United States Patent [11] 3,622,275

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[21] Appl. No. 807,413
[22] Filed Mar. 14, 1969
[45] Patented Nov. 23, 1971

[54] **FLAME PHOTOMETRIC METHOD FOR ANALYZING BODY FLUIDS**
3 Claims, No Drawings

[52] **U.S. Cl.** .... **23/230 PC,** 23/230 B, 23/230 R, 356/39
[51] **Int. Cl.** **G01n 33/16,** G01n 21/58
[50] **Field of Search** 23/230, 232, 253, 254, 255, 259; 356/223, 224, 39

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ABSTRACT: Method for determining sodium, potassium and calcium simultaneously in blood serum by flame photometry using a single dilution of the serum sample. By suitable choice of dilution and by using a novel set of emission wavelengths both linearity and sensitivity are achieved to a degree previously undemonstrated. As a result, direct digitally displayed readout of all three blood metals becomes practical for the first time.

FLAME PHOTOMETRIC METHOD FOR ANALYZING BODY FLUIDS

BACKGROUND OF THE INVENTION

The determination of sodium (Na) and potassium (K) has long occupied a place near the top of the frequency list of required tests in clinical laboratories. A third blood metal, calcium (Ca), also important, has not been found as high in the list primarily because of its considerably greater difficulty of determination. The preferred method of determination of Na and K for reasons of speed, ease and accuracy is by means of the flame photometer. In this instrument, after diluting, the sample is excited to optical emission in a flame and the intensity of this emission is read photometrically as a measure of the concentration of the Na and K. On the other hand Ca is much harder to excite in a flame and is therefore in the absence of a suitable flame photometer generally determined by the Clark-Colip method, by fluorescence or by atomic absorption. None of these methods are as fast or simple as flame photometry. The advent of ion-selective electrodes had not solved this dilemma either—while the Na electrode is reasonably fast the Ca electrode is slow and the K electrode lacks specificity.

Several commercial flame photometers have been devised to date to determine Na and K simultaneously with a single dilution. These typically use a low-temperature, air-gas, laminar flame burner which is inherently subject to drift and therefore lithium (Li), which is not a normal serum constituent, is excited as a known added constituent to "standardize" the results despite the drifting of the flame output. This approach does not permit addition of Ca as a third determined constituent as the emission of the Ca at serum levels in the low-temperature flame is below measurable intensity; furthermore the Li would interfere optically with the Ca determination.

A high-temperature burner burning acetylene or hydrogen with oxygen is capable of exciting Ca. However, the hazard of both fuels, banned by fire laws from hospitals in many communities, has inhibited the use of such a burner in clinical flame photometers. This drawback is not present for the high-temperature burner described in U.S. Pat. No. 3,015,983 issued to one of the present applicants and assigned to Coleman Instruments, Inc., now a division of the present assignee. Said burner operates on natural or city gas, propane or butane (as does a low-temperature flame), but by turbulent combustion with oxygen achieves a very high temperature that is easily capable of exciting suitably diluted serum Ca to a degree adequate for photometric measurement. A commercial instrument using this burner has been the most used flame photometer in the field for over a decade despite the awkward fact that it only determines one element, Na, K or Ca, at a time. This instrument will also do Na and K with a single dilution but requires a filter change during the procedure.

It would seem logical, then, to extend the above instrument to the much desired single dilution determination of Na, K and Ca. However, this is not the simple matter of providing three suitable photometric systems. The current insistance in the instrumental field on digitized solid state circuitry and digital readout makes such a procedure prohibitively expensive unless the emission/concentration relationships are linear. But this has not been the case with this instrument as will presently be shown.

In flame photometry conventional procedure is to dilute serum or plasma a predetermined amount by adding a suitable diluent. This diluent may contain constituents added to minimize instability of readings due to fluctuations in sample delivery to the flame. Constituents may also be added to compensate for or reduce optical or chemical interferences which would affect the emission/concentration relationships adversely. The greater the dilution factor the less will be the necessary volume of the original serum, a desirable feature. However, the less also will be the emission intensity and the greater the demands on the photometric system.

Prior art systems generally have observed Na at the intense yellow doublet at 589 nanometers (nm.). Other Na lines have been recognized but only as possible spectral interferences. All current commercial flame photometers of which we are aware utilize 589 nm. for Na. K generally is observed at the 766/770 nm. line pair in the deep red. Ca has been a problem because the 558/560 nm. line group in the green is relatively weak and occurs in the region of high-flame background emission, hence the molecular band heading at 622 is more effective, particularly with filter instruments.

THE PRESENT INVENTION

We have discovered that by using an unusual combination of emission wavelengths never employed by prior art flame photometers, a filter flame photometer will read serum Na, K and Ca as linear direct readouts in concentration from a single dilution of the sample. Apparently, the universal use of the intense 589 doublet of Na has precluded realization of this objective. This doublet is a resonance line which means that the same wavelengths that are emitted are also absorbed, in part, in the flame. Hence, the flame is partially opaque to the Na emission it is producing. As a result, the emission is not a linear function of concentration since as the concentration increases the flame absorbs an ever increasing percentage of the emission. Thus the emission curve tends to level off at high concentrations.

We have also determined that a high-temperature flame in excess of 2,500° K. is required to produce the desired emission. When operating with a low-temperature flame the nonlinearity due to flame absorption is less than in a high-temperature flame, being negligible up to about 10 p.p.m. of Na. By using relatively high dilution factors, 1/100 or 1/200, users of low-temperature flame equipment have been able, with acceptable inaccuracy, to ignore the nonlinearity in serum measurements. However a low-temperature flame will not excite Ca measurably.

When a high-temperature flame is used, Na nonlinearity starts at 2 p.p.m. Since a 1/25 dilution is required with this flame to secure an adequate ratio of Ca response over flame background, the serum Na would be at about a 130 p.p.m. level and Na would not read out linearly. (When the dilution is much greater than 1/25 the emission intensity of Ca falls off to an unsatisfactory level for sensing because of a high noise to signal ratio; the preferred dilution ranges from 1/10 to 1/50. Below 1/10 the viscosity is too high to atomize effectively.) We have found that by sacrificing Na response by using emission lines other than 589 nm. we can still secure adequate signal over background ratio and at the same time obtain linearity of Na emission as a function of concentration. Two of these lines found to be satisfactory are 330 nm. and the doublet at 818/819 nm. The former is down in emission about 4 orders of magnitude from the 589 doublet; the latter is yet 2 orders lower, which explains why these lines have been rejected by prior art. The traditional approach in flame photometry is to use the most "sensitive" wavelength, i.e., the one with the highest emission over background ratio. By breaking with this tradition we unexpectedly find adequate sensitivity, greatly improved linearity and several other added advantages as well as the previously unrealized single dilution capability.

SPECIFIC EXAMPLE

An 8 microliter sample of blood serum was diluted with 200 microliters of distilled H_2O containing a small amount of wetting agent. The diluted sample was burned in a natural gas flame (burner described in U.S. Pat. No. 3,015,983) having a temperature of 2,800° K. at a rate of 1 to 2 ml./minute. Three interference filters appropriately peaked with blocking filters to reject optical interference from the other two metals were selected to pass 818/819 nm. for Na, 766/770 nm. for K. and the band at 662 nm. for Ca. These wavelengths give adequate sensitivity for serum or plasma measurements. The photosensors had S–1, S–10 and S–4 surfaces for Na, K and Ca, respectively. These surface designations refer to the spectral response of the photosensor and are well known in the art as reference to red (S–1), extended blue (S–10), and blue (S–4) sensitivity. The response over the flame background was adequate to detect the metals and the emission as a function of metal concentration was linear. The output of the phototubes was read on a digital voltmeter calibrated to read directly in p.p.m. of desired metal.

The photosensors for K also may have an S–10 surface and for Ca may have an S–10 or S–4 surface.

Selection of the lowest output line of the three cited for Na, while unusual by standards of prior art, provides an unexpectedly high ratio of sodium response to background as this line occurs at the region where the background of the oxy-gas high-temperature flame is low. We have found that this background is very high below 600 nm. to about 300 nm. It drops above 600 nm. to a low value and maintains this low value from about 610 nm. to over 850 nm. where it starts rising again. Thus all the three recommended wavelengths are in the only low background region afforded by the flame that is accessible to ordinary phototubes and glass optics. Not only does this improve accuracy and stability of instrumental response but it also considerably reduces the cost of instrumentation because extraordinary means of reducing the effect of fluctuations in flame background are not necessary nor are quartz optics or special hard glass phototubes.

As a further advantage of our invention we have found that the linearity of the calibration relationship (emission/concentration) at the 818/819 Na line pair is superior to that previously found at 589 nm. even by the low-temperature flame instruments, which cannot do Ca. We have run calibration curves from 0 to well above the 130 p.p.m. which represents normal serum Na diluted 1/25 with less than 1 percent departure from linearity. In comparison measurement at 589 nm., this amount of linearity cannot be had above 10 p.p.m. Thus Na for the flame photometer of our invention shows a linearity comparable with that previously only found for K and Ca. This linearity makes solid state circuitry possible either in the digital or analog form at a minimum of circuit cost. It further simplifies any cross connection circuits that may be provided to correct for inter element interferences such as the enhancement of K by Na and the optical interference between Na and Ca.

We claim:

1. A flame-photometric method for analyzing blood serum for Na, K and Ca simultaneously in a single dilution which comprises

a. diluting said fluid to a concentration within the range of about 1:10 to about 1:50,

b. burning said diluted fluid in a gas flame having a temperature greater than 2,500° K. to cause emission of spectral lines from the flame including those characteristic of the three metals present in said fluid,

c. isolating from the spectra emitted predetermined wavelengths characteristic of Na, K and Ca, which wavelengths provide a linear relationship between emission intensity and metal concentration, the intensity of the wavelength attributable to said metals being high as compared with the intensity of the flame background, and

d. photometrically sensing said wavelengths to provide a read out indicative of the quantity of each of said three metals present in said fluid.

2. The method of claim 1 in which said predetermined wavelength for sodium excludes the doublet at 589 nanometers.

3. The method of claim 1 in which said predetermined wavelengths in nanometers are as follows: Na 330 or 818/819, K 766/770 and Ca 622.

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