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SHORT COMMUNICATIONS

IMPROVED FLAME PHOTOMETRY

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Flame photometry is now the usual means of measuring sodium and potassium, and commercially made flame photometers will measure about 0·2 μmol of either ion in about 2 ml of solution to about 1%. However, the prior dilution of biological samples is necessary and the minimum quantity for measurement is set by the time constant of the measuring circuit. Specialized flame photometers have been described which extend the detection limit to 10^{-14} mol (Müller, 1958; Ramsay, Brown & Falloon, 1953), but these are hardly suitable for routine work. This communication describes inexpensive modifications for use with commercial flame photometers which avoid prior dilution of the samples and give a tenfold improvement in the detection limit, defined as the smallest quantity of an ion which may be detected with a 95% certainty.

The output of the selenium photocell of the flame photometer is integrated electronically (Fig. 1). Stabilized power supplies (± 15 V) supply decoupling capacitors (10 nF ceramicons), and input offset preset potentiometers have been omitted for clarity. C1 and the reed relay should be low leakage components, and care must be taken to avoid leakage currents around the very high impedance inputs of IC 1 and 2. IC 1 forms an input buffer isolating the selenium cell from the discharge current of C1 on reset, R1, C1 and IC 2 form the integrator when the reed relay is open, and the

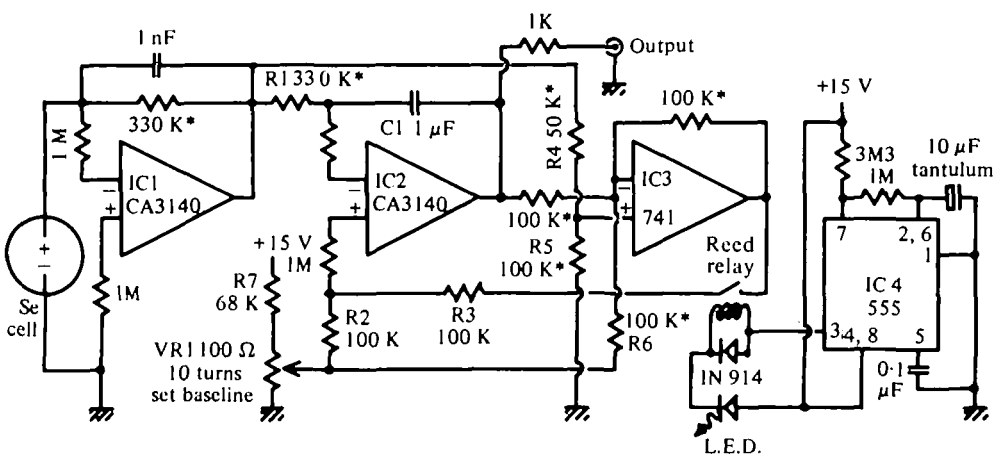

Fig. 1. Integrator circuit diagram. Resistors marked * should be 1% high stability types.

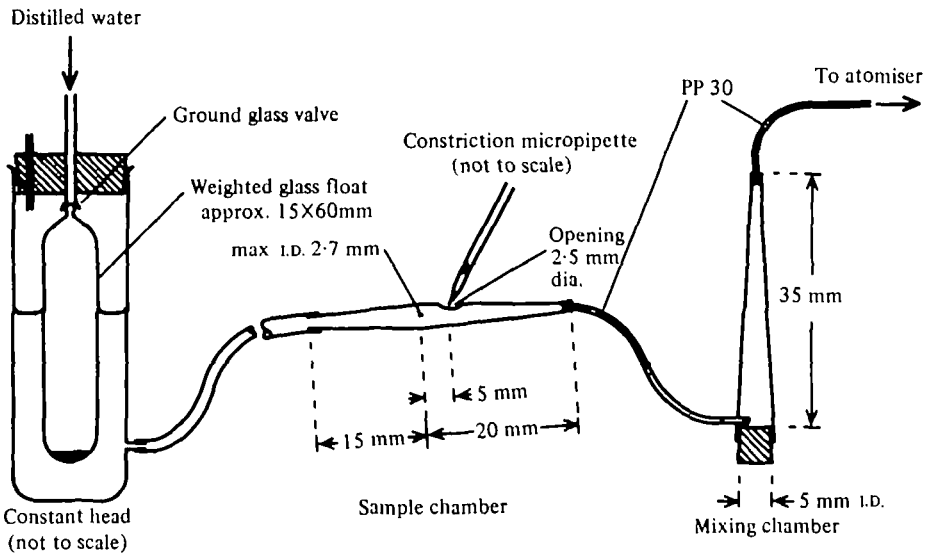

Fig. 2. Sample injection assembly.

integration baseline is set by VR 1. The integrator cycles continuously between integration periods lasting 30 s and reset periods of 7 s, when the L.E.D. is illuminated, being controlled by timer IC 4. During the reset period IC 4 closes the reed relay when C1 discharges with a 1 s time constant (3C1R1). The summing circuit around IC 3 makes the output return to zero on reset, independent of the input and VR 1. The output is recorded by a multirange potentiometric recorder (100 mV to 10 V).

At the start of an integration period (L.E.D. off) a sample of undiluted biological fluid or standard solution is delivered into the distilled water flow to the photometer by a micropipette of 1 μl or less. Such pipettes may be made by microforge techniques (Prager, Bowman & Vurek, 1965) and if the same one is used for sample and standard its precise volume need not be known. Distilled water from a constant head device flows through the sample chamber (Fig. 2) which is a biconical plastic chamber with an opening where the meniscus is held slightly concave by adjustment of the height of the constant head. The sample chamber may be readily made by welding sections from two 'Oxford Sampler' pipette tips back to back. Sample and water then pass by a short length of polythene tubing (Portex PP30, 1 mm O.D.; 0·5 mm bore) to enter the conical mixing chamber tangentially and then to the atomizer. Functioning of the fluid system may be checked by dye injection; this should clear the sample chamber in 5 s and the mixing chamber by 20 s after injection.

If injection of the sample is delayed more than 5 s after the start of an integration period a reduced reading will result. However, this presents no difficulty as the length of the reset period when the L.E.D. is on provides a suitable time for positioning of the pipette.

The integrated output is read from the recorder, relative to the mean of blank (no sample) integrations before and after the sample measurement.

The integrating technique gives results which are not critically dependent on the atomizer flow rate, unlike the unmodified instrument. However, air and gas flow

Table 1. *Calibration data for potassium, using 1 mM-NaCl as ionization buffer*

K^+ n-mol ...	0	0·357	1·644	4·209	14·20	42·62	144·5	362·0
Reading in mV								
Mean	0·03	11·56	54·04	137·0	458·7	1383	4489	9482
S.D.	2·50	2·15	2·92	2·2	5·2	12	59	274
N	27	14	10	10	10	10	10	10
mV/n-mol	—	32·43	32·87	32·55	32·30	32·45	31·06	26·20
S.E.	—	1·61	0·56	0·16	0·12	0·09	0·13	0·24

rates alter the flame conditions and hence affect the sensitivity. The problems of selectivity and interference (Dean, 1960) are still present, but where interference may be controlled by specific inhibitors or 'radiation buffers', such as the use of sodium to suppress ionization of potassium in the flame, such agents may be conveniently incorporated in the distilled water supply to the constant head.

Table 1 shows the results of calibration tests of potassium using an elderly EEL flame photometer, similar to the current model 100, taken using 1 mM-NaCl as a 'radiation buffer'. The response is linear up to more than 40 n-mol, and the scatter of readings is 2–3 mV S.D. up to about 4 n-mol (137 mV), above which it remains about 1%, S.D. presumably reflecting variability of pipette delivery. Thus potassium in a biological fluid at 4 m-equiv/l can be measured to about 2% S.D. in a 1 μl sample, or better still in a larger sample. The detection limit (95% certainty) is that sample giving a 4·9 mV response, or 0·15 n-mol, and this is the confidence limit (95%) for small samples. For very large samples the accuracy declines when linearity fails also.

For sodium without any radiation buffer the calibration is linear up to about 100 n-mol, and the confidence and detection limits are about 0·21 n-mol for small samples. The variability is 1% S.D. for samples above 15 n-mol. Thus sodium may be measured to 1% S.D. in a 0·1 μl sample of biological fluid containing 150 m-equiv/l, or to 2% in a 30 nl sample.

The author has not had opportunities to test the modifications with other makes of flame photometer, but the only likely changes necessary for similar instruments would be alteration of the size of the mixing chamber to suit different nebulizer flow rates. For use with photomultiplier based photometers the input may be taken from the recorder output through a suitable input resistor (e.g. 39 kΩ for 10 mV); the modifications have been used with similar advantages with a Pye Unicam SP 90 II atomic absorption spectrophotometer for assaying calcium and magnesium.

If the best detection limit is not needed, as where sample volume is not limiting, the integrator may be made to set its own baseline whilst resetting, by replacing R2 by a low leakage 10 μF capacitor to ground, and deleting VR 1, R6 and R7. R3 and R4 become 470 K and 100 K respectively and the timer circuit modified to provide a reset period of 50 s. When the reed relay closes the output returns to zero by decaying oscillations of 8·1 s period and 4·7 s decay constant, and when it opens again the buffered input voltage, averaged by the 4·7 s time constant is left on the 10 μ F capacitor to serve as baseline for the next integration period. The detection limit was about 0·7 n-mol for the prototype thus modified, and provides a convenient instrument, especially if equipped with a digital read-out.

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