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CHEMICAL DIFFERENCE FLAME PHOTOMETRY: A NEW APPROACH FOR CLINICAL ANALYSIS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
BETTY LOU SCHOEPKE
1975

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ABSTRACT

CHEMICAL DIFFERENCE FLAME PHOTOMETRY: A NEW APPROACH FOR CLINICAL ANALYSIS

By

Betty Lou Schoepke

Although there have been great improvements in emission flame photometers over the past years, there still remains a need for increased accuracy and sensitivity in certain areas of clinical analysis. A unique concept for a flame photometer, based upon a system of continuous standardization, has been developed. This system may alleviate some of the inherent problems of emission flame analysis while providing improved accuracy, precision and sensitivity. The feasibility of the Chemical Difference Flame Photometric measurement has been demonstrated and comparisons have been made with other systems which are commercially available.

CHEMICAL DIFFERENCE FLAME PHOTOMETRY:
A NEW APPROACH FOR CLINICAL ANALYSIS

By

Betty Lou Schoepke

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

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1975

DEDICATION

To John Morris Schoepke,
my companion, my friend, and my lover

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With gratitude and appreciation, the author wishes to thank the multitude of people who have made generous contributions towards the completion of this investigation, as follows:

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Last but most, my husband John, for his continued encouragement towards my professional and personal growth. And my children Shannon and Kyle, whose expressions of pleasure and excitement that mom works with such "neat" things in the lab and finally finished her degree have been most encouraging.

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INTRODUCTION

Flame Emission Theory

Flame emission spectrometry (flame photometry) is a useful analytical method in many fields and is of particular importance in the area of applied biology and medicine. While more than 70 metallic elements can be evaluated by this method, requests for analysis of the blood electrolytes, sodium (Na^+) and potassium (K^+) outnumber all others in the clinical laboratory.

Although the methods of flame photometry have an inherent sensitivity, precision and rapidity, the actual measurement depends upon complex, thermally dependent processes. The thermal energy required for the emission phenomenon to occur is supplied by the exothermic, oxidative reactions occurring within the flame.

Normally, at room temperature the electrons of an atom are at their lowest energy level, the ground state. As the sodium atom, symbolized in the ground state as Na_o,[a] is placed in an environment of sufficient thermal energy, the electrons will be raised to an allowed higher energy level, the excited state of which is symbolized as Na^*. The process of raising an electron from one energy level to

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[a] This is a simplified version of the notation used by spectroscopists. An explanation of these symbols can be found in most quantum mechanics texts and in the manual prepared for the American Society for Medical Technology (ASMT) Instrumentation Workshop Program (1).

another is called excitation. Mathematically, this process is represented as

$$Na_o + \Delta E = Na^*$$

where E symbolizes energy. In the excited state the Na^* atom is unstable and immediately loses its excess energy by either a nonradiative or a radiative process. For example, excess energy may be transferred from one atom to another in a sequence of collisions that in essence dissipate the excess energy in the excited atom. This is an example of nonradiative deactivation. Flame spectroscopy utilizes the radiative deactivation process in which the excess energy is given off as electromagnetic radiation. The radiated light has a distinct set of frequencies (wavelengths) specific for the element from which it arises. For example, the light emitted by sodium is yellow with the most intense emission at a wavelength of 589 nm. Wavelength (λ) and frequency (ν) are related as given in the following equation:

$$\lambda_o = \frac{c}{\nu_o}$$

λ_o = length of wave in nanometers

c = light velocity

ν_o = frequency of wave in hertz (Hz)

The process whereby an electron passes from a high energy level to a lower energy level by a radiative process is called emission. The mathematical representation of this process is

$$Na^* \longrightarrow Na_o + \Delta E = Na_o + h\nu$$

where h is a constant derived by Planck (2) in his radiation law which establishes the proportionality between energy and frequency. Relaxation is another term given any process by which an atom loses energy.

Figure 1 illustrates the excitation and emission energy levels of sodium. One should note that the ground state can be excited to any permitted excited state as indicated by the energy lines above it. Also, with sufficient energy, as found in high temperature flames, the sodium atom can become ionized. In other words, if the atom is excited enough to lose an electron, the sodium ion (Na^+) is left behind. This is an undesirable process for flame photometric measurements as ions have a different relaxation pathways and the spectrum becomes more complex. Usually the emitted wavelengths resulting from transitions between the ground state and the excited state, resonance transitions, are used for analysis whenever possible.

Up to this point only one atom involved with one transition has been discussed. Unfortunately, when large numbers of atoms are involved there are many chemically complex and thermally dependent processes taking place at one time. Each of these atoms has numerous allowable transitions back to the ground state. In order to better understand and describe these interrelated processes, various laws have been formulated. Most important of these is the Boltzmann distribution law (2). This law states the relationships of the number of atoms in the excited state [Na_o] as a function of the energy difference between the two states. Normally, in the flame only a small portion of the atoms are excited.

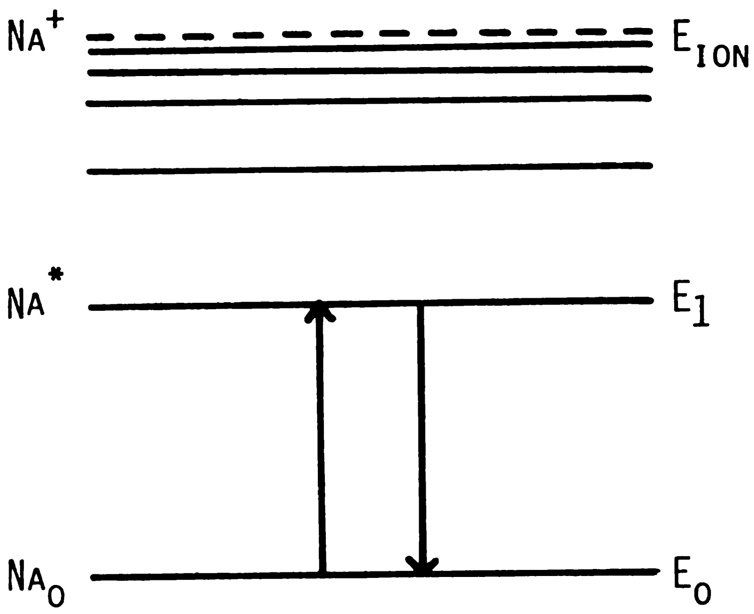


Figure 1. Excitation and emission energy levels for sodium.

$$[Na^*]/[Na_o] \approx 1\%$$

Keep in mind that this 1% may represent tens of billions of atoms. Each transition which involves a radiative deactivation will emit a discrete wavelength or energy. If these energies are sorted and recorded for visual inspection, the result is the emission spectrum for a particular element and will appear as bright lines against a dark background. The emission spectrum is unique for each metal and forms the basis of qualitative analysis. Consequently, components of mixtures of metals can be identified by interpretation of their characteristic emission spectra.

In addition to the allowability of a specific transition, there is a probability function (2) which describes how often that transition will occur. Since allowed transitions do occur with different probabilities, the intensity of various spectral lines for one element will vary. For any specific spectral line, the intensity will vary with the concentration of that metal in the flame. Obviously, this feature forms the basis for quantitative analysis. The intensity of a specific spectral line is dependent upon several factors, the major ones being:

1. Energy of excitation available, i.e., flame temperature
2. Nature of the metal and other species excited
3. Nature of the flame gases
4. Concentration of the metal.

For good quantitative analysis it is essential that the variation due to the first three factors be minimized!

Historical Account of Flame Emission Spectrometry

Melville (1752) first described the yellow light of sodium in flames. Kirchhoff and Bunsen (1856) correlated the colors of the spectrum with specific emission lines. Their work laid the foundation for the use of flames in qualitative analysis. The Bunsen spectroscope was one of the first devices used in an attempt to quantitate the emission phenomenon. Again, visual inspection was required to identify spectral lines while peering through an eyepiece. Then in 1928-30 Lundegardh (3) developed the first photoelectric flame photometer for quantitative analysis in application to medical problems. The first commercial flame photometer was developed by Waibel-Zeiss in 1938 (4). However, it was not until 1945, motivated by the medical needs during World War II, that Barns and co-workers (5) developed the clinical methods of flame photometry as they are practiced today.

The reader is referred to the biennial reviews in the *Journal of Analytical Chemistry* April issue. Alternate years are concerned with applications and instrumentation. Both contain excellent references for flame emission spectrometry.

Typical Instrument System

Although there are nearly 80 different models of flame photometers or flame spectrophotometers available, they all can be related to the block diagram of a typical flame instrument system as depicted in Figure 2.

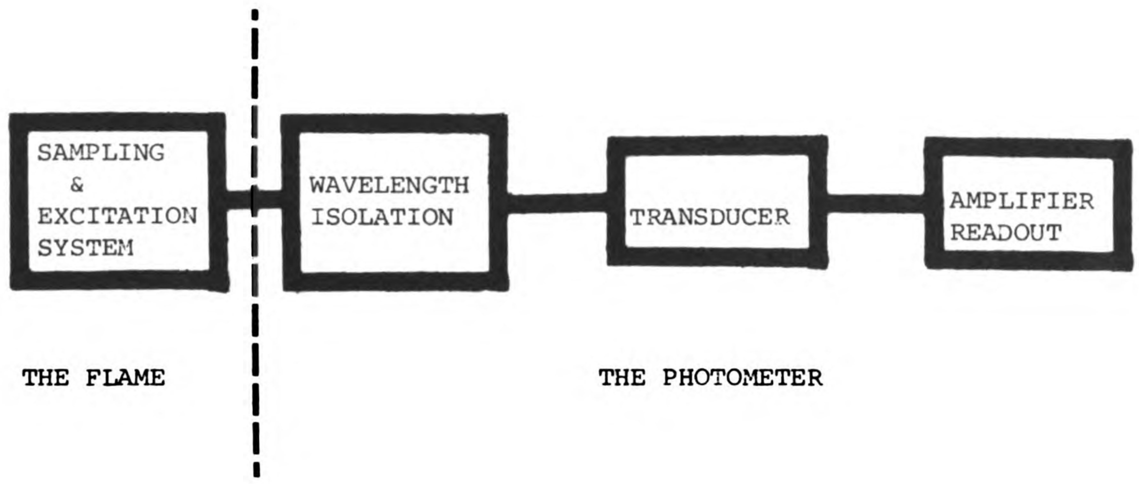


Figure 2. Block diagram for an emission flame photometer.

The Flame

The sample-excitation system is comprised of an aspirator (sprayer), burner and flame. The aspirator is merely a capillary tube placed in a suitable aqueous sample. It serves as a means of solution transport from the sample cup to the flame.

The burner-flame may be one of two types, a turbulent flow (total consumption) or a laminar flow (pre-mix). Figure 3 pictures a total consumption burner. The sample is aspirated (nebulized) and carried into the flame by a Venturi effect created by the oxidant (at A) as it passes over the capillary resulting in a partial vacuum. A distinction is made between aspiration and atomization in flame photometry. Aspiration applies to the mixing of a liquid and a gas or gases which results in a mist. Atomization means the breaking of bonds to form individual atoms. Fuel enters the burner at B and is mixed with the sample mist and oxidant at the orifice of the burner. The turbulence of this system is due to the mixing of the mist and gases, the velocity at which the gases are forced from the burner, and the rate of combustion. As its name implies, the total sample is consumed in the flame.

The major advantages of this burner are the small area in which the flame is concentrated, the efficiency with which the spray enters the flame, the high sensitivity attainable, and ease of cleaning. The disadvantages include the production of varying sized droplets which affect vaporization and atomization efficiency, the change in turbulence when spraying the sample which disturbs flame equilibrium, and the flame background which affects the signal to noise ratio to

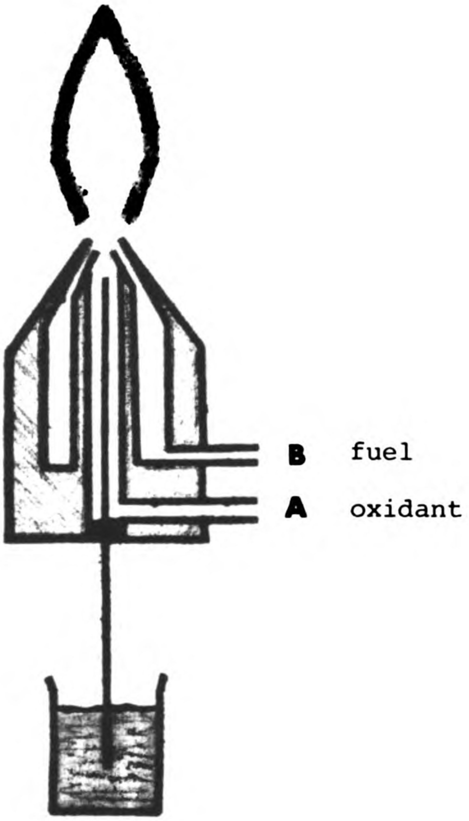


Figure 3. Total consumption burner and sample cup.

attain reproducible results. The flame turbulence may allow some sample droplets to pass through the flame unaffected. It also affects the mixing of flame gases with the ambient air which may alter the flame temperature, and users may complain about the audible noise created by the burner. Consequently, flame temperatures with a total consumption burner often may be lower than that for a pre-mix Méker type burner due to incomplete combustion, mixing of flame gases with ambient air, and spraying of sample into the flame (6).

The pre-mixed flame uses a chamber type nebulizer for mixing of the sample and combustion reactants resulting in a flame with a stable appearance and having considerably less flame background and audible noise. The diagram in Figure 4 shows the pre-mixing chamber for a Méker burner (7). While the air jet nebulizes the sample, the larger mist particles impinge on the chamber wall and are drained away. The remaining, rather uniform, droplets are mixed with the fuel and pass into the flame.

The major disadvantage to the chamber type pre-mixed flame is that 90% or more of the aspirated sample goes "down the drain." Also, cleaning is more of a chore than with the total consumption burner. Table 1 from Alkemade (8) summarizes the comparison of properties of the two burner types.

The fuel-oxidant requirements are dependent upon the burner type and the element(s) to be analyzed. Table 2 lists a few fuel-oxidant combinations and the temperatures they produce. The most common mixture in clinical flame photometers where sodium and potassium are the elements of interest is propane-air.

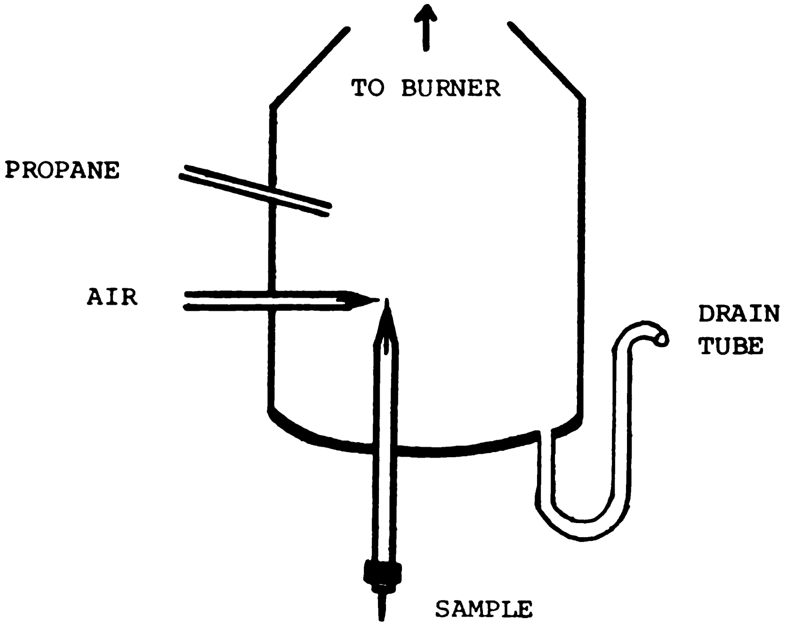


Figure 4. Pre-mixing chamber for a Méker burner.

Table 1. Comparison of properties for two burner types

Property	Premixed flame	Un-premixed flame
Structure	distinct zones	blurred zones
Character of gas	laminar	turbulent
Noise	no	yes
Mixing with ambient air	moderate	strong
Homogeneity	good	bad
Equilibration of the flame gas	fairly good	fairly good
Suitable for gas-mixtures with high burning velocity	no	yes
Spraying efficiency	1-15%	10-100%
Influence of spraying on flame properties	weak	marked
Suitable for spraying inflammable solvents	no	yes
Desolvation of droplets in flame	(nearly) complete	(usually) incomplete

Table 2. Temperatures for various fuel and oxidant mixtures

Fuel	Oxidant	Temperature, °C
Propane	air	1925
Acetylene	oxygen	3050
Propane	oxygen	2800
Hydrogen	air	2100

In the burner-flame numerous processes occur as the sample is converted from a spray into an optical signal. If one were to follow a single droplet containing only water and a compound such as sodium chloride as it is carried upward through the flame, the following processes would be observed:

NaCl-desolvation of solute
Na gas-vaporization of solute
Na_o-atomization
Na^*-excitation
$h\nu$ 589 nm-emission

The significance of these processes will be explained later. Alkemade (9) has designed a diagrammatical representation of the flame process listed above, as shown in Figure 5. In addition, many other flame processes, not necessarily advantageous to emission, may occur. Among these are ionization, formation of compounds with flame gases and self-absorption.

Table 3 lists the ionization percentages at different temperatures for three elements. Ionization is determined by the bond energy and the ionization potential of the element. The amount of ionization increases with an increase in flame temperature but is a reversible process as given by the equation

$$Na_o \rightleftarrows Na^+ + e^-$$

Consequently, if an excess of electrons (e^-) can be introduced into the flame the population of ground state atoms will be increased. An excess of electrons will occur upon addition of an element to the sample solution which is more easily ionized than the metal to be

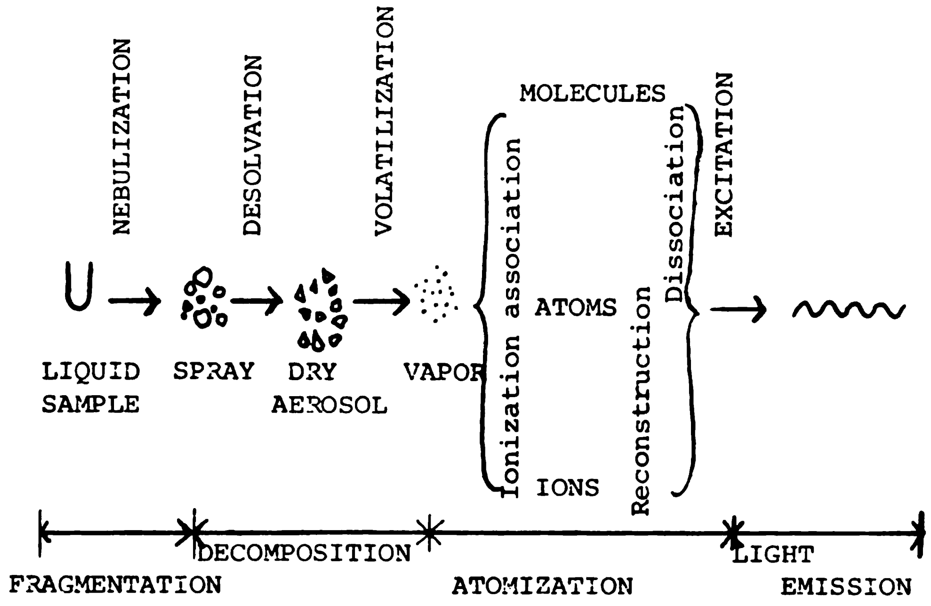


Figure 5. Flame processes.

Table 3. Wavelength, ionization potential and ionization percent for Na, K and Li in two different flame temperatures

			% Ionized	
Element	λ, Å	Ionization Potential, eV	Air-propane 1950°C	Oxygen-acetylene 3050°C
Li	6708	5.39	0.01	16
Na	5890	5.14	0.3	26
K	7665	4.34	2.5	86

measured. From Table 3 one can determine that an addition of potassium to a sodium sample will provide a supply of electrons from the ionization of potassium. As a result the loss of ground state sodium atoms by ionization will be reduced or eliminated and a more accurate sodium signal will be observed.

Compounds such as metal hydroxides and refractory oxides are readily formed with the flame gases. As an example, CaO has a high melting point and takes away free calcium atoms which are essential for the flame analysis of calcium. A hard-to-dissociate compound such as $Ca_3(PO_4)_2$ has a strong bond association energy. This compound ties up free calcium atoms making them unavailable for analysis. Fortunately, the effects of a chemical reaction such as the bonding of the phosphate radical to calcium can be negated by adding a complexing agent to the sample before aspiration. This agent binds the phosphate radical leaving the calcium atom free for analysis.

Self-absorption is the process by which ground state atoms in the cooler outer fringes of the flame reabsorb light emitted from atoms nearer the center. Particularly at high sample concentrations, self-absorption is the primary cause of nonlinearity of a working curve. Although atomic absorption spectrometry is dependent upon this phenomenon, self-absorption can be a disaster for flame emission analysis.

A delineation point in the diagram of the flame photometer is that between the flame and the photometer. It is from this point that the optical signal produced in the burner-flame will be changed into an electrical or analog signal.

The Photometer

The wavelength isolation device must be able to sort the desired spectral line from the flame background. In more sophisticated instruments this is usually accomplished by means of a monochromator. A monochromator consists of a wavelength dispersion component (prism or grating) and a system of mirrors and slits (fixed or variable) as pictured in Figure 6 (10). By rotating the prism or grating the desired wavelength can be directed on the "exit" slit.

In clinical flame photometers, a glass filter is usually used to obtain wavelength isolation. The use of filters results in a less complex and less expensive instrument. As sodium and potassium have rather simple spectra consisting primarily of resonance lines, filters are adequate for selecting the desired wavelengths.

Once emission has occurred, a means of detecting the isolated radiation is required. This is accomplished by use of an appropriate transducer, which is a device that converts energy from one form to another. In this case a vacuum phototube is used to convert radiant energy into electrical energy. The quantity of electrical energy (current) produced is directly proportional to the intensity of incident radiation.

As shown in Figure 7, the phototube consists of two electrodes, an anode and a cathode enclosed in an evacuated glass envelope. The cathode is coated with a photosensitive element. As a photon (a quantum of light) strikes the photosensitive material, outer electrons from the element are released by a process called photoelectric emission. The resulting photoelectrons migrate or are drawn to the

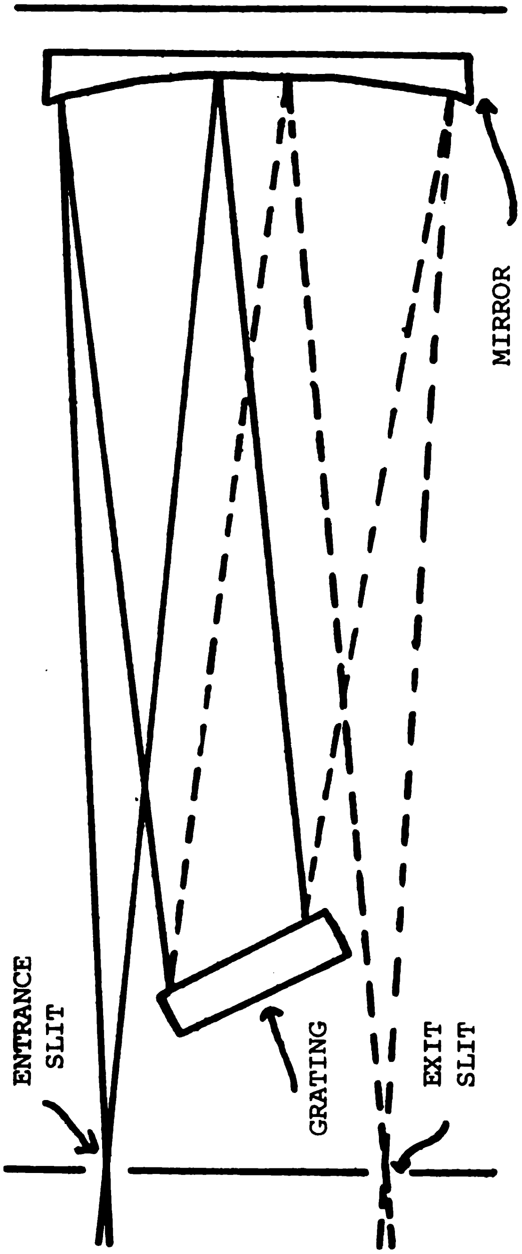


Figure 6. Ebert mount monochromator.

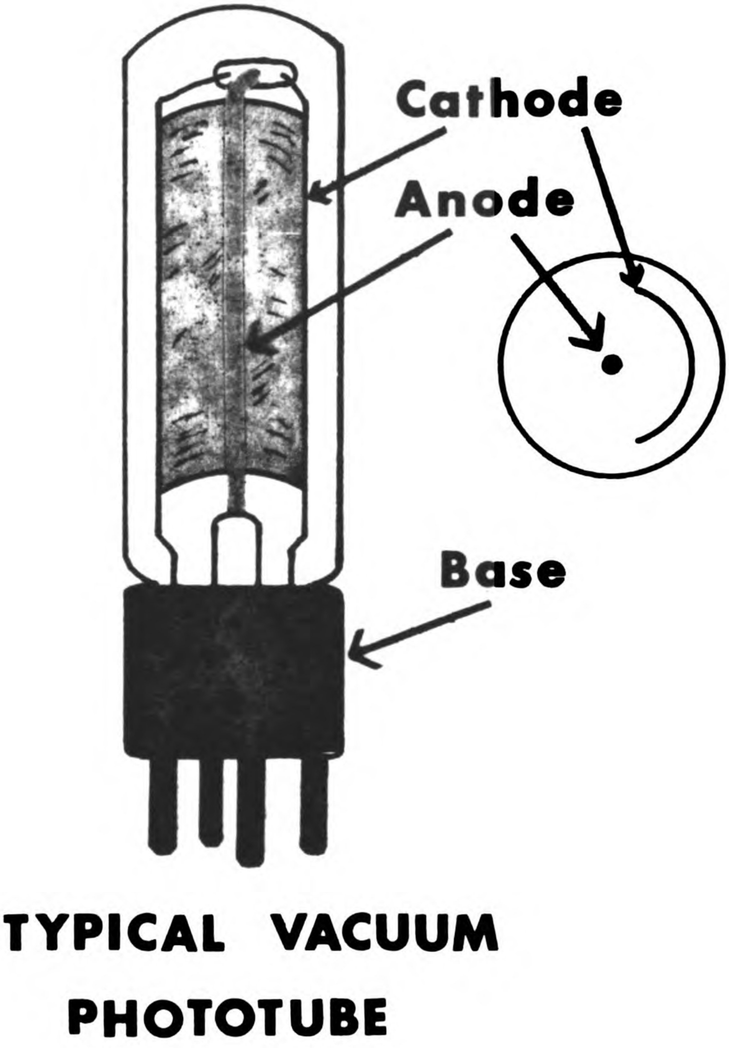


Figure 7. Typical vacuum phototube.

anode which is held at a more positive potential than the cathode. Consequently, a photocurrent is developed by this electron production and movement.

The detector chosen must have an adequate photosensitivity in the wavelength region of the emission. Although phototubes have a linear response to light intensity, they have certain characteristics which may introduce measurement error. The most important of these are dark current, drift and microphonics. Dark current is the current produced in the absence of light. Electrons may be ejected from the cathode surface by processes other than photoemission. Among these processes are thermionic emission and radioactivity, both of which may result in a small but measurable current. Poor electronic stability is reflected by a change or drift in the current output of a tube as a function of time under constant conditions. A sensitivity to mechanical vibrations that cause a significant change in current output is called microphonics. In most cases, phototubes are selected which have a minimum of dark current, drift and microphonic characteristics; however, the characteristic of any specific tube may change during extended usage.

The current developed by a vacuum phototube is very small, usually between 10^{-5} amperes and 10^{-12} amperes. Therefore, amplification of the photocurrent is usually necessary. Normally, one of two general types of circuit is employed in the measurement of this current. Recalling Ohm's law,

$E = IR$

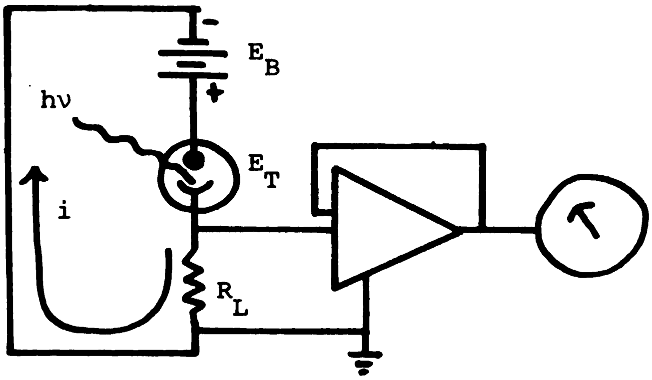
E = voltage or potential
I = current in amperes
R = resistance in ohms,

the circuit in Figure 8A can be explained. A large load resistor ($R_L > 10^{-6}\Omega$) is used to produce a voltage drop (E_{R_L}) from the photocurrent (i). This voltage is amplified and then used to operate a readout device. This voltage type of measurement has two distinct disadvantages. One, the anode to cathode potential (E_T) changes with photocurrent or light intensity and may cause nonlinear detection characteristics. Two, since the large load resistor is directly across the operational amplifier (OA) input terminals, it may act as an antenna and introduce error in the measurement by electronic noise pickup.

In circuit B of Figure 8 a current to voltage operational amplifier replaces the load resistor. This system has two advantages: the non-linear detection is eliminated because the potential from the battery (E_B) equals the potential across the tube (E_T) and is independent of current in the tube; also, since there is virtually no resistance across the amplifier inputs, the scheme is less susceptible to noise pickup. In both circuits the readout device which concludes the measurement may be a meter, a recorder, a digital display, or a computer printout.

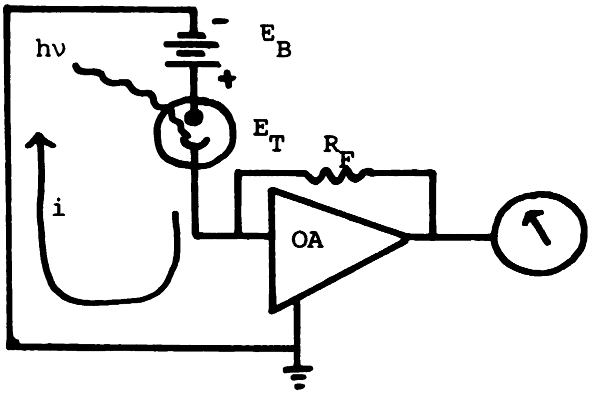
The relationship between the readout and the concentration of an element in solution may be complex. The readout will be proportional to sample concentration resulting in a reproducible working

A.



VOLTAGE MEASURING DEVICE

B.



CURRENT MEASURING DEVICE

Figure 8. Typical circuits for photocurrent measurement.

curve if and only if the following variables are held constant or are optimized.

1. *The transport parameters* such as the flow rate of sample (influenced by oxidant pressure and solution viscosity), the flow rate of unburned gases, and the expansion of flame gases, must be controlled and the effects of varying the concentration of the element in solution must be minimized.

2. *Desolvation and vaporization* of the sample is determined by the aspiration and vaporization efficiency of the burner used. These two variables will influence the sensitivity of the system and obviously must remain uniform.

3. *Atomization* or the fraction of free atoms formed is influenced by numerous processes which will have a great effect on the final readout. These processes were described in the discussion on flame theory. Heiftje and Malmstadt (11) have developed a unique isolated drop technique for pictorial study of the vaporization process leading to the formation of free atoms.

4. *Excitation* occurs only if the temperature of the flame is of sufficient energy to excite the metal atoms. As described above, the Boltzmann distribution law relates the ratio between the number of free and the number of excited atoms as a function of temperature. Therefore, the thermal excitation process converts a number of free atoms into excited atoms.

5. *Emission* results from the radiative deactivation of the excited atom to produce a radiance specific for the particular element. The wavelength of this line is directly related to the energy of

excitation and usually results from resonance transitions. The radiance of a spectral line is defined as the radiant power emitted per solid angle per unit area.

6. *The passage of radiation to an entrance slit* of a monochromator or through a filter occurs following emission. Because the flame emits in all directions, only a small portion is observed at the entrance slit. The fraction of the radiant power entering the monochromator is determined by the area of the propagating spherical radiation that falls on the entrance slit and the solid angle (numerical aperture) that the monochromator can accept.

7. When considering the *passage of light from the entrance slit to the exit slit*, one must assume that the monochromator is set for the appropriate wavelength or that a proper filter has been chosen. Since no optical surface is perfect, some radiation may be lost through the filter or, in the case of the monochromator, from the mirrors and grating or prism. The amount of radiant power at the exit slit is dependent upon the efficiency by which the monochromator optics transmit the light, the relationship of the wavelength setting and spectral band pass to the center of the spectral line and a factor determined by the entrance optics. From the exit slit of the monochromator the radiation is transmitted to a transducer or photodetector.

8. The *sensitivity factor of the photodetector* is determined by the response of the cathode to light photons which impinge on its surface. Usually the cathode surface responds to one out of five photons or has an efficiency of 20%. The photodetector is actually a light measuring device which measures different intensities of

emission and converts the optical signal into an electrical signal. With appropriate amplification of this electrical signal and an accurate readout device, the relationship between sample concentration and readout will be reproducible.

The medical technologist has a choice of commercial flame photometers having a wide range of sophistication, sensitivity and price. Those designed primarily for clinical applications commonly fall into one of two categories: direct reading or internal standard instruments. With the direct reading instrument the readout responds in a manner directly proportional to the radiation intensity of the species being measured. With the internal standard instrument the readout is a ratio between the compound being measured and a compound which has been added to serve as a known reference.

Although direct reading instruments commonly employed in the clinical laboratory offer sufficient precision for sodium and potassium analysis, they are less accurate than internal standard instruments. They are influenced more by fluctuations in critical instrumental parameters and interferences from chemical, spectral or background luminescence and physical variations such as viscosity or burner clogging, the adverse effects of which are minimized by the ratio process employed in the internal standard instrument.
In order to minimize changes of various effects in the flame, the blank and standards should be of similar composition as the sample. A perfect match is not possible. As a result, with direct reading instruments, upon aspiration of the sample the flame is greatly perturbed due to the difference in composition between it and the blank and the standards.

With the internal standard instrument system the lithium, sodium and potassium signals are measured simultaneously. Electronically the sodium and potassium signals are ratioed to the lithium signal. If a change occurs in an instrumental parameter such as the aspiration rate, this ratio process will provide an automatic correction. For example, if the capillary becomes partially clogged, the flow rate will be decreased. This is reflected in a proportional decrease in the lithium, sodium and potassium signals, but the ratio of the signals will remain unchanged. The internal standard method of flame analysis thus has an advantage over direct reading instruments of increased long term readout stability. This enhances both the precision and accuracy of succeeding analysis.

While the use of the internal standard method is widely accepted and practiced in the clinical laboratory, one should be aware that it has certain limitations that affect sodium and potassium analysis. First, the spectral lines selected for the internal standard and the element under analysis should be close together due to emission characteristics which require similar flame temperatures and detector response characteristics which vary greatly with energy. Secondly, both the internal standard and the element of interest should have nearly identical ionization potentials so that the energy fluctuations in the flame will have a similar effect on the percent ionized of each species. Also, an error will be introduced if the sample contains the same element used for the internal standard. For example, a request for a sodium/potassium determination on a psychiatric patient undergoing lithium therapy would be meaningless unless this medication were made known to the laboratory staff.

Lithium has been the element of choice for the internal standard in clinical flame photometry. While it is a satisfactory choice for an internal standard, it has some limitations. Refer to Table 3 for a list of wavelengths and ionization potentials for sodium, potassium, and lithium. Foster and Hume (12) found that a high concentration of sodium enhances the emission intensity of potassium more than that of lithium. Also, they determined that lithium in small amounts is a poor choice as an internal standard for potassium in the presence of elements having low ionization potentials. This is due to the difference in ion equilibrium which is even greater than the difference between the sodium and potassium ion concentrations.

Flame Emission Accuracy, Precision and Sensitivity

Lowered detection limits (increased sensitivity) and increased precision and accuracy reflect progress in flame emission spectrometry. These improvements are directly related to instrumental developments and better control of flame reactions which have resulted in a better understanding of the process involved in flame analysis. Generally, detection limits range from 10^{-5} to 10^{2} ppm for flame emission methods. Table 4 (13) gives a partial list of elements analyzed by flame emission and the best sensitivity obtainable to date. These limits may vary with the type of instrument used, the burner design, the fuel requirements and other factors.

Precision for flame emission methods corresponding to a relative standard deviation of 1-2% is usually claimed (14). Greater precision is possible but it usually requires more elaborate techniques and instrumentation than used in a clinical laboratory.

Table 4. Detection limits for Na, K and Li by emission flame photometry

Element	λ, nm	Detection limit (μg/ml)
K	766	0.0002
Li	671	0.000003
Na	589	0.0001

Accuracy of 5% or better can be expected with flame methods. Accuracy is best improved by optimization of operating conditions, reproducibility of the source radiation and stable electronics. In addition, control of systematic and random errors will contribute to improved accuracy and precision. Parsons and Winefordner (15) have written an excellent paper which discusses optimization of critical instrumental parameters. They discuss not only the parameters which can be systematically varied but also the importance of those which contribute to obtaining the lowest detectable concentrations and the most precise analysis.

Instrument manufacturers have attempted to better meet the needs of the clinician and the medical technologist by providing attractively packaged instruments, more easily used because of their simplicity and "push button" operation and more convenient readout such as digital displays. In addition, many of these instruments are adaptable to an on line automated or semiautomated system. Although these newer instruments reflect greater efficiency in data acquisition and handling than older models, they may not be playing as significant a role in diagnosis as possible. Subtle changes in the normal versus diseased state of the individual have been noted. For instance, narrowing of the normal value range for a given analysis increases the need for better sensitivity, accuracy and precision. Also, a physician may find diagnosis more easily made by comparing the laboratory findings of repeated samples from a patient rather than by comparing them to normal values set by the population in general. In other words, laboratory results may be more significant if they compare an individual's present condition with his past profile rather than by comparison to the "normal" population. The detection of an incipient disease may be possible based upon small changes that result in a value that usually would not be observed using the conventional normal range concept. These measurements will require greater accuracy than is available with today's instrumental methods.

The Standards Committee of the College of American Pathologists (CAP) compared the accuracy obtainable with the accuracy necessary for a clinical test value to be medically significant (16). Among those tests not providing a medically significant level of accuracy was that of sodium. Winstead (17) calculated the present performance as a relative error of ± 4% for a sodium value of 150 mEq/l when it was shown by the CAP to require a relative error of less than ± 2.6%. Performance of tests for potassium was found to meet the needs of the clinician for medically significant results under the existing practice of normal comparison. Winstead also discusses the relationship between fluctuating flame conditions and variations of sample values. Because flame conditions are not constant, the wide variations in results of flame analysis cannot be attributed to systematic error differences in methodology, which is the case in most other clinical laboratory procedures. Therefore, the need for constant monitoring of flame conditions to produce a more accurate measurement is emphasized.

DEFINITION OF STUDY

The purpose of this study is to demonstrate the feasibility of a unique measurement system for a clinical flame photometer. This study may provide information to alleviate some of the inherent problems of flame emission analysis while providing improved sensitivity, precision and accuracy.

The significant features for this measurement system are:

1. The concentration of the reference (blank) solution closely approximates the normal electrolytic values of blood serum.
2. A steady state of flame operation.
3. A difference measurement between the sample and the reference solution.
4. The state of the art electronics.
5. A visual or signed presentation of data for comparison to normal values.

The reference solution approaches the "ideal" blank because it is more similar in composition to the diluted sample than is a water-Sterox SE or a water-lithium blank. Also, upon aspiration of the sample the flame will be less perturbed due to the similarity between the sample solution and the reference solution. In this case the instrument blank is not zero but is the normal value expected for each electrolyte undergoing analysis.

A steady state of flame operation is attained by continual aspiration of the reference solution at a constant flow rate. The constant flow of operation is interrupted only by aspiration of the sample at regular intervals and then returned to the reference state. In this manner there is an immediate check of flame irregularities. Actually the system is under conditions of constant standardization rather than occasional standardization as is the case with other measurement systems. By constantly monitoring the reference solution, one should realize improved accuracy and precision for flame emission analysis.

A chemical difference measurement for this system implies that the measurement is either a positive or a negative deviation from the normal electrolyte concentration represented by the reference solution. While in operation, the instrument base line or zero is set with the reference solution. Upon aspiration of a sample, the automatic zeroing is suspended, a signal greater than, the same as or less than the reference is recorded. At all times except when aspirating a sample, the measurement system automatically adjusts to the reference or zero point for each element under consideration. Figure 9 pictures the measurement as it would appear on a chart recording. The "a" represents the recording of the reference solution (zero) while "b" and "c" identify the upper and lower limits, respectively, of the normal range for each element. The differential type of measurement generally produces a better signal to noise ratio (S/N) than do conventional methods of amplification and offsetting.

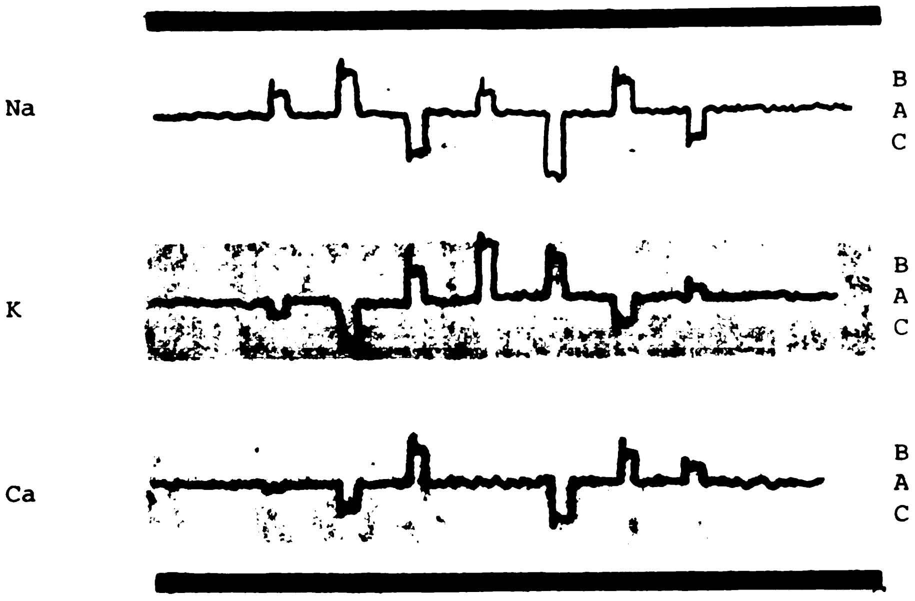


Figure 9. Simulated recorder response for a chemical difference flame photometer measurement.

The maintenance of a high S/N ratio is possible becuase of the "state of the art" electronic components used. Amplification of the small differential signal imposes no particular difficulties. Full scale expansion for the normal range is possible providing increased sensitivity over conventional systems.

Visual data presentation on chart paper provides comparison to values within and outside the "normal" range. Also, the values established as a decision level for a particular electrolyte can be printed or color coded on the chart paper. This system of data presentation provides both the technologist and the physician with an immediate evaluation of a patient's electrolyte status. In addition, this measurement technique is adaptable to an on-line automated mode for either analog or digital instrument systems.

What is gained through the use of the proposed measurement system for flame photometric analysis?

1. An apparent stable operating condition for the flame for increased accuracy.
2. The sensitivity of the system is greatly improved by amplification of the readout.
3. A chemical differentiation system which is much more sensitive to minor differences in the electrolyte concentration than are the usual measurement techniques.

It is the combination of 1, 2 and 3 above which contributes to improved accuracy, sensitivity and precision for clinical flame analysis when using the proposed measurement technique.

MATERIALS AND METHODS

Two types of experiments were completed to compare the traditional measurement of sodium, hereafter referred to as the single deflection (SD) method with the chemical difference flame photometry (CDFP) measurement of sodium. In addition, a third type of experiment was made to establish the feasibility of a CDFP measurement.

Experimental I

Solution Preparation

In the first group of experiments stock sodium standards were prepared according to the Model 21 Coleman flame photometer manual,[a] pages 20 through 24, and did include the appropriate concentrations of potassium and calcium. The working standard concentrations of 136, 140 and 144 mEq/l sodium were prepared according to the volumes given in the Appendix. The diluent used was 15 mEq/l lithium prepared according to the manual for the Instrumentation Laboratory (IL) model 343 flame photometer[b] on pages 14 and 15. The lithium was added in order to make the standards compatible with both instruments.

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[a] Coleman Instruments, Inc., 42 Madison St., Maywood, IL.

[b] Instrumentation Laboratory, Inc., 113 Hartwell Ave., Lexington, MA 02173.

Instrument Operation

Both the Coleman and the IL-343 instruments were operated and standardized according to the directions given in their respective manuals. The Coleman Model 21 Flame Photometer is a well known direct reading filter instrument which may be added as an accessory to the Coleman Jr. spectrophotometer. Standards prepared from dried reagent grade chemicals and samples are diluted 1:200 with a solution of 0.02% sterox SE which serves as a wetting agent to reduce surface tension. Standardization is accomplished by setting the reference state (zero) with a water-sterox blank. The upper limit is usually set on a precalibrated scale with the 150 mEq/l sodium standard.

The standards and diluent for the IL-343 Flame Photometer are prepared from commercial solutions. Both standards and samples are diluted with a 15 mEq/l lithium solution. Standardization is accomplished by setting the lithium response in the reference state to zero while aspirating a water-lithium blank. The upper limit is set at 140 mEq/l sodium and 5 mEq/l potassium while aspirating the standard solution containing the above-stated concentrations of both elements.

Following the initial standardization step, neither instrument was restandardized throughout the duration of data collection from the above prepared standards. In some instances the instruments were under continuous operation for two hours.

Experimental II

Solution Preparation

The second type of experiments is identical to the first type with one exception. In this group of experiments new standards were prepared in the manner presented in the IL-343 manual, page 15. The IL stock standard for 140 Na/5 K mEq/l was appropriately diluted to make working standards of 136, 140 and 144 mEq/l sodium as given in the Appendix. Both instruments were operated as previously described. Data were taken from both instruments as in the first group of experiments.

Experimental III

Instrument System

The third type of experiment was attempted to determine the feasibility of a CDFP measurement and required the fabrication of a new instrument and sampling system. The instrumental setup utilized only the burner and the detector from the Coleman flame photometer. In addition, an amplifier was attached to provide a zero offset, a gain and a sensitivity control.

Sampling System

A simple but effective gravity flow sampling system was fabricated to provide a positive pressure at the burner capillary. The components of the sampling system are identified in Figure 10. A Sargent SR chart recorder was used for the readout. The amplifier circuit is presented in Figure 11.

The IL stock standard for 140 Na/5 K mEq/l was used to prepare working standards in concentrations of 136, 140 and 144 mEq/l sodium. The 140 mEq/l sodium standard was used as a background emission against which the output was continuously aspirated between the samples represented by the 136 and 144 mEq/l sodium standards.

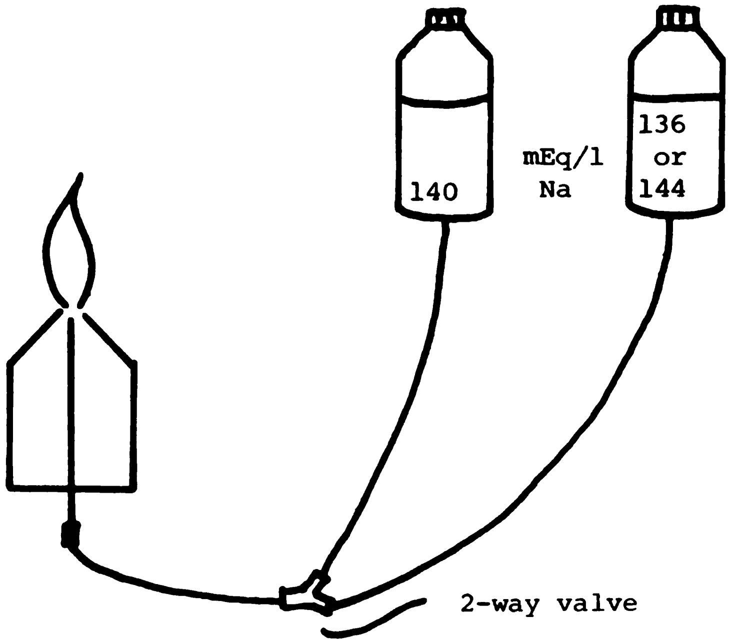

Figure 10. Components of a gravity flow sampling system.

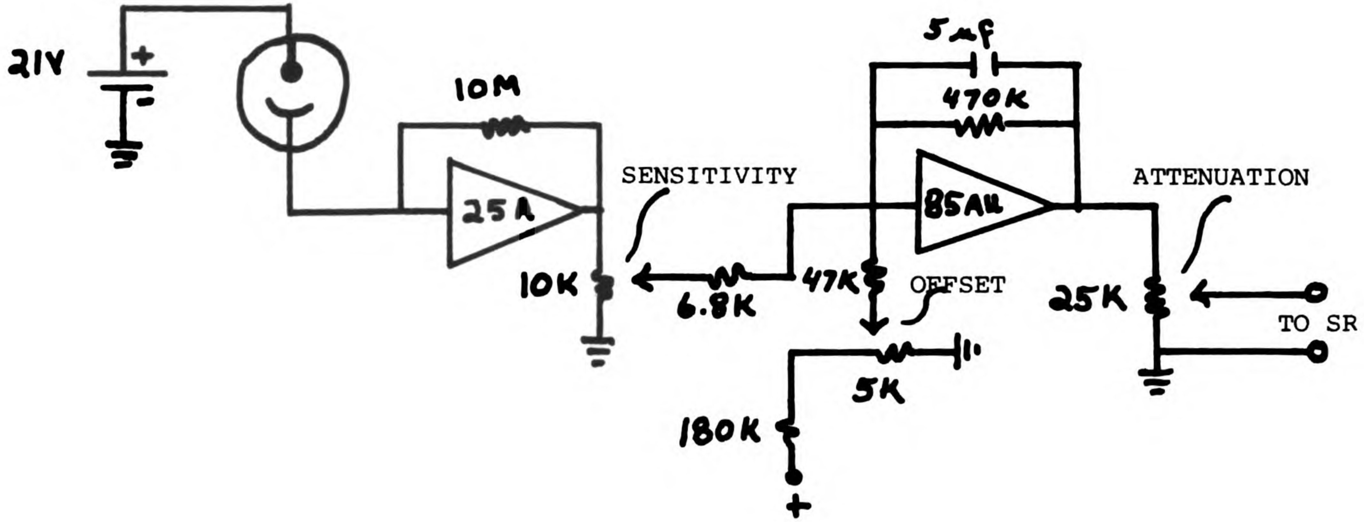

Figure 11. Amplifier schematic for experimental III.

RESULTS AND DISCUSSION

Experimental I, II and III

During the first two types of experiments data were recorded sequentially, at 30-second intervals during a 1- to 2-hour period as shown in Table 5. Mathematical comparisons between the SD and the CDFP measurements were calculated to indicate the amount of improvement of a continuously updated standardization over the SD measurement as follows:

Single Deflection Measurement: For this calculation refer to the first group of bracketed figures in Table 5.

1. Determine the average value of B = 140.5.
2. Subtract the known value from each recorded value:

A. 136.5	B. 140.5	C. 145.0
-136.0	-140.0	-144.0
+0.5	+0.5	+1.0

3. Calculate the average error for A, B and C.

Chemical Difference Flame Photometer Measurement:

1. Determine the average of B for the first two B's = 140.4.
2. Determine the average of B for the second and third B = 140.5.
3. Determine A and C when calculated as a negative or positive deviation from their respective averaged B. As an example:

Table 5. Recording of data for Experimental I and II

Sample	Reading (mEq/l Na)
**B	140.5
*A	136.5
B	140.3
***C	145.0
B	140.7
A	136.5
B	141.4
C	145.4
B	140.6

*Represents the 136 mEq/l sodium standard.

**Represents the 140 mEq/l sodium standard.

***Represents the 144 mEq/l sodium standard.

For A $\bar{B} = 140.4$

$A = 136.5$

A should = $(\bar{B} - 4.0) = 136.4$

But A = $\underline{-136.5}$

the error for A = -0.1

For C $\bar{B} = 140.5$

$C = 145.0$

C should = $(\bar{B} + 4.0) = 144.5$

But C = $\underline{-145.0}$

the error for C = -0.5

4. Calculate the average error for A and C.

A summary of the calculated average errors from both instruments for the two sets of standards and the two types of measurements is presented in Table 8. These figures indicate the improved precision that may be obtainable with the CDFP measurement.

An output of a recording from the third type of experiment is shown in Figure 12. Figure 13 pictures the responses between a 140 and a 141 mEq/l sodium solution using the CDFP instrumental setup.

In all cases the CDFP average error was approximately an order of magnitude better than that for the SD method of measurement. These results represent the worse case situation in operation of an emission flame photometer. However, for acceptable accuracy with a SD measurement, intermittent standardization is required along with the assumption that there is a negligible change in flame characteristics and flow rate. During the long running time, instrumental or base line drift contributed to a larger average error for the SD

Table 6. Comparison of average error for the SD and CDFP measurements

Experimental group	Instrument	Average Error			
		SD		CDFP	
		136 mEq/l sodium	144 mEq/l sodium	136 mEq/l sodium	144 mEq/l sodium
I	Coleman 21	8.74	8.89	0.74	0.63
I	IL-343	3.45	3.88	0.47	0.74
II	Coleman 21	3.87	4.50	0.73	0.65
II	IL-343	5.75	6.38	0.51	0.54

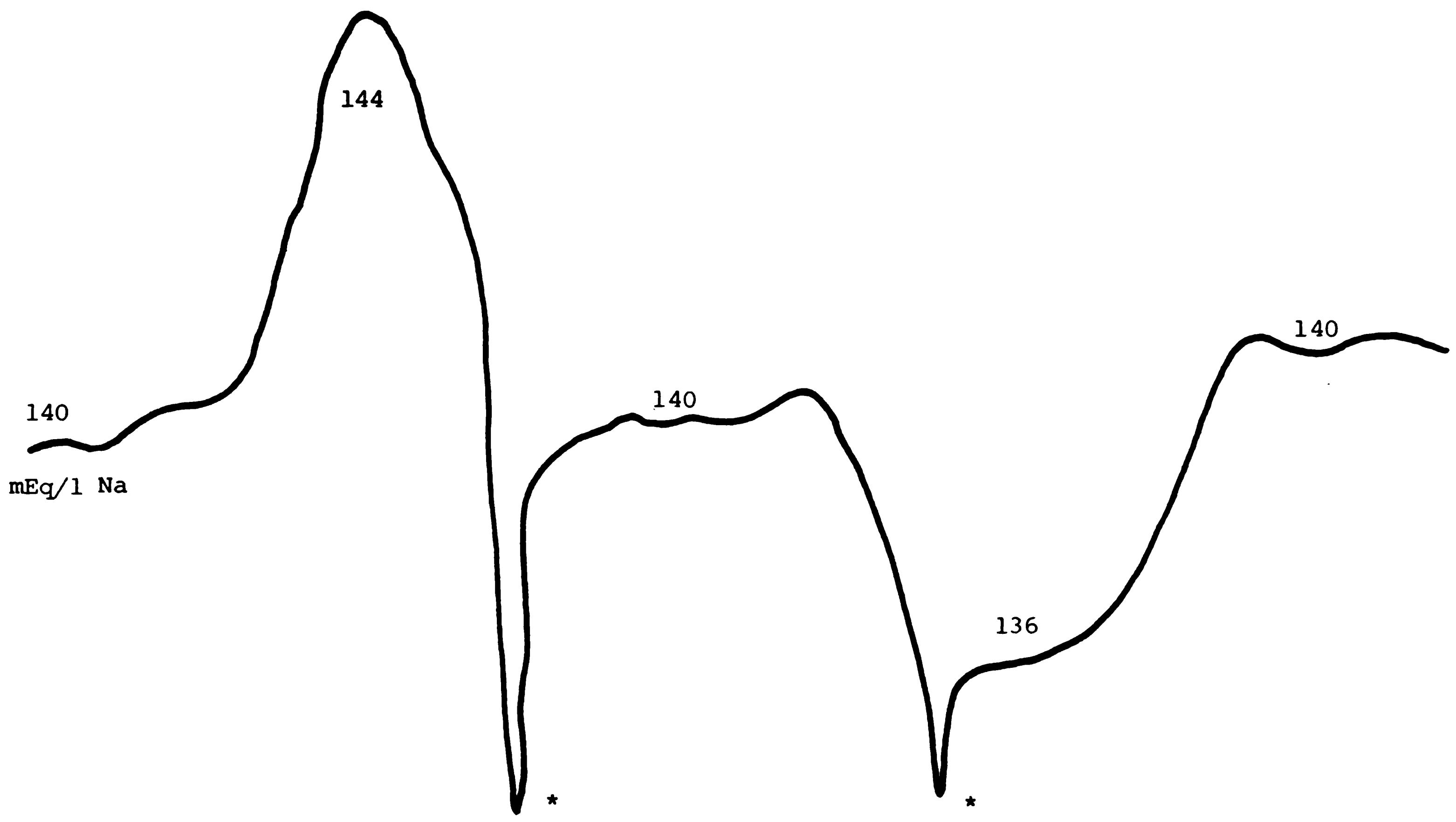

Figure 12. Tracing of the CDFP output.

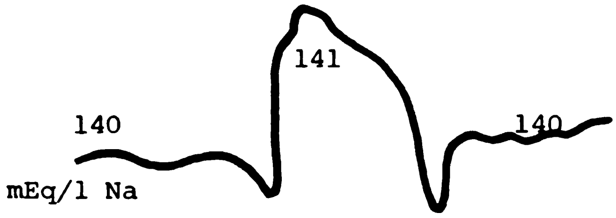

Figure 13. Tracing of the CDFP output for 1 mEq/l sodium difference.

measurement. However, immediately after standardization the error was greater for SD than for CDFP. The instrumental response to the small difference between the reference standard and the sample is a more accurate measurement than the comparison of the large difference between a blank and a standard or sample solution.

The most exciting feature of operating a flame photometer in a differential mode is the apparent increase in sensitivity as represented by the curves in Figures 12 and 13. The signal response from the Coleman 21 instrument was only a difference of approximately three chart divisions between the 140 and the 136 or 144 mEq/l sodium standards when measured and recorded in the conventional manner. The recording in Figure 12 represents a difference of 20 chart divisions for the same standard solutions. The response for a difference of only 1 mEq/l of sodium in Figure 13 is approximately 5 chart divisions. The negative excursions between successive solutions in Figures 12 and 13 (at the *) were produced at times of changing from one sample to another and resulted from the crudely constructed sample introduction system. It is clear that a more efficient sampling system should remove these artifacts. In turn, the resulting output would provide a fail-safe response in regard to the direction the sample concentration differs from the standard background solution.

Although the results of this study have shown that CDFP measurement to be feasible, there remain areas in need of further development. Both a positive pressure reference solution introduction and a rapid automatic sample injection need to be developed. A constant flow rate during continual aspiration of the reference solution should provide a more steady flame. There is an immediate check on flame fluctuations and an automatic adjustment to the reference point when using a rapid sample injection and quick return to the reference point. In addition, the time needed for sample response could be shortened. Also, the development of a micro-burner requiring small sample volumes would be an advantage to the clinical laboratory.

SUMMARY

In summary, it has been found that Chemical Difference Flame Photometry presents an alternate method to flame analysis that has the potential to provide greater accuracy and sensitivity. In addition, it presents the capability to provide a more fail-safe method for determining the relationship of individual samples to the expected normal values. Although the research to date has been done only with sodium, simultaneous multiple analysis is not precluded by any of the theory or hardware. The greatest benefit to the clinician and the patient is the potential to provide accuracy at the decision levels of sodium and other electrolyte concentrations. The CDFP approach is not limited to only emission or atomic absorption flame techniques but should apply to any continuous flow analysis.

APPENDIX

Table A-1. Working standard preparation for Experimental I

Working standard mEq/l Na	Stock 25 mEq/l Na, (ml)	Stock 1 mEq/l Ca, (ml)	Stock 1 mEq/l K, (ml)	1% Sterox (ml)
136	56.0	50	50	40
140	57.6	50	50	40
144	54.4	50	50	40

Each was prepared in a 2 liter volumetric flask and brought to volume with a 15 mEq/l lithium diluent.

Table A-2. Working standard preparation for Experimental II

Working standard mEq/l Na	Stock IS 140 mEq/l Na, (ml)	1% Sterox (ml)
136	4.86	20
140	5.00	20
144	5.14	20

Each was weighed and brought to volume in a 1 liter volumetric flask with a 15 mEq/l lithium diluent.

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VITA

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She was admitted to the graduate program in Clinical Laboratory Science at Michigan State University in September 1970. During her graduate studies she was granted an NIH traineeship from the Department of Pathology, a research grant from Clay Adams, Inc., and American Society for Medical Technology Education and Research Fund and was a graduate assistant in the undergraduate pathology teaching laboratories. In addition she was a primary organizer and coordinator for developing a series of Instrumentation Workshops for ASMT.

Presently she is a technologist for the Clinical Toxicology Laboratory, Department of Pharmacology, Michigan State University.

The author is happily married to John Morris Schoepke. She has a daughter, Shannon S. Bradley, and a son, Kyle N. Bradley.

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