

A FURTHER CONTRIBUTION TO THE DEVELOPMENT OF THE
FLAME-PHOTOMETRIC DETERMINATION OF SODIUM
AND POTASSIUM IN BLOOD SERUM
(Second Communication)

by

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I. APPARATUS*

1 *The flame*

The air-acetylene flame described in the First Communication¹ (abbreviated as F.C.) was chosen on the ground of observations made by WAIBEL on the intensity of atomic line and background emission of several flames. From our experiments as described in the F.C. concerning the air-acetylene flame then used, it may be deduced that this type of flame has the following disadvantages regarding the proposed determinations of K and Na:

a. the high temperature of the flame causes a high degree of ionization of K (and Na) and hence the mutual influence of both elements.

b. in consequence of this ionization there will be a loss of intensity of the spectral atomic lines investigated. This holds especially for K because it is present in smaller amounts and has a low ionization potential.

c. the high temperature also accounts for the disturbing presence of the red Na-doublet (8183–95 Å), which cannot be cut off sufficiently by the glass-filter combination for the K-line (7665–99 Å).

d. the use of acetylene may be a bit risky because of its explosiveness.

So a flame of a lower temperature was thought to be more serviceable. Propane-air and illuminating gas-air mixtures were expected to furnish such a flame. Indeed, when employing these gases—a special burner had to be constructed—the disturbances described above disappeared satisfactorily. The interference of Na (30 mg %) with K (2 mg %) proved to be reduced to within 2% of the K-deflection.

As a consequence of the lowering of the temperature the emission of the alkali atoms is decreased. On the other hand the concentration of these atoms in the cooler flame is increased on account of the reduced degree of ionization. Another factor favouring the intensity of the propane flame is the increased diameter of this flame. By both

* The summary on page 570 may serve as an introduction to this paper.

these factors the loss of emission of the alkali atoms caused by the lowering of temperature may partly be compensated (compare the galvanometer deflections for K with the acetylene and propane flame respectively, see Table VI).

The lower background emission of this cooler flame is, of course, a favourable factor.

For Ca-determinations with the aid of a photo-electric detector the intensity of the red band system (between 6000 and 6300 Å) is often measured. For this purpose the propane-air flame turned out to be considerably less sensitive (see Table VI). In contrast to the case of Na the drop of sensitivity for Ca cannot be satisfactorily explained by the value of the respective excitation potential (2.0 eV).

The agreement regarding the properties of the propane-air and illuminating gas-air flame is remarkable (see table VI).

The burner used for the acetylene-air flame is not suitable for obtaining a stable propane-air flame (blow-off). Therefore a special burner had to be constructed. It was based on the same principles but differed, *e.g.*, in the size and number of the exit holes. The height of the central canal connecting the chamber and the exit holes was enlarged (see Fig. 1). This improved the stability of the flame. This burner can be used for the propane-air as well as for the illuminating gas-air flame. By changing the burner top this burner can easily be made suitable for an acetylene flame*.

In the case of the propane flame, precipitation of alkali salts on the burner top makes cleaning necessary.

If only the part of the flame just above the blue cones is focussed on the photo-detector, the determination is not affected by draught in the surrounding air, and in this case a chimney is superfluous.

Air and gas are supplied in such quantities that the intensity of the flame light to

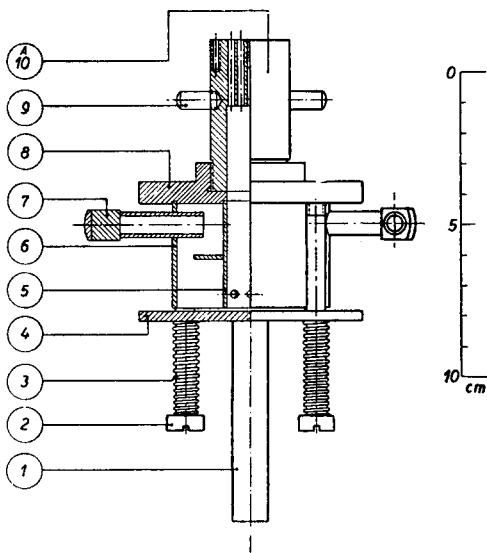


Fig. 1. Construction design of the burner.

- 1 = holder.
- 2 = screw adjusting spring 3.
- 3 = spring pressing plate 4 against burner walls 5 and 6.
- 4 = base plate, also serving as safety cover.
- 5 = wall between inner and outer chamber.
- 6 = outer wall.
- 7 = inlet tube with two openings for gas and air respectively.
- 8 = upper plate.
- 9 = handles facilitating removal of burner top.
- 10 = burner top, changeable for acetylene and propane flames respectively.

be measured is hardly influenced by slight variations of the gas- or air-current. This holds for both the acetylene- and the propane-flame.

The composition of the illuminating gas used is: H_2 :49%; CH_4 and homologues: 20%; N_2 :19% CO :6%; other compounds: 6%.

2. The spray

After some attempts a different type of spray has been elaborated, but still based on the same principles as described in the F.C.

* Burner commercially available on request.

References p. 570.

TABLE I
QUANTITATIVE DATA REGARDING BURNER AND FLAME

	Acetylene	Propane	Illuminating gas
Burner:			
Number of exit holes	71	19	
Diameter of same	0.8 mm	2.0 mm	
Diameter of field covered with exit holes	14 mm	16 mm	
Flame:			
Height of inner cone	3 mm	12 mm	8 mm
Height of flame	25 cm	17 cm	16 cm
Diameter of flame at base	16 mm	20 mm	19 mm
Air supply per minute at 20° C, 1 atm.	17.5 l	18.5 l	18.5 l
Gas supply per minute at same	1.9 l	0.7 l	1.7 l
Temperature measured (line reversal)	2460° K	2180° K	2190° K
Velocity (measured by means of flashes)	9.2 m/sec	5.6 m/sec	—
	Air	Acetylene and propane	Illuminating gas
Manometer:			
Inner diameter of resistance capillary	0.9 mm	0.75 mm	2 mm
Length of same	2.7 cm	8 cm	10 cm
Liquid	mercury	water	water
Excess pressure above atmospheric of gas mixture at inlet of burner	6 mm water		

TABLE II
QUANTITATIVE DATA CONCERNING THE SPRAY

Speed of atomization:	
distilled water	0.59 ml
trichloroacetic acid 5%	0.47 ml
Yield:	
distilled water	(11.0 ± 1.5) %
trichloroacetic acid 5%	(14.0 ± 1.0) %
Aspiratory pressure	260 cm water
Exit diameter of air-inlet capillary	0.65 mm
Volume of chamber	600 cc
Time required for removing the haze from the chamber after shutting of solution supply	15–20 sec
Difference of pressure over the air-inlet capillary	3 atm

The spray chamber has been considerably enlarged. It is placed horizontally and wall (see Fig. 2).

It is expected that by prolonged evaporation, before reaching the glass wall, a larger percentage of the drops will be fine enough to avoid touching the wall. Moreover the

References p. 570.

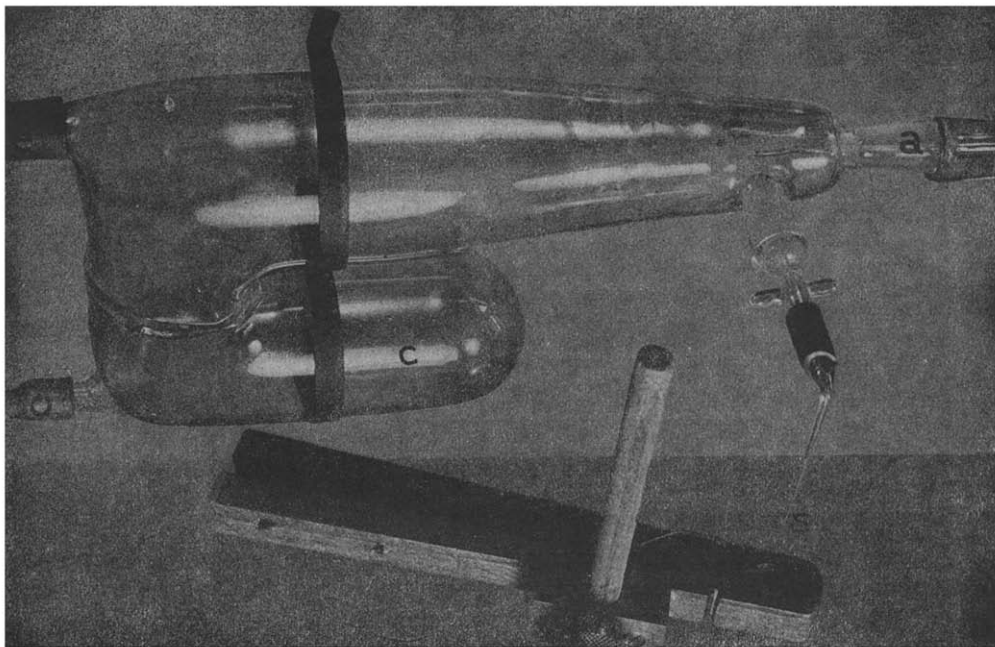


Fig. 2. The spray. Compressed air introduced by *a* atomizes the solution aspirated through *s*; the haze leaves the spray via exit *e*; the remaining liquid is collected in container *c* and removed via *o*.

enlarged content of the spray chamber has a damping effect on inevitable fluctuations of the spray process.

Furthermore a diminishing of the inner diameter of the inlet tube and its exit seemed to cause an increase of yield*. This may be partly due to the effect of lowering the speed of atomization**, which has proved to favour the yield.

The bulb opposite the air capillary has been omitted. It did not give essential improvements.

3. *The spectral selection and the optics*

Just as described in the F.C., the spectral lines are still selected with the aid of glass filter combinations. The more expensive interference filters are only useful when measuring flame intensities which are much lower than the ones we are dealing with. The application namely of interference filters will give a more favourable line to background ratio. Indeed in our experiments an interference filter for the K line has been proved to be three times better in this respect. Moreover, this filter appeared to cut off the red Na-doublet completely. The absolute intensity transmitted was less, however.

The optics consist of one lens focussing a part of the flame on the photo-multiplier.

* Yield is defined as the percentage of consumed solved material leaving the spray chamber. It should be noted that owing to evaporation the yield as defined in this way is not equal to the yield as defined by the percentage of solvent leaving the spray.

** Speed of atomization is defined as the quantity of solution aspirated per minute.

TABLE III
QUANTITATIVE DATA ON SPECTRAL SELECTION AND OPTICS

Filter combination for K line:	Schott and Gen., Jena: RG 9 (2 mm), BG 17 (6 mm).
Filter combination for Na line:	Schott and Gen., Jena: OG 2 (3 mm), BG 18 (2 mm), BG 17 (3 mm).
Filter combination for Ca:	see F.C.
Focussing lens: focus:	40 mm
diameter:	23 mm
Distance flame to lens:	16 cm
Effective area of photocathode of multiplier:	$5 \times 5 \text{ mm}^2$

4. The measuring apparatus

The photo-electric a.c. amplifier combined with a.c. galvanometer, as described in our first paper, has been replaced by a photo-multiplier combined with a.c. galvanometer. The operation of this set has thus become simpler.

The multiplier chosen is still sufficiently sensitive to the red K doublet (7665–99 Å). Its dark current is low enough not to cause a perceptible noise in the frequency range of the a.c. galvanometer response, at room temperature. The spectral sensitivity of the multiplier used seems to be more suitable for cutting off the red Na line than the Cs₂O vacuum phototube described in the F.C.

The sensitivity of the flame-photometer with the multiplier resembles that of the phototube-amplifier combination, though the effective area of the photo-cathode is much smaller for the multiplier.

On the one hand this favourable outcome is due to the high amplification factor of the multiplier in use. On the other hand the sensitivity of the multiplier set is not limited by the (essential) noise of an amplifier but only by the accuracy of reading an index on the galvanometer scale.

A real disadvantage of the use of a multiplier is the fact that at greater light intensities the output of the multiplier is not constant but reaches a limit-value asymptotically, some minutes after exposure of the multiplier. The intensities in the flame light are still so low that the error introduced by this effect in the proposed K- and Na-determination is smaller than $\frac{1}{2}\%$, when our method of reading the deflections, as described in F.C. IV, 2, is followed. For high intensities the light should be weakened.

Possible long time variations in the sensitivity of the multiplier are of minor importance when an external standard method is applied, as described in F.C.

The multiplier, placed in a special metal box, is connected on one side via resistances R_1 up to and including R_{12} (see Fig. 3) to a stabilized high-voltage supply, fed by the a.c. light mains. On the other side it is connected to the a.c. galvanometer by means of a linking box provided with a potentiometer (resistances R_{14} up to and including R_1 , see Fig. 3) controlling the sensitivity of the a.c. galvanometer.

The application of an a.c. galvanometer and of a rotating disk interrupting the flame light in the response frequency of the a.c. galvanometer has been maintained.

Thereby several advantages are obtained:

1. Incident light of the response frequency alone is detected, unless a ripple of this frequency is present in the output of the high-voltage supply*. Leakage light, if not modulated, does not disturb the readings.
2. The dark-current of the photo-multiplier is not measured by the a.c. galvanometer (no ripple being present; see 1). The component of the shot noise of the dark-current within the frequency-range of the galvanometer response is negligibly small.
3. The sensitivity of the whole measuring apparatus can be made independent of slight variations in the a.c. voltage of the mains, feeding both the high-voltage supply and the magnet coil of the a.c. galvanometer. Thus perfect stabilization of the high-voltage is not required.

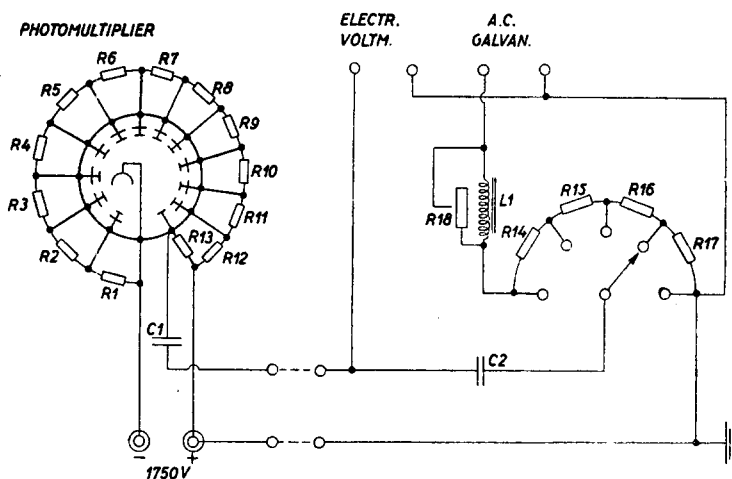


Fig. 3. Simplified wiring scheme. Notice condenser C_1 blocking d.c. output of multiplier.

In the latter case use is made of the possibility of controlling, to a certain extent, the dependence of the sensitivity of the a.c. galvanometer on the a.c. current passing through the magnet coil². This dependence is adjusted in such a way that the dependence of the internal amplification of the multiplier on the a.c. mains is exactly compensated. Generally this adjustment will be accompanied by a slight underdamping of the galvanometer.

In order to perform and to check this adjustment a potentiometer (R_{18} in Fig. 3) and a switch were mounted in the linking box between high-voltage supply and galvanometer.

It may be noted that the output of the multiplier may also be measured by an a.c. electronic voltmeter, instead of by an a.c. galvanometer. In our case the use of a Philips type electronic voltmeter (GM 4I32/05) has been found to cause no loss of sensitivity. Advantages of this device, as compared to the application of an a.c. galvanometer, are higher stability and comfort. The first and second advantage mentioned for the a.c. galvanometer are only partially maintained, the third one not at all.

* The influence of the ripple of the high-voltage supply may be compensated in several manners, e.g. by suitable phase shift of this ripple. An a.c. galvanometer does not detect alternating current of its own frequency, the phase angle of which differs ninety degrees from that of the a.c. magnetic field³.

TABLE IV
QUANTITATIVE DATA CONCERNING THE MEASURING APPARATUS

<i>Photo-multiplier:</i>	
Manufacturer	Vakuumtechnik G.m.b.H. Erlangen (Germany)
Type	VpA 69/d
Number of amplifying stages	11
High voltage supplied	1750 V
Dark current (measured)	$0.37 \cdot 10^{-6}$ A
Infra red limit of spectral sensitivity	11,000 Å
Effective area of photo-cathode	$5 \times 5 \text{ mm}^2$
<i>A.c. galvanometer:</i>	
Manufacturer	Physisch Laboratorium, Utrecht
Sensitivity	100 mm/m. μ A
Indication period	1.4 sec

II. CALIBRATION AND ACCURACY OF THE DETERMINATION

The method of calibration is the same as described in the F.C. The resulting calibration curve now obtained for K is shown in Fig. 4.

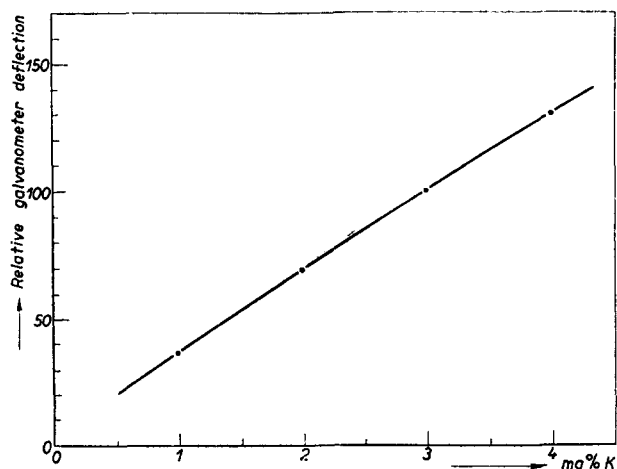


Fig. 4. Standard curve for potassium (no sodium present). Relative galvanometer deflections obtained with K filters, corrected for background, plotted against the K content of the solutions.

The better approach to linearity of the curve in Fig. 4 (K without Na) is characteristic for a decreased degree of ionization (see: F.C. V, 2 and Fig. 4).

The accuracy now obtained for the proposed alkali determinations is somewhat better than the accuracy mentioned in the F.C. It amounts to about 1% for K and 1.5% for Na. It will be sufficient to list the estimated values of the accidental errors. For the meaning and the discussion of this list the interested reader may be referred to the F.C.

References p. 570.

TABLE V
ACCIDENTAL ERRORS IN THE DETERMINATION OF SODIUM AND POTASSIUM

Source of error	Mean error in pCt of	
	K content	Na content
a. difference of temperature of solutions ($\leq 0.5^\circ \text{C}$)	≤ 0.4	≤ 0.4
b. difference of level of solutions	≤ 0.3	≤ 0.3
c. slow variations of emission	0.4	0.95
d. rapid irregular variations of emission	0.5	0.7
e. fluctuations of background emission	≤ 0.1	≤ 0.1
f. fluctuations of zero point	≤ 0.1	≤ 0.1
g. reading of deflection	0.2	0.4
h. non-instantaneous establishment of stationary emission	≤ 0.3	≤ 0.5
i. composition of standard solution	0.5	0.5
j. position of calibration points	0.25	0.4
k. drawing of standard curve	0.15	0.4
l. calculation	0.2	0.4
m. non-instantaneous establishment of stationary multiplier sensitivity	0.2	0.4

The error of reproducibility calculated from the tabulated specified errors agrees with the value experimentally determined.

In Table VI a quantitative comparison of the results now obtained with the application of several flame gases is given.

TABLE VI
QUANTITATIVE DATA CONCERNING THE SENSITIVITY OF THE FLAME PHOTOMETER

	Air-acetylene	Air-propane	Air-illuminating gas
Galvanometer deflection*: for 0.002% K in 5% trichloroacetic acid**	280 mm	230 mm	178 mm
for 0.032% Na in the same	970	320	235
for 0.020% Ca in distilled water	45	5.5	3.5
for background with K filter	16	6.5	8.5
for background with Na filter	7.5	0.7	0.7
for background with Ca filter	1.0	0.2	0.2

* Galvanometer shunted at maximum sensitivity; distance of galvanometer to measuring scale: 55 cm.

** The deflections obtained with solutions in distilled water are about 5% to 15% higher.

ACKNOWLEDGEMENTS

Once again we wish to express our gratitude to Prof. Dr J. M. W. MILATZ AND Dr J. A. SMIT for their interest and help.

SUMMARY

In this paper, sequel to an earlier publication in these *Acta*, a new type of flame-photometer is described, constructed by us in order to improve the determination of K and Na in blood serum. This improvement has been achieved by employing a flame of lower temperature (propane gas and air), a spray with higher yield and a photo-multiplier connected directly to an a.c. galvanometer. In this way annoying disturbances (*e.g.* interference effects between K and Na) are avoided, the consumption of sample is reduced and the handling of the apparatus is simplified. The accuracy and the sensitivity remain unimpaired.

For an explanation of the terms used and the problems discussed the previous communication in this journal should be consulted.

RÉSUMÉ

Dans cette communication qui est la suite d'une publication antérieure dans ces *Acta* nous décrivons un type nouveau de photomètre à flamme construit en vue de perfectionner la détermination de K et de Na dans le sérum sanguin. Nous avons réalisé ce perfectionnement en employant une flamme de température plus basse (propane et air), un vaporisateur à rendement plus élevé et une cellule photoélectrique à multiplication reliée directement à un galvanomètre à courant alternatif. De cette façon des perturbations ennuyeuses (par exemple des effets d'interférence entre K et Na) sont évitées, la consommation de substance est réduite et l'appareil devient plus simple à manier. La précision et la sensibilité ne sont pas diminuées.

Pour une explication des termes employés et des problèmes discutés, voir la communication antérieure dans ce périodique.

ZUSAMMENFASSUNG

In dieser Mitteilung, Fortsetzung einer früheren, in dieser Zeitschrift erschienenen Arbeit, wird ein neuer Flammenphotometer-Typus beschrieben, welcher gebaut wurde, um die Bestimmung von K und Na in Blutserum zu verbessern. Diese Verbesserung wurde erreicht durch Anwendung einer Flamme von niedrigerer Temperatur (Propangas und Luft), eines Zerstäubers mit höherer Leistung und einer Vervielfältigungs-Photozelle, welche direkt mit einem Wechselstrom-Galvanometer verbunden ist. Auf diese Weise werden unangenehme Störungen (z.B. Interferenz-Effekte zwischen K und Na) vermieden, der Verbrauch an Substanz verringert und der Apparat wird leichter zu handhaben. Die Genauigkeit und Empfindlichkeit bleiben unverändert.

Für eine Erklärung der angewendeten Ausdrücke und der erörterten Probleme, siehe die erwähnte frühere Mitteilung in dieser Zeitschrift.

REFERENCES

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Received July 28th, 1951