13
	3	5.8±0.12		3	6.2±0.13
Sauerkraut	1	4.0±0.09			
	2	4.4±0.10			
	3	4.1±0.10			

Source: Moran, 1981.

TABLE 1.4. Pb Content of Canned Foods 1981 Mean

Canned Food	Pb (ppm)	Canned Food	Pb (ppm)
Tuna	0.49	Chili/chili soup	0.35
Chicken gumbo/soup	0.11	Chicken noodle soup	0.08
Vegetable soup	0.17	Mixed vegetables	0.08
Green beans	0.34	Lima beans	0.23
Beans with pork	0.26	Peas	0.20
Corn	0.27	Sweet potatoes	0.43
Asparagus	0.22	Beets	0.21
Tomatoes	0.23	Tomato juice	0.06
Mixed fruit	0.23	Apple sauce	0.04
Apricots	0.17	Orange juice	0.06

Source: Boyer and Johnson, 1982.

Due to the increased toxic effect of Pb to infants and children, infant formulas and baby foods have been examined. Dry baby cereal was found to have between 0.20 to 0.39 μg/g of Pb with a mean of 0.23 μg/g (Gajan et al., 1982). Strained chicken was found to contain 0.09 μg/g of Pb while strained applesauce had 0.18 μg/g of Pb (Holak, 1980).

The Pb Content of fish has also been studied. Oregon groundfish were found to contain between 0.036 to 0.229 μg/g of Pb. The mean Pb content for Northern Coast fish was 0.10 ppm while the mean Pb content of Southern Coast fish was 0.07 ppm (Childs and Gaffke, 1974). Table 1.5 lists the Pb content found in these Oregon groundfish.

TABLE 1.5. Pb Content of Oregon Groundfish

Species	Southern Coast Pb (μg/g wet weight)	Northern Coast Pb (μg/g wet weight)
Rex sole	0.114±0.023	0.229±0.019
English sole	0.130±0.042	0.033±0.004
Dover sole	0.075±0.029	0.105±0.010
Petrale sole	0.044±0.004	0.091±0.009
Orange rockfish	0.092±0.052	0.069±0.005
Lingcod	0.036±0.012	0.070±0.007
Hake	0.004±0.009	
Starry flounder	0.048±0.004	
Sand sole	0.047±0.014	

Source: Childs and Gaffke, 1974.

Albacore muscle fresh from the sea was found to have a Pb concentration of about 0.3 ng/g. The Pb content of albacore muscle from a Pb-soldered can however was found to be 1.4 μg/g (Settle and Patterson, 1980). This again shows that leaching of Pb from the can is a serious problem.

The maximum permitted level for Pb in most foods is set at 2 μg/g by the U.S. Food and Drug Administration (FDA). Although this level is usually not exceeded in fresh foods, foods in Pb-soldered cans, especially if canned in vinegar, can pose severe problems.

Mercury

Mercury is a toxic metal which has no known normal metabolic function. Its presence in the tissues of living organisms is a result of contamination. The level of Hg in the environment, especially in the water, has increased due to improper disposal of industrial wastes.

The chemical form of Hg determines its deposition in the body and its effects. Elemental Hg exists in a vapor state and it is therefore not a problem in food. Inorganic Hg, particularly Hg^{+2}, is highly toxic and results in damage to the kidneys. Inorganic Hg is only minimally absorbed by plants and animals so it is not considered a severe problem (Doulls et al., 1980). Organic Hg however is readily absorbed and accumulated through the food chain and is of main concern. Organic Hg compounds are 95 percent absorbed in the gastrointestinal tract due to their high lipid solubility. Organic Hg, of which methylmercury is the most common and most toxic, accumulates in the brain and blood and causes neurological problems.

Hg has toxic effects involving numerous organs and systems. Methylmercury intoxication causes central nervous system disorders because it can pass through the blood-brain-barrier. Loss of hearing, smell and taste, incoordination, paralysis and abnormal reflexes along with intellectual deterioration also occurs. Inorganic Hg poisoning generally affects the kidneys. There is both glomerular and tubular damage with an increase in the excretion of albumin and low molecular weight proteins. Often up to 80 percent of the absorbed Hg^{+2} will accumulate in the proximal tubules causing severe lesions of the kidney necrosis (Venugopal and Luckey, 1978).

Hg also exhibits teratogenic effects (Venugopal and Luckey, 1978). When administered to rodents, lowered body weight of offspring, cleft palate, abnormal tails, changes in the nervous tissue and death often

occurred. In humans, women suffering from Minimata disease produced offspring who had a cerebral-palsey-like disease with mild spasticity, ataxia, seizures and severe mental retardation.

Hg has a tendency to bioaccumulate in the food chain. Fish accumulate significant amounts of Hg, predominantly methylmercury, and are seen as a major source of Hg in the diet. Methlymercury levels in plants, meats and dairy products are usually quite low, but the use of Hg as a fungicide did result in contaminated grain which led to widespread illness and death (Matson, 1974). In various tests, Hg in the soil appears to be absorbed and translocated to the edible part of the plant. The analysis of various fruits and vegetables however showed very low concentrations of Hg with the highest being 35 ppb (Gomez and Markakis, 1974). The analysis of the major foods except fish in the U.S. also showed that they are essentially free of Hg. None exceeded 50 ppb. Table 1.6 shows the Hg content of some common foods.

Table 1.6. Total Hg Content of Foods

Food	Mean (ppm)	Food	Mean (ppm)
Milk, whole	0.01	Cheese, cheddar	0.02
Chicken muscle	0.02	Chicken liver	0.03
Eggs, whole	0.03	Wheat	0.02
Barley	0.03	Oats	0.01
Rice	0.01	Pork liver	0.03
Beans, dry	0.03	Salt, noniodized	0.06

Source: Gomez and Markakis, 1974

Almost all of the Hg in the diet comes from fish, other seafood and possibly red meat. Although the Hg level of most marine fish is usually less than 0.20 ppm, some large marine fish such as tuna, swordfish, marlin halibut and shark range from 0.20 to 1.50 μg/g and can be as high as 5.0 μg/g (An Assessment of Mercury in the Environment, 1978). Commercial fish from the Seychelles waters of the Indian Ocean were analyzed for Hg (Matthews, 1983). Many of the larger species such as kingfish, becune, carangue balo and bonito had Hg levels exceeding the maximum permissible level of 0.5 ppm set by the FDA. Dogtooth tuna exceeded this level with two fish having levels of 3.3 and 4.4 ppm. Swordfish generally show the highest incidence of Hg with the majority of them exceeding the FDA maximum allowable limit (An Assessment of Mercury in the Environment, 1978). Table 1.7 shows the Hg content reported for some fish.

Table 1.7. Hg Content of Fish From the Seychelles

Species	Hg range (ppm)	Species	Hg range (ppm)
Yellowfin tuna	0.012-0.06	Skipjack tuna	0.026-0.448
Dogtooth tuna	0.38-4.4	Bonito	0.065-1.26
Carangue balo	0.025-1.51	Kingfish	0.055-1.46
Becune	0.26-1.58	Sailfish	0.01-0.86
Bourgeois	0.045-0.69	Vara vara	0.135-0.812
Velle platte	0.13-0.9	Job	0.01-1.035

Source: Matthews, 1983

The high levels of Hg found in fish can be a health hazard as evidenced by the methylmercury poisoning in Minimata Bay in which the total Hg level in fish was estimated at 11 μg/g wet weight (An Assessment of Mercury in the Environment, 1978). The standard of 0.5 ppm set by the FDA is often exceed in many types of fish. Due to this, the continual monitoring on Hg in fish that are to be consumed is necessary.

Arsenic

Arsenic, which is recognized as a cumulative poison and has been implicated as a carcinogen, is present in most food products due to its use in agricultural chemicals. Arsenic is used extensively in rodenticides, insecticides, fungicides, wood preservatives, soil sterilants, defoliants, feed additives, weed killers, pigment and antifouling paints and in the glass, textile and tanning industry. The contamination of soil and water from industrial or agricultural activity can present a serious health hazard if the As is able to get into the food chain.

Arsenite, the trivalent As, is more toxic than arsenate, the pentavalent As. The no-effect level in rats for arsenate is 125 ppm while it is only 62.5 ppm for arsenite (Doull et al., 1980). Generally the naturally occurring As is pentavalent while that added to the environment is trivalent.

Acute As poisoning occurs as a consequence of accidental or homocidal ingestion and generally not from contaminated foods. The ingestion of contaminated food resulting in long term low level exposure, leads to chronic As intoxication. It is characterized by malaise, fatigue, gastrointestinal disturbance, hyperpigmentation and peripheral neuropathy. Anemia, decreased erythrocyte production and leukopenia are also observed. Arsenic also inhibits the functioning of enzymes such as d-amino acid

oxidase, pyruvate oxidase, choline oxidase and transaminase.

It has also been implicated as a possible carcinogen. Skin cancers can appear years after exposure. In 1955 in Japan babies received a formula made from powdered milk contaminated with As. Years later there were incidences of leukomelanoderma and keratosis in these children. Mental retardation, epilepsy and brain damage were also prevalent in these exposed children (Arsenic, 1977).

Arsenic is also thought to have teratogenic effects. The administration of sodium arsenate to hamsters, mice and rats has caused anencephaly, congenital malformations, cleft lip, fused vertebrae, eye defects and forked ribs (Hood and Bishop, 1972). Potassium arsenate however was fed to four ewes during pregnancy at a dose of 5 mg/kg without effect (Jones et al., 1966).

Several mutagenic studies have been done for sodium arsenate. Human leukocyte cultures exhibited chromosomal breakage after short term exposure (Paton and Allison, 1972). Patients who had undergone As therapy more than 20 years earlier had much higher incidences of chromosomal abberations than those who had not been treated with As (Arsenic, 1977).

Due to its various toxic effects, it is necessary to limit exposure to As. A limit of 1 ppm for foods is set in Great Britain while in the U.S. 2.6 ppm is allowed for most foods. Very little As is found in food products other than in fish and fish products. Table 1.8 shows the As concentration reported for a variety of foods. Meat, fish and salt have the highest values. Table 1.9 shows typical levels found in a variety of fish.

TABLE 1.8. Arsenic Content of Foods

Food	As (μg/g)	Food	As (μg/g)
Meats		Vegetables and grains	
Beef, stewing	1.3	Wheat, whole	0.17
Pork, loin	0.06	Rye, seed	0.16
Pork, liver	1.4	Corn	0.11
Pork, kidney	0.0	Corn meal	0.78
Lamb, chop	0.35	Rice, U.S.	0.13
Other		Puffed rice	1.6
Cocoa, Hersheys	0.59	Kellogg's Special K	0.66
Coffee	0.00	Beet greens	0.24
Tea	0.89	Swiss chard	0.56
Salt, table	2.71	Rhubarb	0.48
Salt, sea	2.83	Red pepper	0.06
Sugar, lump	0.10	Garlic, fresh	0.24
Milk, evaporated	0.17	Cherry tomatoes	0.37
Butter, unsalted	0.23	Mushrooms	2.9

Source: Schroeder and Balassa, 1966.

Table 1.9. Arsenic Content of Seafood

Type	As (ppm)	Type	As (ppm)
Haddock	2.17	Kingfish	8.86
Oysters, fresh	2.9	Oysters, frozen	2.7
Scallop, fresh	1.67	Shrimp, shells	15.3
Shrimp, fresh frozen	1.50	Conch, fresh	3.1
Conch, dried whole	5.63	Clams, fresh frozen	2.52

Source: Schroeder and Balassa, 1966.

Arsenic concentrations above 1 ppm are present naturally in most fish, edible seaweed and their products. The United Kingdom total diet survey suggests that 75 percent of the total As ingested originates in fish and shellfish. This points out the necessity for closely monitoring the As concentration in seafood.

Selenium

Selenium differs from the other trace metals mentioned in that it is an essential micronutrient. There is however a very narrow difference between the concentration at which it is considered essential and the concentration at which it is known to be toxic. The nutritional requirement for Se is thought to end at 0.1 ppm while toxicity may begin at 0.4 ppm (Venugopal and Luckey, 1978). The estimated threshold level at which Se will produce physiological, pathological, clinical and lethal effects in animals is listed in table 1.10.

TABLE 1.10. Biological Spectrum of Dietary Se in Animals

Characterization	ppm in Animals
Deficiency	less than 0.03
Physiological	0.03-0.4
Pathological	0.4-3.0
Clinical	3-20
Lethal	greater than 20

Source: Venugopal and Luckey, 1978

Se is present in human blood, urine and tissues. It is thought to play a role as either a replacement for or in working in conjunction with vitamin E. Se has a significant role in the biosynthesis of ubiquinone and it is an essential constituent of glutathione peroxidase, an enzyme which is responsible for the maintainance of erythrocytes. Se is also thought to play a role in DNA-RNA control and in the stimulation of antibody synthesis.

The toxicity of Se varies according to the chemical form and the species involved. Elemental forms of Se are not absorbed that well from the gastrointestinal tract and are considered less toxic. Selenite (Se^{+4}) is considered more toxic than selenate (Se^{+6}). The minimum lethal dose (MLD) of sodium selenite given intravenously to rabbits is 1.5 mg Se/kg of body weight. The MLD of sodium selenate given under the same conditions is 2 to 2.5 mg Se/kg of body weight (Selenium, 1976).

Se intoxication in animals usually occurs due to the consumption of Se accumulator plants grown on highly seleniferous soils. Acute poisoning

which is rare, can occur if sufficient quantities of contaminated plants are consumed. Sudden death or sudden distress which is characterized by labored breathing, prostration, diarrhea and abnormal movement and posture can occur.

Chronic Se poisoning is caused by the ingestion of excess Se over a longer period of time. There are two types of chronic Se poisoning in animals, blind staggers and alkali disease. Blind staggers is characterized by impaired vision, respiratory failure and weakness of limbs. This usually occurs if the livestock consumes plants containing 100 to 1000 ppm of Se. Alkali disease occurs from the ingestion of plants containing about 25 ppm of Se (Doull et al., 1980). Alkali disease is characterized by lack of vitality, loss of hair, hoof malformations, lameness, emaciation, anemia and necrosis of the liver.

Chronic Se toxicity in man can occur with excessive dietary intake of Se. Depression, pallor, gastrointestinal distress, and a garlic odor of the breath are usually the first signs. Loss of hair and nails, discoloration or decayed teeth, chronic dermatitis, anemia and kidney, liver and spleen damage can result after continued intoxication.

Extensive tests have been done dealing with many of the toxic effects of Se in animals, but less is known about its effects in man. Excess Se is known to have an adverse effect on reproduction in animals. Mice given Se produced fewer and smaller litters and deaths before weaning were excessive (Schroeder and Mitchener, 1971). The feeding of 10 ppm of selenite to pigs lowered the conception rate and increased the proportion of piglets that were small, dead or weak at birth (Wahlstrom and Olson, 1959).

There is much controversy over whether Se should be considered a carcinogen. Non-metasticizing hepatic tumors were induced in rats by feeding them grain containing 5 to 10 ppm of Se. Hepatic carcinomas,

hepatic adenomas and precancerous neoplasms were observed in 25 percent of the rats fed 4.3 ppm selenite (Venugopal and Luckey, 1978). Epidemiological studies however have indicated a decrease in human cancer death rates with increasing Se content of crops (Shamberger et al., 1976). Recent experimental evidence also supports the antineoplastic effect of Se with regard to certain tumors (Doull et al., 1980).

Se is known however to have teratogenic effects. The chick embryo is extremely sensitve to Se toxicity. Se concentrations in feed that were too low to produce symptons of poisoning in other farm animals can produce grossly deformed embryos with missing eyes and beaks and distorted wings and feet (Selenium, 1976). Abnormal development of embryos in rats, pigs sheep and cattle were also the result of the teratogenic effect of Se (Venugopal and Luckey, 1978).

Se is also thought to cause fetal toxicity and teratogenesis in humans. Female laboratory technicians exposed to selenite powder experienced miscarriages and a deformed infant with bilateral clubfoot was born (Venugopal and Luckey, 1978). Indian woman in Columbia who ate grains suspected of being contaminated with Se gave birth to malformed babies (Selenium, 1976).

Although Se is an essential element, the excessive intake of Se has been shown to cause toxic effects. We should therefore be concerned with the distribution of Se in foods. Se is usually present in appreciable amounts in meat, seafood and most grains. Se, which is naturally present in the environment , is added to the environment through its industrial and agricultural uses. Se is used in the electronics industry, glass and ceramic manufacturing, steel manufacturing , in paints and varnishes, in fungicides and in insecticides.

The Se content of plants and plant products depend on the kind of

soil on which they are grown and the manner in which the plant takes up and retains Se. Most crop plants, grains and grasses are not primary Se accumulators but they can still contain up to 30 ppm of Se (Selenium, 1976). Wheat usually contains 0.1 to 1.5 ppm but in highly seleniferous areas, levels as high as 18.8 ppm have been found. Corn has been found to contain 1 to 14.9 ppm, barley 1.6 to 15.7 ppm and rye has been found to contain 0.9 to 3.8 ppm when grown near industrial establishments (Selenium, 1976). Highly fertilized agricultural products can contain more than ten times the usual Se content. The more typical concentrations of Se found in grains, cereals, vegetables and fruits are given in Table 1.11.

The Se concentration in meats depends upon the Se content of the feedstuffs used. If animals are grazed on plants grown in highly seleniferous soils, the Se content will be higher. In 1979 the FDA accepted Se as a feed additive in many species due to its recognition as an essential micronutrient. The Code of Federal Regulations Title 21 ¶573.920 specifies that Se can be added as a nutrient in animal feed in the form of sodium selenite or sodium selentate. In complete feed for chickens, swine, sheep, beef cattle, dairy cattle and ducks the level can not exceed 0.1 ppm. In turkeys it can not exceed 0.2 ppm in complete feed. These regulations were passed because the minimum dietary requirements for Se in animals, which range from 0.1 to 0.2 ppm, are not met in 70 percent of animal feeds. The toxic level in poultry and swine is considered to be 3.0 ppm so the addition of 0.1 to 0.2 ppm, of Se to feeds was considered to be safe by the FDA. If excessive amounts of Se are added to feedstuffs however, it could lead to toxicity in the animals or in humans due to the residue of Se. Se levels in meat from cattle raised in seleniferous areas can be much higher and range from 1.17 to 8 mg/kg of body weight (Selenium, 1976). The Se content reported for various meats and dairy products is given in Table 1.12.

TABLE 1.11. Se Content of Vegetables, Grains and Cereal Products

Product	Se (μg/g)	Product	Se (μg/g)
Carrots, fresh	0.022	Turnips, fresh	0.008
Carrots, canned	0.013	Apples, fresh	0.006
Cabbage, fresh	0.023	Apple sauce	0.002
Cauliflower, fresh	0.007	Bananas, fresh	0.010
Corn, fresh	0.004	Oranges, fresh	0.014
Corn, canned	0.005	Peaches, fresh	0.004
Garlic, fresh	0.276	Peaches, canned	0.004
Green pepper, fresh	0.008	Pears, fresh	0.006
Green beans, fresh	0.006	Pineapple, fresh	0.006
Green beans, canned	0.009	Pineapple, canned	0.008
Lettuce, fresh	0.009	Barley cereal	0.676
Mushroom, fresh	0.141	Bread, white	0.280
Mushroom, canned	0.109	Bread, whole wheat	0.676
Onion, fresh	0.015	Corn flakes	0.028
Sweet potatoes	0.007	White flour	0.197
Canned potatoes	0.011	Wheat flour	0.645
White potatoes	0.006	Egg noodles	0.662
Radish, fresh	0.042	Oat breakfast cereal	0.451
Tomatoes, fresh	0.005	Oats, quick	0.114
Tomatoes, canned	0.010	Polished rice	0.334
Brown rice	0.383	Puffed rice	0.026
Wheat cereal	0.241	Wheat breakfast cereal	0.110

Source: Morris and Levander, 1970.

TABLE 1.12 Se Content of Meat and Dairy Products

Product	Se (μg/g)	Product	Se (μg/g)
Cheese		Beef	
American	0.090	Round steak	0.363
Cottage	0.054	Ground	0.208
Swiss	0.108	Liver	0.454
Cream, table	0.006	Kidney	1.69
Cream, substitute	0.034	Pork	
Milk		Chop	0.261
Evaporated, canned	0.013	Kidney	1.90
Skim	0.050	Lamb	
Skim, powdered	0.243	Chop	0.172
Whole, homogenized	0.013	Kidney	1.69
Eggs		Chicken	
Yolk	0.193	Breast	0.125
White	0.057	Leg	0.151
		Skin	0.154

Source: Morris and Levander, 1970.

In fish, Se levels vary widely both within and between species. Even in waters which are low in Se, unusually high accumulations have been reported. Tuna fish were reported as having a range of 3.40 to 6.2 ppm with an average of 4.63 ppm (Kifer et al., 1969). This is considered very high. The Se content reported by Holak (1976) for canned tuna was much lower. The Se concentrations reported for seafood and seafood products are listed in Tables 1.13 and 1.14.

TABLE 1.13. Se Content of Fish Meal

Type	Average (ppm)	Range (ppm)
East Canadian herring	1.95	1.30-2.6
Chilean anchovetta	1.35	0.84-2.6
Tuna	4.63	3.40-6.6
Smelt	0.95	0.49-1.23
Menhaden	2.09	0.75-4.2

Source: Kifer et al., 1969.

TABLE 1.14. Se Content Content of Seafood and Seafood Products

Type	Se (μg/g)	Type	Se (μg/g)
Lobster tail	0.681	Cod, fillet	0.465
Shrimp, shelled	0.604	Flounder, fillet	0.338
Oysters	0.659	Canned tuna	
Perch, fillet		#1	0.30
#1	0.35	#2	1.00
#2	0.23	#3	0.16
#3	0.44		

Source: Morris and Levander, 1970; Holak, 1976.

Although the normal concentrations of Se found in food are not alarming, foods grown and harvested from highly seleniferous soils or waters can contain excessive amounts of Se. Since excessive amount of Se are known to have toxic effects in man and animals it is necessary to monitor the Se concentration in Se accumulator species, primarily fish and grains, or species which are harvested from areas thought to be highly seleniferous.

Conclusion

The occurance of heavy metals in food is well documented. Traces of these heavy metals are obviously a health hazard. Although the concentrations usually found in most foods are not excessive, contaminated areas often show a large increase in trace metal concentrations. Due to an increased awareness of the toxicity of these metals , and to an increase in the concentration of these metals due largely to industrial processes, it is essential that the concentrations of these heavy metals in food be established. Fish, shellfish and other seafood are particularly important because they tend to accumulate higher amount of these metals, especially Cd, Hg, As and Se, as compared to other foods.

II. METHODS OF TRACE ANALYSIS

There are a variety of instrumental methods which are applicable to the analysis of trace metals. These include atomic absorption spectrometry, plasma emission spectrometry, polarography and stripping voltammetry, gas chromatography, fluorescence spectroscopy and neutron activation analysis. Below is a brief description of each of these methods with a review of their application to the analysis of trace metals, with particular reference to the analysis of arsenic and selenium, in food.

Atomic Absorption Spectrometry

Atomic Absorption Spectrometry (AAS) is one of the most widely used techniques for quantitatively determining trace elements. It has been established for the determination of more than sixty elements in the concentration range of parts per billion (ppb) for flame atomization and picograms (pg) for electrothermal methods.

An atomic absorption spectrometer consists of an atomization system, an optical system and a detector system. The atomizer produces free atoms from the introduced sample. Since atoms will absorb radiation only at wavelengths characteristic of its species, this gives a qualitative estimate of the species. The amount of radiation absorbed is proportional to the concentration of the element being analyzed. This is the basis for quantitative analysis.

The atomizer is usually a flame or electrothermal device. A flame atomizer enables better precision over an electrothermal atomizer. It is usually the first choice unless the sample volume is very small or the amount to be measured is very low. Air-hydrogen, nitrogen oxide-acetylene and air-acetylene flames are commonly used depending on the temperature

needed to atomize the element of interest.

Electrothermal atomizers consist of graphite furnaces and heated rods. To eliminate interference problems, atomization should be obtained under isothermal conditions. Electrothermal atomizers have a small sample capacity, usually 10 to 50 microliters of solution. Detection limits reported are often three orders of magnitude better than with flames. Relative detection limits are usually on the order of 0.1 ppb while absolute detection limits are measured in terms of picograms.

The optical system employed generally consists of a double beam system in which the light from the radiation source is split into two components. A sample beam passes through the atomizer and the reference beam passes around the atomizer. These two beams can then be compared to eliminate instabilities in the radiation source.

The detector system usually consist of a photomultiplier whose full spectrum range extends from 193.7 nm, the As line, to 852.1 nm, the Cs line. The amount of light energy falling on the detector will be electronically converted to an absorbance reading.

The application of AAS for trace metal analysis in food is extensive. With AAS it is necessary for the sample to be in a liquid form. To ensure complete dissolution of the sample, a dry or wet ashing procedure is necessary. Great care must be taken to prevent loss of the element during the sample digestion. Sample digestion procedures are discussed in Chapter VI.

AAS is not very suitable for the direct determination of As and Se. The principal wavelengths for absorption of these elements are in the ultraviolet region. For As it is 193.7 nm and for Se it is 196.0 nm. The formation of a hydride followed by introduction of the hydride into the AAS is a much better procedure for determining As and Se.

Samples are first wet oxidized to convert As and Se to arsenate and selenate respectively. The acidified solutions are then reduced with sodium borohydride. Once formed the hydride is swept from the reaction vessel by inert gas and decomposed thermally. The atomizer is usually an argon-hydrogen flame or a silica cell heated by a flame or an electric heater in which hydrides are decomposed to the atomic species and hydrogen.

The detection limits using hydride generation with an argon-hydrogen flame were reported to be 5 ng or 0.1 ppb for As and 50 ng or 1.0 ppb for Se (Schmidt and Royer, 1973). An air-acetylene flame was found to be less sensitive. With a hollow cathode lamp source and an air-acetylene flame commonly attained sensitivities for As range from 1 to 1.5 ppm with detection limits between 0.25 to 1 ppm. The sensitivity is defined as the concentration of analyte which will produce 1 percent absorption. Sensitivity for Se is 1 ppm with a detection limit between 0.2 to 1 ppm (Kirkbright and Ramon, 1971). The use of a premixed nitrous oxide-acetylene flame allows for a lower sensitivity than the air-acetylene or argon-hydrogen flame, but the background absorbance and noise limits its applications.

Another version of AAS determination of volatile hydrides utilizes cold trap collection and flameless AAS. The hydrides of As, Sb, Bi and Se are collected in a liquid nitrogen cold trap and then volatilized into a graphite furnace. Flameless atomization results in a tenfold lower detection limit as compared to flame atomization due to lower noise levels, more efficient atomization and longer analyte residence time in the optical path. Matrix effects however are more serious and an ion exchange treatment is often needed. Sensitivities for this method are reported as 1.0 ng for As and 10 ng for Se. Detection limits are reported as 0.2 ng for As and 10 ng for Se (Schmidt and Royer, 1973).

A method for measuring nanogram quantities of As in samples with an unknown matrix was also developed. Instead of using two inlet ports, the gas was directed into the interior of the graphite tube via one port. Three traps were used to collect water during the arsine generation procedure to insure that the gas was completely dry. Water reduces the efficiency of revolatilizing the hydride since the hydride is soluble in water. The sensitivity and absolute detection limit of this method were found to be 0.11 ng and 0.14 ng respectively (Uthus et al., 1981). The As concentration of various NBS standards was determined and the results agreed with the certified values.

A semi-automatic hydride generator utilizing a sodium borohydride and sodium hydroxide solution to reduce an acid-oxidized food sample was found to give a detection limit of 7 ng or 0.35 ng/ml for As and 16 ng or 0.80 ng/ml for Se. The relative recoveries were good with 98±5 percent for As and 106±6 percent for Se being reported (Fiorino et al., 1976). In this variation the hydrides were quantitatively reduced and then carried directly into a shielded, nitrogen-diluted, hydrogen diffusion flame. The apparatus used was much simpler than those using a peristaltic pumping system to provide a continuous mixing of the reactants.

Plasma Emission Spectrometry

Inductively coupled argon plasma emission spectrometry (ICAP-ES) is becoming one of the most important techniques for the analysis of trace elements. Although its detection limits are poorer as compared to electrothermal atomic absorption and instrumentation is very expensive, it allows for simultaneous multielement analysis and calibration curves are linear over a concentration range of 4 to 5 orders of magnitude.

An ICAP emission spectrometer consists of a radio-frequency generator, a torch circled with copper induction coils, a spray chamber and nebulizer, an argon supply and flow system, a spectrometer with an entrance slit and exit slits, photomultipliers and a measuring device.

Plasmas are very hot gases in which atoms are present in large part as ions. ICAPs are plasmas that derive their sustaining power by induction from high-frequency magnetic fields. Since argon gas is nonconducting at room temperature, it is necessary to produce argon ions and electrons that will interact with the magnetic field in the radio-frequency coil.

As argon flows through a quartz tube inside a copper coil, the coil is energized by a radio-frequency generator which will create a charging magnetic field. A Telsa coil is used to obtain argon ions and electrons. These ions and electrons accelerate but also experience resistance to their flow. Heating occurs which results in the ignition of a plasma in the coil region above the torch. The temperature of the plasma near the coil is about 8,000 - 10,000 °K. Another argon stream acts as a coolant flow to prevent the plasma from melting the torch. A third argon gas flow carries the sample into the central part of the torch. The sample is usually injected through a spray-chamber nebulizer. Once in the plasma the sample atoms emit radiation which can be detected, quantified and resolved with a spectrometer.

Since the plasmas have temperatures that are twice as hot as flames and the residence time of the sample species is much longer, the chemical interferences are greatly reduced. The degrees of atomization are also more complete and can approach 100 percent.

ICAP-ES has been applied to the simultaneous analysis of trace metals in food, but the sensitivity obtainable is not sufficient for the direct analysis of As, Bi, Ge, Pb, Sb, Se and Sn. They are usually present in the

ng/g range in foods while detection limits are usually in the 0.5 to 3 ng/ml range (Wolnik et al., 1981). The formation of volatile hydrides, which was used for AAS determination, can be applied to ICAP-ES to improve its sensitivities and detection limits.

The hydrides are formed in the same manner as with AAS. A system for the continuous generation of the hydride with sodium borohydride and subsequent introduction into an ICAP-ES was reported to give an order of magnitude improvement in detection limits over those obtained with a conventional nebulizer (Wolnik et al., 1981). The determination of As and Se in standard reference materials by hydride evolution ICAP-ES was found to agree with the certified values of these samples (Nygaard and Lowry, 1982).

The use of liquid nitrogen as a condensation trap for the collection and subsequent revaporization of the hydride which was used in AAS has also been applied to ICAP-ES (Hahn et al., 1982). To minimize argon condensation in the trap and plasma instability, helium was used during the initial stages of the reaction, with a brief purge of argon just prior to revaporization. This hydride generation condensation system improved the detection limits for the ICAP-ES.

The reported detection limits for hydride generation condensation ICAP-ES are 0.02 ng/ml for As and 0.1 ng/ml for Se. Hydride generation without condensation gave detection limits of 0.8 ng/ml for As and Se. Conventional nebulization, which is much less reliable, gave detection limits of 20 ng/ml for As and Se (Hahn et al., 1982).

Polarography and Stripping Voltammetry

Differential pulse polarography (DPP) and stripping voltammetry are two electrochemical techniques that have been applied to the analysis of trace metals in food. A discussion of the theory behind these methods and the

instrumentation is given in Chapter IV.

The analysis of Se is usually carried out using differential pulse cathodic stripping voltammetry (DPCSV) with a hanging mercury drop electrode (HMDE). This allows a much lower detection limit than DPP or CSV used alone. Se, specifically Se(IV) which is the electroactive species, will interact with a mercury electrode in the presence of chloride ion to form a complex which will strip from the surface of the electrode at cathodic potentials. The detection limit for this procedure is reported as 3 ng/g with a working range of 3 to 10,000 ng/g (Blades et al., 1976). A DPCSV method employing hydride generation for the determination of Se gave a detection limit of 1 ppb (Dennis et al., 1976). In this method the gaseous hydrogen selenide which is produced by the reduction of Se by sodium borohydride, is trapped in an alkaline cell solution where it is analyzed at an HMDE.

Se analysis can also be carried out using anodic stripping voltammetry (ASV), but a solid electrode must be employed as the working electrode. A rotating gold disk electrode and a gold plated glassy carbon electrode have been used for ASV. The gold disk electrode gives a better detection limit and a smaller residual current (Adeloju et al., 1983). A combination of DPP and ASV gives a much better sensitivity than ASV alone. A detection limit of 4 ppb is reported for DPASV (Andrews and Johnson, 1976). The DPASV and ASV methods do have several disadvantages however. Since a solid electrode is used, pretreatment of the electrode surface is necessary and variations of the electrode surface will lower its reproducibility. The DPCSV method is usually preferred when analyzing large numbers of complex samples as in the analysis of foods.

As can also be determined by ASV or CSV. With ASV it is necessary to use a solid electrode. A gold working electrode was found to be

preferable over a platinum electrode, but the difficulty of pretreatment of the electrode still exists. The use of DPASV gave a detection limit similar to ASV with 0.02 ng/ml or 100 pg of As(III) in 5 ml of 1 M perchloric acid being reported (Forsberg et al., 1975). The deposition time required for ASV however is significantly longer than that required for DPASV.

A method for high-speed ASV of As at a gold-film electrode was developed in which the sample was digested with mineral acids followed by the reduction of As(V) to arsenic(III) chloride by copper(I) chloride (Davis et al., 1978). The volatile arsenic(III) chloride was then transferred by a stream of nitrogen to a trap solution for analysis. A polar planimeter was used to evaluate the peak area and a digital readout of As concentration was obtained. The procedure was found to be accurate and reliable at the ng level. NBS reference materials were analyzed and the results using this method were in general agreement with the certified values.

Computerized potentiometric stripping analysis was also used to determine As(III). In potentiometric stripping analysis As is deposited on the working electrode surface in the same manner as in ASV, but the As is stripped chemically at a controlled rate and a potential versus time curve is recorded. The detection limit reported for this technique is 4.3 mM which is about five times better than that obtained with the high speed ASV (Jagner et al., 1981). CSV using an HMDE has also been carried out for As determination. The HMDE is advantageous because it does not suffer from the disadvantages of a solid electrode. When a trace amount of Se is added, in the range of 50 ng/ml, the reduction of As(III) to As(0) is shifted toward a more positive potential. This increases the As cathodic stripping peak heights and gives a detection limit of 2 ng/ml (Holak, 1980).

The determination of As by differential pulse polarography has also

been successful. A detection limit of 0.3 μg/ml or 4 X 10^{-9} M in a 1 M HCl supporting electrolyte is attainable (Holak, 1980). Interferences experienced due to the reduction of Pb and Sn can be eliminated by use of a purifying technique with an ion-exchange resin. About 0.4 μg/g of As can be determined using this method (Holak, 1976).

Gas Chromatography

Gas chromatography (GC) has excellent separation capabilities and it is highly suited to trace element analysis as long as the trace elements can be transformed into thermally stable and volatile derivatives.

A GC consists of a carrier gas which must pass through pressure regulators to control the flow rate, a sample injection chamber, a separation column, a detector, and an oven to heat the injector, column and detector. Liquid samples are usually injected from a microsyringe while solid samples are usually dissolved in a solvent. The sample chamber is heated for rapid volatilization and the inert carrier gas sweeps the sample directly into the column. The column can be made of a variety of materials depending on the elements or compounds to be analyzed. The columns will act to separate and resolve complex mixtures into their component parts. Various types of detectors can then be used to sense and record separations. A peak is obtained and the area under the peak is proportional to the concentration of the analyte.

GC has been applied to the determination of both As and Se. Total As can be determined by GC if the As is first collected and converted to phenylarsine. The collection and conversion procedure involves sample digestion, crystallization of As with thionalid, followed by filtration and phenylation of the precipitate. The triphenylarsine formed is then separated on a GC column and quantitatively detected by a microwave emission

detector which is attached to the GC column outlet. The relative sensitivity for this method is reported as 30 ng/g (Talmi and Norvell, 1979).

For Se determination, the sample is digested followed by a reaction with 1,2-diamino-4-nitrobenzene to form 5-nitropiazselenol. The 5-nitropiazselenol is extracted into toluene and injected into the GC for determination.

Stable isotope dilution gas chromatography-mass spectrometry has been applied to the determination of Se in biological materials (Shimoishi, 1976). Se reacts with O-phenylenediamine to form the piazselenol. Samples are spiked with enriched ^{82}Se and the isotopic ratio of ^{80}Se to ^{82}Se is measured by combined gas chromatography-mass spectrometry using dual ion monitoring. Determinations at the ppb level are possible with this method. The results of the analysis of standard reference materials show excellent agreement with the certified values.

Electron capture detection has been applied to the GC determination of Se in marine sediments. The digestion procedure and reation with O-phenylenediamine followed by injection into the GC gave a detection limit of 20 ng/g of Se (Siu and Berman, 1983).

An electron-capture detector was also used for the determination of Se in milk and milk products. A procedure was developed to determine total Se and specifically Se(VI). Se(IV) is first analyzed using an extraction procedure that is specific for it. Total Se is then analyzed with the difference between the two being the Se(VI) content. The detection limit for this method is reported as 0.005 μg of Se (Shimoishi, 1976).

X-ray Fluorescense Spectroscopy and Flourometry

The most widely used x-ray technique for quantitative analysis is x-ray fluorescense. In x-ray fluorescense an incident beam of high energy x-rays is used to excite the target atoms and to knock an electron off the target atom. After a short time, the excited ion returns to the lower energy shell, producing a fluorescence spectrum similar to the emission spectrum. Only the line spectrum appears however, not the continuous background. This gives fluorescence spectra a much greater signal-to-background level which is preferred for analytical work.

An x-ray fluorescence spectrometer consists of an x-ray source, a method of dispersing the x-rays and a detection device. The Coolidge tube is the most frequently used x-ray source. It consists of a heated tungsten cathode which generates electrons that are accelerated to high velocities in an electric field. When the electrons strike the target anode their rapid deceleration causes 1 to 2 percent of the kinetic energy to be converted to x-rays. The x-rays are then dispersed into the target atoms causing excitation. The fluorescent radiation that is given off is sensed by a detector which will measure the intensity of the emitted light.

X-ray fluorescence could be used to quantitatively determine As and Se without prior sample destruction, but these methods are not very sensitive (Selenium, 1976). A decomposition and preconcentration step is mandatory for trace analysis.

An x-ray fluorescence technique to determine trace amount of Se in the ng/g and μg/g range was developed. Following a wet digestion or incineration digestion procedure, a two step reduction process using 5 N HCl and NaI reduces the selenite to elementary Se. The Se is then deposited on a small membrane filter and determined using x-ray fluorescence. The

detection limits for this method are 0.13 μg/g and 0.08 μg/g of Se for the wet digestion and the incineration sample decomposition procedures respectively (Raptis et al., 1980). The result from the analysis of standard reference materials using this method are in excellent agreement with the certified values.

A fluorometric method can be used to analyze Se concentration down to the submicrogram range. The determination of selenium in sulfide samples was done using this method. Following a wet digestion method, selenium is reacted with a complexing agent such as 3,3'-diaminobenzidine, 1,8-diaminonaphthalene or 2,3-diaminonaphthalene. This results in the formation of a piazselenol. The fluormetric determination of this selenium-2,3-diaminonaphthalene complex gives a detection limit of 0.002 μg/ml (Michael and White, 1976).

Neutron Activation Analysis

Neutron activation analysis (NAA) involves measuring the radioactivty induced in the sample after bombarding it with neutrons. NAA can be done without destruction of the sample which eliminates chemical treatment of the sample. Trace level determinations can be made and the simultaneous analysis of multielements are easy to perform.

Although NAA is a very sensitive and selective method, in many cases the elements to be determined must be separated selectively from the neutron activated matrix. An inorganic ion exchange coupled to a solvent extraction system allows for rapid isolation of the radioisotopes without many chemical steps (Gallorini et al., 1978). A combustion technique which does not involve any previous dissolution of the matrix has also been shown to be satisfactory (Orvini et al., 1974).

The absolute sensitivity is 0.1 ng of arsenic for a thermal neutron flux of 10^{12} neutrons/square cm-sec (Arsenic, 1977). A detection limit of 20 ppb is observed for Se (Gladney et al., 1978). At submicrogram levels of Se however, this method can give inaccurate results (Selenium, 1977).

Other Methods

Chemiluminescence, which is the emission of light from excited molecules induced by chemical energetics, has had rare applications in trace metal analysis. A gas-phase chemiluminescence procedure based on the reaction between ozone and the hydrides of the analytes was investigated for As, Sb, Sn and Se. The detection levels were reported as 0.15 ng, 10, ng, 35 ng and 110 ng respectively (Fujiwara et al, 1982). Although application of this method for the determination of As in NBS orchard leaves agreed with the certified value, the contents of Sn, Sb and Se were too low for this method to detect.

Spectrophotometric methods have been one of the most reliable methods for determining small quantities of As and Se. When arsenate reacts with acidified molybdate, arsenomolybdic acid is formed. This can be reduced to give a blue color which is stable and free from interferences. Arsine could also be bubbled through a 0.5 percent solution of the silver salt of diethyldithiocarbamate in pyridine (Arsenic, 1977). This produces an intense red color. Selenium can be reacted with aromatic amines such as 3,3'-diaminobenzidine and 2,3-diaminonaphthalene to produce a color (Crosby, 1977). The intensity of the color formed is proportional to the concentration of the analyte. A spectrophotometer is used to measure the absorbance of the solution at the analyte's maximum absorption wavelength. The absorption measured is proportional to the concentration of the analyte. Although this method is simple, quick and relatively inexpensive, its

detection limit is not low enough to permit it to be used for trace determinations. The detection limit, for example, of the selenium-3,3'-diaminobenzidene complex in spectrophotometry is 50 µg/l (Selenium, 1977).

Chemical and titrimetric analyses are also not practical for trace determination. Besides requiring long sample preparation time and large samples, they usually are incapable of measuring down to a few ppm (Matson, 1974).

Spark source mass spectrometry has also been proposed for trace metal analysis. After the sample is ashed it is mixed with pure graphite and sparked in the spectrometer. Multielement analysis is possible and ppb detection levels are observed for over 60 elements (Matson, 1974), but it has limitations of speed and cost.

Conclusion

AAS is highly specific and enables low limits of detection, but the direct measurement of As and Se is not feasible. Hydride generation followed by AAS has proven to be an effective technique for trace analysis although it is not without problems. The formation of arsine from As is subject to interferences from excessive salts, acids from the digestion and various ions including Cu^{+2}, Co^{+2}, Ni^{+2}, and NO_3^-. The recovery of small quantities of AsH_3 is difficult. Although the detection limit is reported in the ng range, analysis is reliable only at levels of As above 0.2 µg (Davis et al., 1978).

The generation of hydrogen selenide is also subject to interferences from elements like Ag, Fe, Bi, Cu, As, Sb, Sn, Co, Ni, Pt and Mn. Although some detection limits are reported as low as 0.005 µg, great care is needed to avoid metal interferences which would prevent volatilization (Fiorino et

al., 1976). Hydride formation conditions vary depending on the manufacturer and shelf age of the sodium borohydirde which is used as a reductant. This usually necessitates varying the acidity of the analyte until an optimal signal is reached. Freshly crystallized sodium borohydride would prevent this, but the large quantities necessary for this reaction make it infeasible.

AAS is not a simultaneous multielement technique, it requires considerable operator supervision and the instrumentation is relatively complex and costly.

ICAP-ES also requires the formation of the respective hydrides for As and Se, due to the insensitivity of their emission lines. The problems with hydride formation mentioned above also apply here. Although ICAP-ES is capable of simultaneous multielement analysis, detection limits are poorer than with AAS and instrumentation is also expensive.

X-ray fluorescence is less sensitive than the hydride evolution technique, but it is more accurate and is not subject to many of the interferences in the chemical preparation step. The sample preparation procedure however is still a problem and relatively large sample sizes are necessary (Gruenwedel and Whitaker, eds., 1984). Fluorometric methods suffer from interferences from strong oxidizing and reducing agents, Cu and Fe. The formation of a piazselenol is a time consuming process and the instability of the piazselenol requires that the fluorescence measurement be made within five minutes of extraction (Blades et al., 1976).

The GC determination of As is long and time consuming because it requires the collection and then conversion to triphenylarsine. The determination of Se usually requires the formation of a piazselenol which is subject to the restrictions mentioned above. GC for the analysis of foods is generally time consuming and expensive.

NAA does not require sample digestion but it requires much time and elaborate expensive equipment which make it impractical especially in a small laboratory.

Electrochemical methods require pretreatment of the sample and it is subject to interferences from some metal ions. Solid electrodes, which must be used with the ASV of both As and Se are subject to several problems. The response of the electrode depends on its past history, its pretreatment and its ability to form oxide films. Cu can interfere severely with solid electrodes and its removal is complex and time consuming. Because of the care required in handling solid electrodes, they are unsuitable for routine analysis.

The use of a mercury electrode eliminates these problems. CSV which uses a HMDE, and DPP which uses a dropping mercury electrode (DME) are suitable for As and Se analysis. DPSCV is very sensitive for Se and it is the electrochemical method of choice. DPP enables low detection limits for As, although ion-exchange separation or the addition of trace amounts of Se is necessary to achieve maximum sensitive and reliable peaks.

The polarograph has several advantages in that it costs less than most instruments, it allows for the simultaneous determination of several metals and it is very versatile in that many other materials can be determined with it. Although other methods allow for comparable or slightly lower detection limits, the speed, sensitivity, selectivity and the small sample requirements make DPP and SV excellent techniques for trace analysis.

III. POLAROGRAPHY AND STRIPPING VOLTAMMETRY

Voltammetry is the branch of electroanlytical chemistry that measures the effect of an applied potential on the current that flows through a cell in which the solution under investigation is undergoing electrolysis. By gradually increasing the applied voltage, changes in the current, caused by oxidation or reduction of a substance in the solution, can be measured resulting in a current voltage curve (voltammogram). The potential at which the current is one-half of the total wave height is known as the half-wave potential ($E_{1/2}$). This is illustrated in Figure 3.1. Each species is reduced or oxidized at a characteristic half-wave potential in a given solvent and temperature. The $E_{1/2}$ enables the qualitative analysis of a solution. The magnitude of the current is proportional to the concentration of the electroactive species which in undergoing oxidation or reduction. This enables the quantitative analysis of the electroactive species.

A three electrode system, consisting of a working electrode, a reference electrode and a counter electrode, is generally used in polarography. A potentiostat controls the voltage across the working electrode and the counter electrode. Figure 3.2 illustrates the three electrode potentiostat. The reference electrode is used to measure the potential of the working electrode. It is isolated by the voltage-follower amplifier so that very little current is drained from it. The working electrode potential measured by the reference electrode is fed into the control amplifier along with various input voltages. If the potential of the working electrode differs from the desired value, as indicated by a difference between the potential measured by the reference electrode and the sum of the other input voltages, the control amplifier will compensate

FIGURE 3.1. Current-Voltage Curve Illustrating the Half-Wave Potential, Diffusion Current and Charging Current

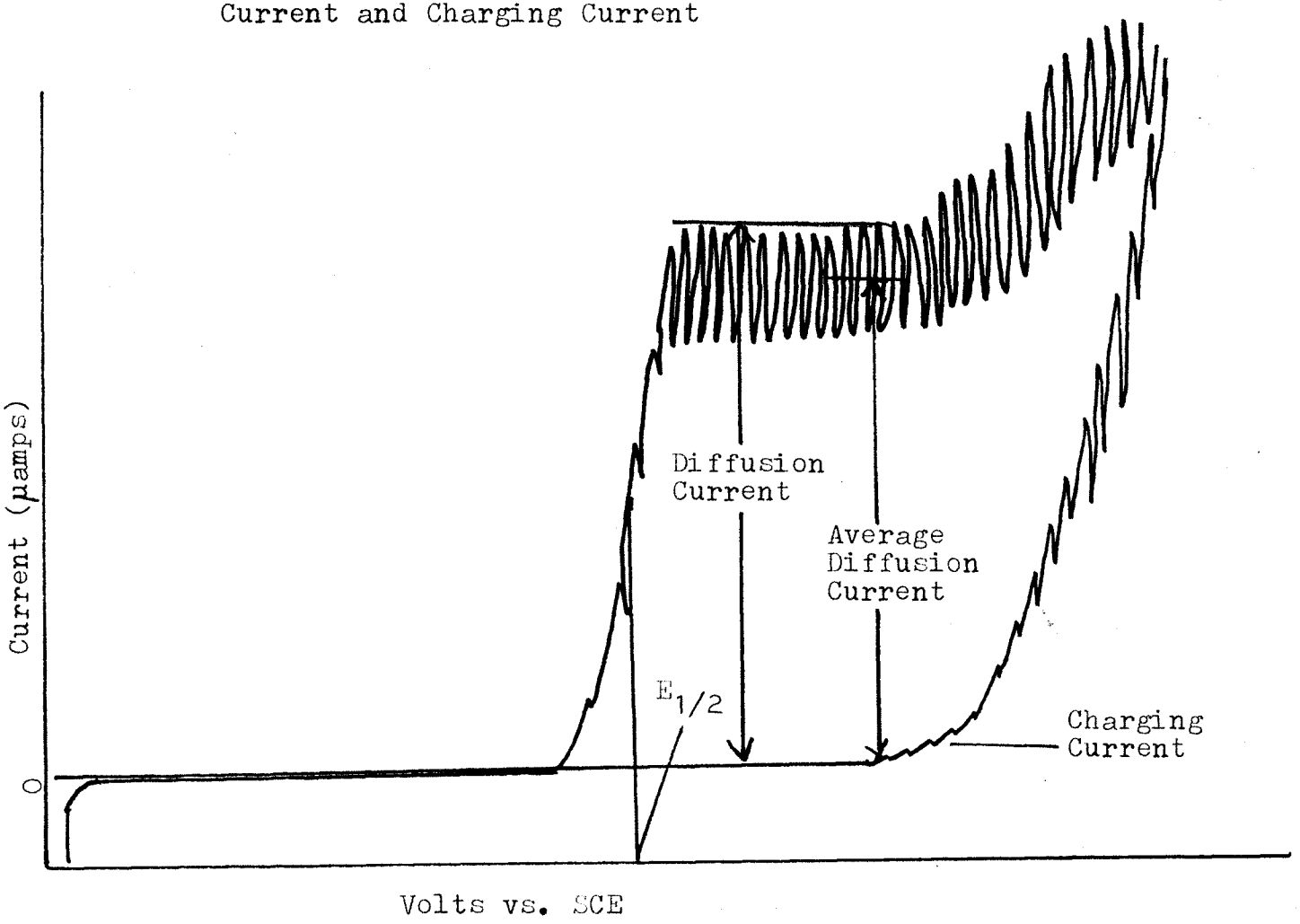
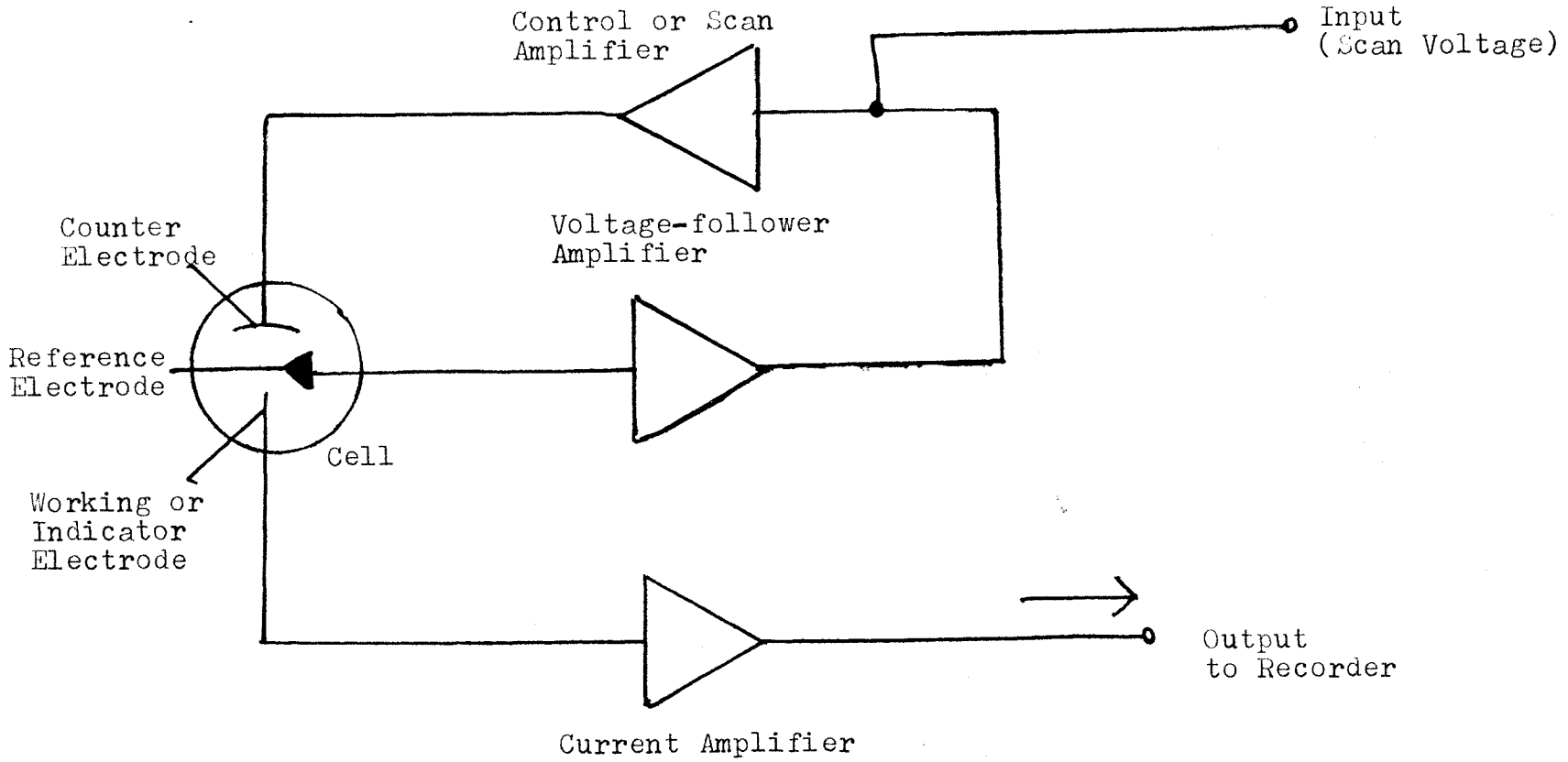


FIGURE 3.2. Schematic Diagram of a Three Electrode Potentiostat



Source: Bauer et al., 1978.

for this by supplying a corrective voltage at the counter electrode. The current flow is between the counter electrode and the working electrode. The current amplifier will convert the current flowing at the working electrode to a voltage that is proportional to the magnitude of the current. The voltage will then be amplified and presented to a recorder.

Polarographic Currents

The current that flows through a cell depends on the rate of the electrode reaction and the rate of transport of the electroactive species to the electrode surface. Diffusion is the only mode of transport to the electrode surface if migration is eliminated (by use of a supporting electrolyte) and convection is eliminated (solution is not stirred). Since the rate of this diffusion is the slowest step in the process, the height of the limiting current will be proportional to the rate of diffusion.

$$i_d = nFAD(dC/dX)_{x=0} \tag{3.1}$$

where:

i_d = diffusion current, A = area of electrode,

D = diffusion coefficient of the electroactive species,

$(dC/dX)_{x=0}$ = the concentration gradient of the electroactive species at the electrode surface.

The Ilkovic equation shows a linear relationship between the diffusion current i_d and the concentration of the electroactive species.

$$i_d = 708nCD^{1/2}m^{2/3}t^{1/6} \tag{3.2}$$

where:

i_d = diffusion current in μamps

C = concentration in mM/liter

D = diffusion coefficient in cm^2/sec

n = number of electrons transported.

Due to the growth of each mercury drop, the observed diffusion current varies with the growth of the surface area of each drop. The current will therefore oscillate between well defined upper and lower limits at applied potentials. The maximum current is given by the Ilkovic equation with the average current being 6/7 of the maximum current. The average current is represented by the modified Ilkovic equation. It shows a linear relationship between the average diffusion current and the concentration of the electroactive species.

$$i_d = 607nCD^{1/2}m^{2/3}t^{1/6} \qquad (3.3)$$

The diffusion current is proportional to the concentration of the electroactive species as long as the hydrodynamics and the mechanism of the mass-transfer process is independent of the concentration of the electroactive species and the supply of the electroactive species at the electrode surface is not affected by a chemical or physical process whose rate is not first-order. The diffusion current is used for quantitative analysis of a species.

Other types of currents can exist and these will interfere with the quantitative analysis of a species. A charging current is consumed in charging the electrical double layer of the mercury electrode in order to insure that the dropping electrode achieves the potential determined by the applied voltage. If the current is measured while the charging current is quite large, it creates problems in obtaining a true diffusion current and it can give rise to significant errors. The current measured would not be due to the diffusion current, but due to the charging of the double layer.

The charging current is limited by the rate at which the fresh electrode surface is formed.

$$i_c = 0.00567 C_i (E_z - E) m^{2/3} t^{-1/3} \quad (3.4)$$

where:

i_c = charging current

C_i = integral capacitance

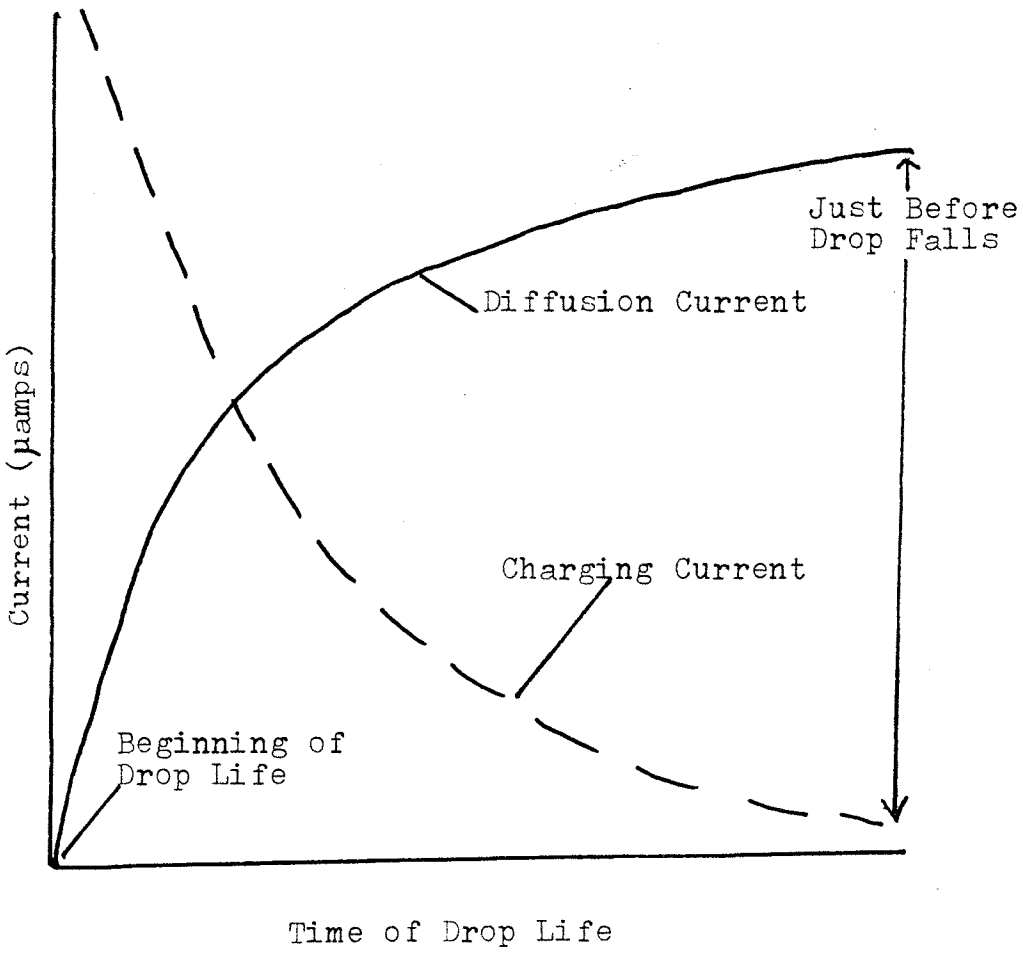
E_z = the potential relative to the point of zero charge

Since the charging current is proportional to $t^{-1/3}$ (the charging current will decrease over time) and the diffusion current is proportional to $t^{1/6}$ (the diffusion current will increase over time), measurement of the current late in the life of the drop will give a better indication of the true diffusion current. This is illustrated in Figure 3.3.

Kinetic currents occur when an electroactive substance is formed from an electroinactive form at the given potential. The rate of this chemical reaction will govern the polarographic current. The behavior of formaldehyde is a classic example of this. The diol, which predominates in aqeous solutions, is not reducible, but the aldehyde form is. A wave is obtained whose height reflects the rate of transformation of the diol to the reducible aldehyde form. The kinetic current is usually proportional to the concentration of the electroactive species, but it varies markedly with pH or temperature changes. Kinetic currents can be used in analytical procedures, but the experimental conditions must be carefully controlled.

Adsorption currents occur if an electroactive species or the product of the electrode reaction is adsorbed onto the surface of the working electrode. The current would then be limited by the available surface area of the electrode. The adsorption current is independent of the electroactive species concentration and can not be used in analytical work.

FIGURE 3.3. Variation of the Charging Current and the Diffusion Current with Time During the Life of a Single Drop



Catalytic currents are obtained when a substance which acts as a catalyst is present in the solution. The catalyst will cause a shift in the oxidation or reduction potential of the electroactive species. Since a catalyst is regenerated, a small concentration of a catalyst can cause much higher limiting currents. Application of catalytic currents to analytical work is limited.

In quantitative analysis it is important to know which factor is limiting the current. Table 3.1 shows the dependence of polarographic currents on several parameters. Varying these parameters and observing the effects can give a good indication of which current is the limiting current.

TABLE 3.1. Characteristics of Polarographic Currents

	Variation With				
Current	Concentration	Height of Hg column (h)	Time	pH	Temp.
Diffusion(i_d)	kc	$kh^{1/2}$	$t^{1/6}$	independ.	+1.3%/°C
Kinetic(i_k)	kc	independ.	$t^{2/3}$	depend.	+5-20%/°C
Adsorption(i_a)	to a limit	kh	$t^{-1/3}$	usually independ.	different
Catalytic(i_c)	to a limit	varies	$t^{2/3}$	strongly dependent	-

Source: Bauer et al., 1978.

Observed currents are sometimes considerably larger than the diffusion current. These large currents, called maxima, arise because of spontaneous streaming of the solution near the mercury electrode. During streaming a vigorous motion of the solution can be observed with a microscope. The streaming occurring with maxima of the first order is believed to be due to the motion of the double layer caused by the asymmetry of the electrical field around the drop. The streaming that occurs with maxima of the second order is due to the turbulence arising inside the drop because of the flow of mercury into it. The streaming will increase as the rate of the flow of mercury increases. This streaming brings more electroactive particles to the surface of the electrode than can be transported by diffusion.

Maxima of the first order are associated with the reduction of inorganic species. The current will increase almost linearly with a change in applied potential and cause the current to be much higher than the true diffusion current. Then quite suddenly the current will fall to a steady normal diffusion current. This results in the formation of a pronounced peak on the current-voltage curve. The occurrence of such maxima interferes with the determination of the half-wave potential and can prevent the resolution of different waves.

Maxima of the second kind are usually observed with organic components. A second order maxima appears as a rounded hump on the plateau of the wave. These maxima cover a wide potential range and can interfere considerably.

Maxima of the first order are more prominent in dilute supporting electrolytes while maxima of the second kind will increase as the concentration of the supporting electrolyte increases. Maxima are least likely to occur if a low rate of mercury flow (less than 0.5 mg/sec) and a low concentration of electroactive species (less than 10^{-3} M) is used. A

maxima supressor, such as gelatin or Triton-X-100, will remove the interference and restore the wave to its expected shape. These surface-active substances adsorb to the surface of the mercury drop and prevent streaming. Used in excess however, the supressors can also distort, lower or shift the position of the polarographic wave.

Electrodes

A polarographic cell usually contains three electrodes which are immersed in the solution to be analyzed. The electrode whose potential is varied is known as the working or indicator electrode. The indicator electrode can be made from a number of materials including platinum, gold, mercury, graphite and glassy carbon among others. When a dropping mercury electrode (DME) is used as the indicator electrode, the technique is referred to as polarography. In stripping voltammetry the indicator electrode must be stationary, therefore a DME is not used. Solid electrodes made of platinum, gold, graphite or glassy carbon could be used, but the hanging mercury drop electrode (HMDE) and the mercury film electrode (MFE) are generally preferred due to their versatility. A reference electrode and a counter electrode are also used.

A DME consists of a fine glass capillary connected to a mercury reservoir by rubber tubing. The position of the mercury reservoir must be constant to insure that the mercury pressure will be constant. The mercury flow rate and drop time will then be reproducible. The contact between the mercury reservoir and the electrical circuit is achieved by the insertion of a platinum wire sealed in a glass tube into the mercury reservoir.

With a DME, drops of mercury fall from the orifice of the capillary at a reproducible, constant rate. Each drop is the working electrode while

attached to the column of mercury in the capillary. The current depends on the drop time and the rate of mercury flow through the capillary. The drop time is determined by the length of the capillary, the height of the mercury column which determines the mercury pressure, and the inner diameter of the tip of the capillary. The drop time is proportional to the length of the capillary and inversely proportional to the height of the mercury column above the capillary tip. The drop time is usually between 5 to 30 drops per minute. The rate of mercury flow depends on the diameter and the length of the capillary tube. Outflow is usually one to four mg/sec.

The current-voltage curves obtained with a DME are very reproducible as long as the drop time and flow rate are constant. Each new mercury drop has a fresh, clean surface that is practically unaffected by the electrolysis at the previous drops. Each drop represents a point on the polarogram so vibrations will only affect one point rather than the whole measurement.

Since a slow rate of voltage scanning is used, the change of the DME potential during the life of a single drop can be neglected. The current measured on each drop can therefore be considered to be obtained under constant potential.

The small dimensions of the DME enables the analysis of small volumes of solution and results in only a small amount of electrolysis. The DME however is a discontinuous electrode. The drop is constantly changing in size and falling off, which results in large capacitor currents. The sensitivity of the DME is limited in the analysis of dilute solutions.

The HMDE consists of a small mercury ball the size of a drop hanging from a thin mercury thread in the capillary tube. A drop of known and reproducible size can be obtained by means of a micrometer-feeder HMDE. The HMDE is used for stripping analysis. The entire stripping analysis is

performed on one drop. After the stripping analysis is performed the drop is dislodged and replaced for the next analysis. This eliminates the problem of the large charging current which is obtained with the periodic growth and fall of the mercury drop in a DME. The HMDE has a reproducible surface and all of the characteristics of the DME which make it suitable for electroanalysis apply to the HMDE.

A MFE is prepared by depositing a mercury film on a glassy carbon electrode. The MFE is very sensitive because a very concentrated amalgam is formed during the deposition step due to the small amount of mercury in the film. A MFE however can only be used for anodic stripping and it is generally not good for routine analysis because of the care required to insure that the surface of the MFE is as reproducible as possible. This is usually done by a conditioning step in which a potential which is positive with respect to the $E_{1/2}$ of the electroactive species is applied to the MFE to insure the oxidation of the metal back into the solution.

Mercury is chemically inert in most aqueous solutions but it is oxidized at potentials between 0 and +0.4 V versus the saturated calomel electrode. Therefore, substances which react positively at these potentials cannot be determined with a mercury electrode due to the excessively high currents from the mercury oxidation. Mercury electrodes however can be used to determine practically all reducible metals because of the high overpotential for evolution of H_2 at a mercury electrode. Electrodes of other material can not be used at these very negative potentials.

The most common reference electrode is the saturated calomel electrode (SCE). A silver-silver chloride reference electrode is also commonly used. The purpose of a reference electrode is to provide a stable potential with which the potential of the working electrode is compared.

The potential of the reference electrode must be unchanged during the voltage change, so the reference electrode must be unpolarizable under conditions where a small current flows for a period of time.

Reference electrodes have potentials that are known and that are independent of the composition of the electrolyzed solution, the presence or oxidants on surface-active substances, and the current flowing through it. Any error or variation in the potential of the reference electrode will lead to a corresponding uncertainty on the potential of the dropping electrode.

A counter electrode is necessary in solutions of high resistance or when the reference electrode may be unstable because there can be a difference between the apparent applied potential and the potential actually occurring at the working electrode. When a counter electrode is used, the current will be passed between the working electrode and the counter electrode. The counter electrode will measure the potential difference between the working electrode and the reference electrode. Since no current will pass through the reference electrode, its potential will remain constant and equal to its open-circuit value. The resistance contribution to the measured potential will also be much smaller. The counter electrode is a conductive material that is chemically inert such as platinum.

Supporting Electrolytes

The solution to be electrolyzed must be sufficiently conducting so supporting electrolytes are used to increase the conductivity and decrease the resistance of the electrolyte. A supporting electrolyte will eliminate the movement of ions due to migration in the electric field. In order to quantitatively interpret the current-voltage curve, movement due to migration must be eliminated so a true diffusion current can be obtained.

The choice of the supporting electrolyte depends on the potential at which the electroactive compound gives a wave and on the properties of the sample. Electrolytes which do not cause depolarization in the widest possible potential range are used to prevent interference with the wave of the electroactive substance. Electrolytes that do not react with the electroactive species in the solution or with mercury should be used.

The concentration of a supporting electrolyte is usually between 0.1 M to 1.0 M. Lower concentrations cause resistance to be larger while higher concentrations can raise the level of impurities. Impurities can cause polarographic waves to occur which would interfere with the analysis if they occurred in the range of potentials that was being studied.

Strong acids, such as hydrochloric or sulfuric acid, strong bases, such as sodium or lithium hydroxide, neutral salts, such as chlorides, perchlorates, sulfates of alkali metals, or tetraalkylammonium ions, or buffering solutions of complexing agents, such as tartates, citrates, cyanides, fluorides or amines, can be used. The supporting electrolyte must be chosen carefully because some electroactive species can lose their electrochemical activity in certain media. An extensive list of supporting electrolytes for various electroactive species is given in Meites' Polarographic Techniques.

Deaeration

Oxygen is normally present in solutions open to the air at amounts of 0.0001 M. Oxygen is reducible over most of the ranges of potentials and will give two waves which could overlap and interfere with other waves. Oxygen undergoes two steps of two electron reduction. In the first step O_2 is reduced to H_2O_2 at an $E_{1/2}$ of -0.05 V versus SCE. The second two electron reduction to water occurs at an $E_{1/2}$ of -0.5 to -1.3 V versus SCE.

Oxygen can be removed by bubbling an inert gas through the solution before the analysis and then passing the inert gas over the solution during the analysis to prevent re-solution of air. Nitrogen is normally used because it is not reducible.

Applications of the Polarographic Theory

There are a variety of polarographic techniques that are available for quantitative analysis. In dc polarography the voltage scan is applied as a linear ramp as shown in Figure 3.4. This technique is not very sensitive and the detection limit is about 1 ppm. In trace analysis normal pulse polarography, differential pulse polarography and stripping analysis are commonly used.

In normal pulse polarography a square wave voltage pulse of about 40 microseconds is applied to the electrode during the last quarter of the drop life. The amplitude of the voltage pulse is gradually increased for each drop as shown in Figure 3.5. The drop time must be incorporated into the electrode system to synchronize the drop time of the DME with the frequency of applied pulses.

The effect of the charging current is minimized by measuring the current just before the drop falls. During the 20 microseconds of the second half of the pulse the charging current is quite small but the faradaic current (that due to the electrolysis) is still large. The curve resembles waves in dc polarography but it has a staircase appearance as shown in Figure 3.6 because the current is sampled once during each drop life and stored until the next sample period. A plateau is reached in the overall wave when the current remains practically constant. After polarization each successive drop is polarized and achieves a limiting current equal to the previous one. When one polarographic wave is preceded

FIGURE 3.4. Programming Waveform for DC Polarography

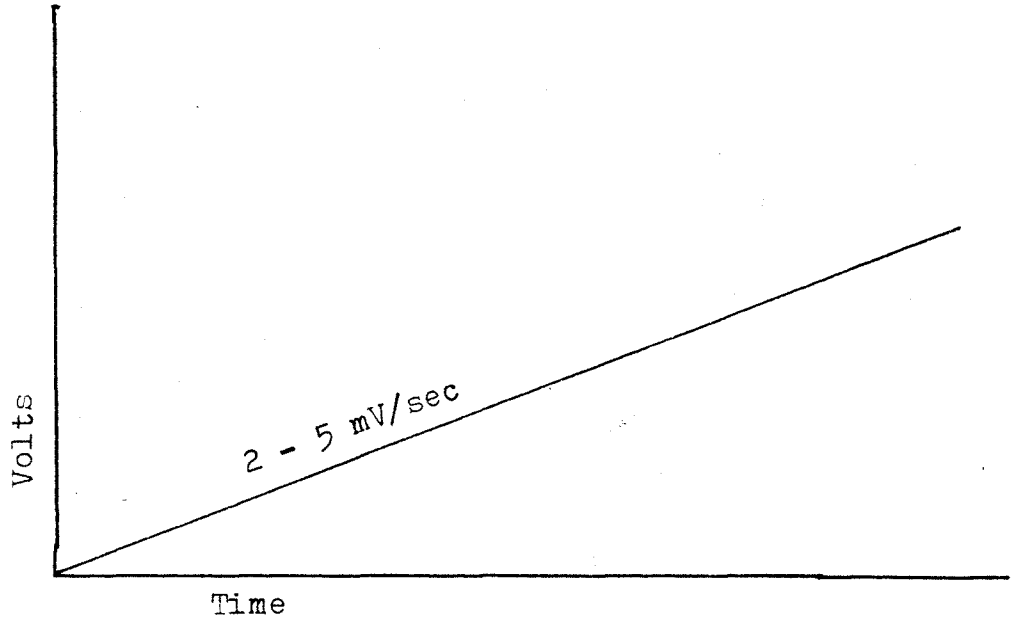
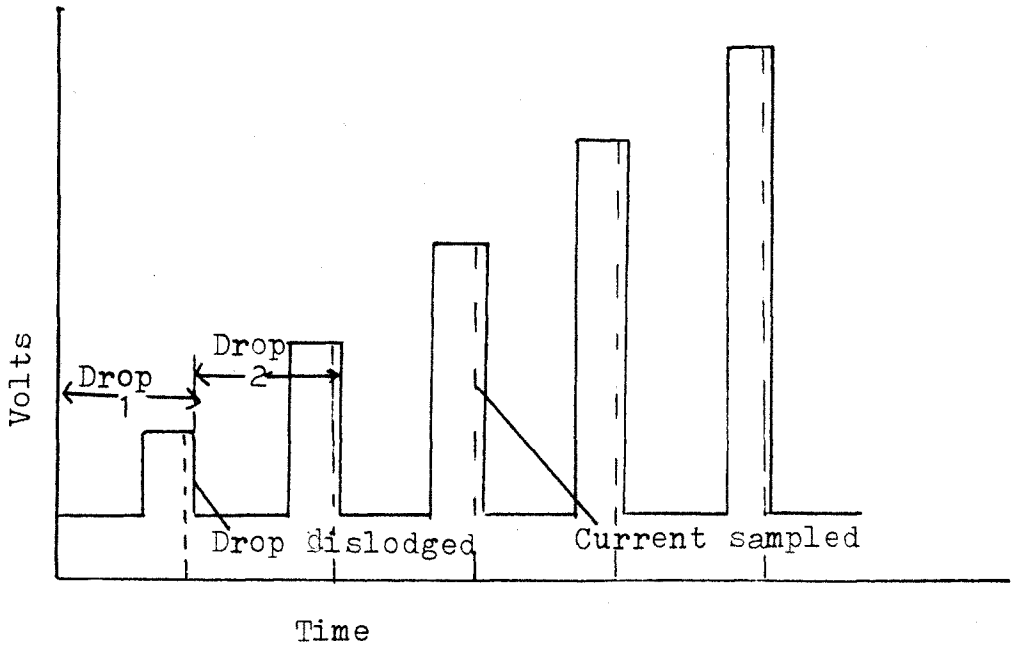


FIGURE 3.5. Programming Waveform for Pulse Polarography



by another the limiting current is measured as the difference in plateaus because the wave heights are usually additive.

In differential pulse polarography the base potential applied is steadily changed in small increments. A pulse height of 10 to 100 mv is maintained at a constant level with respect to the base potential as shown in Figure 3.7. The current is measured twice during each drop life, once immediately before the pulse is applied and the other late in the pulse just before the drop is dislodged. This minimizes the charging current. The current difference of the second value minus the first value measured is plotted against the base potential.

If there is no electrolysis occurring, the difference of the two currents will remain constant and a horizontal line will be recorded. When the potential enters a range where electrolysis occurs the difference in current will increase as evidenced by a rising portion on the polarogram. The peak shape as shown is Figure 3.8 results from the measurement of the current difference. The pulse height can be varied to manipulate sensitivity. As pulse height increases the peak potential increases but resolution of closely spaced peaks decreases.

Stripping voltammetry consists of a preconcentration step, equilibration step and stripping process. A stationary electrode, usually a HMDE or MFE, is used. Stripping voltammetry can be used for cations which form amalgams or for anions which form slightly soluble compounds with mercury.

During the preconcentration step the solution is stirred to maximize analyte-electrode contact, while a potential is applied to the indicator electrode to cause the species of interest to deposit on the surface of the mercury electrode. The potential chosen for the deposition phase depends on the species being analyzed.

FIGURE 3.6. Resultant Current-Voltage Curve for Pulse Polarography

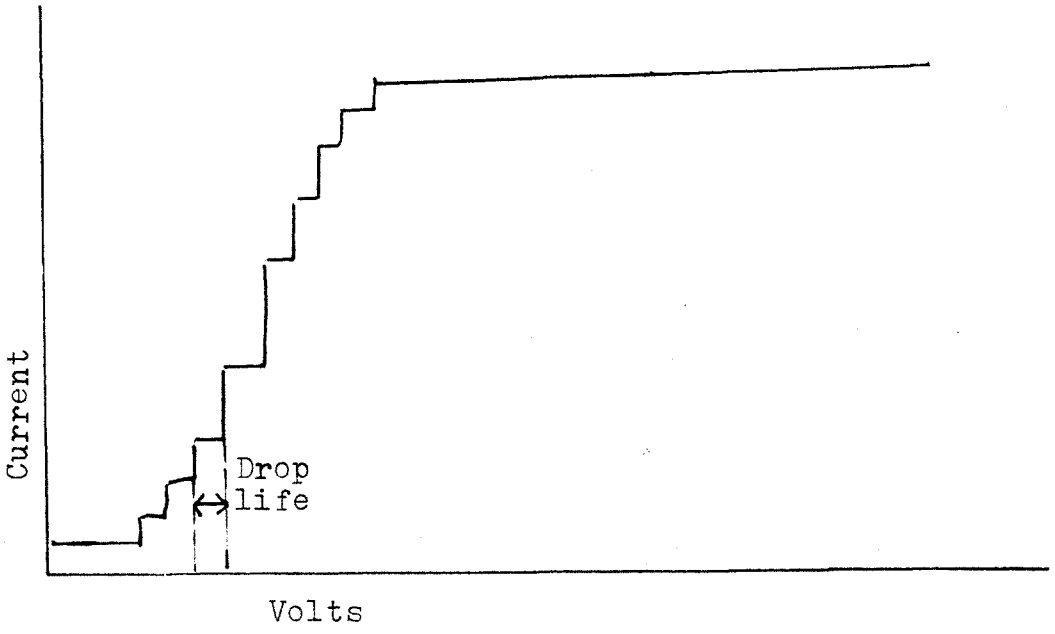


FIGURE 3.7. Programming Waveform for Differential Pulse Polarography

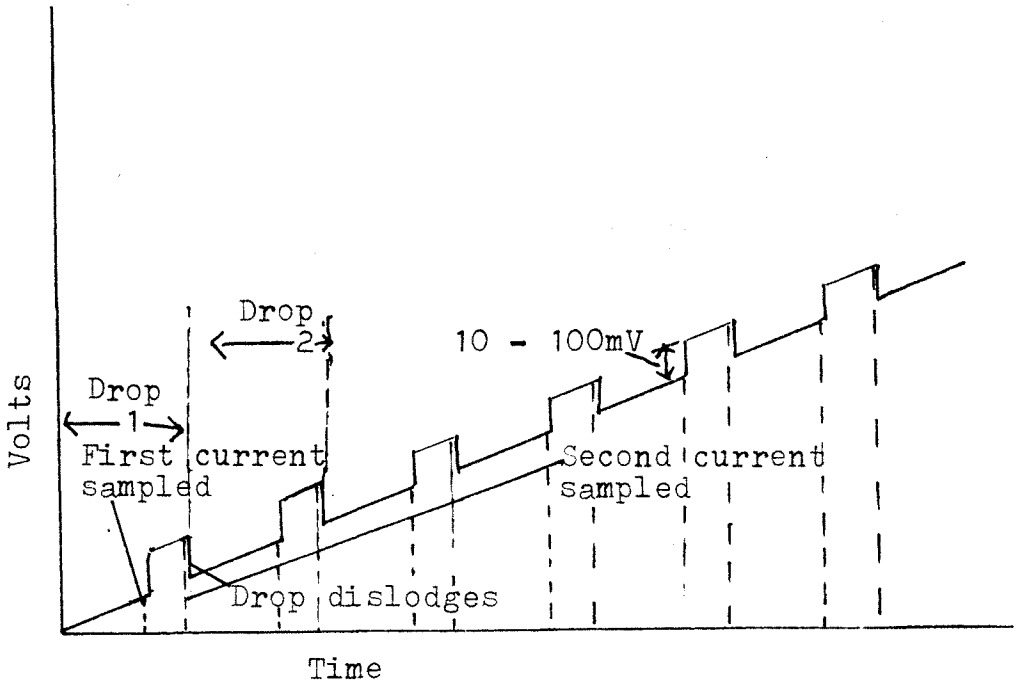
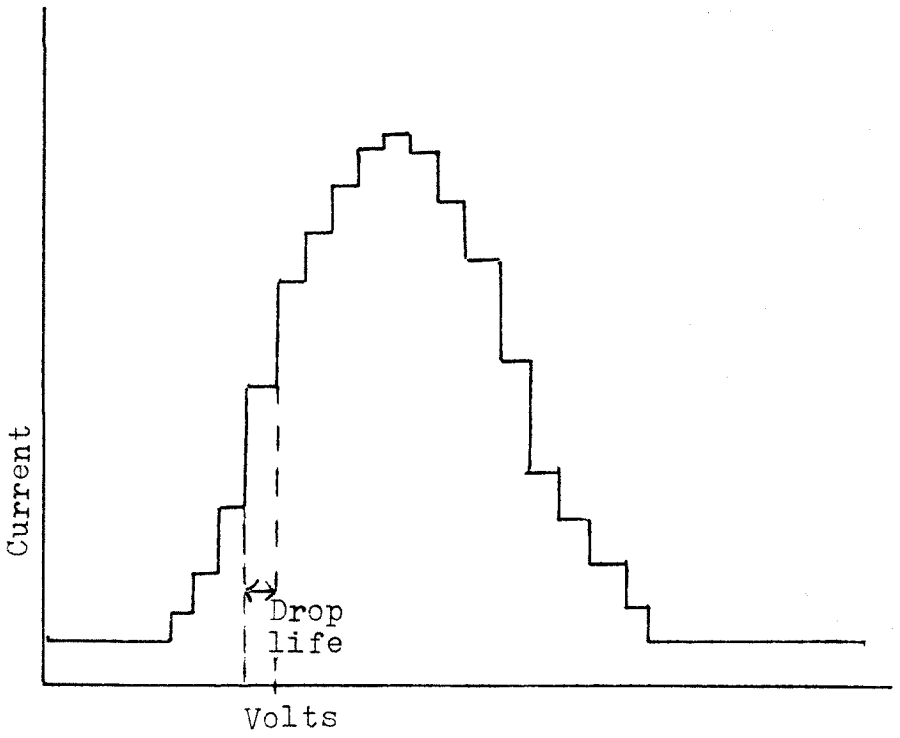


FIGURE 3.8. Resultant Current-Voltage Curve for Differential Pulse Polarography



During the deposition phase in anodic stripping voltammetry (ASV) the reducible metal forms an amalgam with the mercury on the electrode. The deposition potential should be 0.3 to 0.4 V more negative than the $E_{1/2}$. ASV can only be used for those metals that are soluble in mercury.

During the deposition phase in cathodic stripping voltammetry (CSV) the metal will form an insoluble salt with the mercurous ions in which will result in the formation of a film on the surface of the mercury electrode. Since CSV involves the formation of a film on the surface of the electrode, the calibration curve will not be linear at higher concentrations. The deposition potential in CSV should be negative with respect to the $E_{1/2}$.

The deposition step, which serves to concentrate the electroactive material from the relatively large solution volume into the much smaller electrode volume, is followed by an equilibration step. The stirring is stopped so the amalgam concentration may become more homogenous throughout the drop. Convection in the solution also stops.

The equilibration step is followed by a voltage scan. In CSV a negative scan results in the cathodic reduction of mercury from the mercury salt. In ASV a positive scan results in the anodic dissolution of the metal amalgam. The species will strip back into solution at its characteristic potential which is very close to its $E_{1/2}$. The current will be proportional to the concentration of the species. A differential pulse wave form is used because it is very sensitive and it allows a very low detection limit.

The sensitivity and reproducibility of the stripping process is influenced by several factors. The deposition time is proportional to the amount of material deposited in the electrode, except with very high concentrations. In this case, differential pulse polarography can be used. The amount of material deposited also depends on the mass transport which

is affected by the convection pattern around the microelectrode. It is therefore essential to insure that the deposition time and the stirring rate be reproducible from analysis to analysis.

The surface of the electrode must also be reproduced exactly because the limiting current is proportional to the effective surface area. This is best done with a micrometer-driven HMDE. Trace metal impurities, surface-active agents and complex-forming agents can greatly influence the stripping process. The extent of the interference depends on the type of the metal being analyzed.

The polarographic technique used depends on the species being analyzed and the detection limit desired. In pulse polarography the limit of detection is in the 10^{-5} M or 0.1 ppm range. Differential pulse polarography usually gives a detection limit of an order of magnitude lower than this. Stripping voltammetry combined with differential pulse polarography enables quantitative analysis down to 10^{-9} M or 1 ppb.

IV. EXPERIMENTAL PROCEDURES

Apparatus

A Sargent-Welch Polarograph Voltammetric Analyzer Model 4001 was used throughout the study. Figure 4.1 is a line diagram showing the electrical connections of the polarograph.

The cells used were standard cells equipped with a SCE reference electrode, a platinum wire counter electrode and a working electrode. The HMDE used for the DPCSV determination of Se was Metrohm Model E 410. The DME used for the DPP determination of As consisted of a Hg reservoir connected to a glass capillary equipped with a Sargent-Welch drop timer. A stopcock connected to a nitrogen tank was used to deaerate the solution prior to analysis and to maintain nitrogen over solution during analysis.

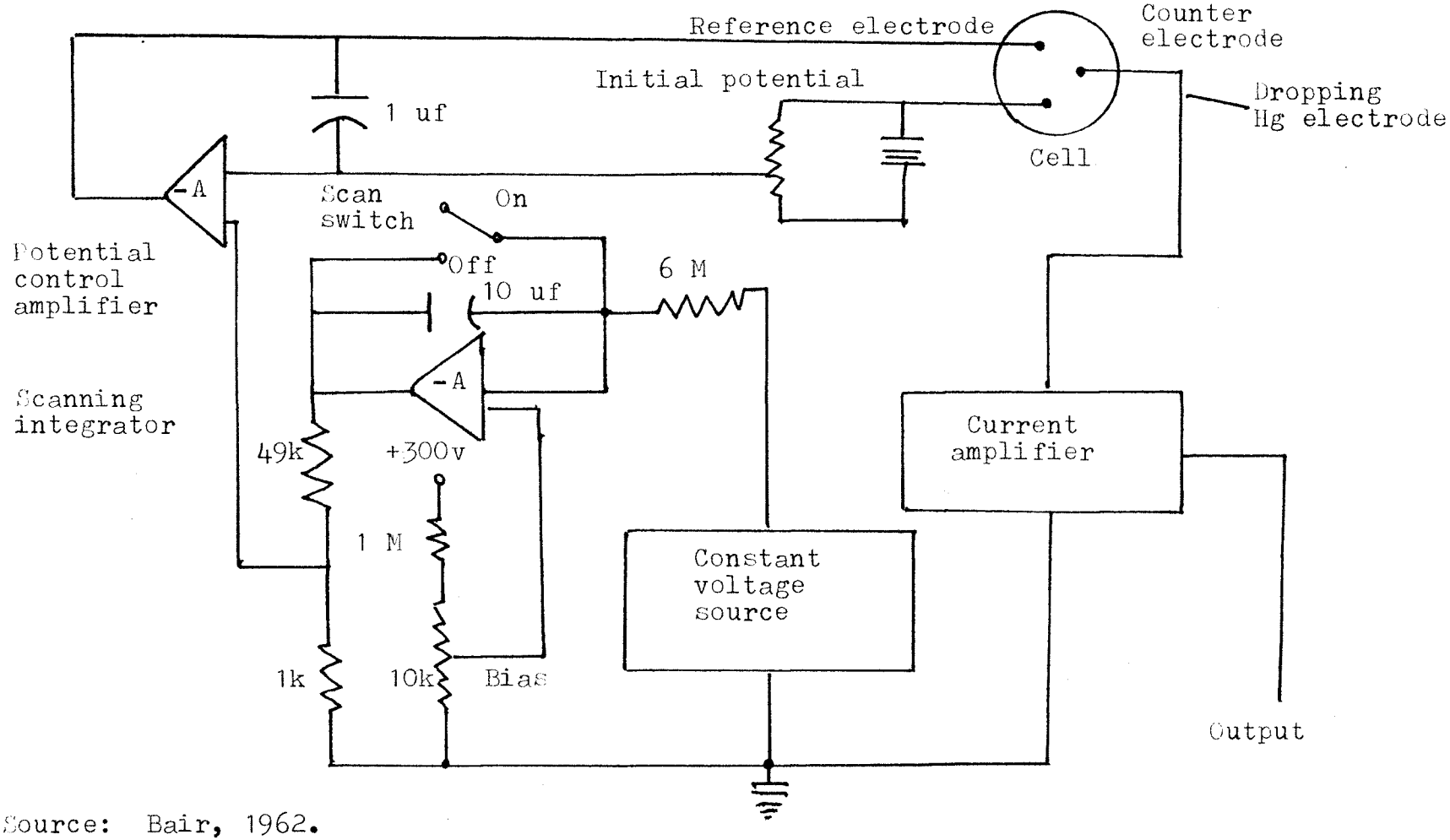
A Precision Scientific Company Mag-Mix Model 65904 with ten stir speeds was used to stir the solution with an A.H.T. Company 1/4 X 7/8 inch stirring bar.

A Thermolyne furnace Model 1400 and a Sybron Thermolyne Nuova II stir plate with ten heat settings was used for sample preparations.

Reagents

SeO_2 (99%), As_2O_3, H_2O_2 (30%), $Mg(NO_3)_2.6H_2O$, HNO_3 (69.5%), HCl (37.6%) and H_3PO_4 (85.8%) were 'Baker Analyzed' reagents. H_2SO_4 meeting ACS specifications was from MCB Reagents. Na_2SO_4 (anhydrous) was from Matheson, Coleman and Bell. Amberlite IRA-400 (Cl) analytical grade synthetic anion exchange resin (modified amine form) was from Fischer Chemical. Continuous vacuum, triple distilled Hg meeting ACS specifications was used.

FIGURE 4.1. Line Diagram of the Potential Control and Scanning Circuit for the Sargent-Welch Polaragraph Voltammetric Analyzer Model 4001



Source: Bair, 1962.

Instrument Parameters

The instrument settings were chosen to optimize the size of the peak current and its reproducibility. DPCSV was used for the determination of Se. Table 4.1 lists the instrument settings and conditions used.

TABLE 4.1. Instrument Parameters for the DPCSV Determination of Selenium

Working Electrode	HMDE
Drop Size	3 Scale divisions on E-410
Supporting Electrolyte	0.1 M HCl
Operation Mode	DP
Initial Voltage	-0.35
Final Voltage	-0.70
Scan Rate	0.2 V/min
Scan Direction	"-"
Modulation Amplitude	50 mV
Drop Time	0.5 sec
Chart Speed	2 cm/min
IR Compensation	on
Purge Time	300 sec
Deposition Time	90 sec
Equilibration Time	30 sec
Stir Rate	2 on Mag-Mix 65904
E_p	-0.55 V

The electroactive form of selenium is Se(IV). Se(IV) gives 2 peaks at -0.08 and -0.55 volts versus the SCE as shown in Figure 4.2. The first reduction step results from the conversion of an adsorbed chloro-selenium complex to mercuric selenide. This peak was somewhat deformed and the peak height was not directly proportional to the concentration. The second peak results from the reduction of mercuric selenide. This peak is well defined and was found to be directly proportional to the concentration over a range of 0 to 4 μg/100 ml. The calibration curve is shown in Figure 4.3.

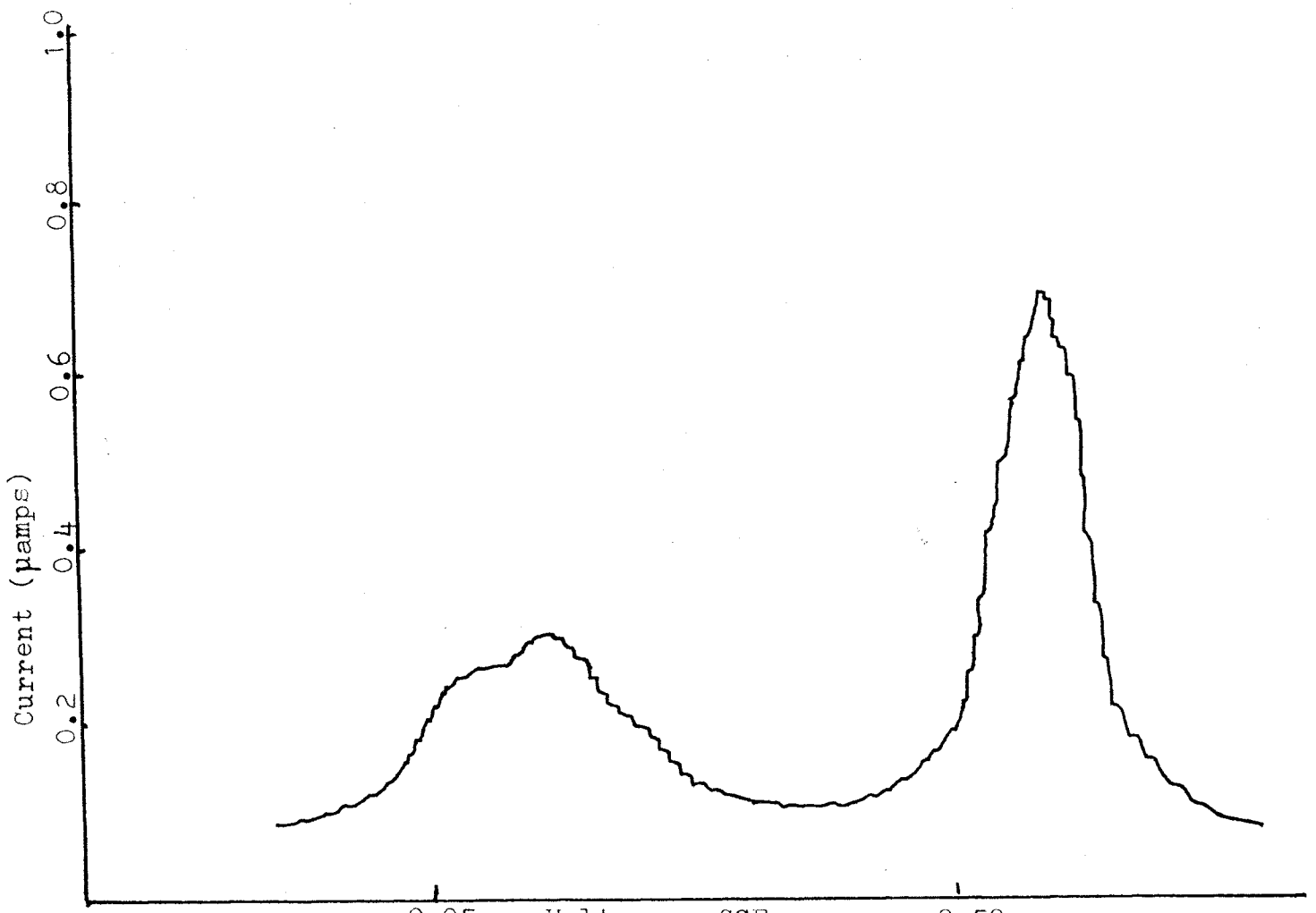
The supporting electrolyte of 0.1 M HCl was chosen because it gave a well defined peak without interferences. A more concentrated supporting electrolyte of 1 M HCl was found to interfere with the resolution of the Se(IV) peak.

The effect of the deposition potential on the peak current was studied. A concentration of 3 μg in 0.1 M HCl was used . The results are illustrated in figure 4.4. A deposition potential of -0.35 was chosen because it gives maximum sensitivity.

A scan rate of 0.2 V/min was found to give good resolution of the Se(IV) peak. As the scan rate is increased the peak becomes distorted and drawn out. A pulse amplitude of 40 mV was found to give both good sensitivity and a well defined peak. As the pulse amplitude increased to 80 mV the peak became distorted.

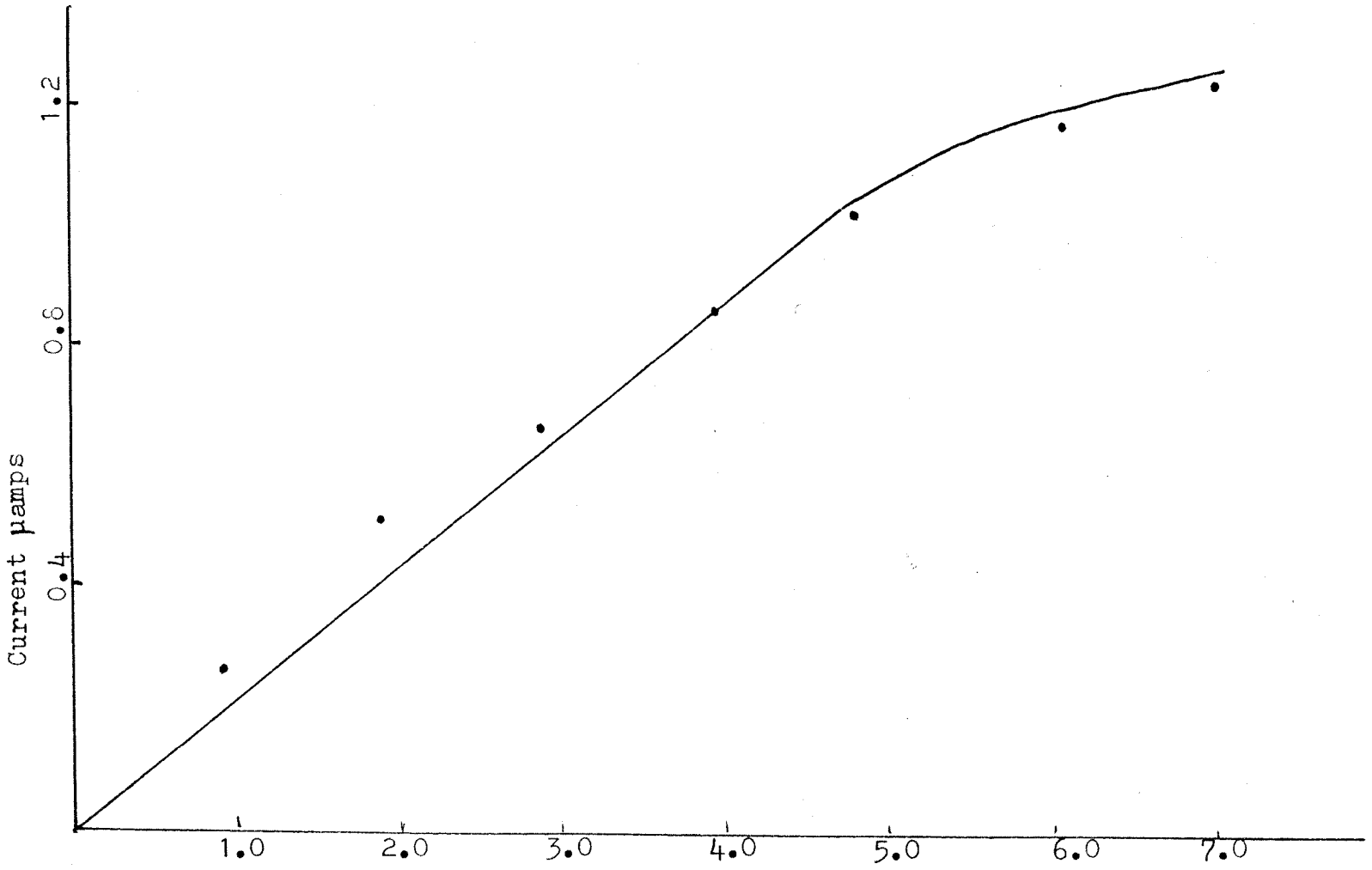
A drop size of 3 scale divisions on the E-410 HMDE is reported by Metrohm to give a drop diameter of 0.76 mm with a drop surface area of 1.80 ± 0.05 mm^2. This was the maximum size that could be used without causing problems of the drop dislodging from the capillary during stirring. A stir rate of 2 on the Mag-Mix was found to give adequate stirring without creating enough turbulence to dislodge the mercury drop.

FIGURE 4.2. DPCSV Current-voltage Curve for Selenium(IV)



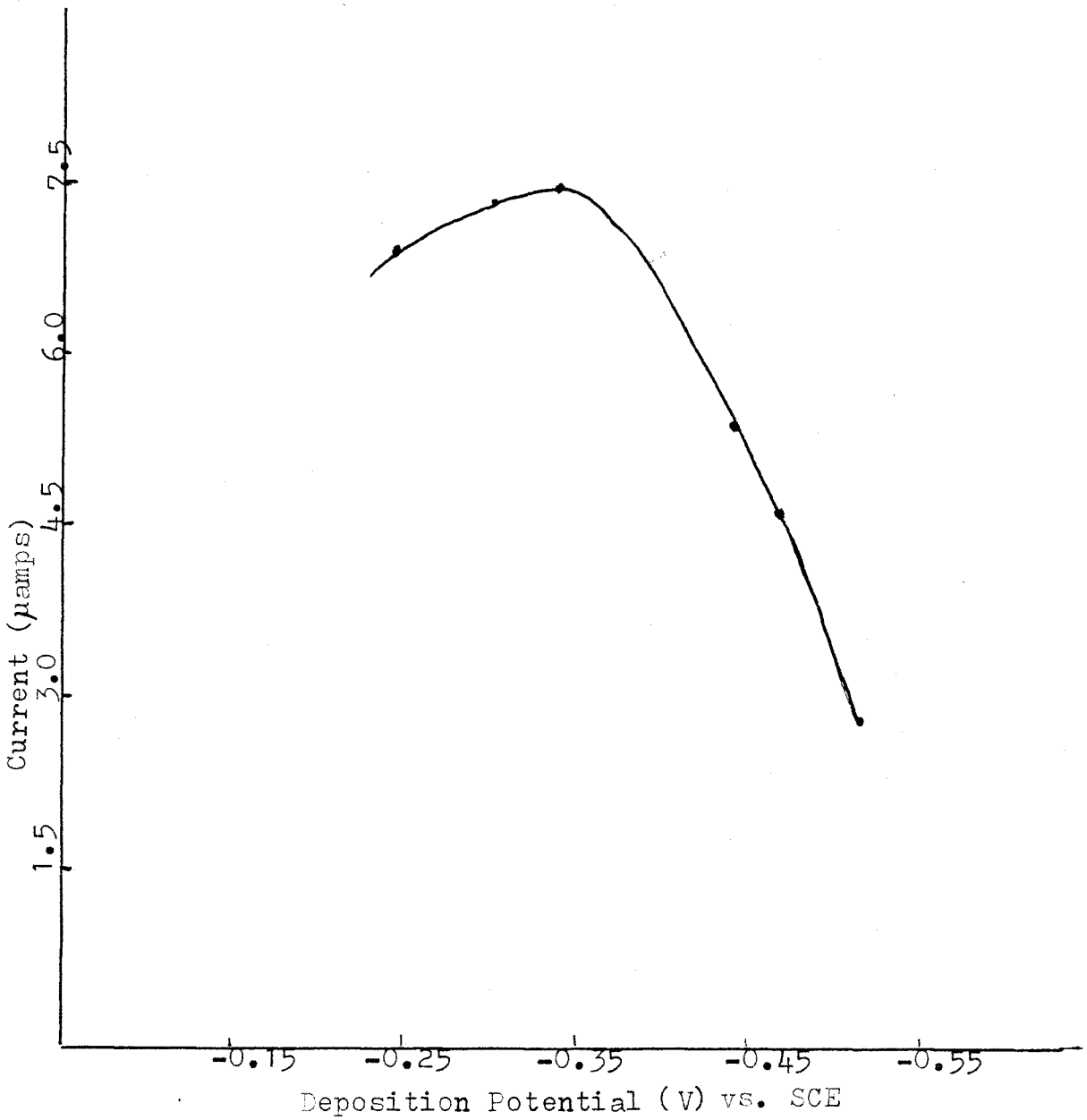
A solution of 3 μg of Se(IV) in 100 ml of 0.1 M HCl was used. Conditions were the same as the experimental conditions.

FIGURE 4.3. Calibration Curve for Selenium(IV)



Conditions used were the same as the experimental conditions.

FIGURE 4.4. Effect of Deposition Potential on the Peak Current for Selenium(IV)



A solution of 3 μg Se(IV) in 100 ml of 0.1 M HCl was used. Conditions used were the same as the experimental conditions.

The electroactive form of arsenic is As(III). Two peaks are observed, one at -0.40 V and the other at -0.639 V versus the SCE, as shown in Figure 4.5. The first peak is due to the reduction to As(0) while the second peak is due to further reduction to AsH_3. The first peak is much sharper than the second and it was found to be directly proportional to the concentration over a range of 0 to 7 μg of As(III)/50 ml. The calibration curve is shown in Figure 4.6.

A supporting electrolyte of 1 M HCl was chosen because it gave a very well defined first peak without giving interferences. A scan rate of 0.2 V/min with a pulse amplitude of 40 mV was found to give a high resolution and a well defined peak. Increasing the modulation amplitude resulted in a broadened peak.

A drop time of 1 second was found to give a well defined symmetrical peak. A purge time of 300 seconds was sufficient to eliminate O_2 from the sample.

Electroanalytical Procedure

For the determination of Se the 25 ml digested sample was placed in a cell with 100 ml 0.1 M HCl. The system was purged with nitrogen for 5 minutes. A deposition time of 60 seconds and an equilibration time of 30 seconds was followed by the stripping scan. After the stripping scan a known amount of standard Se solution was added to the sample cell. The stripping procedure was then repeated after again purging the solution with nitrogen for 5 minutes. This was done twice. The standard addition method was used to determine the Se concentration because it helped to compensate for matrix differences.

The determination of the percent recoveries for Se was carried out in the same manner as above but prior to sample digestion a known amount of Se

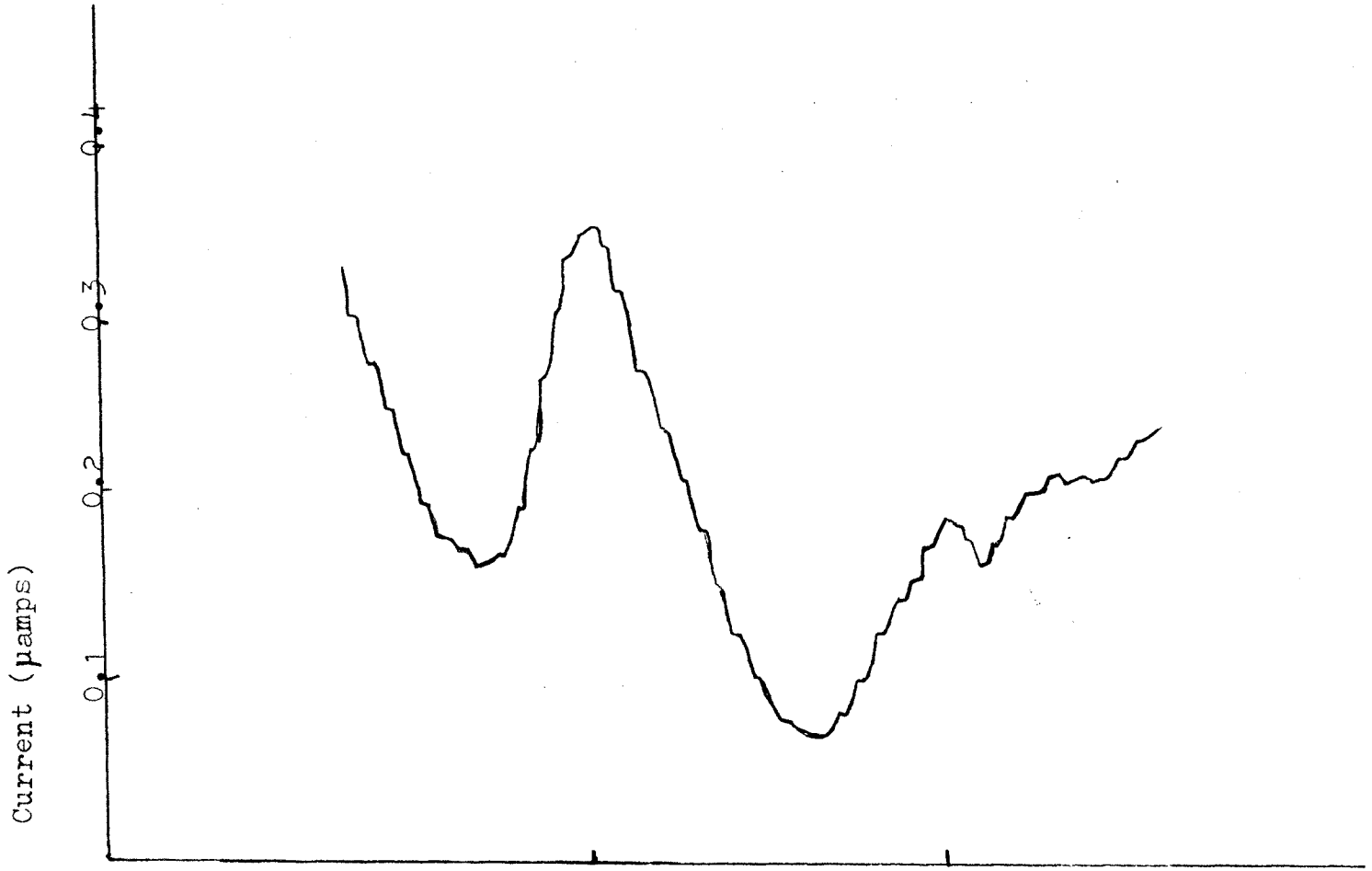
A purge time of 300 seconds was sufficient to remove the O_2 present in the sample. The deposition time of 90 seconds was adequate to form a sufficient amount of Se complex in the range of Se being analyzed. An equilibration time of 30 seconds was sufficient to cease all stirring and convection in the solution prior to stripping.

DPP was used for the determination of arsenic. Table 4.2 lists the instrument parameters and conditions used.

TABLE 4.2. Instrument Parameters for the DPP Determination of Arsenic

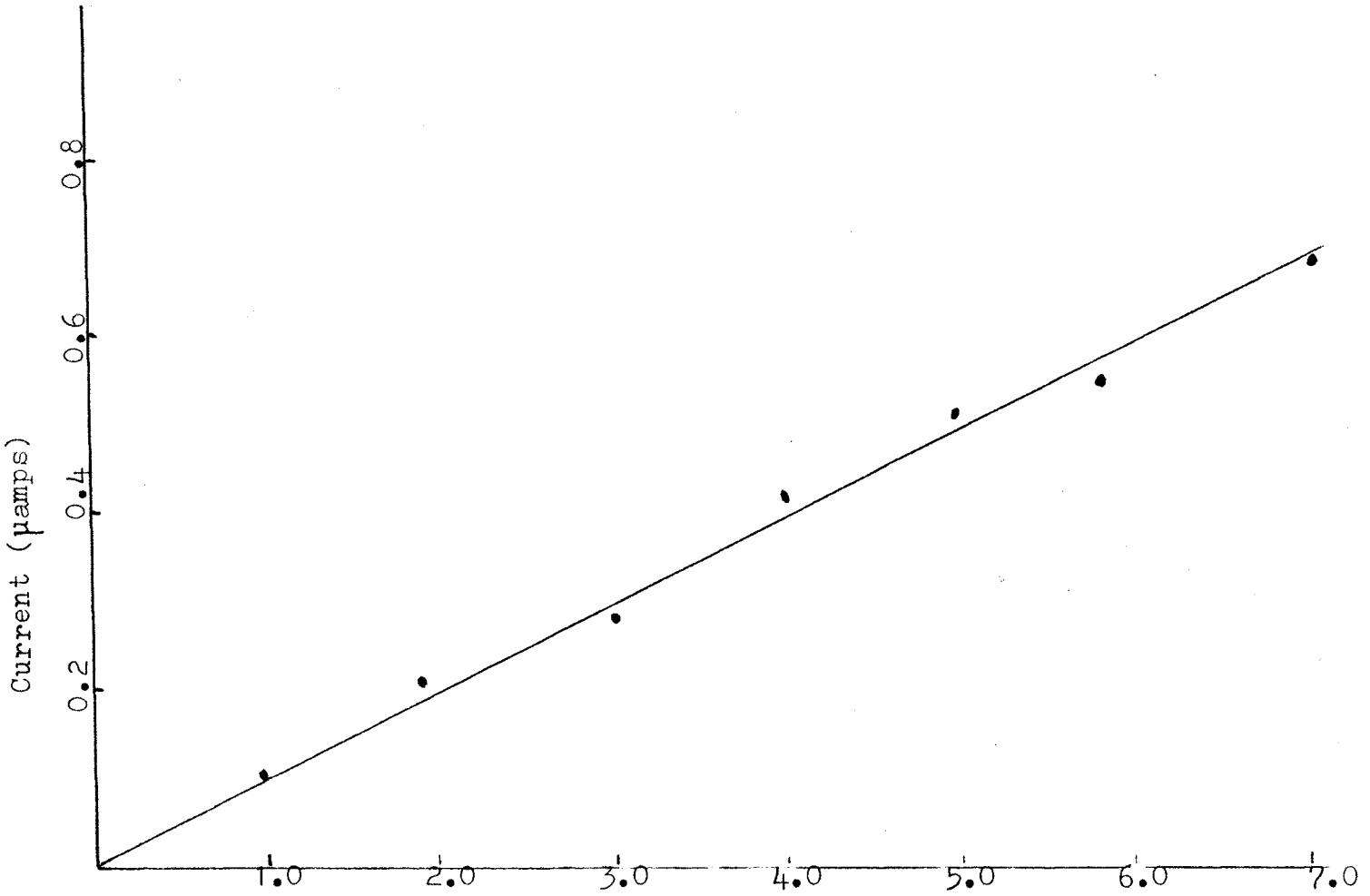
Working Electrode	DME
Supporting Electrolyte	1 M HCl
Drop Time	1 sec
Operation Mode	DP
Initial Voltage	-0.20
Final Voltage	-0.60
Scan Rate	0.2 V/min
Scan Direction	"-"
Modulation Amplitude	40 mV
Chart Speed	2 cm/min
IR Compensation	on
Purge Time	300 sec
E_p	-0.40 V

FIGURE 4.5. DPP Current-Voltage Curve for Arsenic(III)



A solution of 3 μg of As(III) in 50 ml of 1 M HCl was used. Conditions were the same as the experimental conditions

FIGURE 4.6. Calibration Curve for Arsenic(III)



Conditions used were the same as the experimental conditions.

was added to the sample. Only 10 ml of the 25 ml digested sample was used in the determination. This was necessary because the calibration curve for Se is only linear up to 4 μg/100 ml. If the whole 25 ml digested sample was used, the Se concentration would not fall in the linear range.

For the determination of As(III) the removal of inorganic ions such as Pb(II), Sn(II) and (IV) and Tl(I) and (III) is necessary. These ions interfere due to their reduction currents which occur near the As peak at -0.40 v versus the SCE. Pb(II) will give a peak at -0.435 V, Sn(II) at -0.47 V, Se(IV) at -0.52 V, Tl(I) at -0.475 V and Tl(III) at -0.45 V versus the SCE. The presence of these ions will cause a much larger peak to appear near -0.40 V versus SCE which will interfere with the determination of As. An ion exchange purification technique was developed to remove these inorganic ions (Holak, 1976). In an HCl solution, Pb, Sn, Tl and many other metals exist as negatively charged complexes. These negatively charged complexes will be adsorbed by a strongly basic anion exchange resin, thereby effectively removing them from the solution. Arsenic, which is present as H_3AsOH_3, is uncharged and therefore is not adsorbed. This procedure, which was used for the As analysis, is described below.

A 25 ml digested sample is placed in a 100 ml beaker. To this 2 ml of concentrated HCl and 2 grams of anhydrous Na_2SO_4 is added. The beaker is then covered with a watch glass and put on a steam bath for 20 minutes. This is necessary because in samples which have undergone wet ashing procedures, with strong oxidizing agents such as HNO_3 or $HClO_4$, As will exist as As(V). Arsenic(V) is not electroactive in most supporting electrolytes. Therefore, it must be reduced to As(III). Na_2SO_4 acts as the reductant and will quantitatively reduce As(V) to As(III). After cooling, the sample is placed in 50 ml of 1 M HCl. The sample is purged with nitrogen for 15 minutes. Two grams of ion exchange resin is added to

the sample which is then purged with nitrogen for 5 minutes prior to analysis. The DP scan is then performed. The standard addition method is carried out in the same manner as was done for Se.

The determination of the percent recoveries for As was carried out in the same manner as for Se, but prior to sample digestion a known amount of As was added to the sample.

Sample Digestion Procedures

The determination of electroactive species using polarographic methods requires that the sample be in solution. When a solid sample is to be analyzed the sample must first be digested. This can be accomplished in a number of ways including wet digestion with acid or dry ashing in a furnace. Four different digestion procedures using several variations of each method were tested to determine which was the most reliable procedure with the best recovery. All the sample digestion procedures were performed in the hood.

The first digestion procedure was from Metrohm Application Bulletin No. 113 e (1977). It consisted of a wet digestion with H_2SO_4 and HNO_3. A 1 gram sample of the tuna fish was placed in a 100 ml beaker along with 20 ml of distilled water, 1 ml H_2SO_4 (96%) and 10 ml HNO_3 (65%). The beaker was then covered and left standing at room temperature for 18 hours. The sample was then heated on a medium heat level. All of the samples except the blank turned black due to carbonisation. The samples were removed from the heat, allowed to cool and then heated again after adding 10 ml HNO_3 and 1 ml of H_2SO_4. This procedure was repeated twice for each sample. The samples were heated on a medium heat until they were very pale yellow. The heat level was then raised to high until SO_3 mist, characterized by thick white fumes appeared. The sample was cooled.

Although not specified in the procedure because it was developed primarily for the determination of Pb, Cu and Sn, 5 ml of 6 N HCl was added to the sample to convert all of the selenium to the electroactive Se(IV). The sample was boiled for 5 minutes, cooled and then transferred to a 25 ml volumetric flask with distilled water. Upon analysis using DPCSV a recovery of 83.7 percent was attained.

Two variations of the above method were also tested. The amounts of acid added initially were doubled while the addition of the distilled water was eliminated. The samples still turned black and 1 ml H_2SO_4 with 10 ml of HNO_3 had to be added twice. Instead of boiling the sample after the addition of the 5 ml of 6 N HCl, the sample was slowly heated for 30 minutes. This first variation gave a percent recovery of 83.5.

A second variation consisted of heating the sample on a very low heat after being allowed to stand overnight. The total heating time was increased from about 3 hours to 6 hours. The samples again turned black which necessitated the addition of more acid . This was done twice. The samples were slowly heated for 30 minutes after adding the 6 N HCl. This second variation gave a recovery of 86.7 percent.

The second digestion procedure was from Adeloju et al. (1983). To a 0.2 gram sample, 4 ml of H_2SO_4 and 10 ml of HNO_3 was added. Two glass beads were added to the beaker. The sample was slowly heated until all of the sample material dissolved and HNO_3 fumes ceased to appear. The heat was then raised to high until SO_3 mist appeared. The sample was cooled and 12.5 ml of concentrated HCl was added to the sample which was then boiled for 30 minutes. The sample was cooled, diluted to a pH of 1.0 and analyzed. Since this procedure only used 0.2 grams of sample the Se peaks were barely discernible and a recovery of only 43.8 percent was attained.

The above procedure was done using a 1 gram sample. The higher organic content caused the sample to turn black due to carbonisation. Additional acid was added. After the solution turned pale yellow the heat was raised until SO_3 mist appeared. HCl was then added as above. This first variation also gave a poor recovery of 49.4 percent.

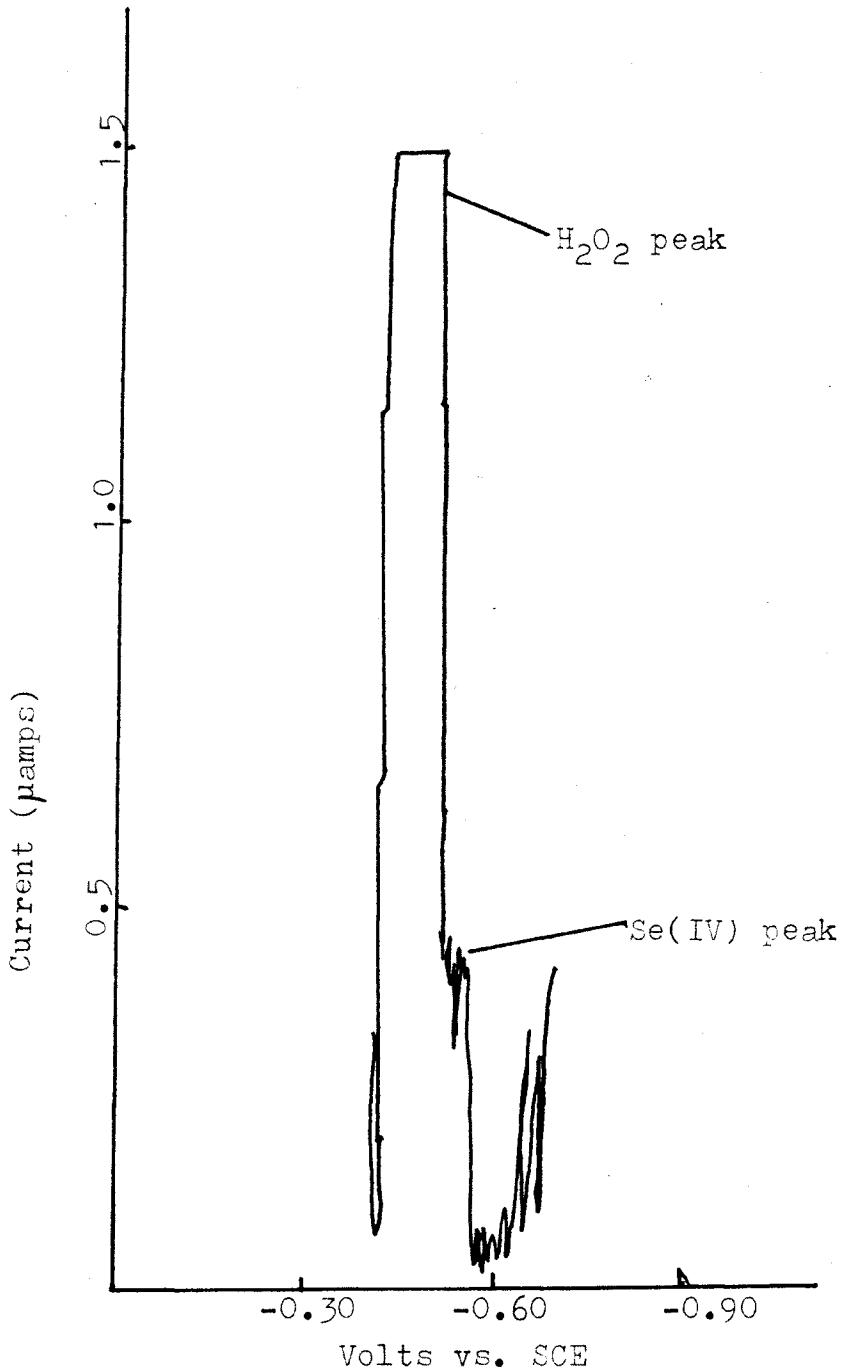
A second variation was tried in which all of the adaptations of the first variation were done but 5 ml of 6 N HCl was added to the sample instead of 12.5 ml of concentrated HCl. A recovery of 68.7 percent was attained. This was significantly greater than the 49.4 percent recovery. The lower recovery of 49.4 percent was probably due to the volatilization of Se as selenium(IV) chloride which can occur in boiling concentrated HCl solutions. The use of the 6 N HCl prevented this.

The third procedure which was from Reamer and Veillon (1981), consisted of wet digestion with H_3PO_4, HNO_3 and H_2O_2. A 1 gram sample was placed in the beaker with 1 ml of H_3PO_4 and 10 ml of HNO_3. The sample was covered and allowed to stand for 18 hours. The sample was the boiled until it turned pale yellow. Thirty percent H_2O_2 was added slowly until the sample solution cleared. The sample was then boiled for 5 minutes. It was then transferred to a 25 ml volumetric flask with distilled water and analyzed. A large peak occurred near the potential at which a Se(IV) peak should have appeared as shown in Figure 4.7. This peak appeared in all of the samples including the blank. It was found to be due to the presence of H_2O_2 in the digestion procedure.

The above procedure was carried out again, this time eliminating the addition of the H_2O_2. However the recovery for this variation was low at 64.7 percent.

Although the procedure specifies that the sample is to be boiled after standing overnight, it was felt that this resulted in a loss of Se.

FIGURE 4.7. Interference Peak Due to the Presence of H_2O_2 in the Digestion Procedure



In variation 2, the sample was slowly heated for 5 hours instead of boiled for a half hour. Although slowly heating the sample added greatly to the digestion time, it significantly increased the recovery to 81.6 and 79.0 percent for the two samples that were tested.

The fourth procedure, which was from Holak (1976), consisted of digestion with HNO_3 and $Mg(NO_3)_2.6H_2O$. A 1 gram sample was placed in a beaker with 10 ml of HNO_3 and 4 grams of $Mg(NO_3).6H_2O$. The sample was then heated on a low heat until it became dry. This took about 5 to 6 hours. After the sample was dry the heat level was raised to a maximum until all of the HNO_3 fumes were given off. The sample was then placed in a muffle furnace for 30 minutes at about 500 °C. After cooling the sample, 5 ml of 6 N HCl was added to the sample and it was placed on a steam bath until the white residue dissolved. The solution was then transferred to a 25 ml volumetric flask with distilled water. A recovery of 83.0 percent was attained.

A variation of the method was tried in which after the addition of the HNO_3 and $Mg(NO_3)_2.6H_2O$ the sample was covered and allowed to predigest for 18 hours. The procedure was then carried out as specified above. This predigestion step significantly increased the recovery to 95.5 and 96.9 percent which was obtained for the analysis of two cans.

Other digestion procedures are also available. Although dry ashing methods are commonly used, they often result in losses of the analyte due to the high temperature at which they operate. This is particularly severe with As and Se. The combination of wet digestion and dry ashing of Holak (1976) prevented these losses. Other dry ashing methods however generally result in huge losses.

There are a variety of other wet digestion procedures available. Many digestion procedures have been developed in which $HClO_4$ is used in

combination with other acids (Fiorino et al.,1976; Ihnat and Miller, 1977). Digestion procedures using HNO_3 alone or HNO_3 in combination with $K_2S_2O_8$ have also been developed (Adeloju et al., 1984). Blades et al. (1976) developed a procedure involving extraction of a 3',4'-diaminophenylpiazselenol into benzene with subsequent back extraction into dilute acid for analysis.

The sample digestion procedure using HNO_3 and $Mg(NO_3)_2.6H_2O$ with an 18 hour predigestion step was found to give an excellent recovery. It also eliminated the use of $HClO_4$ and benzene, two potentially dangerous compounds which are commonly used in digestion procedures. This procedure was therefore used for the digestion of all of the tuna fish samples in determining the Se concentration.

This procedure was also tested for the DPP determination of As. Coupled with the ion exchange purification technique previously mentioned, a recovery of 94.3 percent was attained. No interferences were observed. This procedure was therefore used in the As determinations.

Table 4.3 gives a summary of the sample digestion methods tested and the percent recoveries attained for each. Figures 4.8 to 4.17 are the voltammograms that were obtained for each method. The voltammograms for the Holak Variation 1 digestion procedure are presented in Figures 5.4, 5.6 and 5.16.

TABLE 4.3 Percent Recoveries for Various Sample Digestion Procedures

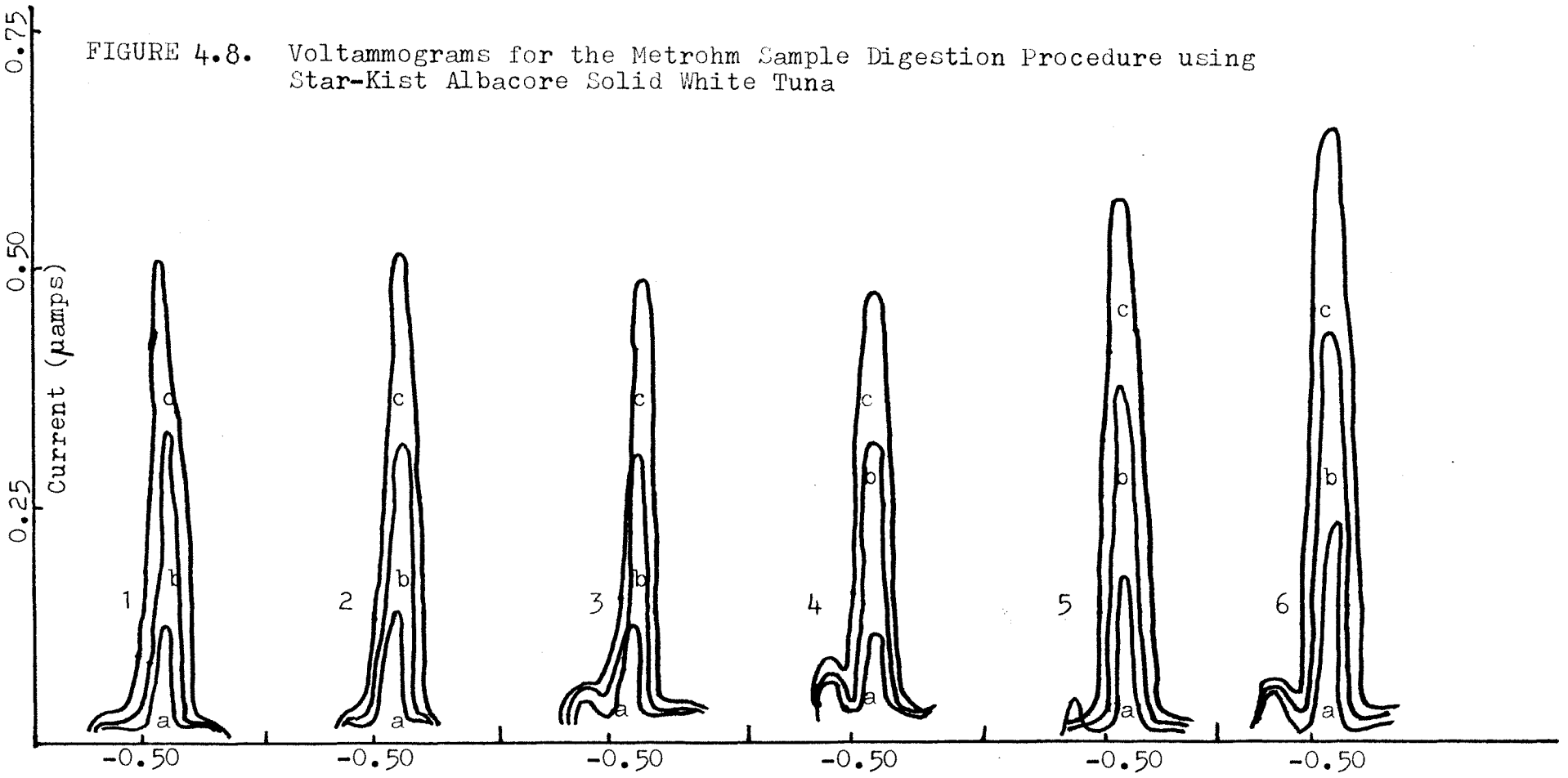
Method	Brand of Tuna Fish	Se Found ($\mu g/g$)[a]	Percent Recovery[a]
Metrohm (1977)	Star-Kist albacore solid white	0.583±0.0121	83.7
Variation 1	Empress chunk light	0.760±0.0110	86.8
Variation 2	Empress chunk light	0.713±0.0082	83.5
Adeljou, Bond, Briggs & Hughes (1983)	Key Food chunk light	0.280±0.0089	43.8
Variation 1	Key Food chunk light	0.300±0.0126	49.4
Variation 2	Chicken of the Sea chunk light	0.655±0.0259	68.7
Reamer and Veillon (1981)	Bumble Bee chunk light	could not be determined due to interference from H_2O_2	
Variation 1	Bumble Bee chunk light	0.520±0.0126	64.7
Variation 2	Bumble Bee chunk light	0.667±0.0250	81.6
	Bumble Bee chunk light	0.652±0.0335	79.0
Holak (1976)	Chicken of the Sea chunk light	0.757±0.0216	83.0
Variation 1	Chicken of the Sea albacore solid white	0.555±0.0±75	98.5
	Bumble Bee chunk light	0.773±0.0121	96.9

TABLE 4.3 Percent Recoveries for Various Sample Digestion Procedures

Method	Brand of Tuna Fish	As Found (μg/g)[a]	Percent Recovery[a]
Holak			
Variation 1			
(for arsenic)	Deep Blue chunk light	1.615±0.0367 ug/g of As	94.3

a- the average of three samples with two standard addition determinations for each sample.

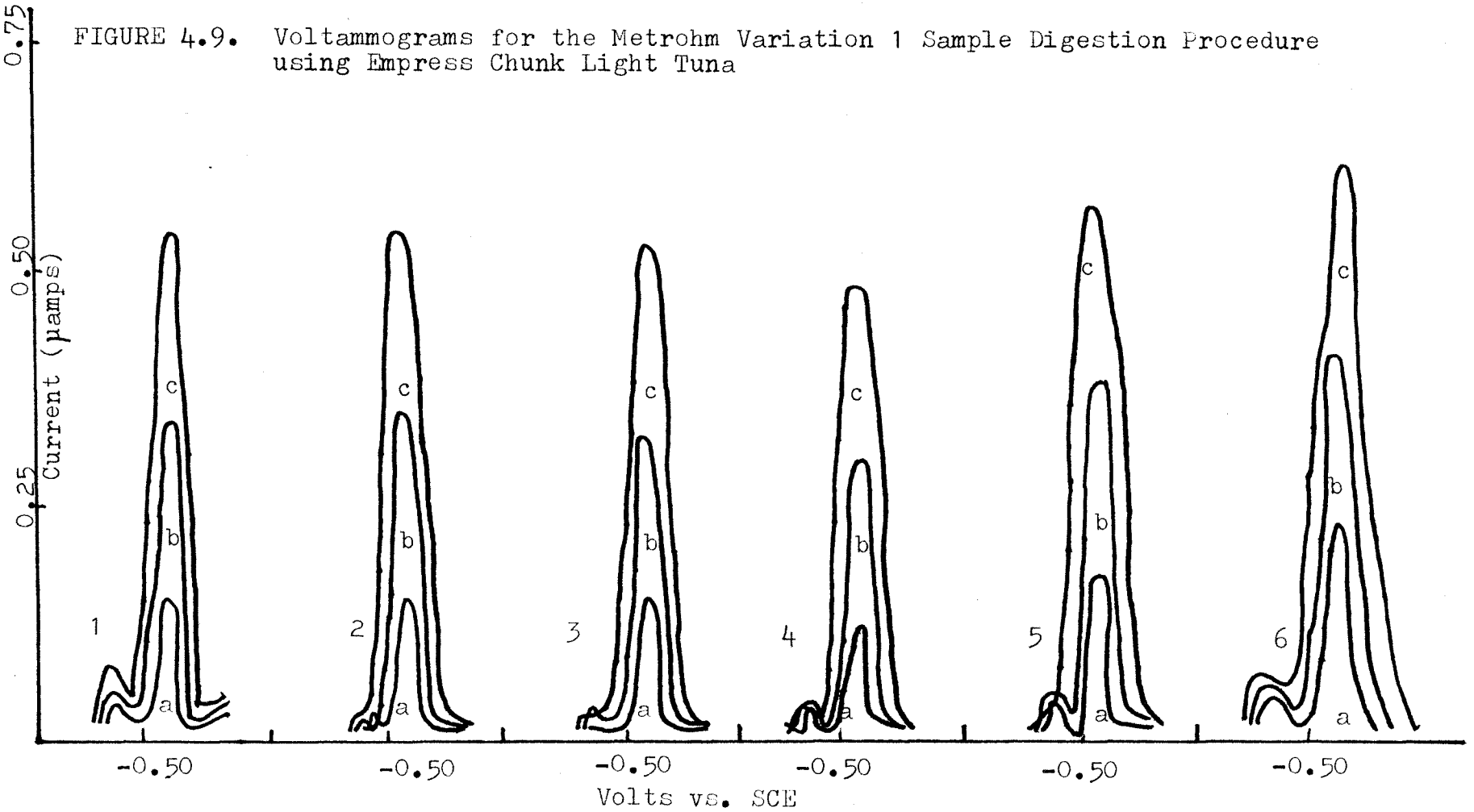
FIGURE 4.8. Voltammograms for the Metrohm Sample Digestion Procedure using Star-Kist Albacore Solid White Tuna



1. (a) 1.00136g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 1.08526g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 1.00927g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 1.00125g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 1.09221g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 0.99858g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

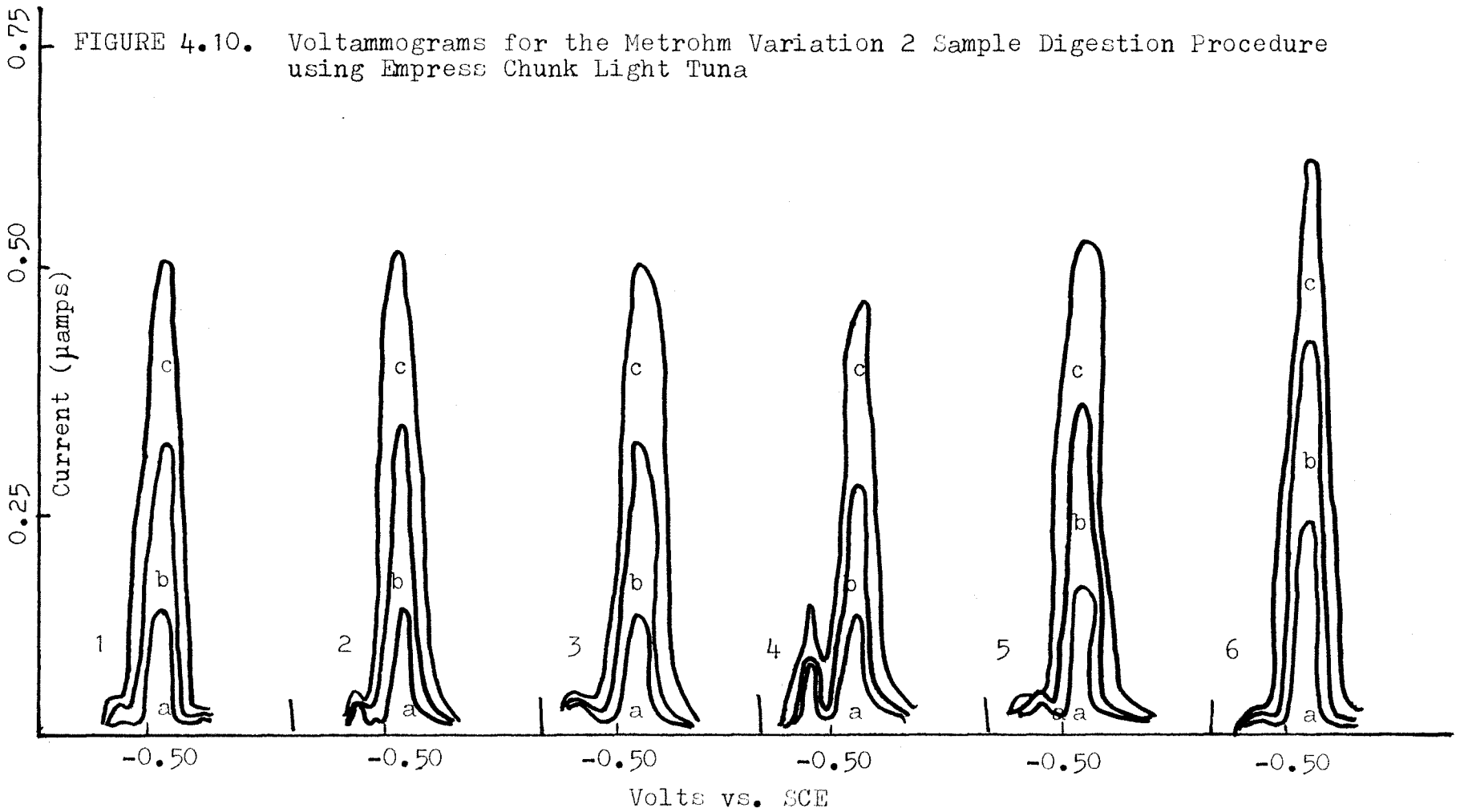
FIGURE 4.9. Voltammograms for the Metrohm Variation 1 Sample Digestion Procedure using Empress Chunk Light Tuna



1. (a) 1.00098g of tuna (b) 1.0 µg of Se added (c) 2.0 µg of Se added
2. (a) 1.00358g of tuna (b) 1.0 µg of Se added (c) 2.0 µg of Se added
3. (a) 1.00013g of tuna (b) 1.0 µg of Se added (c) 2.0 µg of Se added
4. (a) 1.07331g of tuna with 1.0 µg of Se added (b) 1.0 µg of Se added (c) 2.0 µg of Se added
5. (a) 0.95962g of tuna with 2.0 µg of Se added (b) 1.0 µg of Se added (c) 2.0 µg of Se added
6. (a) 1.00079g of tuna with 3.0 µg of Se added (b) 1.0 µg of Se added (c) 2.0 µg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

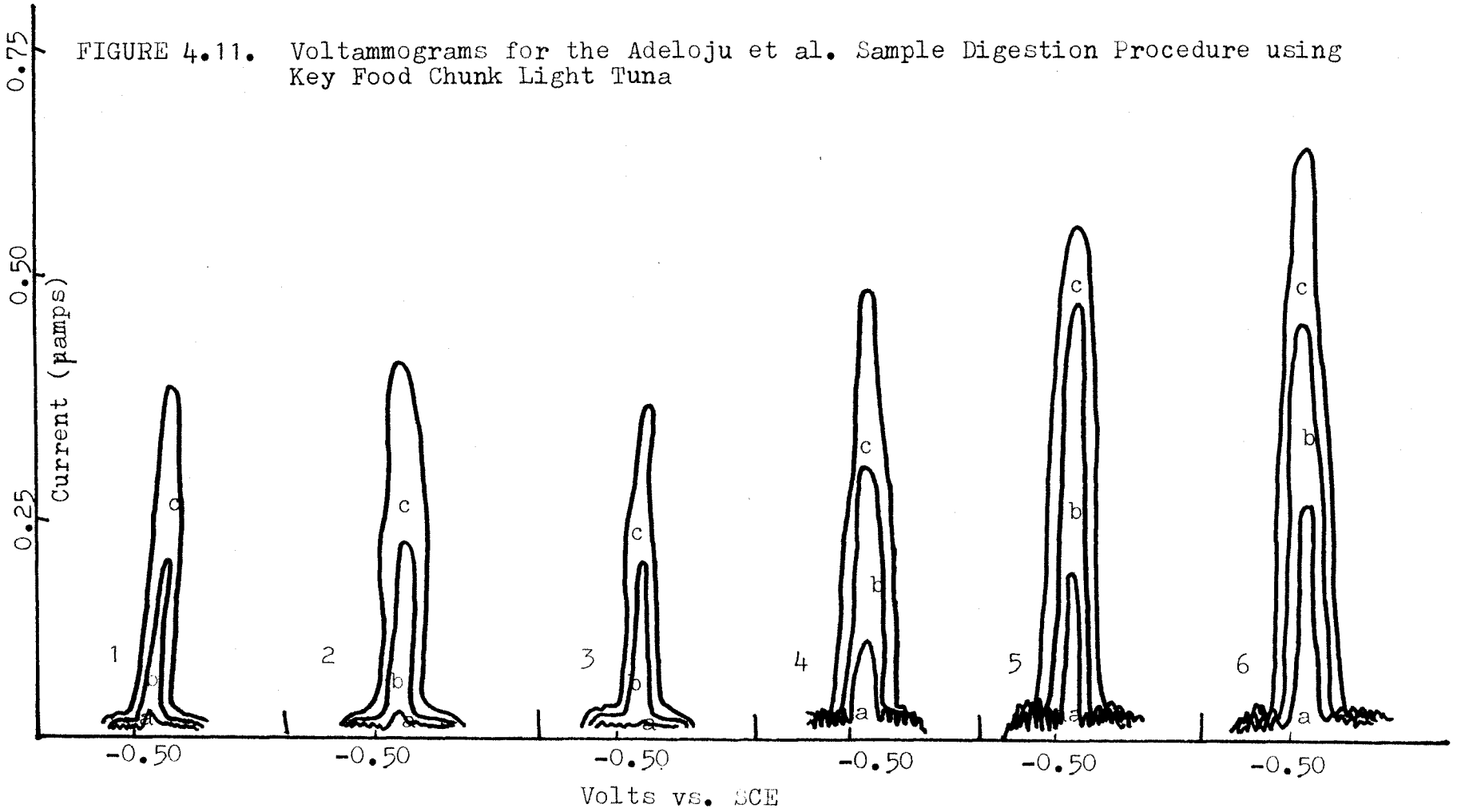
FIGURE 4.10. Voltammograms for the Metrohm Variation 2 Sample Digestion Procedure using Empress Chunk Light Tuna



1. (a) 1.03560g of tuna (b) 1.0 µg of Se added (c) 2.0 µg of Se added
2. (a) 1.05031g of tuna (b) 1.0 µg of Se added (c) 2.0 µg of Se added
3. (a) 1.03129g of tuna (b) 1.0 µg of Se added (c) 2.0 µg of Se added
4. (a) 1.07117g of tuna with 1.0 µg of Se added (b) 1.0 µg of Se added (c) 2.0 µg of Se added
5. (a) 1.03224g of tuna with 2.0 µg of Se added (b) 1.0 µg of Se added (c) 2.0 µg of Se added
6. (a) 1.03701g of tuna with 3.0 µg of Se added (c) 1.0 µg of Se added (c) 2.0 µg of Se added

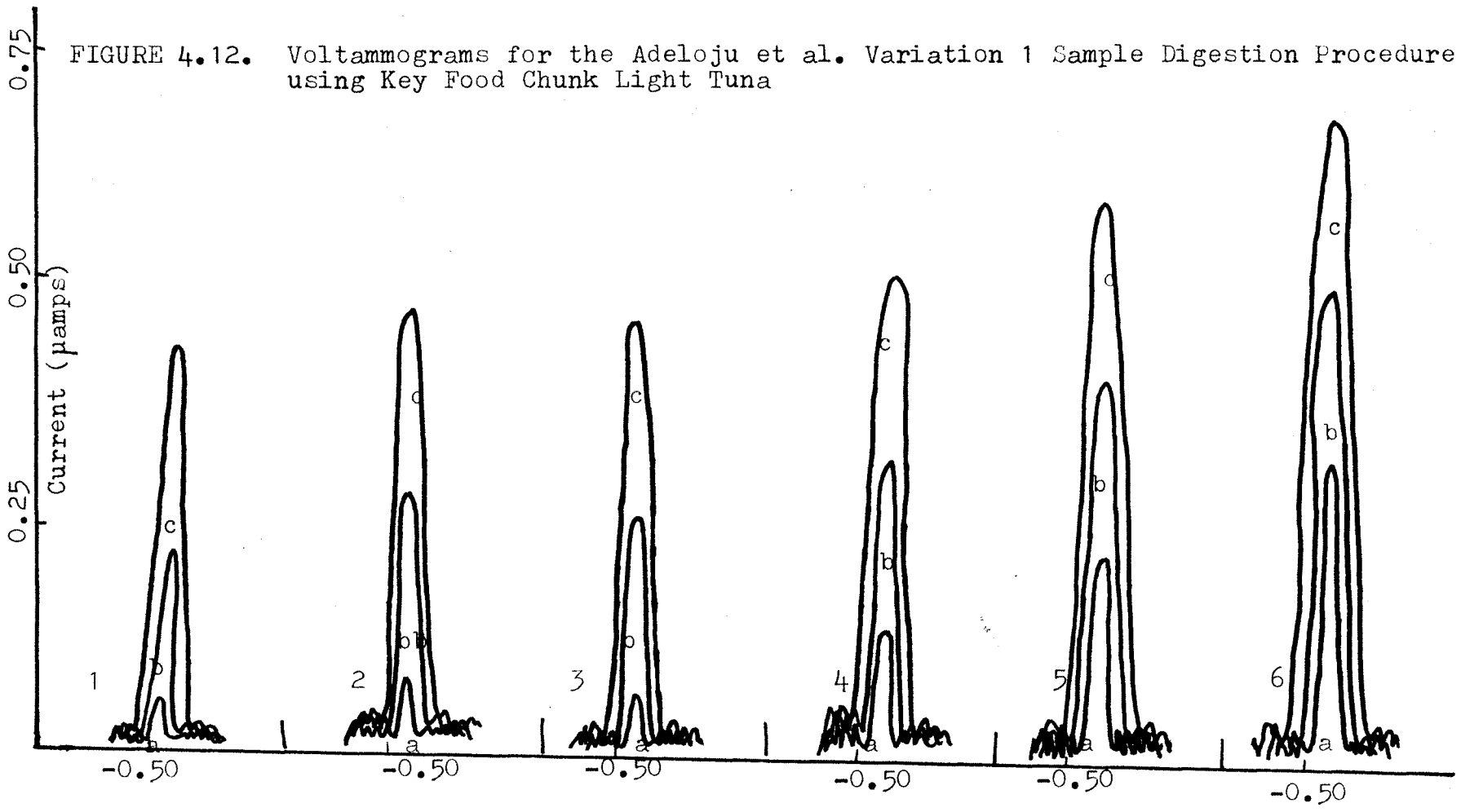
4, 5 and 6 are 10 ml of a 25 ml sample

FIGURE 4.11. Voltammograms for the Adeloju et al. Sample Digestion Procedure using Key Food Chunk Light Tuna



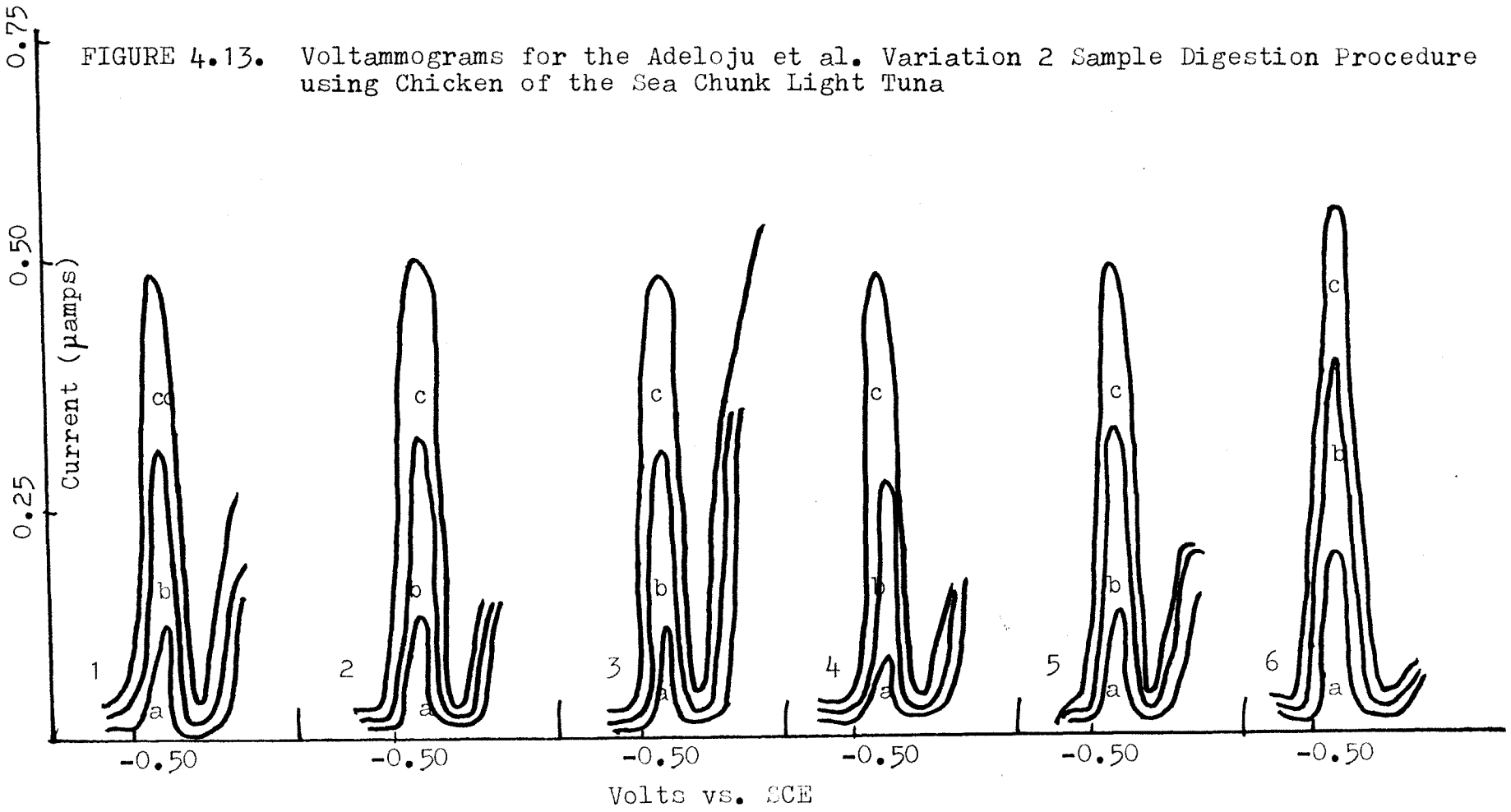
1. (a) 0.21003g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 0.28633g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 0.19914g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 0.20115g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 0.25510g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 0.20081g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

FIGURE 4.12. Voltammograms for the Adeloju et al. Variation 1 Sample Digestion Procedure using Key Food Chunk Light Tuna



1. (a) 0.96635g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 0.97044g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 0.95962g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 1.05061g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 1.06772g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 1.00348g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

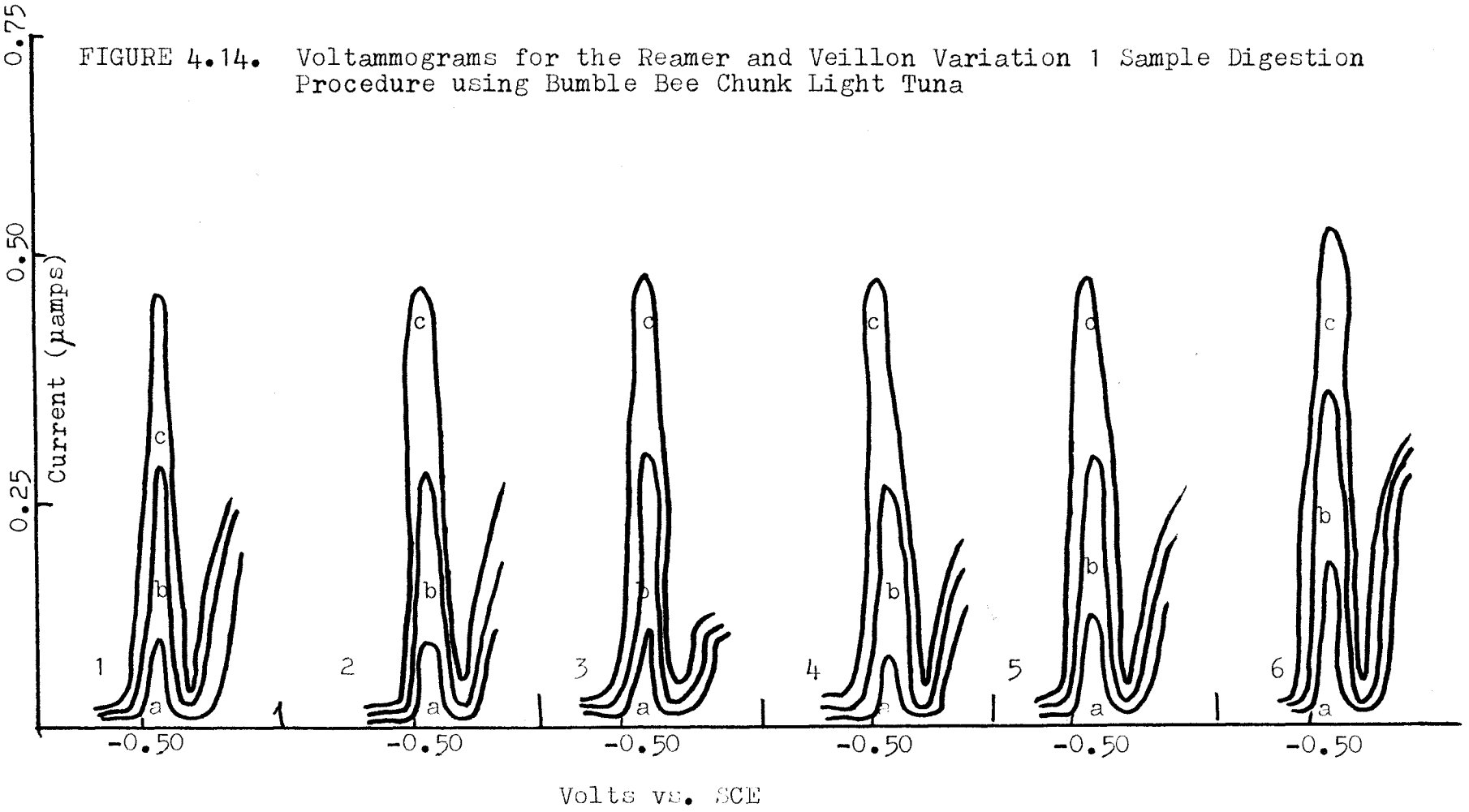
FIGURE 4.13. Voltammograms for the Adeloju et al. Variation 2 Sample Digestion Procedure using Chicken of the Sea Chunk Light Tuna



1. (a) 0.96656g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 0.97993g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 0.98481g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 0.93538g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 0.92269g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 1.00030g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

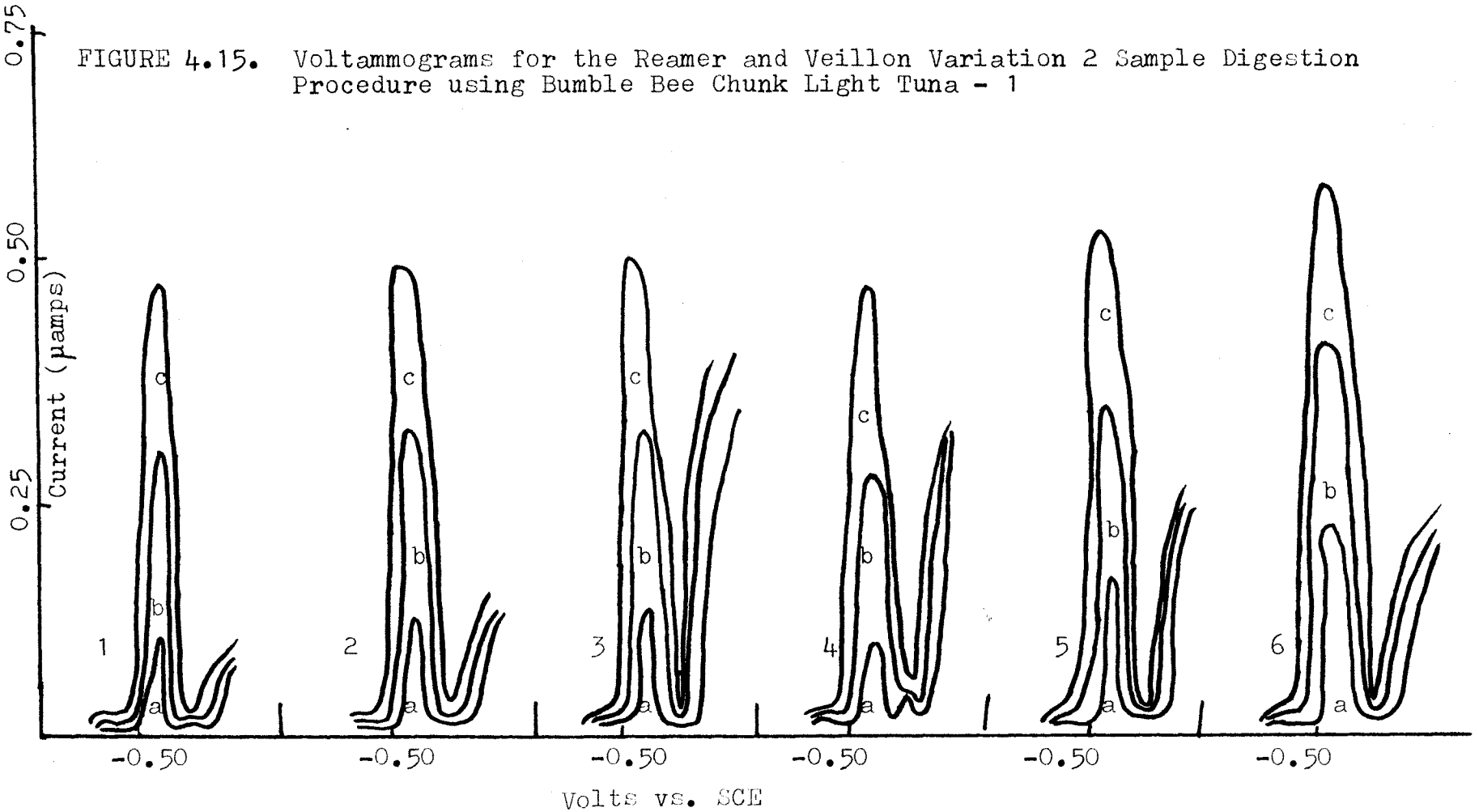
FIGURE 4.14. Voltammograms for the Reamer and Veillon Variation 1 Sample Digestion Procedure using Bumble Bee Chunk Light Tuna



1. (a) 1.00113g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 0.96931g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 1.00078g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 1.00052g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 0.99755g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 1.00271g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

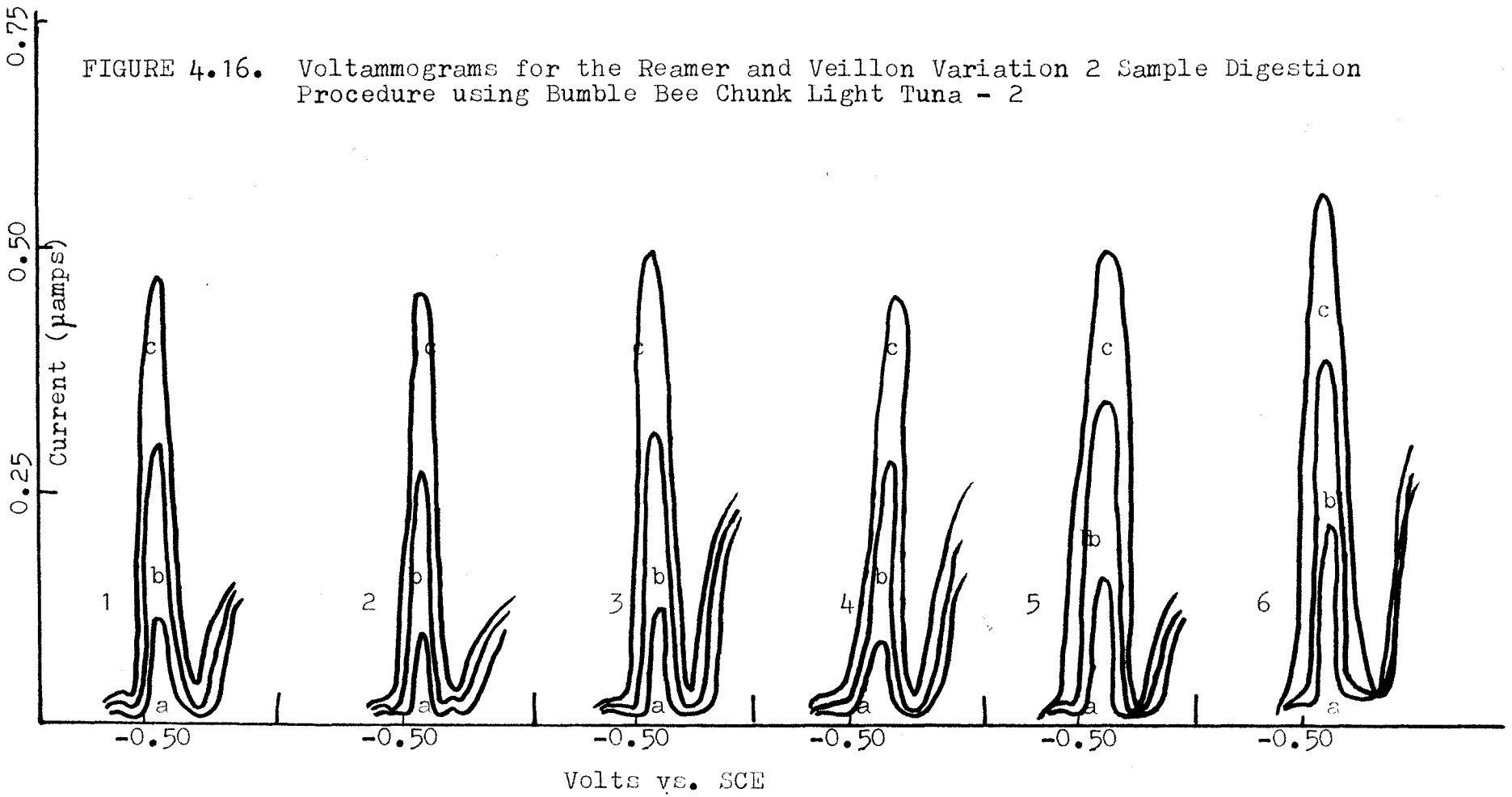
FIGURE 4.15. Voltammograms for the Reamer and Veillon Variation 2 Sample Digestion Procedure using Bumble Bee Chunk Light Tuna - 1



1. (a) 0.96634g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 1.00176g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 0.99989g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 0.97005g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 0.93726g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 1.00258g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

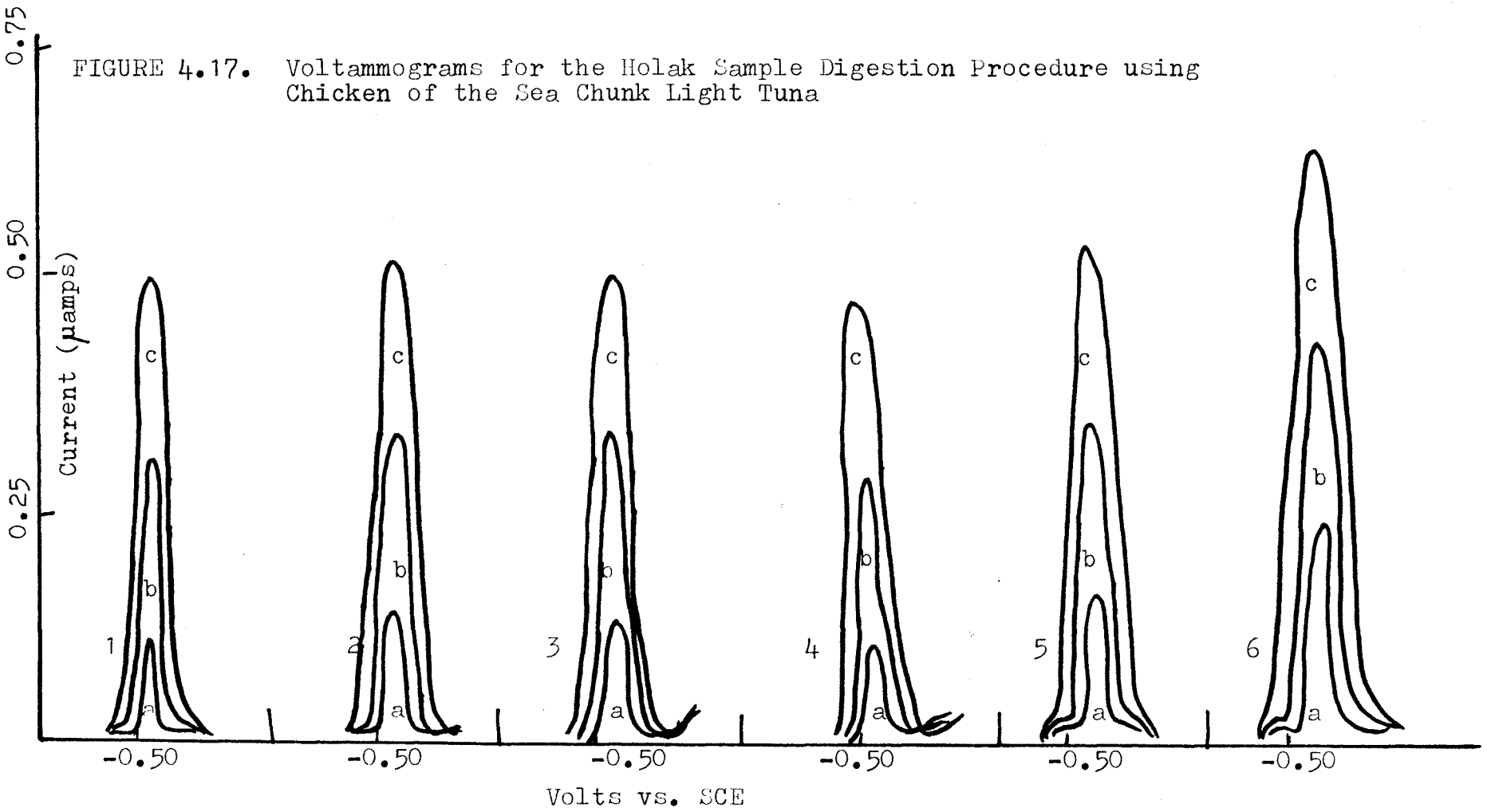
FIGURE 4.16. Voltammograms for the Reamer and Veillon Variation 2 Sample Digestion Procedure using Bumble Bee Chunk Light Tuna - 2



1. (a) 0.98942g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 0.92279g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 1.00377g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 0.99937g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 1.00346g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 0.91255g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

FIGURE 4.17. Voltammograms for the Holak Sample Digestion Procedure using Chicken of the Sea Chunk Light Tuna



1. (a) 1.00365g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
2. (a) 0.98813g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
3. (a) 0.96630g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
4. (a) 0.98134g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
5. (a) 0.91136g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
6. (a) 1.03571g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

Sources of Tuna Fish

The four main species of tuna are albacore, yellowfin, skipjack and bluefin. These four species account for more than 95 percent of all tuna commercially caught. The albacore is the most sought after tuna because of its delicate white meat. It is the only tuna that can be labeled as white. Albacore or Thunnus alalunga, are usually found from the coast of southern California to mid-Mexico. In late summer they travel to Puget Sound, Washington.

Yellowfin and skipjack comprise 95 percent of all of the light tuna. Yellowfin or Thunnus albacores, is the most popular species of light tuna. Yellowfin usually live between the Gulf of California and the north coast of Chile. Skipjack or Katsuwonus pelamis are the smallest of the commercially sought light meat tunas. They are found in most of the warm waters of the world's oceans.

Bluefin or Thunnus thynnus, comprise only about 5 percent of the worldwide commercial tuna catch. They tend to be seasonally available in the surface waters off the West Coast of the U.S. They are also found in the Atlantic Ocean.

Most species of tuna prefer temperate or tropical waters which accounts for the best fishing areas being off the coast of California, Mexico, South America and off the tropical west coast of Africa. Tuna are very sensitive to water temperature and do not tolerate changes of more than 10 to 15 °F. The tuna travel the oceans on a highly irregular course because they follow the warm ocean currents.

Tuna are the most migratory fish known and are found in oceans all over the world. Tuna are fast swimmers with their speed estimated to exceed 25 miles/hour. Albacore and bluefin tune that have been tagged off the coast of California have been recaptured off the coast of Japan, a

distance of over 5000 miles. Skipjack and yellowfin tuna also travel great distances, the records being in excess of 5900 and 3100 miles respectively.

Tuna have a high rate of metabolism and spend their whole lives traveling the warm waters. To feed their high energy needs, tuna will consume massive amounts of crab, smaller fish, shrimp-like crustaceans, squid and other small sea animals. A young tuna will actually triple its weight in its first year of life.

The U.S. Government in the Code of Federal Regulations Title 21 ¶161.190 specify four different pack styles of tuna. Solid or solid pack consists of loins cut in transverse segments placed in the can with the planes of their transverse cut ends parallel to the end of the can. Chunk, chunks or chunk style consists of a mixture of pieces of tuna in which the original muscle structure is maintained. These pieces may vary in size. Flakes consist of pieces of tuna in which 50 percent of the contents will pass through a 1/2-inch-mesh screen. The muscular structure of the flesh is retained. Grated tuna consists of a mixture of particles of tuna that have been reduced to a uniform size that will pass through a 1/2-inch-mesh screen.

The terms solid, chunk, flake or grated relate to the size of the loin and have no relation to the variety of fish in the can Any species can be canned as either solid, chunk, flake or grated. The color designation of white is limited to the albacore species while light includes the skipjack, yellowfin and bluefin species.

Canned tuna can be packed in a variety of media including vegetable oils, olive oil or water. Canned tuna may be seasoned with salt, monosodium glutamate, hydrolyzed protein, spices, vegetable broth, garlic or lemon.

Table 4.4 lists the sources of the tuna fish analyzed. The type of tuna refers to the above mentioned restrictions. The source refers to the area in which the fish was most probably caught as identified by the supplier. All of the tuna fish were purchased in supermarkets throughout Brooklyn, NY.

Tuna fish have been analyzed for various toxic metals including Cd, Pb, Hg, As and Se. Table 4.5 lists the average concentrations that have been reported. In the analysis of tuna fish from Great Britain, Cd concentrations in 48 samples were all below 0.2 μg/g. The Pb content of fresh albacore muscle was also found to be low at 0.3 ng/g. Albacore muscle from a can was found to have 1.4 μg/g of Pb. The main problem with Pb is not accumulation from the environment, but leaching from the can.

Hg content was found to be higher, but it varies widely between species. While yellowfin tuna were found to have between 0.012 to 0.06 μg/g of Hg, dogtooth tuna were found to have between 0.38 to 4.4 μg/g of Hg. Skipjack tuna were in the middle with 0.026 to 0.448 μg/g of Hg being reported. Hg does have a tendency to bioaccumulate in the food chain and these high levels of Hg can be a health hazard.

Arsenic concentrations in tuna fish also vary widely with a range of 0.71 to 4.6 μg/g being reported. Very little As is found in foods other than fish and seafood. A limit of 2.6 ppm is set for most foods in the U.S. The range of As reported indicates that this limit is exceeded in some tuna fish. Arsenic has various toxic effects and it is necessary to limit exposure to it.

Se concentrations reported for tuna fish range from 0.16 to 6.6 μg/g. Although no limit for Se in foods is set in the U.S., Se is recognized as a toxic metal. Trace quantities of Se are essential, but there is a very

narrow difference between the concentration at which it is considered essential and the concentration at which it is known to be toxic.

Se is normally present in seawater at 4 ppb, white As is normally present in seawater at 5 ppb (Venugopal and Luckey, 1978). They are both also widely used in industry and agriculture which could lead to increasing concentrations in the environment. Once in the water, As and Se are taken up by fish in normal metabolic processes. The As and Se can deposit in the tissues of the fish and accumulate over time. The As and Se content of the fish can therefore be much greater than the concentrations of these metals in the water.

Trace quantities of heavy metals are present in most foods. Usually the levels are not excessive, but fish and seafood tend to accumulate higher amounts of these metals. Tuna fish have been reported to contain a wide range of As and Se. Due to an increased awareness of the toxicity of these metals, it is important that the levels of As and Se in tuna fish be accurately determined.

TABLE 4.4 Sources of the Tuna Fish Analyzed

Brand	Type	Code #	Source
Star-Kist	Chunk light	999X1-D5200	-
	Albacore solid white	791F3-ES557	-
Chicken of the Sea	Chunk light	C120A-RW620	West Coast of Central and South America
	Albacore solid white	SMAIZ-W4K2H	West Coast of U.S.
Bumble Bee	Chunk light	CLA4S-1XS41	Solomon Islands near New Guinea
	Albacore solid white	SWP9J-L4362(for Se)	Mid-Atlantic
	Albacore solid white	SWP1J-L7VHI(for As)	Mid-Atlantic
Key Food	Chunk light	SGB-40610	-
	Albacore solid white	M5LB-0028C	Atlantic
Deep Blue	Chunk light	SCB-40504(for Se)	West Coast of Central and South America
	Chunk light	SCB-40507(for As)	West Coast of Central and South America
American	Chunk light	M54-XB-32230	West Coast of Central and South America
CHB	Albacore white flakes in oil	M3LU-N09AB	West Coast of U.S.

TABLE 4.4 Sources of the Tuna Fish Analyzed

Brand	Type	Code #	Source
Season	Albacore solid white	751F8-ES646	-
Empress	Chunk light	41118-1TK	-
Progresso	Light in olive oil	RKM16-A7842	-
Genova	Solid light in olive oil	TLHAZ-53KBF	-

Note: - Source not available

Unless otherwise noted all were canned in water

TABLE 4.5 Concentration of Toxic Metals in Tuna Fish

Metal	Concentration (μg/g unless noted)	Source
Cd	less than 0.2	Fribergh et al., 1974
Pb		
fresh albacore muscle	0.3 ng/g	Seattle & Patterson, 1980
canned tuna fish	1.4	"
Hg		
yellowfin tuna	0.012-0.06	Matthews, 1983
skipjack tuna	0.026-0.448	"
dogtooth tuna	0.38-4.4	"
As	0.71-4.6	Arsenic, 1977
Se		
average	4.63	Kifer et al., 1969
range	3.4-6.6	"
canned tuna	0.49	Holak, 1976

V. RESULTS

Fifteen different brands and types of tuna fish were analyzed for Se using DPCSV. Three samples from each can with three percent recoveries for each can were analyzed. These results are presented in Table 5.1. Figures 5.1 to 5.15. are the voltammograms that were obtained for each sample.

The types of tuna fish with the highest and lowest Se concentration were analyzed for As using DPP. Three samples with three percent recoveries were also performed for each can. These results are presented in Table 5.2. Figures 5.16 to 5.17 are the voltammograms that were obtained for each sample.

Table 5.3 summarizes the average Se concentration for each brand and type of tuna fish. The standard deviations and relative standard deviations are also given. Table 5.4 summarizes the average As content of the two brands of tuna fish with the standard deviations and the relative standard deviations.

TABLE 5.1 Results of the Selenium Analysis of Tuna Fish

Brand and Type	Sample	Se Found[a] (μg/g)	Sample	Se Added (μg)	Se Recovered[a] (μg)	Recovery Percent
Star Kist						
Chunk light	1	0.50	4	1.00	1.46	97.3
	2	0.51	5	2.00	2.25	90.0
	3	0.48	6	3.00	3.33	95.1
Albacore solid white	7	0.62	10	1.00	1.15	68.5
	8	0.72	11	2.00	2.70	101.0
	9	0.70	12	3.00	3.43	93.1
Chicken of the Sea						
Chunk light	13	0.91	16	1.00	2.03	108.0
	14	0.90	17	2.00	2.91	101.0
	15	0.90	18	3.00	3.85	98.5
Albacore solid white	19	0.55	22	1.00	1.52	98.1
	20	0.57	23	2.00	2.45	96.1
	21	0.55	24	3.00	3.27	92.1

TABLE 5.1 Results of the Selenium Analysis of Tuna Fish

Brand and Type	Sample	Se Found[a] (μg/g)	Sample	Se Added (μg)	Se Recovered[a] (μg)	Recovery Percent
Deep Blue						
Chunk light	25	0.37	28	1.00	1.28	95.5
	26	0.32	29	2.00	2.21	94.4
	27	0.33	30	3.00	3.14	94.6
Bumble Bee						
Chunk light	31	0.78	34	1.00	1.70	96.0
	32	0.79	35	2.00	2.75	98.9
	33	0.76	36	3.00	3.60	95.7
Albacore solid white	37	1.20	40	1.00	2.17	98.2
	38	1.25	41	2.00	3.13	97.8
	39	1.16	42	3.00	4.13	98.1
Key Food						
Chunk light	43	0.67	46	1.00	1.58	96.3
	44	0.65	47	2.00	2.57	97.0
	45	0.65	48	3.00	3.56	97.2

TABLE 5.1 Results of the Selenium Analysis of Tuna Fish

Brand and Type	Sample	Se Found[a] (μg/g)	Sample	Se Added (μg)	Se Recovered[a] (μg)	Recovery Percent
Key Food						
Albacore solid white	49	0.84	52	1.00	1.79	96.8
	50	0.86	53	2.00	2.77	96.9
	51	0.86	54	3.00	3.74	96.9
American						
Chunk light	55	0.69	58	1.00	1.65	98.2
	56	0.66	59	2.00	2.50	93.6
	57	0.69	60	3.00	3.65	98.9
CHB						
Albacore white flakes						
in oil	61	0.47	64	1.00	1.40	95.2
	62	0.46	65	2.00	2.37	96.0
	63	0.49	66	3.00	3.36	96.8

TABLE 5.1 Results of the Selenium Analysis of Tuna Fish

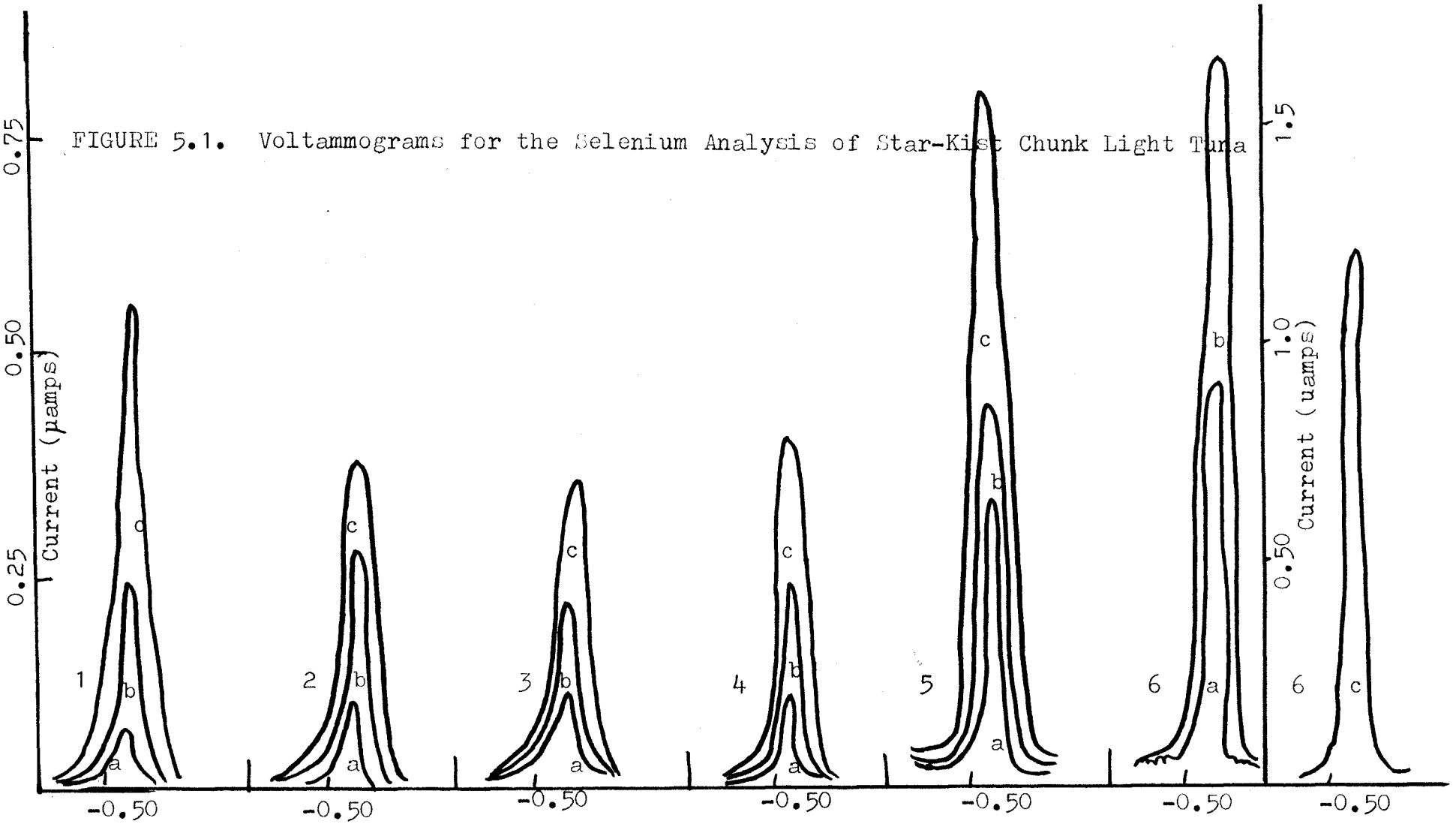
Brand and Type	Sample	Se Found[a] (μg/g)	Sample	Se Added (μg)	Se Recovered[a] (μg)	Recovery Percent
Season						
Albacore solid white	67	0.59	70	1.00	1.58	98.8
	68	0.59	71	2.00	2.57	98.9
	69	0.59	72	3.00	3.52	97.7
Empress						
Chunk light	73	0.87	76	1.00	1.85	96.9
	74	0.92	77	2.00	2.84	95.6
	75	0.90	78	3.00	3.72	95.9
Progresso						
Chunk light in olive oil	79	0.77	82	1.00	1.75	98.3
	80	0.77	83	2.00	2.73	98.6
	81	0.79	84	3.00	3.80	100.5

TABLE 5.1 Results of the Selenium Analysis of Tuna Fish

Brand and Type	Sample	Se Found[a] (μg/g)	Sample	Se Added (μg)	Se Recovered[a] (μg)	Recovery Percent
Genova						
Solid light in						
olive oil	85	0.39	88	1.00	1.36	97.8
	86	0.40	89	2.00	2.33	97.5
	87	0.38	90	3.00	3.29	97.3

a- the average of two standard addition determinations for each sample.

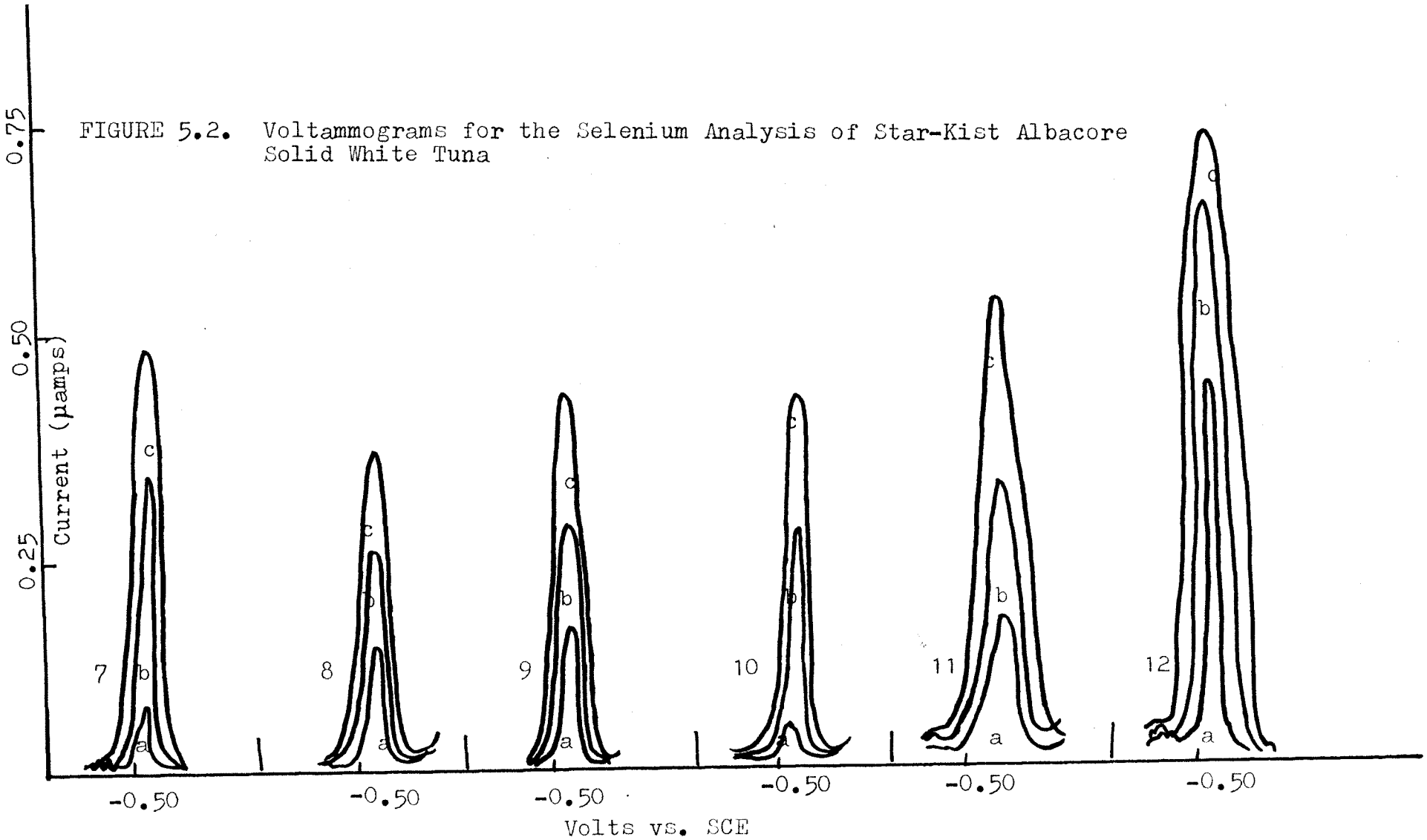
FIGURE 5.1. Voltammograms for the Selenium Analysis of Star-Kist Chunk Light Tuna



1. (a) 1.00806g of tuna (b) 1.0 μg of Se added (c) 3.0 μg of Se added
2. (a) 1.01179g of tuna (b) 1.0 μg of Se added (c) 1.5 μg of Se added
3. (a) 0.98962g of tuna (b) 0.5 μg of Se added (c) 1.0 μg of Se added
4. (a) 0.99884g of tuna with 1.0 μg of Se added (b) 1.5 μf of Se added (c) 3.0 μg of Se added
5. (a) 0.99203g of tuna with 2.0 μg of Se added (b) 0.5 μg of Se added (c) 2.0 μg of Se added
6. (a) 0.99984g of tuna with 3.0 μg of Se added (b) 1.5 μg of Se added (c) 3.0 μg of Se added

4, 5 and 6 are 10 ml of a 25 ml sample

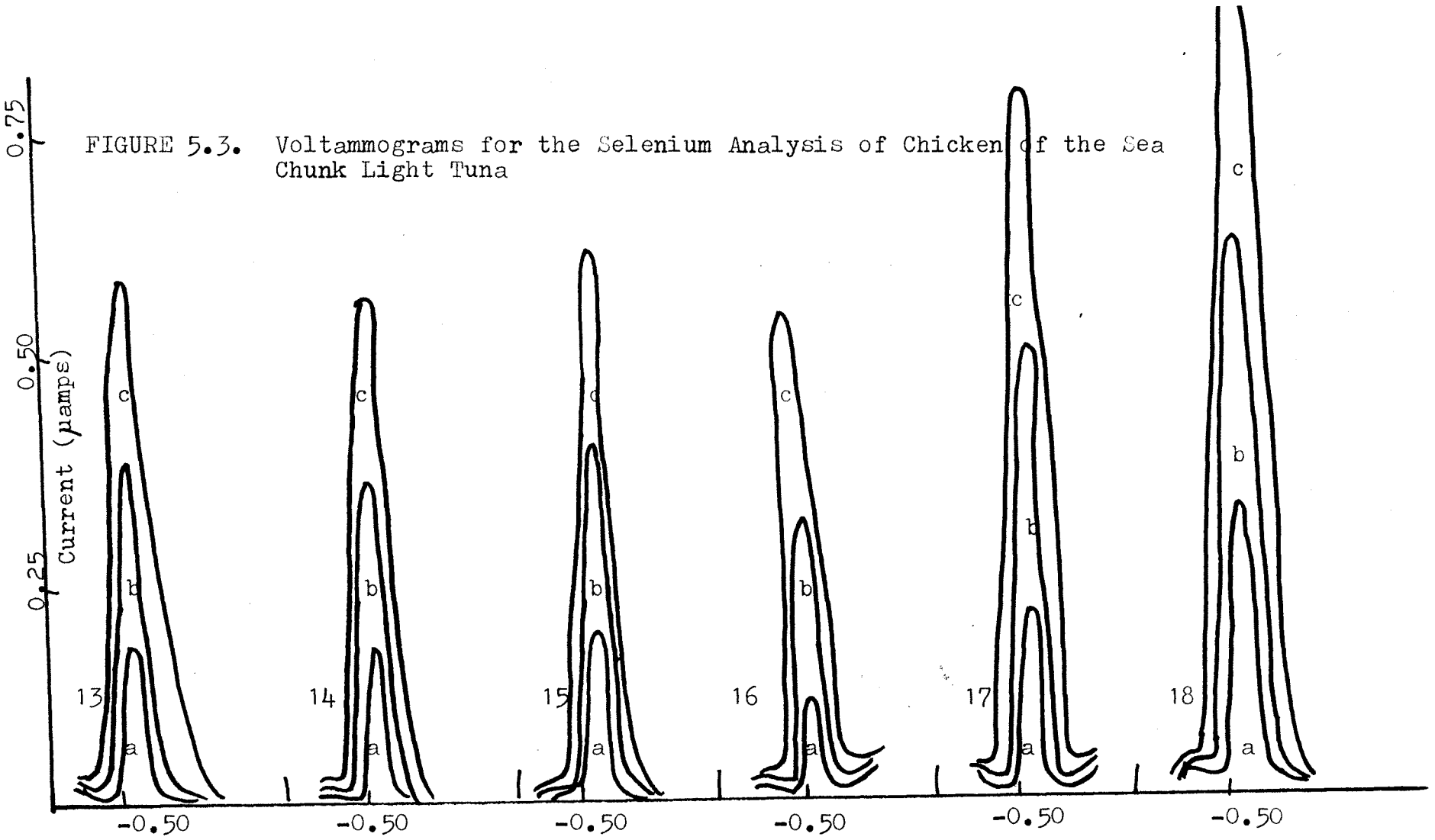
FIGURE 5.2. Voltammograms for the Selenium Analysis of Star-Kist Albacore Solid White Tuna



7. (a) 0.98912g of tuna (b) 2.0 μg of Se added (c) 3.0 μg of Se added
8. (a) 0.97072g of tuna (b) 0.5 μg of Se added (c) 1.0 μg of Se added
9. (a) 1.00207g of tuna (b) 0.5 μg of Se added (c) 1.0 μg of Se added
10. (a) 1.00153g of tuna with 1.0 μg of Se added (b) 2.0 μg of Se added (c) 3.5 μg of Se added
11. (a) 0.98215g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.5 μg of Se added
12. (a) 1.01305g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 1.5 μg of Se added

10, 11 and 12 are 10 ml of a 25 ml sample

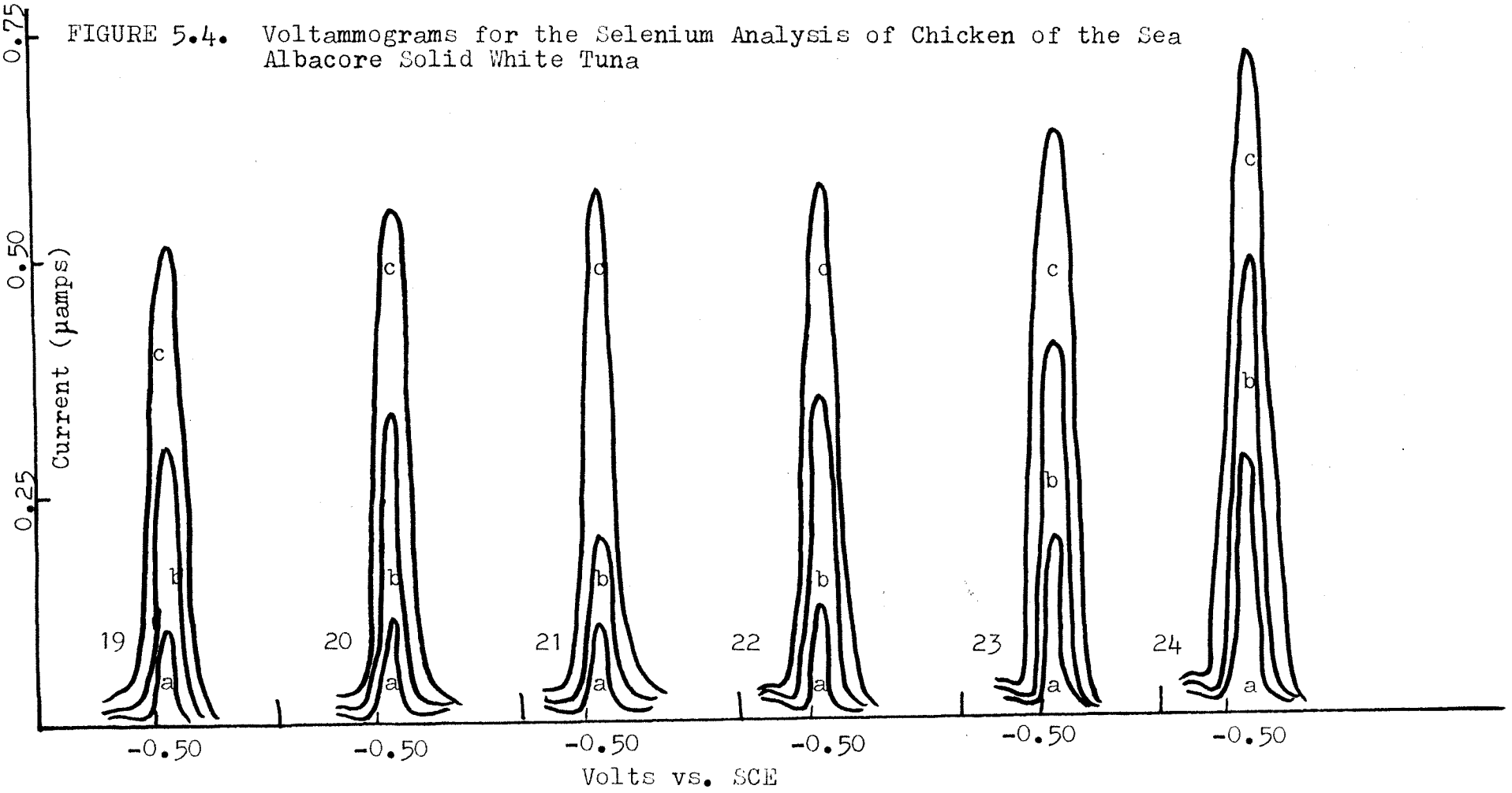
FIGURE 5.3. Voltammograms for the Selenium Analysis of Chicken of the Sea Chunk Light Tuna



13. (a) 0.99171g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
14. (a) 0.99133g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
15. (a) 0.97133g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
16. (a) 0.98209g of tuna with 1.0 μg of Se added (b) 1.5 μg of Se added (c) 3.0 μg of Se added
17. (a) 0.98301g of tuna with 2.0 μg of Se added (b) 1.5 μg of Se added (c) 3.0 μg of Se added
18. (a) 1.00606g of tuna with 3.0 μg of Se added (b) 1.5 μg of Se added (c) 3.0 μg of Se added

16, 17 and 18 are 10 ml of a 25 ml sample

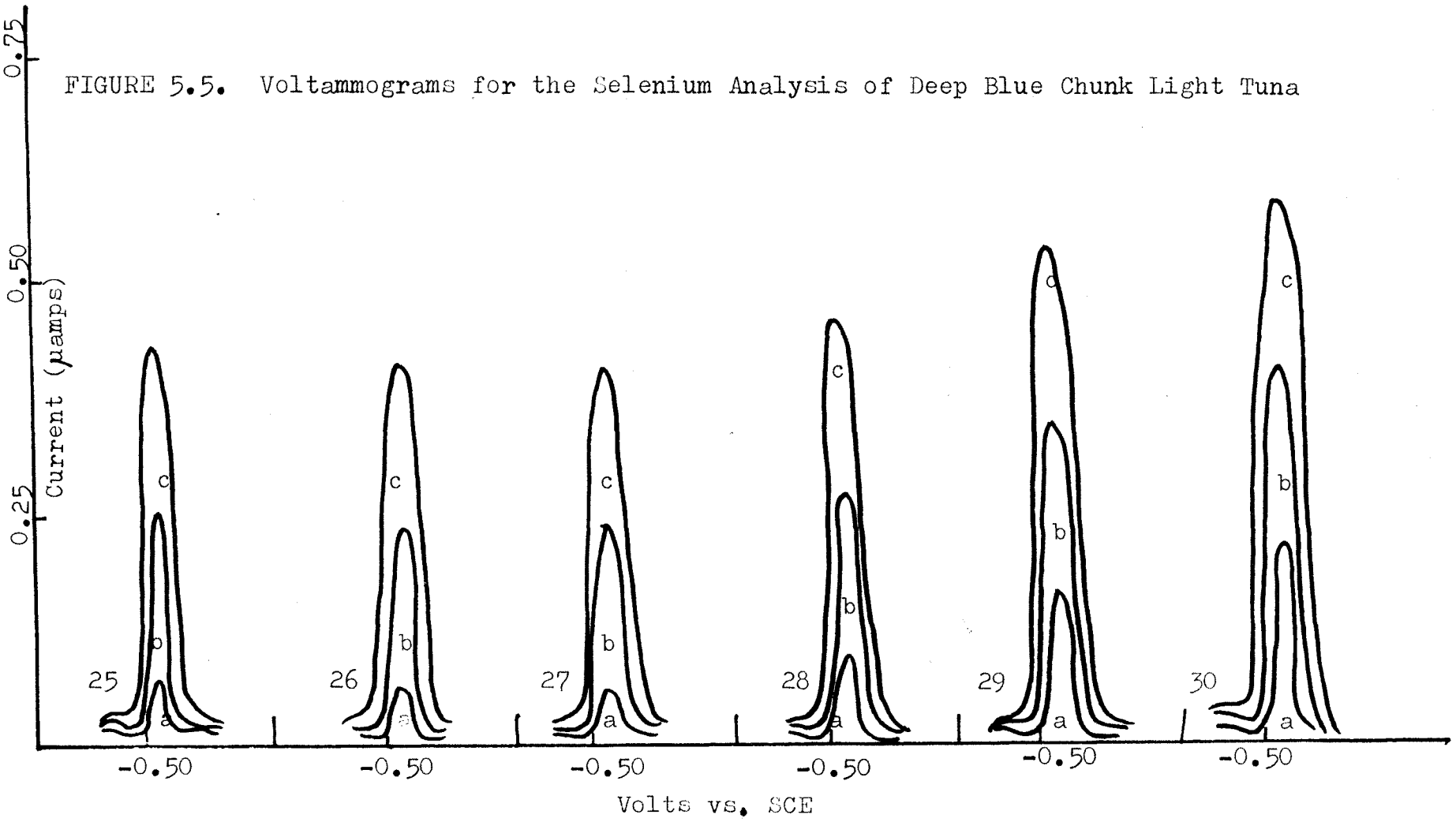
FIGURE 5.4. Voltammograms for the Selenium Analysis of Chicken of the Sea Albacore Solid White Tuna



19. (a) 0.96979g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
20. (a) 0.99540g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
21. (a) 0.99332g of tuna (b) 1.0 μg of Se added (c) 2.5 μg of Se added
22. (a) 0.99016g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
23. (a) 0.98735g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
24. (a) 0.98529g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

22, 23 and 24 are 10 ml of a 25 ml sample

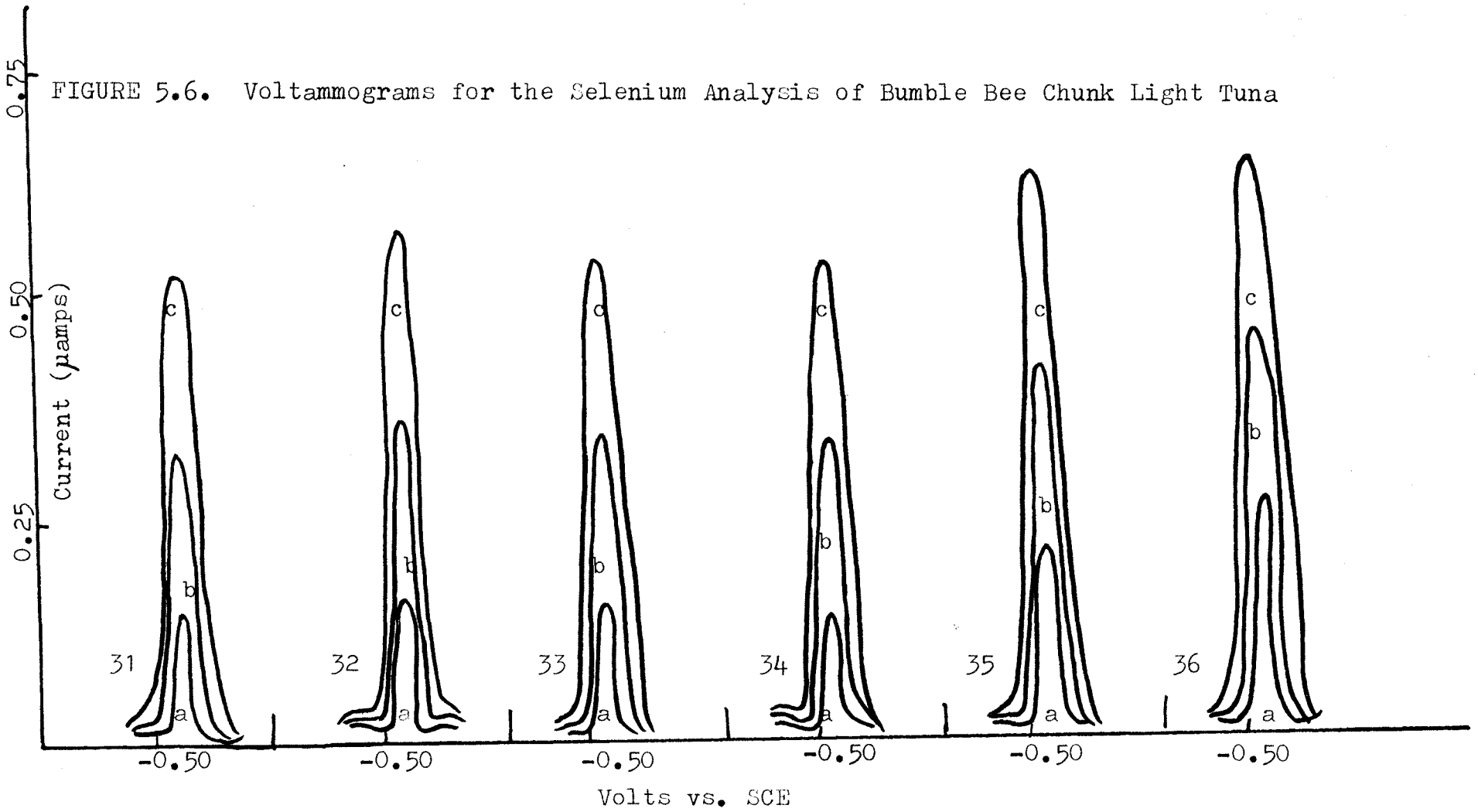
FIGURE 5.5. Voltammograms for the Selenium Analysis of Deep Blue Chunk Light Tuna



25. (a) 1.02024g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
26. (a) 0.98579g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
27. (a) 0.98954g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
28. (a) 1.00087g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
29. (a) 0.98823g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
30. (a) 1.01394g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

28, 29 and 30 are 10 ml of a 25 ml sample

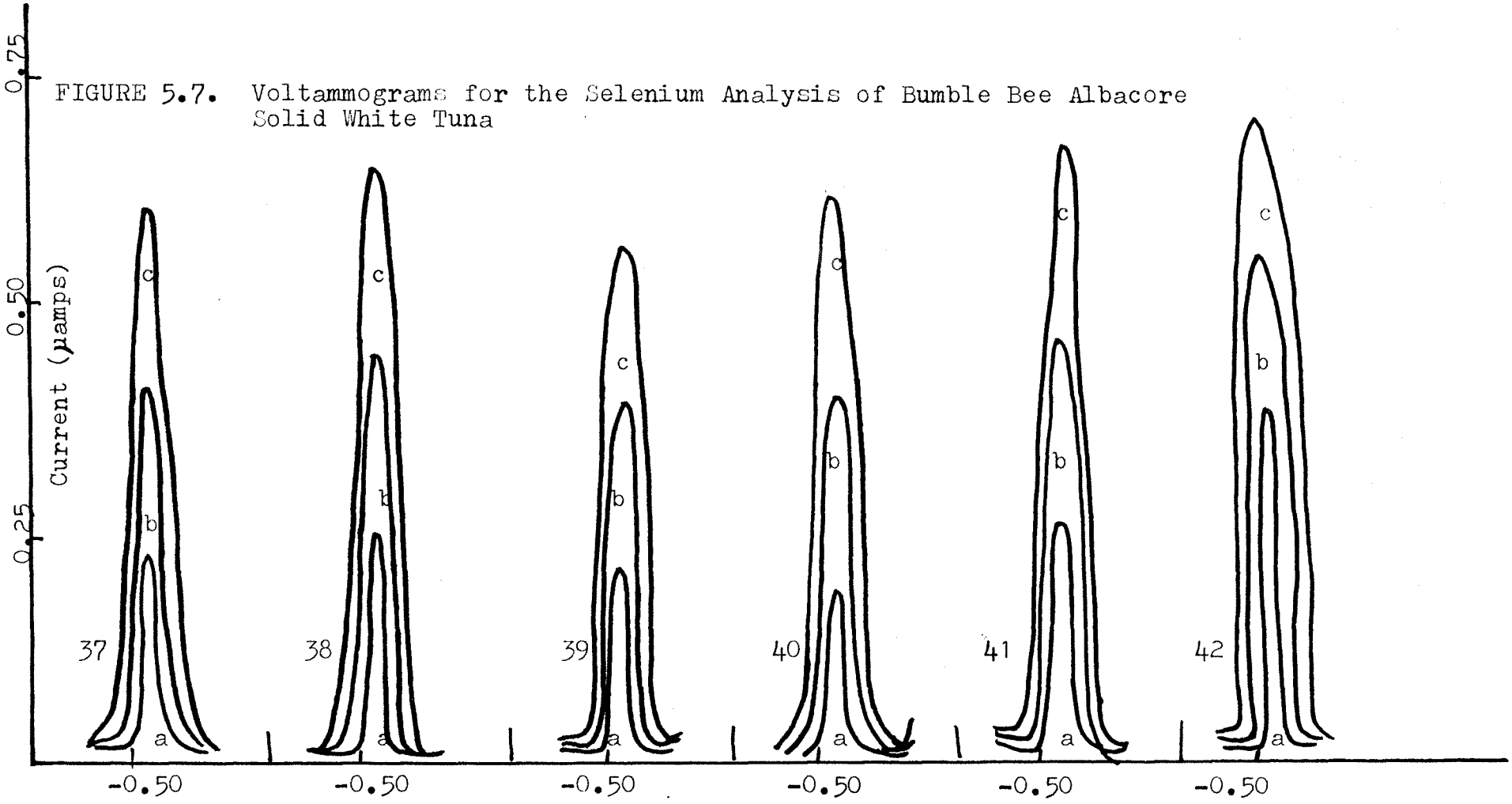
FIGURE 5.6. Voltammograms for the Selenium Analysis of Bumble Bee Chunk Light Tuna



31. (a) 0.99936g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
32. (a) 0.99807g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
33. (a) 1.02073g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
34. (a) 0.99395g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
35. (a) 1.01175g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
36. (a) 0.98941g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

34, 35 and 36 are 10 ml of a 25 ml sample

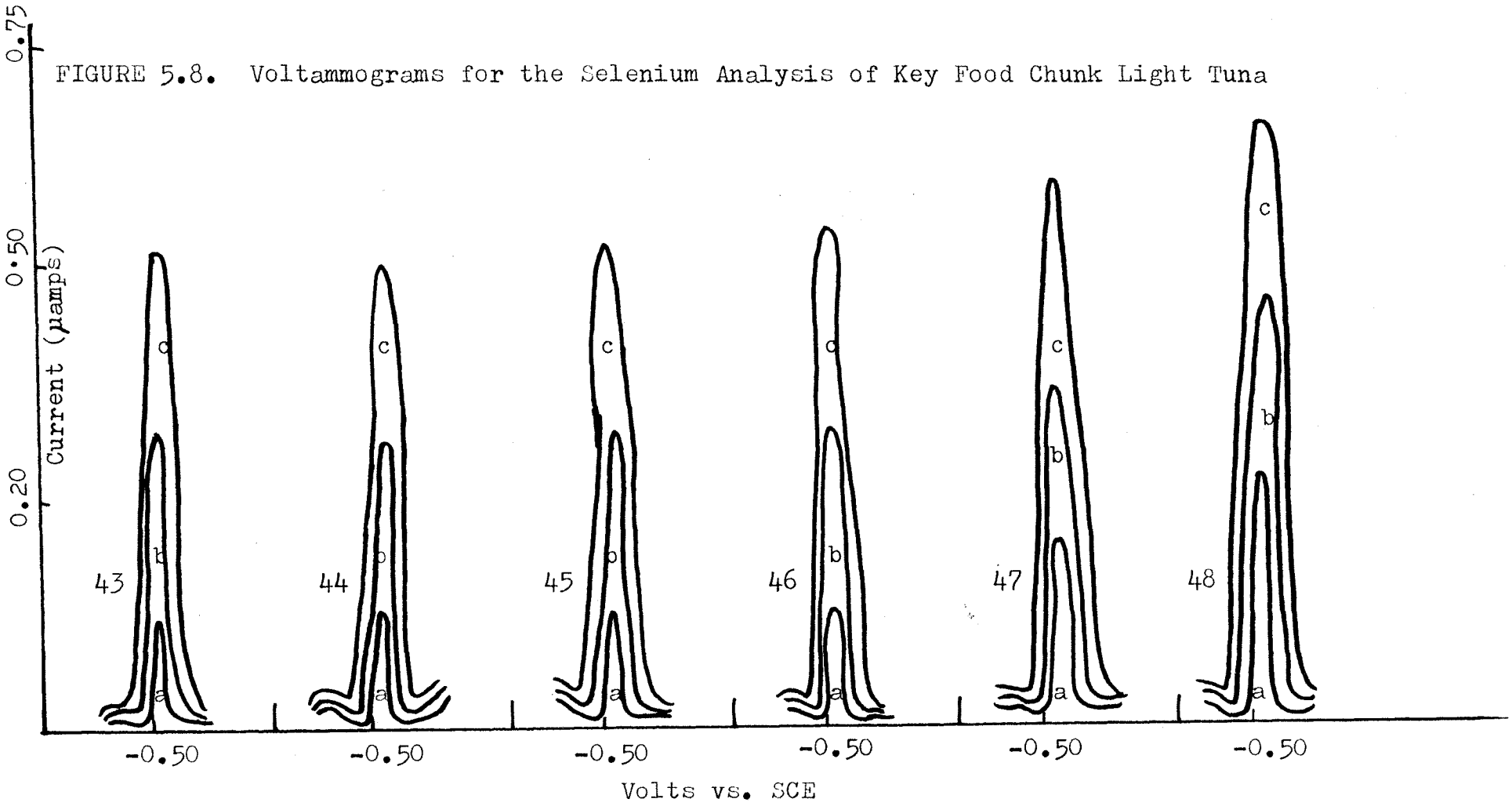
FIGURE 5.7. Voltammograms for the Selenium Analysis of Bumble Bee Albacore Solid White Tuna



37. (a) 0.99967g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
38. (a) 1.00127g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
39. (a) 1.01016g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
40. (a) 1.00852g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
41. (a) 0.99859g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
42. (a) 1.00774g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

40, 41 and 42 are 10 ml of a 25 ml sample

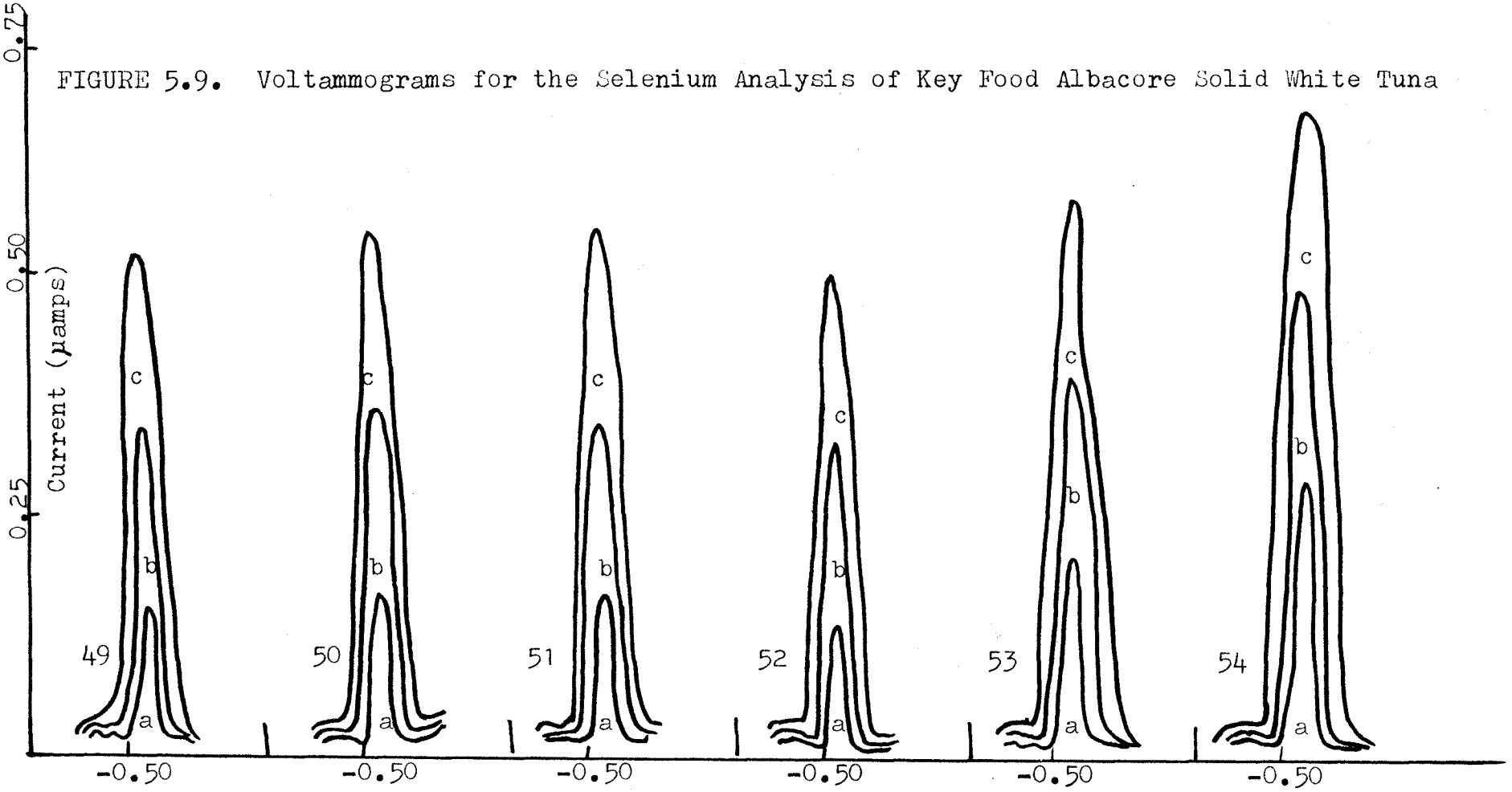
FIGURE 5.8. Voltammograms for the Selenium Analysis of Key Food Chunk Light Tuna



43. (a) 1.02642g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
44. (a) 0.98816g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
45. (a) 1.01300g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
46. (a) 0.99225g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
47. (a) 0.99412g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
48. (a) 1.01572g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

46, 47 and 48 are 10 ml of a 25 ml sample

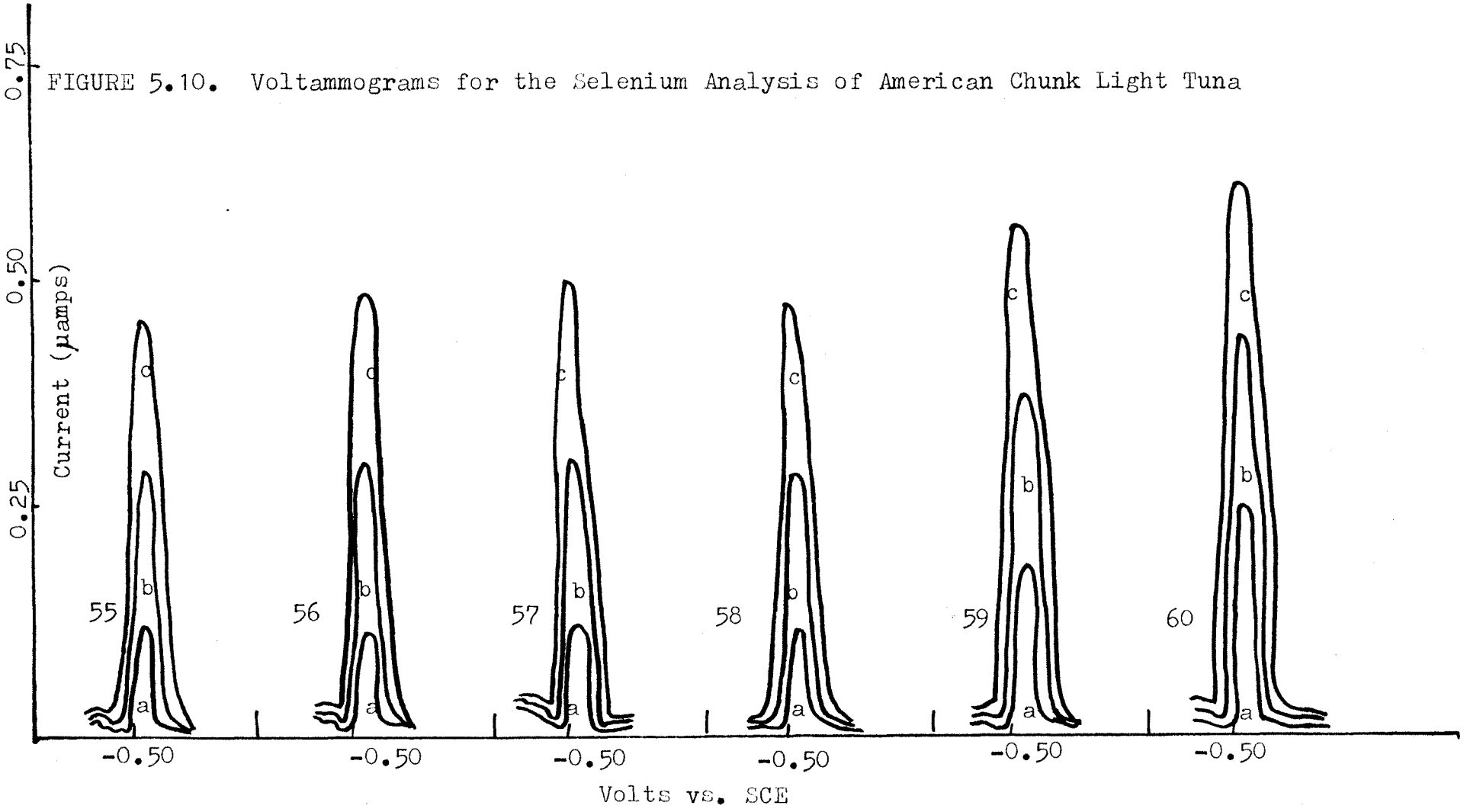
FIGURE 5.9. Voltammograms for the Selenium Analysis of Key Food Albacore Solid White Tuna



49. (a) 0.99154g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
50. (a) 0.99668g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
51. (a) 1.00151g of tuna (b) 1.0 μg of Se added (c) 2.0 μf of Se added
52. (a) 0.99960g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
53. (a) 1.00663g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
54. (a) 1.00626g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

52, 53 and 54 are 10 ml of a 25 ml sample

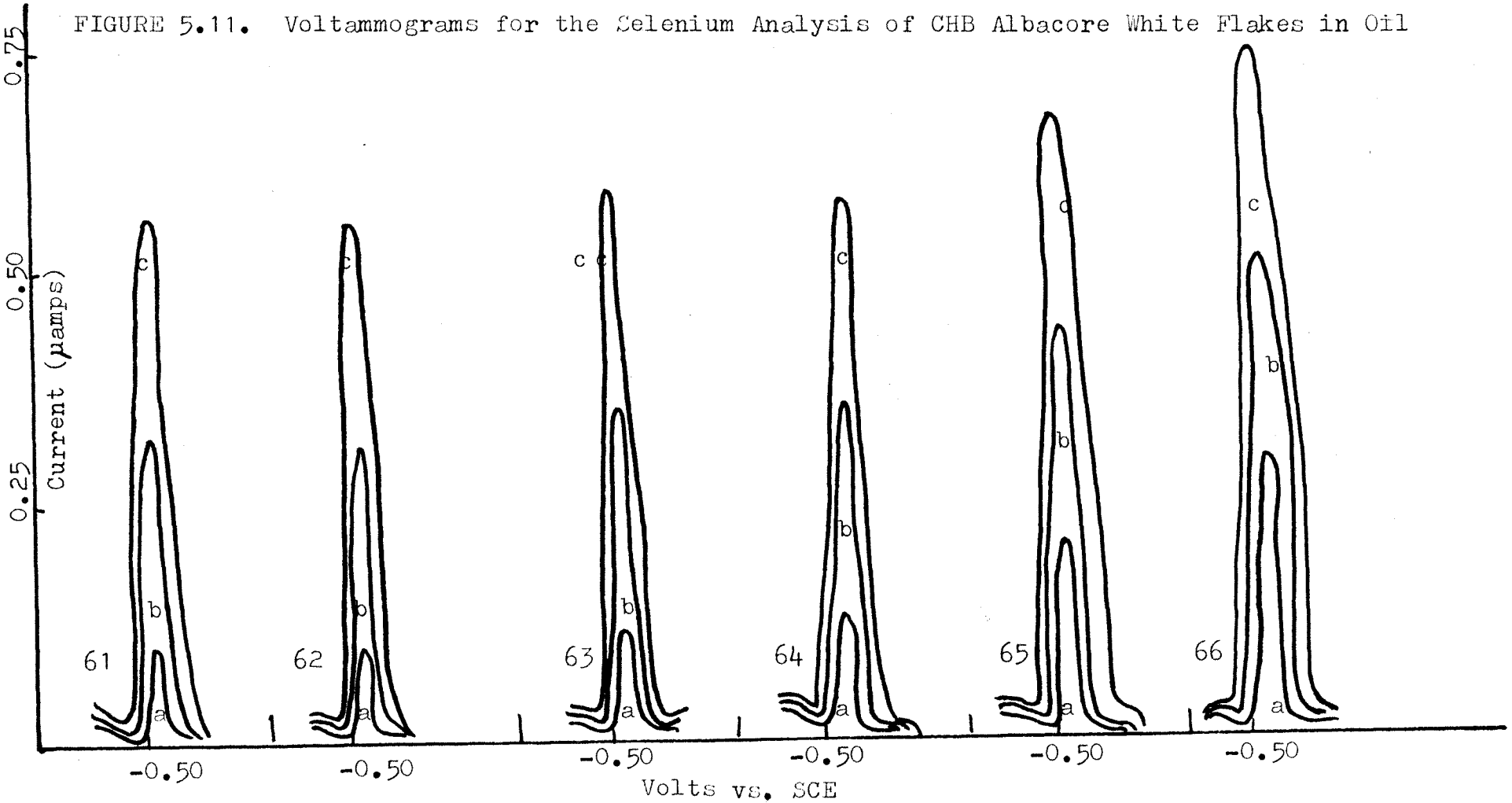
FIGURE 5.10. Voltammograms for the Selenium Analysis of American Chunk Light Tuna



55. (a) 1.02024g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
56. (a) 0.98570g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
57. (a) 0.98954g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
58. (a) 1.00087g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
59. (a) 0.98823g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
60. (a) 1.01394g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

58, 59 and 60 are 10 ml of a 25 ml sample

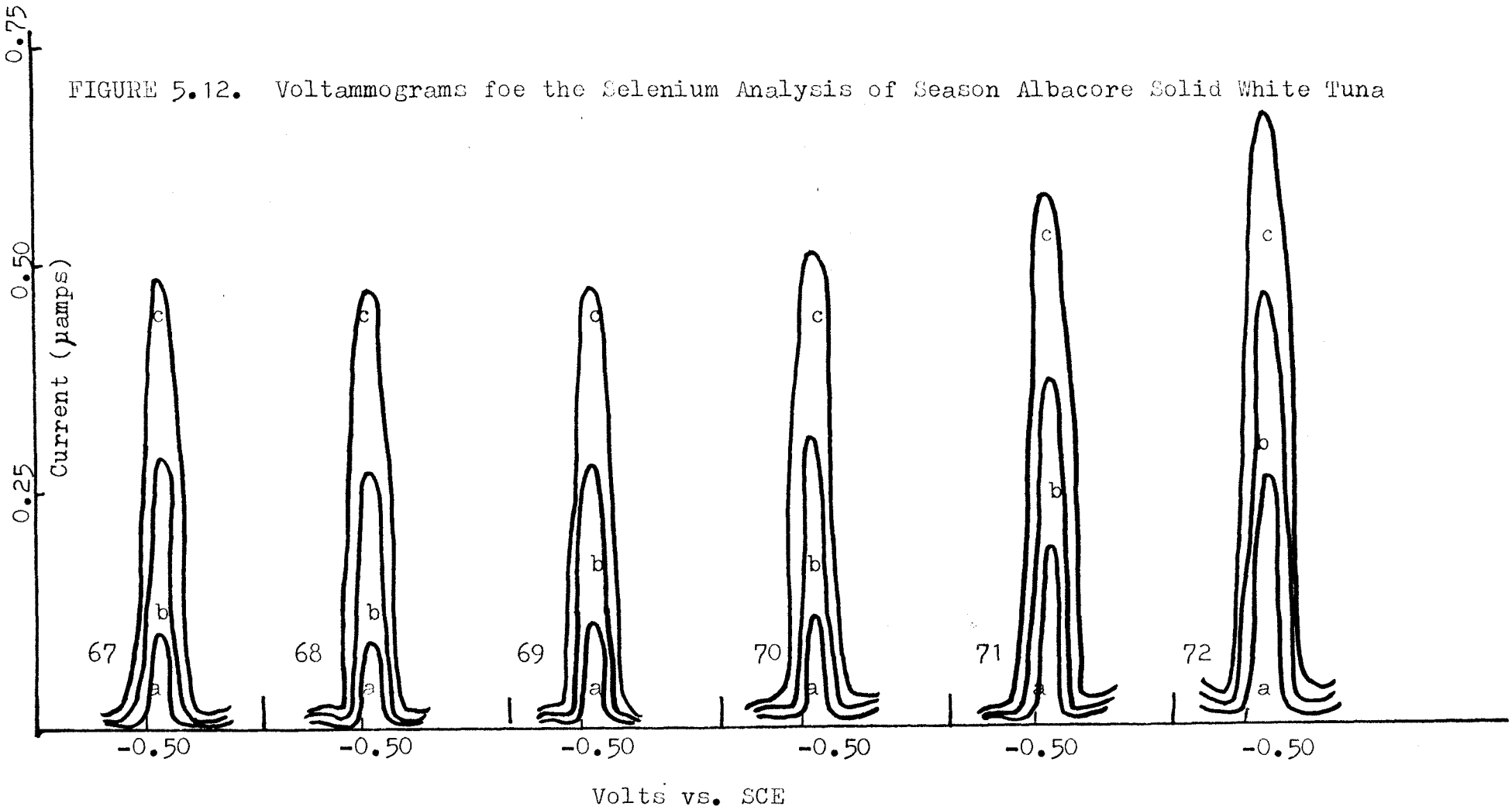
FIGURE 5.11. Voltammograms for the Selenium Analysis of CHB Albacore White Flakes in Oil



61. (a) 1.00917g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
62. (a) 0.97750g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
63. (a) 1.01157g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
64. (a) 1.00182g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
65. (a) 1.00403g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
66. (a) 0.99100g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

64, 65 and 66 are 10 ml of a 25 ml sample

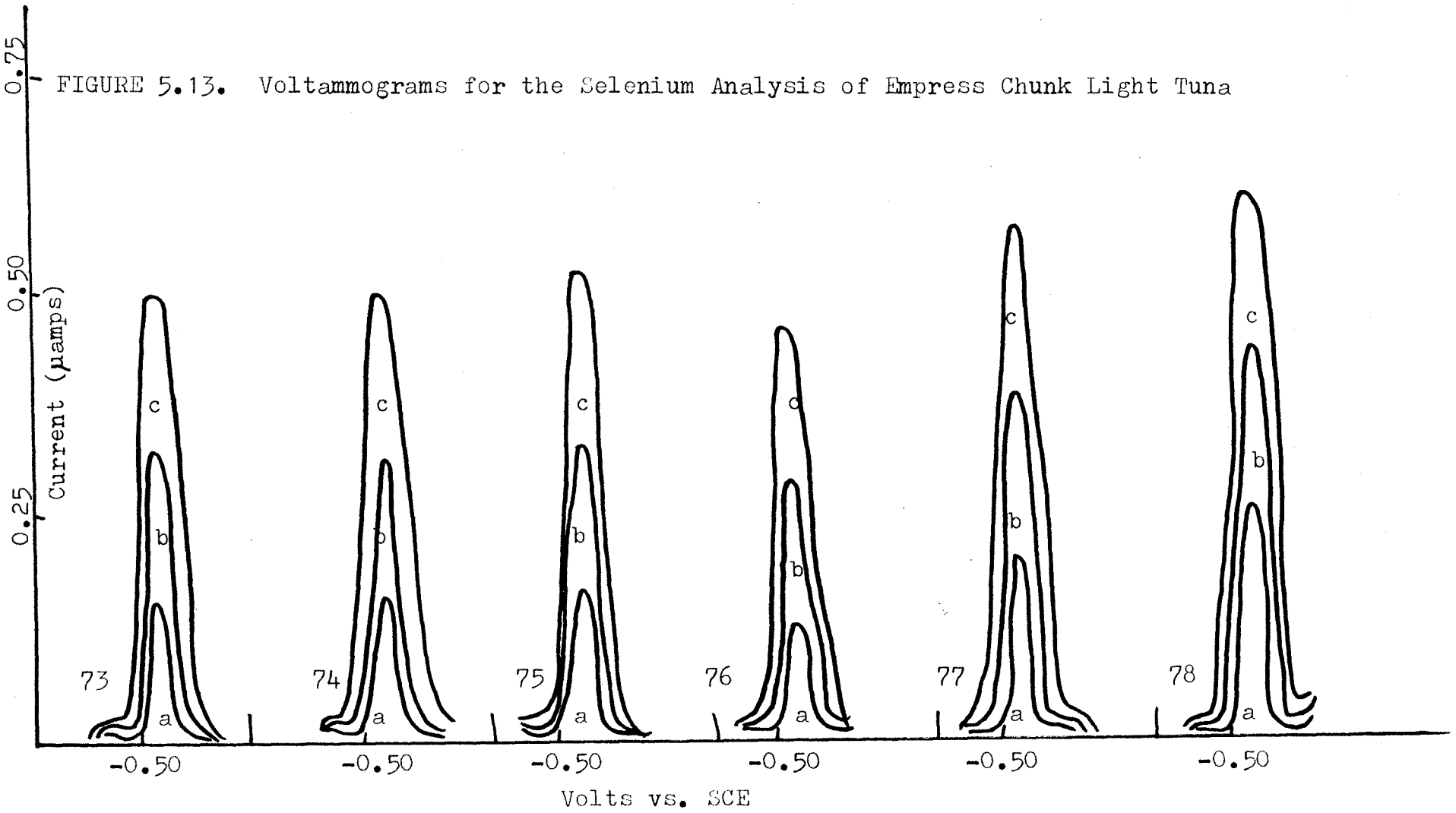
FIGURE 5.12. Voltammograms foe the Selenium Analysis of Season Albacore Solid White Tuna



67. (a) 0.99025g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
68. (a) 0.97193g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
69. (a) 1.01062g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
70. (a) 1.03224g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
71. (a) 1.01303g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
72. (a) 1.01734g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

70, 71 and 72 are 10 ml of a 25 ml sample

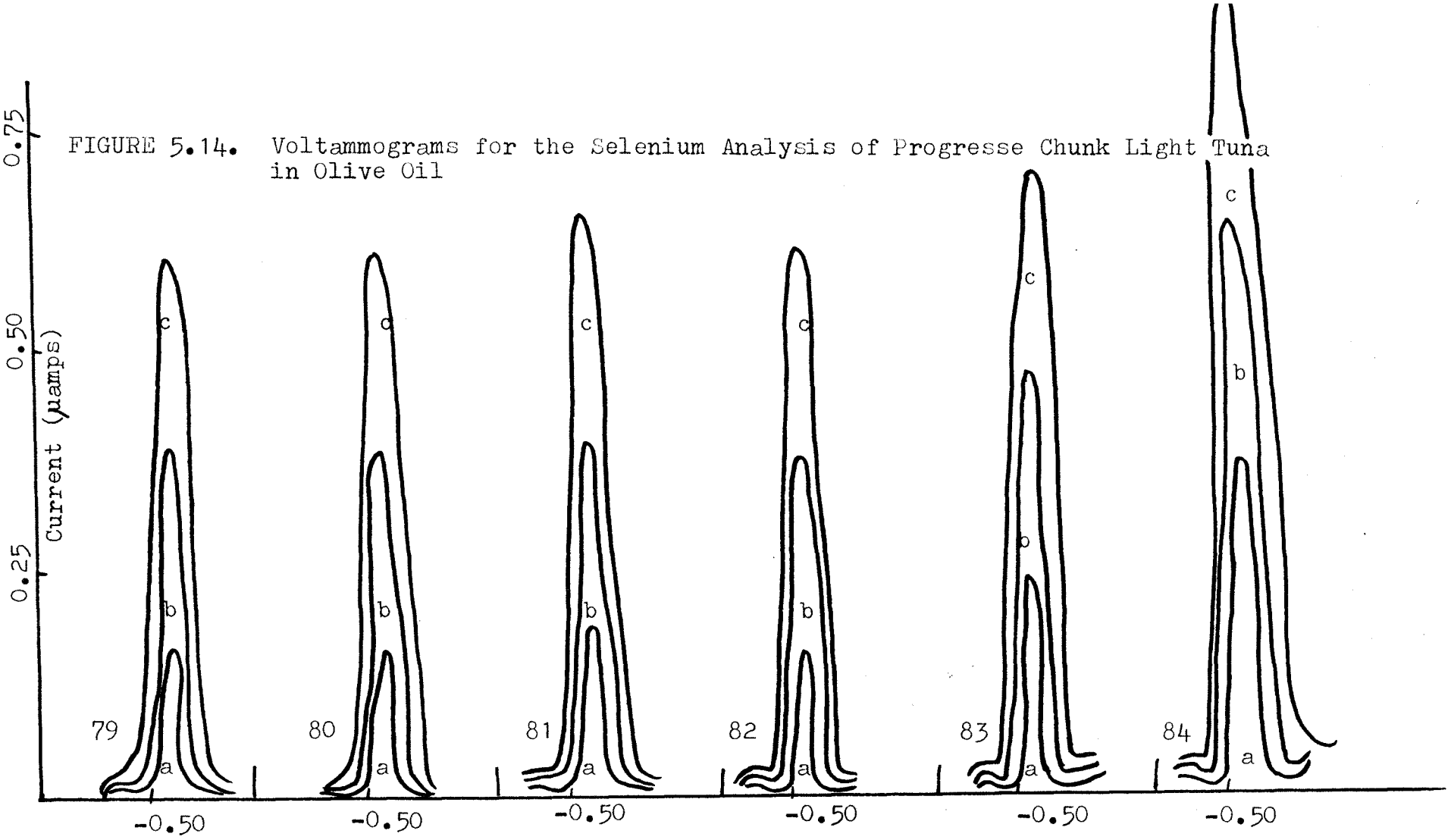
FIGURE 5.13. Voltammograms for the Selenium Analysis of Empress Chunk Light Tuna



73. (a) 1.00778g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
74. (a) 1.01280g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
75. (a) 1.00495g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
76. (a) 1.01869g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
77. (a) 1.01764g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
78. (a) 0.99206g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

76, 77 and 78 are 10 ml of a 25 ml sample

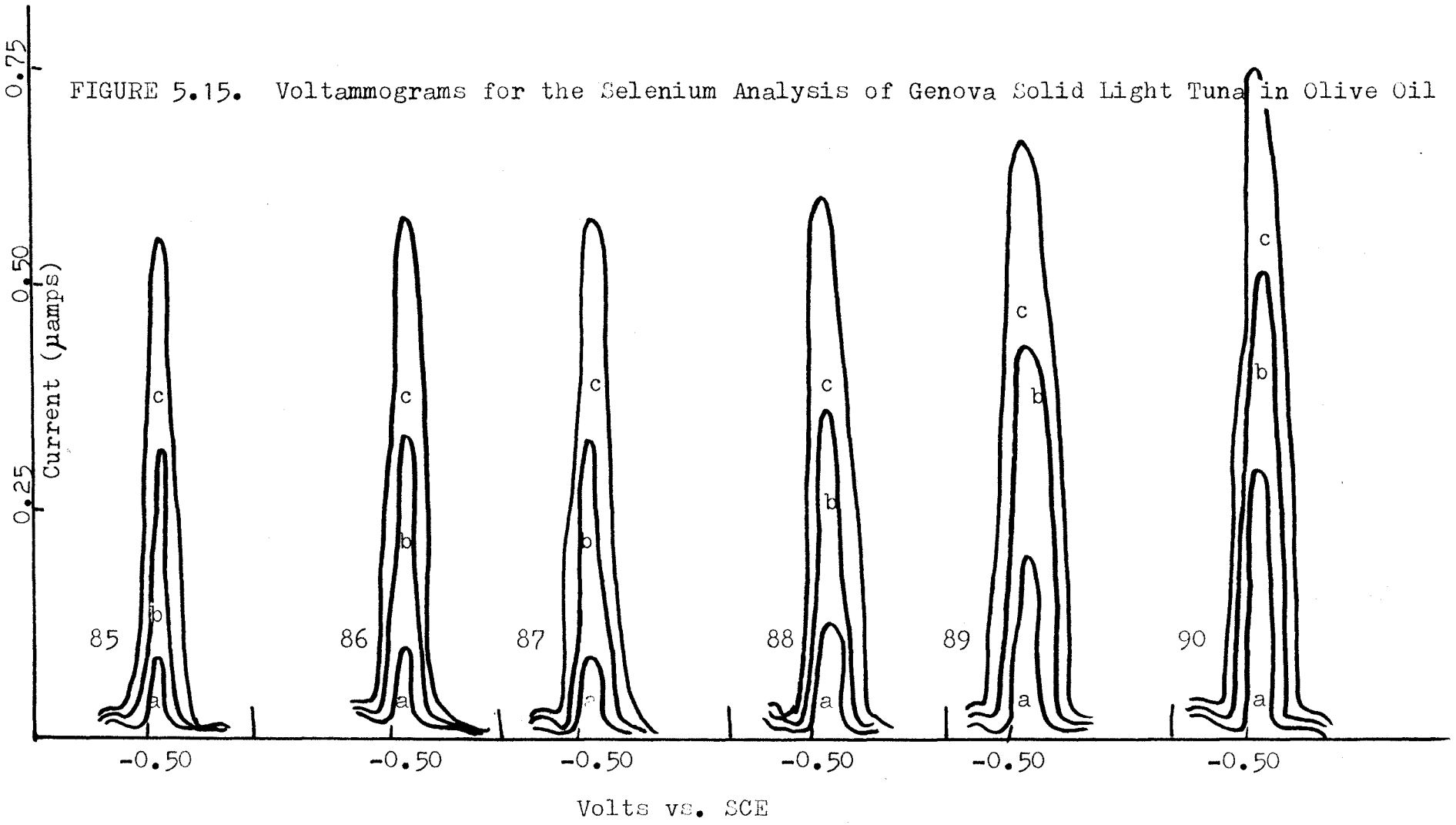
FIGURE 5.14. Voltammograms for the Selenium Analysis of Progresse Chunk Light Tuna in Olive Oil



79. (a) 0.98265g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
80. (a) 1.00047g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
81. (a) 1.01934g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
82. (a) 1.00878g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
83. (a) 1.00607g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
84. (a) 1.01262g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

82, 83 and 84 are 10 ml of a 25 ml sample

FIGURE 5.15. Voltammograms for the Selenium Analysis of Genova Solid Light Tuna in Olive Oil



85. (a) 0.99627g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
86. (a) 0.99759g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
87. (a) 1.00119g of tuna (b) 1.0 μg of Se added (c) 2.0 μg of Se added
88. (a) 1.00819g of tuna with 1.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
89. (a) 0.99079g of tuna with 2.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added
90. (a) 0.97173g of tuna with 3.0 μg of Se added (b) 1.0 μg of Se added (c) 2.0 μg of Se added

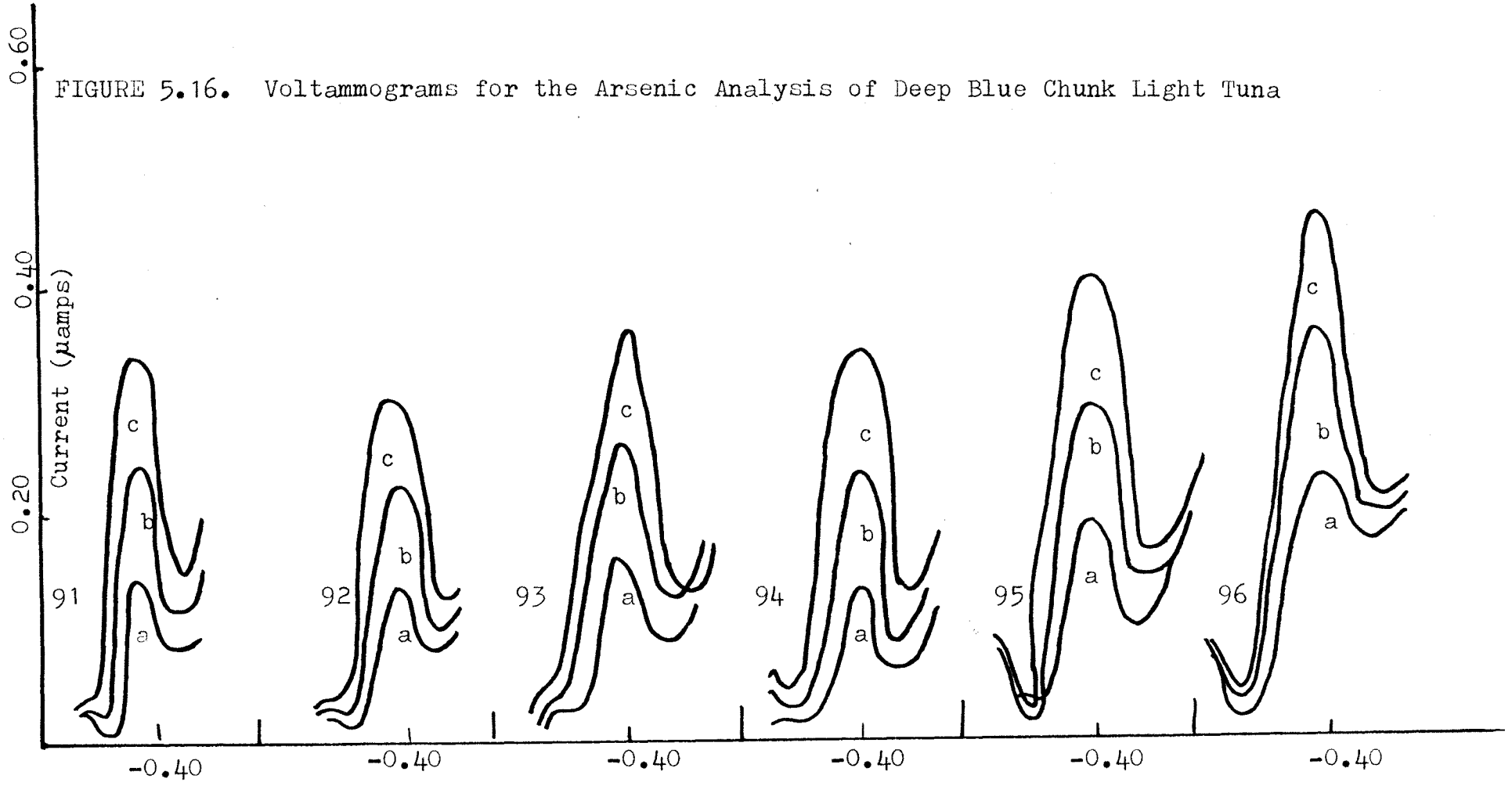
88, 89 and 90 are 10 ml of a 25 ml sample

TABLE 5.2 Results of the Arsenic Analysis of Tuna Fish

Brand and Type	Sample	As Found[a] (μg/g)	Sample	As Added (μg)	As Recovered (μg)	Recovery Percent
Deep Blue						
Chunk light	91	1.62	94	1.00	2.46	93.2
	92	1.58	95	2.00	3.49	94.6
	93	1.65	96	3.00	4.39	95.0
Bumble Bee						
Albacore solid white	97	2.37	100	1.00	3.20	95.8
	98	2.45	101	2.00	4.15	94.7
	99	2.43	102	3.00	5.22	96.3

a- represents the average of two standard addition determinations for each sample.

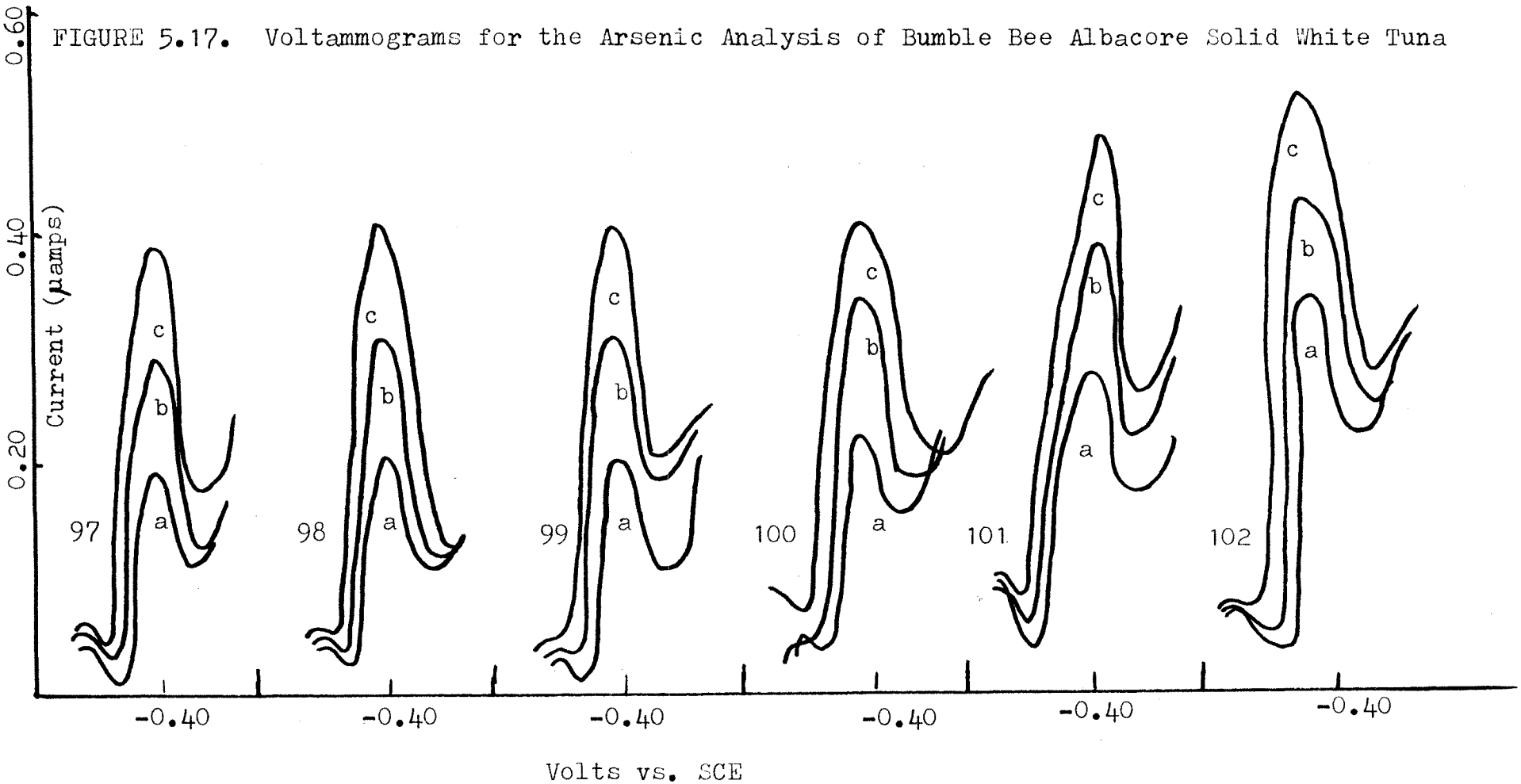
FIGURE 5.16. Voltammograms for the Arsenic Analysis of Deep Blue Chunk Light Tuna



91. (a) 1.00973g of tuna (b) 1.0 μg of As added (c) 2.0 μg of As added
92. (a) 0.99347g of tuna (b) 1.0 μg of As added (c) 2.0 μg of As added
93. (a) 0.97855g of tuna (b) 1.0 μg of As added (c) 2.0 μg of As added
94. (a) 1.00504g of tuna with 1.0 μg of As added (b) 1.0 μg of As added (c) 2.0 μg of As added
95. (a) 1.01347g of tuna with 2.0 μg of As added (b) 1.0 μg of As added (c) 2.0 μg of As added
96. (a) 0.99314g of tuna with 3.0 μg of As added (b) 1.0 μg of As added (c) 2.0 μg of As added

94, 95 and 96 are 10 ml of a 25 ml sample

FIGURE 5.17. Voltammograms for the Arsenic Analysis of Bumble Bee Albacore Solid White Tuna



97. (a) 1.00655g of tuna (b) 1.0 μg of As added (c) 2.0 μg of As added
98. (a) 1.00564g of tuna (b) 1.0 μg of As added (c) 2.0 μg of As added
99. (a) 0.99857g of tuna (b) 1.0 μg of As added (c) 2.0 μg of As added
100. (a) 0.98894g of tuna with 1.0 μg of As added (b) 1.0 μg of As added (c) 2.0 μg of As added
101. (a) 1.00821g of tuna with 2.0 μg of As added (b) 1.0 μg of As added (c) 2.0 μg of As added
102. (a) 1.00018g of tuna with 3.0 μg of As added (b) 1.0 μg of As added (c) 2.0 μg of As added

100, 101 and 102 are 10 ml of a 25 ml sample

TABLE 5.3. Selenium Content of Fifteen Brands and Types of Tuna Fish

Brand and Type	Se found[b] (μg/g)	Standard Deviation	Relative Standard Deviation
Star-Kist			
Chunk light	0.50	±0.025	5.0
Albacore solid white	0.68	±0.054	7.9
Chicken of the Sea			
Chunk light	0.90	±0.015	1.7
Albacore solid white	0.56	±0.018	3.3
Deep Blue Chunk light	0.34	±0.022	6.5
Bumble Bee			
Chunk light	0.77	±0.012	1.6
Albacore solid white	1.20	±0.040	3.2
Key Food			
Chunk light	0.65	±0.010	1.5
Albacore solid white	0.85	±0.010	1.2
American Chunk light	0.68	±0.015	2.2
CHB Albacore white flakes in oil	0.47	±0.017	3.6
Season Albacore solid white	0.59	±0.006	1.0
Empress Chunk light	0.89	±0.025	2.8
Progresso Chunk light in olive oil	0.77	±0.013	1.7
Genova Solid white in olive oil	0.39	±0.026	6.7

Average of all determinations: 0.68±0.268 μg/g of Se

b-the average of three samples with two analysis for each sample.

TABLE 5.4. Arsenic Content of Two Brands of Tuna Fish

Brand and Type	As found[b] (μg/g)	Standard Deviation	Relative Standard Deviation
Deep Blue			
Chunk light	1.62	±0.037	2.3
Bumble Bee			
Albacore solid white	2.41	±0.037	1.5

Average of the two samples: 2.02±0.559 μg/g

b-the average of three samples with two analysis done for each sample

Calculations

For measurement of the peak current:

$$i_p = P \times CSF \qquad CSF = Cs/D$$

where:

i_p = peak current in μamps

Cs= full scale current sensitivity

D= total # of chart divisions

CSF= current scale factor

P= peak height in # of chart divisions

The standard addition method was used to determine the concentration of the sample. The equation used to calculate the concentration from the peak current is:

$$C_{sample} = \frac{C_{std} \times i_p}{(i_p' - i_p) \times Sg}$$

where:

C_{sample} = concentration of the analyte in the sample

C_{std} = μgs of analyte added to the cell

i_p = peak current before addition of the analyte

i_p' = peak current after the addition of the analyte

Sg= grams of sample in solution

For each sample the standard addition procedure was done twice. The concentration of the analyte in the sample was taken as the mean of the two determinations.

The equation used to calculate the mean is:

$$X' = \frac{\sum_{i=1}^{K} (C1_i + C2_i)}{n}$$

where:

X'= mean concentration for the particular type of tuna fish

C1= concentration of analyte determined by the first standard addition

C2= concentration of analyte detetmined by the second standard addition

n= total # of analyses

K= total # of samples

The percent recovery was determined by adding a known amount of analyte to the sample prior to digestion, then analyzing the sample to determine the amount of analyte recovered. The equation used to calculate the percent recovery is:

$$\% \text{ Recovery} = Cf/Ce \times 100$$

$$Ce = (Sg \times X') + Aa$$

$$Cf = Sg \times Cs$$

where:

Ce= µg of analyte expected

Cf= µg of analyte detected

Aa= µg of analyte added prior to digestion

Cs= concentration of analyte detected in the sample (µg/g)

Sample Calculations

For the analysis of Deep Blue chunk light tuna the current sensitivity was 1 μamp. The total # of chart divisions was 100.

Sample 25 wt= 0.98579g P= 5.5 CSF= 0.01

$i_p = 5.5 \times 0.01 = 0.055$

C_{std}	P	CSF	ip'
1.0 μg	22.5	0.01	0.225
2.0 μg	39.5	0.01	0.395

$$C_{sample} = \frac{1.0\mu g\ (0.055\mu amps)}{(0.255\mu amps - 0.055\mu amps)\ 0.98579g} = 0.32\ \mu g/g$$

$$C_{sample} = \frac{2.0\mu g\ (0.055\mu amps)}{(0.395\mu amps - 0.055\mu amps)\ 0.98579g} = 0.32\ \mu g/g$$

Sample 26 wt= 1.02024g P= 6.5 CSF= 0.01

$i_p = 6.5 \times 0.01 = 0.065$

C_{std}	P	CSF	ip'
1.0 μg	24.0	0.01	0.240
2.0 μg	41.0	0.01	0.410

$$C_{sample} = \frac{1.0\mu g\ (0.065\mu amps)}{(0.240\mu amps - 0.065\mu amps)\ 1.02024g} = 0.36\ \mu g/g$$

$$C_{sample} = \frac{2.0\mu g\ (0.065uamps)}{(0.410\mu amps - 0.065\mu amps)\ 1.02024g} = 0.37\ \mu g/g$$

Sample 27 wt= 0.98954g P= 5.5 CSF= 0.01

i_p = 5.5 x 0.01 = 0.055

C_{std}	P	CSF	i_p'
1.0µg	21.5	0.01	0.215
2.0µg	39.5	0.01	0.395

$$C_{sample} = \frac{1.0\mu g \ (0.055\mu amps)}{(0.215\mu amps - 0.055\mu amps)\ 0.98954g} = 0.034\ \mu g/g$$

$$C_{sample} = \frac{2.0\mu g \ (0.055\mu amps)}{(0.395\mu amps - 0.055\mu amps)\ 0.98954g} = 0.032\ \mu g/g$$

$$X' \text{ for Deep Blue Chunk light} = \frac{(0.36 + 0.37 + 0.32 + 0.32 + 0.32 + 0.34)}{6}$$

X'= 0.34 µg/g Se

For percent recovery:

Sample 28 wt= 1.00087g Aa= 1.0 µg of Se

P= 9.0 CSF= 0.01 i_p = .090

C_{std}	P	CSF	i_p'
1.0 µg	26.5	0.01	0.265
2.0 µg	44.5	0.01	0.445

$$C_{sample} = \frac{1.0\mu g\ (0.090\mu amps)}{(0.265\mu amps - 0.090\mu amps)\ 1.00087g} = 1.28\ \mu g/g$$

$$C_{sample} = \frac{2.0\mu g\ (0.090\mu amps)}{(0.445\mu amps - 0.090\mu amps)\ 1.00087g} = 1.28\ \mu g/g$$

$$\% \text{ Recovery} = \frac{1.00087g \times 1.28\mu g/g}{(1.00087g \times 0.34\mu g/g) + 1.0\ \mu g} \times 100 = 95.5$$

Error Analysis

The standard deviation was calculated as follows:

$$d^2 = (X_i - X')^2$$

$$S = \sqrt{d^2/(n-1)}$$

where:

d^2= sum of the squares of the error for the sample

X_i= analytical value of th ith sample

X'= mean value for that sample group

n= number of analyses

S= standard deviation

The relative standard deviation was calculated as follows:

$$RSD = (S/X') \times 100$$

For Deep Blue chunk light tuna X' was 0.34 μg/g, while the individual analyses were 0.36, 0.37. 0.32, 0.32, 0.34 and 0.32 μg/g.

$$d^2 = (0.34 - 0.36)^2 + (0.34 - 0.37)^2 + \ldots\ldots\ldots + (0.34 - 0.32)^2$$

$$d^2 = 0.0025$$

$$S = 0.0025/5$$

$$S = \pm 0.022 \text{ μg/g}$$

$$RSD = (0.022/0.34) \times 100 = 6.5\%$$

The standard deviation and relative standard deviation for each type of tuna fish is listed in Table 5.3 and 5.4. The average standard deviation for the Se determinations was ±0.021 μg/g. The average relative standard deviation was 3.1 percent. The average standard deviation for the As determinations was ±0.037 μg/g. The average relative standard deviation was 1.9 percent.

VI. DISCUSSION

The analysis for trace quantities of metals requires a sensitive and reliable technique. Although many methods for trace analysis are available, differential pulse polarography and stripping voltammetry have many advantages which make them excellent techniques. DPCSV is a very sensitive method for Se analysis, while DPP enables very low detection limits for As analysis. The detection limits reported are 3 ng/g and 0.3 ng/ml respectively. The speed, sensitivity, selectivity, versatility and the small sample requirements for DPCSV and DPP are some of the other advantages.

Analysis using DPP and DPCSV requires digestion of the sample. Many sample digestion procedures are available, but often they result in loss of the analyte. Dry ashing procedures usually result in huge losses due to the high temperature at which they operate. Wet digestion procedures can also result in huge losses if the sample is heated too vigorously. The sample digestion procedure using 10 ml of HNO_3 and 4 grams of $Mg(NO_3)_2.6H_2O$, with an 18 hour predigestion step, followed by slow heating and subsequent introduction into a furnace set at 500°C, prevented such losses. It gave an average recovery of 98.2 percent. This was found to be an excellent sample digestion procedure.

The average Se concentration in all of the analyses was 0.68±0.268 µg/g. The average Se concentration for light tuna which consists of skipjack, yellowfin and bluefin species was 0.65±0.205 µg/g with a range of 0.34 to 0.90 µg/g. The average Se concentration for white tuna, which consists only of the albacore specie, was 0.73±0.266 µg/g with a range of 0.47 to 1.20 µg/g.

The albacore tuna, except in the case of Chicken of the Sea, contained a higher Se content than the same brand of light tuna. In the three brands in which both albacore and light tuna were tested, the albacore tuna contained an average Se concentration of 0.27 μg/g greater than the light tuna. In Chicken of the Sea however, light tuna contained 0.90 μg/g while albacore tuna had 0.56 μg/g.

Bumble Bee solid white tuna was found to contain 1.20 μg/g of Se, which is 75 percent greater than the average Se concentration in all tunas, and 65 percent greater than the average Se concentration in the albacore tunas. The Bumble Bee albacore was identified as being caught in the mid-Atlantic region. Key Food albacore white tuna also had a higher Se concentration with 0.85 μg/g being detected. This was 16 percent higher than the average content of the white tunas and 25 percent greater than the average content of all tunas analyzed. The Key Food albacore was also identified as being caught in the Atlantic Ocean. The two other albacore tunas in which the sources are identified were caught off the west coast of the U.S. These were Chicken of the Sea and CHB albacore. They had an average Se concentration of 0.52 μg/g.

The three light tunas which were identified as being caught off the west coast of Central and South America were found to contain 0.34, 0.68 and 0.90 μg/g of Se. The Deep Blue tuna contained only skipjack and had the lowest concentration of 0.34 μg/g. The type of the other brands of tunas could not be identified. Bumble Bee light tuna which was caught off the Solomon Islands near New Guinea contained 0.77 μg/g of Se which was not very different from the range found off the coast of Central and South America.

The brands with the highest and lowest Se concentration were also analyzed for As. Deep Blue light tuna which had the lowest Se

concentration of 0.34 μg/g, also had the lower As concentration of 1.62 μg/g. Bumble Bee albacore which had the highest Se concentration of 1.20 μg/g, also had the higher As concentration of 2.41 μg/g. The Deep Blue skipjack tuna was caught off the west coast of Central and South America while the Bumble Bee was from the mid-Atlantic.

The 15 cans of tuna fish tested contained much less Se than reported by Kifer et al. (1969). He reported tuna having an average Se concentration of 4.63 ppm (μg/g) with a range of 3.40 to 6.20 ppm (see Table 1.13). The Se content of these fish was determined with fluorometry. The sample was digested in a nitric-sulfuric-perchloric acid mixture using disodium ethylene diaminetetraacetate to prevent interferences. The Se was then complexed with 2,3-diaminonapthalene and extracted into cyclohexane. Samples were then analyzed in a spectrophotofluorometer. Although these fish were reported to be from waters which were low in Se, tuna fish are highly migratory fish and the area in which they are caught is not necessarily the area in which they lived. Since all of the fish tested had excessively high Se concentrations, it is also possible that there was undetected Se contamination.

The concentration of Se in other seafood and seafood products (see Table 1.14) was found to be much lower than that reported by Kifer. Lobster, shrimp, cod, flounder and perch had a range of 0.23 to 0.681 μg/g. Three cans of tuna fish were reported to have 0.30, 0.16 and 1.00 μg/g of Se. The fifteen cans of tuna fish analyzed fell well within this range with only Bumble Bee albacore having a higher concentration.

Vegetables and fruits contain much lower Se concentrations with garlic containing the highest amount with 0.276 μg/g Se being reported. Whole wheat flour and bread had appreciable amounts of Se with 0.645, 0.241 and 0.676 μg/g being reported.

White flour and bread had lower concentrations with 0.197 and 0.280 μg/g Se being reported (see Table 1.11). Dairy products contain little Se but beef, pork and lamb kidneys contained higher amounts of Se than found in the tuna fish (see Table 1.12). Table 6.1 compares the Se content of the canned tuna fish with the Se content reported for other foods.

The As concentrations reported for most foods excluding seafood (see Table 1.8) is much lower than that found in the tuna fish. Mushrooms, puffed rice, table salt and sea salt with 2.9, 1.6, 2.71 and 2.83 μg/g As are the only exceptions. Much higher concentrations of As are reported for seafood (see Table 1.9). Shrimp shells have been reported to contain up to as much as 15.3 μg/g of As, kingfish up to 8.86 μg/g and dried conch up to 5.63 μg/g. As concentrations of the other seafood fall below this and range from 1.50 to 3.1 μg/g with and average of 2.37 μg/g of As. The As concentration of the Deep Blue skipjack tuna was below the average with 1.62 μg/g. The As concentration of the Bumble Bee albacore fell close to the average with 2.41 μg/g being found. Table 6.2 compares the As content of the canned tuna fish with the As content reported for other foods.

Although some species of fish have been reported to contain 5.0 μg/g of Hg (An Assessment of Mercury in the Environment, 1978), that reported for most fish (see Table 1.7) is much lower. Dogtooth tuna, which is generally not an important commercial tuna was found to contain from 0.38 to 4.4 ppm of Hg. Skipjack contained up to 0.448 ppm and yellowfin up to 0.6 ppm. These are still considered rather high when one considers that the FDA maximum allowable concentration is 0.5 ppm of Hg. Although As and Se are found in higher concentrations in tuna fish, Hg is more toxic. It has been suggested that the presence of Se in tuna fish may actually decrease the toxicity produced with high concentrations of Hg (Ganther et

TABLE 6.1 Comparison of the Se Content of the Canned Tuna Fish with the Se Content Reported for Other Foods

Type of Food	Average Se(μg/g)	Source
Canned tuna	0.68	-
East Canadian herring	1.95	Kifer et al., 1969
Chilean anchoveha	1.35	"
Smelt	0.95	"
Menhaden	2.09	"
Tuna	4.63	"
Lobster tail	0.681	Morris & Levander, 1970
Shrimp, shelled	0.604	"
Flounder, fillet	0.338	"
Cod, fillet	0.465	"
Perch, fillet	0.34	Holak, 1976
Canned tuna	0.49	"
Beef, pork and lamb kidneys	1.76	Morris & Levander, 1970
Dairy products	0.078	"
Vegetables	0.012	"
Fresh Fruits	0.007	"
Garlic	0.276	"
Mushrooms	0.125	"
Whole wheat products	0.521	"
White grain products	0.239	"

TABLE 6.2 Comparison of the As Content of the Canned Tuna Fish with the As Content Reported for Other Foods

Type of Food	Average As(μg/g)	Source
Canned tuna	2.02	-
Haddock	2.17	Schroeder & Balassa, 1966
Oysters, fresh	2.9	"
Scallop, fresh	1.67	"
Shrimp, fresh frozen	1.50	"
Conch, dried whole	5.63	"
Kingfish	8.86	"
Shrimp shells	15.3	"
Clams, fresh frozen	2.52	"
Beef, stewing	1.3	"
Pork, liver	1.4	"
Pork, kidney	0.0	"
Pork loin	0.06	"
Lamp chop	0.35	"
Salt, table and sea	2.77	"
Mushrooms	2.9	"
Garlic, fresh	0.24	"
Whole wheat grains	0.17	"
Butter	0.23	"

al., 1972). Hg is rarely found in appreciable quantities in other foods (see Table 1.6).

Lead does not seem to accumulate to any great degree in fish (see Table 1.5) or other fresh food. Rex sole from the northern coast had the highest concentration reported with 0.229 μg/g Pb. Albacore muscle was found to contain very little Pb with 0.3 μg/g being measured. As and Se tend to accumulate in foods to a much greater degree.

The Pb found in foods is generally due to the leaching of the Pb from Pb-soldered cans. The Pb content of many canned foods however (see Table 1.4) is well below the limit set by the FDA of 2 μg/g. The foods in which Pb seems to be a problem is those which are canned in vinegar (see Table 1.3). All of these food exceeded the 2 μg/g standard. The Pb concentrations in these foods ranged from 4.0 to 10.0 μg/g.

The normal Cd content of food is generally low. Oregon groundfish were found to contain little Cd as well as most other types of food (see Table 1.1 and 1.2). Shellfish, liver and kidney are the only foods that tend to accumulate Cd to any high degree (Doull et al., 1980) with up to 10 μg/g being reported. Except for in shellfish, liver, kidney or contaminated areas, As and Se seem to accumulate to a much greater degree that Cd in most food and fish.

The FDA maximum allowable limit for As in most fish and seafood is set at 2.6 ppm. This limit was not exceeded in either the Deep Blue skipjack or Bumble Bee albacore. The Deep Blue skipjack was found to contain 1.62 ug/g of As which is sufficiently below this standard. Bumble Bee albacore however was found to contain 2.41 μg/g which is closely approaching the FDA level of 2.6 ppm. Both cans do in fact exceed the limit of 1 ppm which is set in Great Britain. The Great Britain standard however does not take

into account that most of the As exists as As(IV) and not As(III) which is more toxic.

The FDA also has specifications regarding residues for arsenic drugs used in animals (Code of Federal Regulations, Chapter 21, ¶556.60). In edible tissues and eggs of chickens and turkeys 0.5 ppm is allowed in uncooked muscle tissue and eggs while 2 ppm is allowed in uncooked edible by-products. In edible tissues of swine 2 ppm is allowed in uncooked liver and kidney while 0.5 ppm is allowed in uncooked muscle tissue and other by-products. These levels are considerably lower than allowed for tuna fish and are probably due to the fact that As residues in animals as the result of a drug is controllable while there is no control over the natural accumulation of As in fish and other seafood.

The FDA does not have any guidelines or regulations dealing with the maximum allowable Se concentration. The FDA however permits the addition of up to 200 μg Se per tablet as a dietary supplement.

The FDA also allows the addition of Se as a supplement in animal feedstuffs (Code of Federal Regulations, Chapter 21, ¶573.90). The levels of 0.1 ppm allowed to be added to the feed of swine and chickens and the 0.2 ppm allowed to be added to the feed of turkeys was shown not to significantly increase the Se concentration in the edible products of chickens, turkeys and swine ("Food Additive and Pesticide Petitions and Proposals", 1973). The animals tested absorbed the dietary Se in proportion to their physiological need and excesses were rapidly excreted. This may occur at low concentrations of Se, but animals raised on higher concentrations of Se tend to accumulate the Se which can lead to toxic effects. Prolonged misuse of Se as a feed supplement can lead to toxicity.

The Se concentrations found in the tuna fish analyzed do not seem to pose a hazard to man. The FDA considers 200 μg of Se to be safe, so even

the highest level of Se found, 1.20 μg/g, would not appear to pose a problem. Concern might arise however if Se toxicity already exist. The consumption of tuna fish in this case would give an additive effect.

Conclusion

DPCSV and DPP proved to be excellent techniques for the determination of trace quantities of Se and As. The sample digestion procedure using HNO_3 and $Mg(NO_3)_2.6H_2O$ prevented losses of the analyte. It was found to be an excellent digestion procedure.

Se and As accumulate in tuna fish to varying degrees. Albacore tuna tend to accumulate more Se than skipjack, yellowfin or bluefin. Arsenic was also more concentrated in albacore. The higher concentrations are probably due more to environmental conditions rather than to the ability of certain species to be Se or As accumulators. Higher concentrations of Se and As were found in the albacore that was caught in the Atlantic as compared to those caught in the Pacific. Tuna however are highly migratory fish so the area in which they are caught are not necessarily the area in which they lived.

The Se and As concentrations found in the tuna fish exceeded those found in most other foods except for seafood. Se and As seem to accumulate in fish, and food in general, more so than the other heavy metals except in the case of contamination. Fish and seafood do tend to accumulate As and Se to greater degrees than most other foods.

The concentrations of Se found in the tuna fish should not be of concern unless Se toxicity already exists. Although the As concentrations were below the FDA standard of 2.6 ppm, the Bumble Bee albacore from the mid-Atlantic approached this level with 2.41 μg/g.

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