Technical note

An atomic-absorption ultramicrospectrophotometer for calcium determination*

Keywords—*Atomic absorption spectroscopy, Calcium determination*

Principle of measurement

THE SAMPLE volumes are of the order of a few nanolitres, the concentration in calcium being between 50 and 200 parts in 10^6. Atomic absorption is used. However, the flame requires sample volumes 10 000 times larger than are available, and sample dilution is not possible since, at a 50 parts in 10^6 concentration, the solvent itself will significantly increase the apparent calcium content to be measured.

For this reason, we decided to use a very small ($2 \times 15 \times 10$ mm) graphite crucible, on which the sample is deposited. An electrical pulse then heats the crucible and vaporises the sample. A temperature of approximately 3000 K is attained. The optical absorption of the atomised vapour thus created is then used to determine the calcium content. Proper vaporisation of the sample has been found experimentally to require the crucible to be placed under a partial vacuum.

This method presents many advantages:

(*a*) Determination takes only 1–2 min.

(*b*) No preparation is required for the sample disposition. After its removal from rat kidney, the sample can be deposited with a micropipette on the crucible without undergoing any treatment except a possible pH modification through the addition of hydrochloric acid to the sample.

(*c*) There is no matrix effect: the white-hot crucible vaporising away all trace of the deposit.

(*d*) The crucible is very cheap and can be replaced at a low cost in the case of an accidental burnout.

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On the other hand, the vacuum requirement is a slight drawback. However, the optimum pressure is not critical, a compromise pressure between 0·1 and 1 torr. Hence, a primary vacuum pump is generally sufficient for the purpose.

Description of the apparatus (Fig. 1)

The beam from an hollow cathode light source is focused into the sample vaporisation volume. This light source is electronically modulated via its power supply. A second optical system then sends the beam into a photomultiplier through a narrowband interference filter ($\Delta\lambda = \lambda/1000$).

The dioptic elements are made of silica in order to make it possible to determine metals having their resonance line in the ultra violet. Adjustments of the dioptric elements are provided to compensate for the defocusing of the beam due to chromatic aberration. In the same way, the whole of the optics has been designed to prevent the light from the white hot crucible to reach the photomultiplier.

The crucible is held between two clamping blocks, also of graphite, in order to ensure proper electrical and mechanical contacts. The sample is deposited into a 0·2 mm crater drilled into the upper part of the crucible. Baffles are used to prevent sublimated graphite from reaching the insulating parts of the crucible holder. The crucible power supply can be activated, either manually or automatically, whenever the vacuum reaches a preset value. The electrical pulse duration is adjustable between 0·1 and 1 s.

Provision has been made to measure the crucible resistance, from which its remaining life can be estimated. When this reaches a predetermined threshold value, the

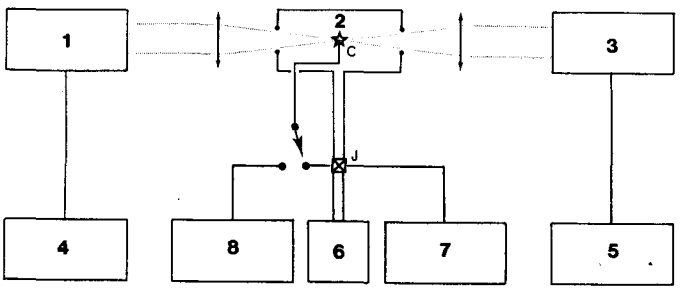

Fig. 1 Block diagram of the apparatus

1 Source (hollow cathode)
2 Atomisation cell with crucible (C)
3 Detection (photomultiplier and interference filter)
4 Oscillator ($f \simeq 1$ kHz)
5 Fast-response recorder
6 Vacuum pump
7 Automatic crucible power supply controlled by vacuum gauge (J)
8 Manual heating of crucible

crucible should be replaced. The photomultiplier current is modulated from both the light source ('carrier' modulation) and the calcium-vapour absorption ('useful' modulation). After proper amplification and rectification, the useful modulation is separated from the carrier and fed into a fast-response chart recorder.

Sample processing

Essentially the principles of LECHENE *et al.* (1969) are followed. The solutions used are test solutions simulating biological liquids, such as: Na—140 mM, K—3·5 mM, Ca—2·5 mM (60 parts in 10^6 of Ca).

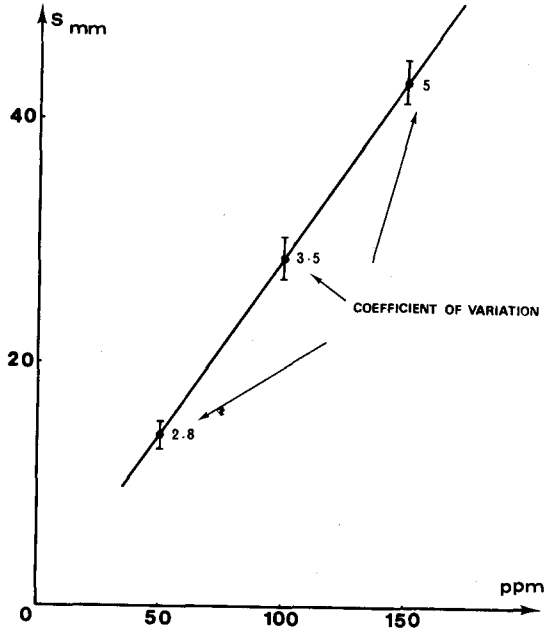

Fig. 2 Linearity of signal against p.p.m. concentration with 7 nl samples

These solutions are deposited on a stainless-steel plate protected by a silicon layer (8% Rhodorsil). Above the solution, a water-saturated mineral-oil layer prevents evaporation. With the help of a binocular lens, a micropipette is lowered by means of a micromanipulator into the solution, and a given volume of liquid drawn up into it.

The micropipette consists of a capillary tube a few tenths of millimetres long stretched with a Fonbrune microforge. The sampling volume is contained between the tip of the pipette and an internal narrowing of the capillary. That volume is then measured by filling it with a solution of known radioactivity. The internal and external walls of the pipette are silicon coated to ensure a progressive drawing of the sample. This coating also prevents any wetting of the pipette by the solution, thus making it possible to sample successively with the same pipette various solutions without a risk of contamination.

The binocular lens and micromanipulator are located on the same mount and can be moved together from the sampling zone to the crucible.

Results obtained from test solutions

Fig. 2 presents results obtained on 7 nl samples of various calcium content (50–100–150 parts in 10^6). Each point corresponds to about 100 determinations. The coefficient of variation of these determinations is less than 5% in all cases.

Fig. 3 shows, with a constant concentration (100 parts in 10^6), the results obtained relative to sample volumes of 7 and 13 nl. These first results showed a quite reasonable linearity and no zero offset. Thus, we proceeded to the second stage of our experiments, which called for determinations of biological samples collected inside rat kidney or blood vessels. In all experiments, calibration of the methods was accomplished by means of test solutions.

Results from biological solutions

Plasma ultramicrofiltrates

Our first results were quite disappointing. We found a calcium concentration equal to 0·6 mM/l, about half of what was to be expected. At first we looked for a possible phosphate effect by using various test solutions to which $K_2H\,PO_4$ has been added in different quantities: 1, 2, 3, 4 and 5 m mol/l. No effect of the phosphate could be detected.

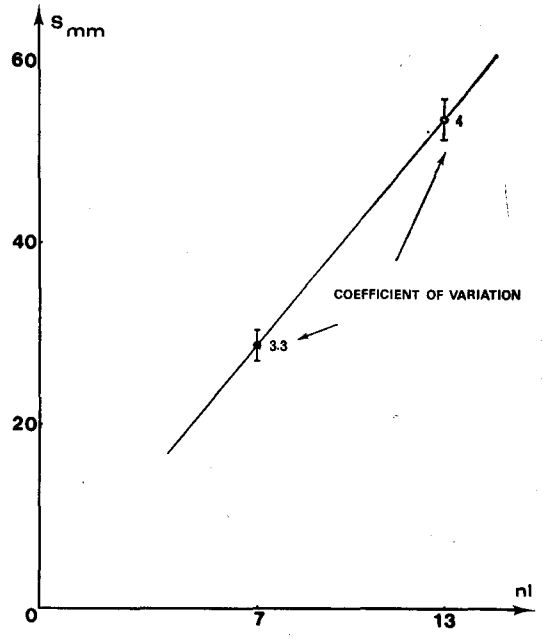

Fig. 3 Linearity of signal against volume sample with a 100 p.p.m. solution concentration of calcium

Then, we thought that the ultrafiltrate CO_2 partial pressure certainly decreased with successive manipulations of the samples. That could in turn account for an increasing of the sample pH, resulting in a partial precipitation of calcium as salts.

We checked this hypothesis by acidifying biological samples, and this technique led us immediately to reproducible and coherent values for the calcium content in ultrafiltrates.

Samples from tubular micropuncture

Whereas samples collected from the end of the proximal tubular yield the same calcium concentration with or without acidification, this treatment has the same effect on the early proximal tubular samples as it has on ultrafiltrates.

This is easily explained by the fact that the tubular liquid pH is about the same, in the early proximal portion, as that of the ultrafiltrate, whereas at the proximal end the pH reaches 6·5–6·8.

The microspectrophotometer just described has been designed and built as a prototype in the Ecole Polytechnique, Laboratoire d'Optique et Physique Moléculaire, Paris.

Various tests on synthetic and biological liquids have been conducted in the Laboratoire de Microponction, headed by Prof. Amiel in the Tenon Hospital, Paris.

The whole equipment is now under industrial development by SORO Electro Optics SA, and will be available commercially in 1974.

References

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