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A Rapid Pulse Nebulization Technique for Flame Atomic Fluorescence Spectrometry

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A simple and rapid pulse nebulization technique in which 200 μl sample volume is injected and aspirated into a flame-nebulizer is described for the determination of cadmium by line source atomic fluorescence spectrometry (AFS) and for magnesium and other elements by continuum source AFS. The volume of blood and serum samples required for replicate analysis is reduced below 1 ml; nebulizer clogging by heavy sample matrices is reduced; small volumes of rare samples can be analysed in the same batch as bulky samples using this technique. The technique is also applicable to flame AES which can also be performed with the instrumentation used in this work.

In the flame atomic fluorescence spectrometric determination of cadmium, the high sample uptake rate of about 9 ml min^{-1} requires large sample volumes for replicate analysis. In the nitric acid deproteinisation procedure already reported for the determination of low cadmium levels in blood,[1] a 2 ml minimum blood volume was required for replicate analysis by continuously aspirating the analytical sample into the nitrogen-separated air-acetylene flame. This demand on sample is unsatisfactory for rare samples especially in cases like cadmium determination where sample dilution must be minimised to improve analytical sensitivity. The possibility of using a pulse nebulization technique with small sample volumes has, therefore, been explored in the line source AFS determination of cadmium and the continuum source AFS determination of other elements as exemplified by magnesium in clinical samples.

The application of pulse nebulization to flame spectrometry has been reported by various workers[2–11] and reviewed by Berndt *et al.*[11] This includes micro-sampling techniques employing small Teflon funnels[9] and a Teflon sampling manifold for use with small sample injections.[10] Applications specific to flame AFS have also been reported.[2,8]

Experimental

Chemicals and Samples. The procedure reported was applied to whole blood, serum, and urine samples after appropriate pretreatment, Whole blood and serum samples were deproteinised by shaking the sample and 2 M† nitric acid in equal volumes and centrifuging. The supernatant was analyzed for cadmium by line source AFS without further dilution; it was, however, further diluted 100 times with deionised water before determining magnesium by continuum source AFS. Urine samples were only acidified to 0.04 M with hydrochloric acid for cadmium determination, while they were diluted 200 times with deionised water and similarly acidified for magnesium analysis. It has previously been shown that hydrochloric acid enhances the measurement sensitivity for both cadmium[12] and magnesium.[1]

Instrumentation. The spectrometer used for these studies was the laboratory-constructed one already described.[1,12] It consisted of a SPEX double monochromator with a photomultiplier detector and a Brookdeal 5C14 photon counter for signal processing. A nitrogen-separated air-acetylene flame was used as atom cell. A thermostated electrodeless discharge lamp excited by microwave in a 3/4 wave Broida cavity was used as a line source. The continuum source was a 300 W Xenon arc lamp. For line source AFS, a mechanical chopper was applied for background correction by intensity modulation while, for continuum source AFS, the rotating circular quartz plate with different quadrant thicknesses[13] was used for background correction by wavelength modulation. Though the continuum source was used as a primary source in continuum source work, it was also employed as a secondary source for effecting scatter correction in line source AFS work, operating at low power. The layout of the spectrometer for line source and continuum source AFS is presented in Figs. 1 and 2 respectively.

To modify the spectrometer for pulse nebulization, a polypropylene micro-pipette tip was attached to the nebulizer capillary tubing as shown in Fig. 3 to serve as a sample pulse reservoir, taking care to obtain a leakproof connection. It was ensured that the end of the capillary tubing did not project into the pipette tip so that each sample pulse injected into it was completely uptaken into the nebulizer without any dead volume. Micro-pipettes of various volumes were employed to deliver sample pulses into the reservoir. Figure 3 is not drawn to scale but typical dimensions for the device are indicated.

Procedure. The instrument was aligned and optimised for line source AFS, continuum source AFS, or flame AES as required. The measurement cycle time, usually 1 s, for collecting the signals was selected on the photon counter as explained later and the optimum uptake rate was measured. An aliquot of the sample to be measured was injected rapidly into the sample reservoir from a micro-pipette so that it was aspirated into the nebulizer in a single unbroken stream. The highest signal displayed soon after the injection was recorded. This was repeated for 3 or 4 aliquots of each sample and the mean signal for the sample was obtained as the average of the 3 or 4 replicate results. Between injections, deionised water or an appropriate blank solution was continuously aspirated to prevent inter-sample memory effects. A sample blank was also analyzed by the same procedure and its signal was subtracted from all sample results.

Alternatively the signal from each aliquot was traced on a chart recorder. It was then necessary to determine the appropriate measurement cycle time to obtain a representative signal trace.

Optimization of Measurement System. The optimum measurement cycle time constant was selected from a range of options provided on the photon counter. The optimum time constant, however, depended on other system para-

† 1 M=1 mol dm^{-3}.

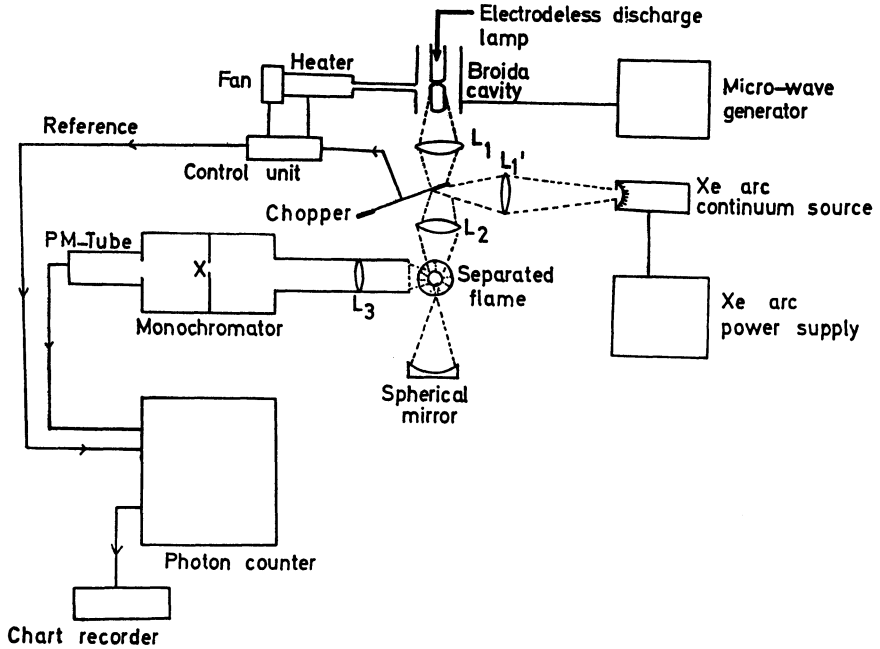

Fig. 1. The AFS instrumentation. (X)=Rotating quartz chopper. L=Lenses.

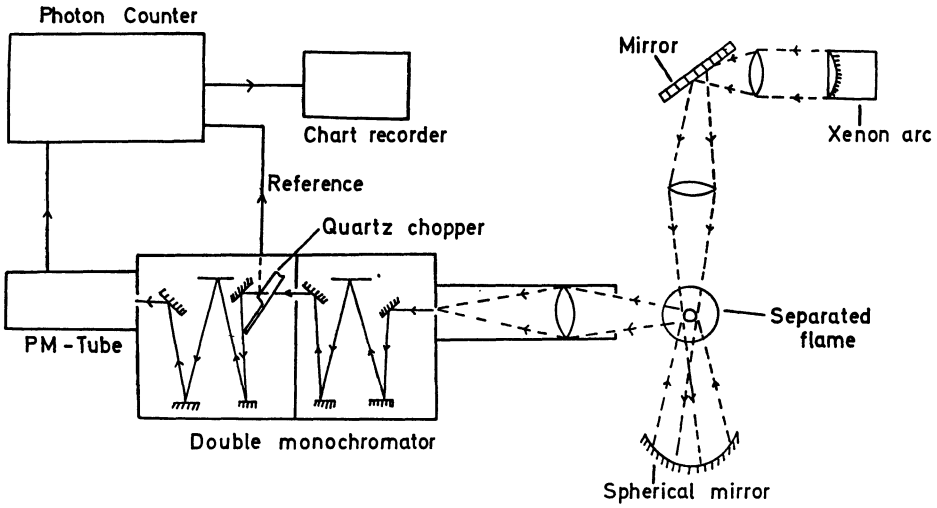

Fig. 2. The modified instrumentation for continuum source AFS.

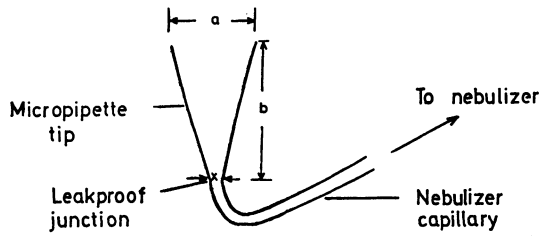

Fig. 3. Reservoir for sample injection. (a)=0.6 cm (b)=5 cm x=0.5 mm

meters defined below for purposes of this pulse nebulization technique.

Uptake Rate (R_u): The optimum uptake rate was similar to that obtained for continuous sample aspiration. It was measured at the nebulizer screw setting that gave the highest signal for pulses of a suitable standard solution.

Sampling Time (t_s): This was the time required for a sample pulse to be completely sucked up from the reservoir. It was measured approximately with a stopwatch and averaged for a large number of equal pulses; it was directly related to the sample uptake rate and pulse volume as

$$t_s = \frac{V_p}{R_u}, \qquad (1)$$

where V_p is the pulse volume; and R_u is the uptake rate.

Peak Time (t_p): This was the total time required to attain a peak analyte concentration in the measurement zone of the flame, after sample injection into the reservoir. Usually this time was slightly longer than t_s as in the expression

$$t_p = t_s + x.$$

where x is the time between the complete uptake of the pulse and the attainment of a stable peak signal level in the flame.

Readout Time (t_r): This was the period between sample injection and the digital display of the relevant signal. The value of t_r depended on the measurement cycle time constant selected on the photon counter.

For a given sample pulse volume and uptake rate, some measurement cycle time constants were more suitable than others. The option selected for a given application was determined by the way in which the signal was obtainable from pulse operation.

From a measurement of the uptake rate (R_u), Eq. 1 could be used to derive t_s for any pulse volume used. t_r could be measured directly for a given uptake rate and pulse volume. Usually t_r exceeded t_s by a fraction that could be estimated, the excess depending solely on the measurement cycle time selected.

At the measurement zone, the atoms from a pulse just injected arrived according to the profile illustrated in Fig. 4 and a peak signal was observed for a pulse only if t_r bracketted t_p.

In selecting the measurement cycle time (t_{mc}), three possibilities could be applied as follows:

I. $t_{mc} \ll t_p$: During the period t_p, when the pulse is passing through the measurement zone, a counting cycle is executed and repeated many times with progressively increasing total counts as t_p is approached and progressively decreasing totals as t_p is exceeded. These signals which are digitally displayed in sequence can be plotted to obtain a peak profile as shown in Fig. 4. Alternatively the signals accumulated during t_p may activate a chart recorder to plot a peak as in the profile. For a pulse volume of 200 μl and the 9 ml min^{-1} uptake rate routinely employed in these measurements, the 0.1 s measurement cycle typified this condition.

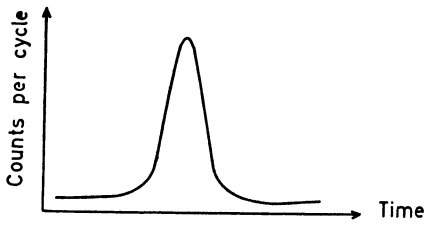

Fig. 4. The Peak Profile when $t_{mc} \ll t_p$.

II. $t_{mc} \gg t_p$: In this case the sample passes through the flame rather quickly while the counting continues for much longer. The signal collected during a short period is ascribed to a much longer period during most of which sample is not passing through the measurement zone. Sensitivity is poorer and higher chances exist of noise signals being counted. The 10 s measurement cycle typified this condition.

III. $t_{mc} \approx t_p$: In this option, sample presentation may get so locked to the measurement cycle that signal collection takes place only at the time when sample is passing the measurement zone. The signal is registered as one total at the end of the measurement cycle and offers increased sensitivity. The 1 s count time was typical.

It is to be noted that in case I where a definite peak can be traced as the measurement is repeated many times while sample is passing through the flame, the peak value does not follow the sample presentation immediately. There are a number of lower signals before the peak value. Therefore the peak signal needs to be selected from others that arise from the same sample pulse. Alternatively the signal is traced on a chart recorder and the peak height measured from the trace. In the other cases, the signal displayed immediately following the injection of the sample pulse is the peak signal. Only one measurement cycle is executed while sample is passing through the measurement zone and only one signal is output at the end of the cycle. This signal, however, is much higher than even the peak value in case I.

In this instrumentation, all three options are possible but only option III provides good sensitivity and precision. Option I suffers from reduction in sensitivity because of a reduced counting time, while option II suggests lower precision.

Results and Discussion

Line Source AFS. The experimental conditions of the flame are shown in Table 1. The sample uptake rate and other parameters for signal retrieval were measured with a stopwatch for a measurement cycle time of 1 s. Pulses of a 10 μg l^{-1} Cd standard solution were injected while adjusting the uptake rate until a maximum signal was obtained. The uptake rate was then measured by continuous nebulization. The sampling time, t_s, was measured by noting the time between the sample delivery into the reservoir and sample disappearance from the reservoir into the nebulizer capillary tubing. The calculated values also shown in Table 2 were based on the measured uptake rate of 9.2 ml min^{-1}. The readout time, t_r, was measured by noting the time between the delivery of the pulse into the reservoir and the display of the relevant signal on the photon counter readout. The mean values of these parameters were based on 15 measurements of each and are reported in Table 2.

The Optimum Pulse Volume and Measurement Cycle. Measurements were repeated for various pulse volumes of an aqueous 4 μg l^{-1} cadmium standard solution and the results shown in Table 3 were obtained for measurement times of 1 s and 0.1 s. The trends were similar for both measurement cycle times and also for a 10 μg l^{-1} cadmium standard solution.

In the 20—200 μl range of pulse volumes, the 200 μl pulse produced the highest sensitivity. As indicated in Table 3, it also yielded a more satisfactory detection limit than smaller sample volumes investigated. So

Table 1. Optimised flame conditions

Factor	Optimum values
Flame type	Nitrogen-separated air-acetylene flame
Flame composition	Stoichiometric-fuel rich
Nitrogen pressure	1 psi
Acetylene back pressure	12 psi
Acetylene feed pressure	8 psi
Air feed pressure	40—45 psi
Flame height	28 mm

Table 2. Parameters for signal retrieval

Parameter	Pulse volume/μl	Measured	Calculated
t_s	200	1.31 s	1.30 s
t_s	50	—[a)]	0.33 s
t_r	200	1.58 s	
R_u		9.2 ml min^{-1}	

a) t_s was too short to measure accurately for 50 μl pulses.

TABLE 3. VARIATION OF Cd FLUORESCENCE SENSITIVITY WITH PULSE VOLUME AND COUNTING TIME CONSTANTS

Pulse volume	Signal (counts/cycle)		Detection limit/$\mu g\ l^{-1}$	
μl	1 s cycle	0.1 s cycle	1 s cycle	0.1 s cycle
200	226	42	0.76	1.36
100	65	35	2.66	1.63
50	54	21	3.21	2.72
20	51	8	3.41	7.14

the 200 μl pulse volume was selected for routine analysis. For similar reasons, a measurement time of 1 s was preferred to 0.1 s. In this case, the measurement time was similar in magnitude to t_s and t_p. That t_s, t_p, and t_r exceeded the measurement cycle time, t_{mc}, of 1 s offered optimum sensitivity as it ensured that sample was present in the measurement zone throughout the counting cycle. Otherwise there would be no sample in the flame for some part of the counting cycle and reduced sensitivity would result.

Measurements With a Chart Recorder. The smallest pulse volume that gave a trace with well defined peaks was the 200 μl pulse volume. Only the 0.1 s time constant yielded peak traces. The precision and the linearity of calibration curves were satisfactory but the detection limit was three times worse than that obtained when the signal was obtained directly from the photon counter display using a 1 s measurement time constant. The value increased from 0.7 $\mu g\ l^{-1}$ to 2.0 $\mu g\ l^{-1}$ for cadmium. Samples could still be compared by this procedure if they contained more than the 2 $\mu g\ l^{-1}$ level.

Comparison of Procedures. Pulse nebulization was compared with continuous nebulization by applying each sampling technique to exactly the same solutions, instrumental settings and analysis conditions. A measurement time of 1 s and 200 μl sample were employed for all pulse nebulization measurements. Parameters considered for comparison included overall sensitivity, precision, accuracy, detection limit, and linear range of calibration curve.

To compare overall sensitivity, a number of samples were measured using both techniques and the signal levels were compared. The detection limits calculated for each sample introduction technique were also compared.

Pulse nebulization generally provided 40—52% less sensitivity than continuous nebulization when 200 μl sample pulses were applied. As shown in Table 4, detection limits were twice worse for pulse nebulization than for continuous nebulization.

Precisions calculated as relative standard deviations for 15 replicate measurements were compared for pulse and continuous nebulizations. Very similar results were obtained for the two techniques.

The calibration curve obtained for pulse operation was linear up to 8 ppm, while the linear range for continuous nebulization was only up to 2 ppm.

Larger Pulse Volumes. The disparity indicated between pulse and continuous nebulization was further investigated by observing pulse volumes above 200 μl.

TABLE 4. COMPARISON OF DETECTION LIMIT FOR CADMIUM

Sample	Detection limit/ppb	
	Pulse nebulization	Continuous nebulization
Aqueous	0.11	0.05
Water diluted blood	0.48	0.24
Blood Deproteinate	0.22	0.11
Urine	0.16	0.08

TABLE 5. DEPENDENCE OF ANALYTICAL FIGURES OF MERIT ON PULSE VOLUMES

Pulse volume	Signal	Detection limit	Precision
μl	(counts/s^{-1})	$\mu g\ l^{-1}$	%
100	80	0.39	4.9
200	223	0.11	3.6
400	285	0.08	3.1
600	401	0.05	2.9
700	398	0.05	3.1
800	396	0.05	3.1
1000	409	0.05	2.4
1200	420	0.05	2.9
1500	406	0.05	3.2

The respective signals for an aqueous 4 $\mu g\ l^{-1}$ cadmium standaed solution and the corresponding detection limits are shown in Table 5; the measurement precision provided by each pulse volume is also indicated. A 1 s measurement cycle was employed in all cases.

As can be seen from Table 5, the sensitivity was improved with the increase of pulse volume and became constant over about 600 μl. There was a progressive improvement in both measurement precision and detection limit until 600 μl. Thereafter these parameters attained identical values to those obtained by continuous nebulization. Then, the nebulization ceased to be strictly pulse but semicontinuous, as the sample volume was enough to maintain a steady signal level over several counting cycles. Instead of achieving reasonable sensitivity and precision using a microvolume of rare samples, 600 μl of sample, when continuously aspirated, became insufficient for three replicate measurements as achieved by the pulse technique.

Comparison of Accuracy. The same whole blood samples were analyzed for cadmium by flame AFS employing pulse and continuous nebulization in turn and the results are campared in Table 6. A comparison of other results for cadmium in whole blood obtained by this pulse AFS technique with those obtained independently in a different laboratory by carbon furnance AAS showed satisfactory agreement. Whole blood samples which had been analyzed for cadmium by many laboratories were also analyzed by this pulse AFS technique and the results were in good agreement with the laboratory means.

Continuum Source AFS. The application of pulse nebulization to the continuum source AFS technique was also investigated in detail for the determination of magnesium. Various pulse volumes of a 400 $\mu g\ l^{-1}$ Mg aqueous standard were measured by the con-

Table 6. Determination of cadmium in whole blood by pulse and continuous nebulization

Sample No.	Concentration/μg l^{-1}	
	Pulse nebulization	Continuous nebulization
1	15.4	21.4
2	10.8	8.0
3	10.0	8.8
4	14.8	14.8
5	27.0	29.2

Table 7. Comparison of analytical results for magnesium by pulse continuous nebulization

Sample	Mg results/μg ml^{-1}	
	Pulse nebulization (y)	Continuous nebulization (x)
Blood 1	40.5	40.5
Blood 2	40.7	40.8
Blood 3	47.5	47.3
Blood 4	44.6	44.5
Urine 5	99.8	99.5
Urine 6	97.9	98.6
Urine 7	114.0	113.4
Urine 8	111.2	110.9
Urine 9	108.9	109.6
Serum 10	20.5	20.2
Serum 11	17.3	17.6
Serum 12	18.5	18.7
Serum 13	24.1	23.9

Regression line: $y=x+0.01$; $r=0.9999$.

tinuum source AFS procedure using the measurement cycles of 0.1 and 1 s. From the results, the 1 s counting time provided higher sensitivity than the 0.1 s counting time. The 200 μl pulse volume was also superior to smaller pulse volumes in sensitivity and precision. As in the determination of cadmium by pulse nebulization, the detection limit, overall sensitivity, and precision for magnesium were improved with increasing pulse volume and were similar to values obtained by continuous nebulization for pulse volumes larger than 600 μl.

Using 200 μl pulses and a measurement cycle of 1 s, the pulse technique was employed to determine magnesium in various samples which were also measured by continuous nebulization. A comparison of the results is presented in Table 7. The blood, serum, and urine samples were appropriately diluted and acidified with hydrochloric acid as already described. The calibrations were achieved for all samples using aqueous magnesium standards acidified to 0.04 M with hydrochloric acid. The linear range extended from 4 μg l^{-1} to 10 μg ml^{-1} for pulse nebulization while it was in the range of 2 μg l^{-1}—2 μg ml^{-1} for continuous nebulization.

Other Applications. A broader scope for pulse nebulization in AFS and other techniques developed with the present instrumentation was demonstrated in other application as summarized in Table 8. A pulse size of 200 μl was employed in all cases. The technique was suitable for the determination of both major and minor elements in clinical samples.

Table 8. Other applications of pulse nebulization

Element	Technique	Detection limit μg l^{-1}	% Precision at (aqueous level)
Mg	CSAFS	4	2.5 (400 μg l^{-1})
Zn	CSAFS	80	2.3 (500 μg l^{-1})
Fe (248.3 nm)	CSAFS	10	2.6 (500 μg l^{-1})
Na (589 nm)	FAES	3	1.8 (10 μg ml^{-1})
P (246.4 nm)	FMFS	21000	2.3 (200 μg ml^{-1})

(FMFS) = Flame molecular fluorescence spectrometry.

Conclusion

The pulse nebulization device was very simple and easily attached to the pneumatic nebulizer, yielding zero dead volume during operation. The sensitivity and analytical precision were comparable to those attainable with large sample volumes aspirated continuously. The procedure was rapid and the volume of sample required for replicate analysis was effectively reduced. Employing the deproteinisation procedure for blood sample pretreatment, this sample introduction technique reduced system clogging with heavy sample matrices, compared to the normal continuous nebulization since less total amount of matrix per sample was introduced into the system. Accuracy was not impaired by the pulse technique.

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