Selenium determinations in archeological bones by Differential Cathodic Stripping Voltametry.

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ABSTRACT

A method is described to determine selenium in archaeological bones. Following the wet ashing and complete disolution of the sample, selenium is determined by Differential Cathodic Stripping Voltammetry Technique.

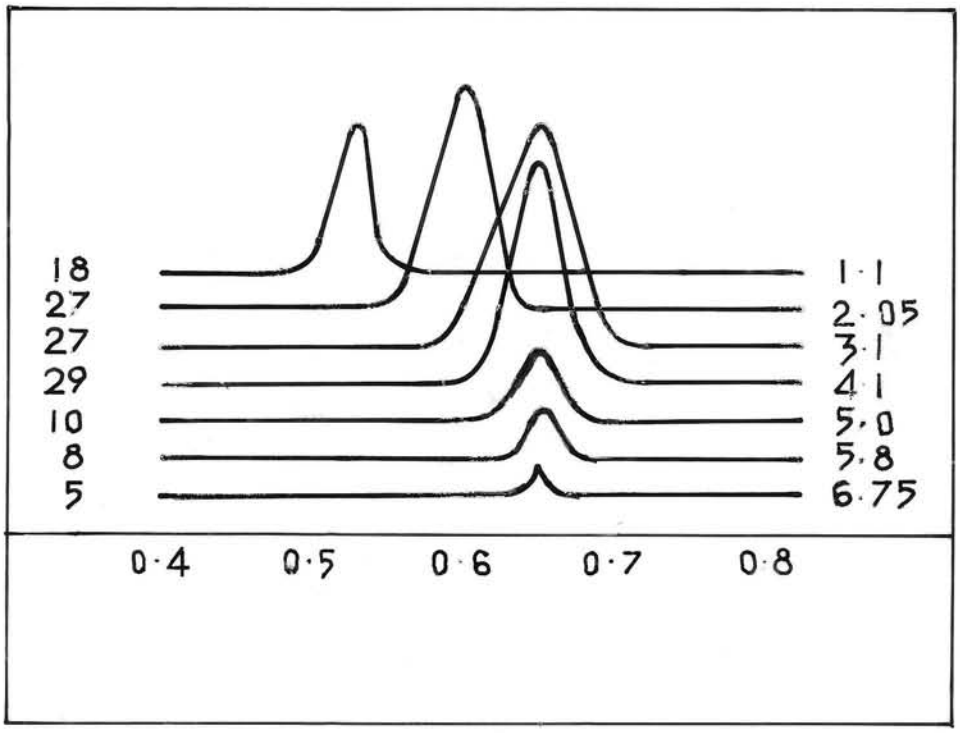

Fig.1 The recovered quantity of 30 ppb selenium at different pH.Ppb Se to the left and pH to the right. X:axis showing the potential.

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It has been established that selenium is an essential micronutrient for plants and animals (Stadtman, 1974). It is also known to possess toxic properties when present in amounts greater than microgram level. As such it has attracted the attention of agriculturists and nutritionalists, for its estimation of toxicological and nutritional levels. This report describes the method for determination of selenium lower than the microgram level in archaeological bones to get some information about the dietary habits of the ancient people. No such attempt has been made for archaeological remains, though Hadjimarkos (1962) has tried to use fluoride and selenium levels in contemporary and ancient Greek teeth in relation to the dental caries. Chemical analysis of the excavated bone often provides an information about the palaeoenvironments, its fossilization process, and sometimes about relative dating by fluorine analysis (Oakley, 1969). Further, the trace element composition of it is a useful approach for obtaining dietary information (Brown, 1973). Bone strontium has been studied for palaeo-dietary research.

Schrauzer and White (1978) have studied the selenium in human nutrition by dietary intakes and effects of supplementation. They observed that the highest intakes were seen in individuals subsisting on diets rich in whole wheat grain cereal products and seafoods. They correlated selenium concentrations in whole blood of the subjects under study with the dietary selenium intakes directly. Various fish and fish products contain by the greatest quantity of selenium generally greater than 1 mg/g dw. (Prasad, 1976).

The relation between selenium and bone metabolism was also studied by Wang et al. (1982). They have shown that the accumulation of selenium in the bone and cartilage reached a peak value after 24 hrs and thereafter maintained a high concentration for a longer period of time. It may therefore be said that selenium possibly is accumulated in human bones when the diet is rich of this element. Obviously selenium content in archaeological bones may be higher in such condition than in the other bones from another archaeological site where dietary pattern is different.

At present, several methods are available for determination of trace amounts of selenium at sub-nanogram levels. The choice of a particular method depends on the problem studied, and the accessible equipment. Selenium in biological materials like tissue and blood have been determined by fluorometry (Beal, 1975, Schrauzer and White, 1978), by anodic stripping voltammetry (Dennis et al. 1976, Blades et al. 1976), or by x-ray fluorescence (Raptis et al. 1980).

For the extraction of selenium from bones, no dry ashing is carried out since selenium is lost by volatilization. Wet ashing technique at about 200°C is ideal for trace amounts of selenium which is also satisfactory for the destruction of organic matter in the bone. Digestion mixture consisting of sulphuric-perchloric-nitric acid was suggested by Fogg and Wilkinson (1956) and Christian (1965). The main

disadvantage associated with the use of sulphuric acid is its tendency to form insoluble compunds and its high boiling point. The insolubility of alkaline earth sulphates cause difficulties when dealing with the samples like bone which is rich in calcium.

Szpunar et al. (1978) have described the method for a complete dissolution of excavated bone instead of digesting bone sample. This method of dry ashing at elevated temperature is rather time consuming. In the present study, we have found a method in which the digestion mixture consists of nitric perchloric-hydrochloric acid. By this, complete dissolution of bone is achieved and all organic matter is destroyed.

In Cummins (1964) a rapid method for determination of selenium in biological samples is suggested; a digestion mixture of sulphuric-perchloric acid with sodium molybdate for the conversion of organic selenium to selenium IV, with no loss by volatilization, to the microgram level. However this mixture did not work for extraction of selenium from bones lower than this level.

The method for biological samples as describes by Blades et al. (1976) was wet ashing followed by extraction of selenium into benzene using 3-3 diaminobenzene and back-extraction in dilute acid. This was tried for bones, however, it gave low results.

The electrolyte used by Blades et al (1976) was a mixture of 0.2 N HCl and 0.3 N $HClO_4$. It was found that the results are not reproducible for standard selenium solutions above 60 ppb. Ebhardt and Umland (1982) and Henze et al. (1969) suggested a mixture of ammonium sulphate, EDTA and Cu^{+2} for determination of Selenium and tellurium. Guentar (1981) has discussed the use of copper in the electrolyte. He stated that selenium presumably is deposited with copper on the surface of the mercury drop, and are redissolved in the determination step by further reduction to the anionic state (Se^{2-}). We have used the same mixture in the present experiment since it can be worked for 10 to 1000 ppb selenium (Table:1).

The present report describes a method for determination of selenium over a 10 - 1000 ppb range, using cathodic stripping voltammetry and its application to the bone. It is found, however, that the method does not work for burnt archaeological bone, perhaps since selenium is associated with proteins and these are lost by heating. The experiment was performed in two steps:

1. Digestion of the sample with nitric-perchloric-hydrochloric acid mixture in Kjeldahl's digestion flask.

2. Polarogram of the solution obtained by the digestion. The stripping analyzer used in the polarographic analysis, model 374, Princeton Applied Research Corporation, Princeton, NJ.

Reagents :

1. Selenium stock solution: 150 ppm - dissolve 330 mg Na_2SeO_3 in 1000 ml distilled water. Serially dilute to 1.5 ppm and 15^{-3} ppb.

2. 0.5M ammonium sulphate + 4 x 10^{-3}M ethylene diamineteracetic

acid + 1 ppm Cu^{+2}: Dissolve 66.07 g $(NH_4)_2SO_4$, 1.49 g EDTA and 3.92 mg $CuSO_4$. SH_2O in 1000 ml distilled water.

Determination :
The bone sample was rinsed, dried at 50°C and crushed in a morter. Weigh accurately 100 mg of the bone sample and put into Kjeldahl's digestion flask. Add 2 ml conc. nitric acid, 1 ml perchloric acid and 0.5 ml hydrochloric acid. Heat the mixture on digestion unit for half an hour, stirring occassionally to get a clear solution. Transfer the content with 2 ml distilled water to a 10 ml beaker. Adjust the pH to 1.8 - 2.0 with ammonia. Dilute to 10 ml in a volummetric flask. Pipette out 1 ml of this solution and dilute to 10 ml with an electrolyte. Introduce this sample solution into the polarographic cell and insert the electrodes. The instrumental settings are followed according to Henze et al. (1969) and Ebhardt and Umland (1982).

The instrumental settings are:

Initial potential	- 0.300
Final potential	- 0.800
Cond. potential	pass
Deposition time	60
Scan rate	fast
Replication	one
Sensitivity	medium
Peak potential	0.57
Purge time	5´
Drop size	medium
Peak sensitivity	high
Blank	electrolyte
Standard	30 ppb Se(IV) Na_2SeO_3

Dennis et al. (1976) have determined selenium in the presence of different ions and have concluded that selenium is recovered almost completely in the presence of calcium, phosphate, carbonates etc. To study the effect of pH and of the other constituents of bones on the determination of selenium at ppb level, we tested a selenium 30 ppb solution in the presence of calcium, phosphate carbonate (approximately in the same amounts present as main constituent in bone) at different pH (fig 1). We found no interference of these ions, and selenium is recovered at pH 2 to pH 4 with some change in the peak potential.

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