United States Patent [19]

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[11] **3,708,228**

[45] **Jan. 2, 1973**

[54] SAMPLING TECHNIQUE FOR ATOMIC ABSORPTION SPECTROSCOPY

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[22] Filed: **April 30, 1971**

[21] Appl. No.: **138,943**

[52] **U.S. Cl.** **356/87,** 356/36, 356/39, 356/246

[51] **Int. Cl.**.....**G01j 3/30,** G01n 33/16, G01n 1/10

[58] **Field of Search**.....356/36, 39, 87, 244, 246

[56] **References Cited**

UNITED STATES PATENTS

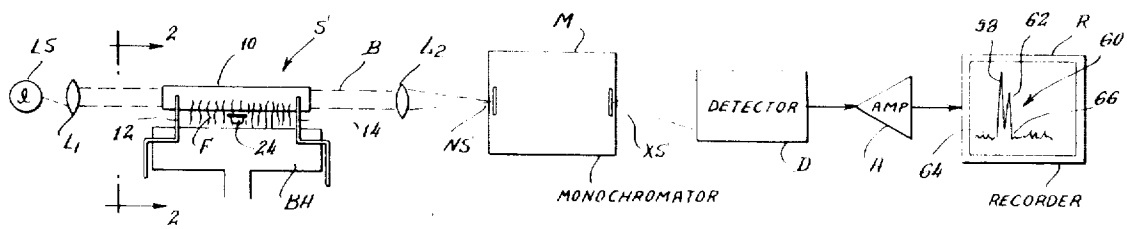
3,565,538 2/1971 Kahn et al. 356/246

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[57] **ABSTRACT**

A sampling technique for atomic absorption spectroscopy uses a very small quantity (e.g., 10 μl) of, for example, an organic fluid (e.g., blood) for determining small relative quantities of a reasonably volatile metal (e.g., lead). The sample is placed in a small cup-shaped holder and dried by heating; the organic components are then at least partially oxidized as by adding 20 μl of 100-volume (30 percent) hydrogen peroxide solution and heating until completion of reaction and redrying, both heating steps being at about 140° C by means of a hot plate. The sample holder is then placed under a central aperture in a relatively long absorption tube located in the conventional flame so as to surround the conventional radiation beam of an atomic absorption spectrometer. The relative diameter of the sample holder and of the absorption tube aperture and their relative position are such that a small proportion of the flame enters the aperture so that the sample material is caused to enter the absorption tube but is not flushed out of the open ends thereof for at least a few seconds. Both improved reproducibility and a sensitivity gain factor of about 5 is obtained relative to the use of somewhat larger non-symmetrical (e.g., boat-shaped) sample holders without an absorption tube. Blood lead levels of less than 0.1 μg per ml (i.e., less than 0.001 μg in a 10 μl sample) can readily be determined at an analysis rate of up to 50 samples per hour.

12 Claims, 3 Drawing Figures



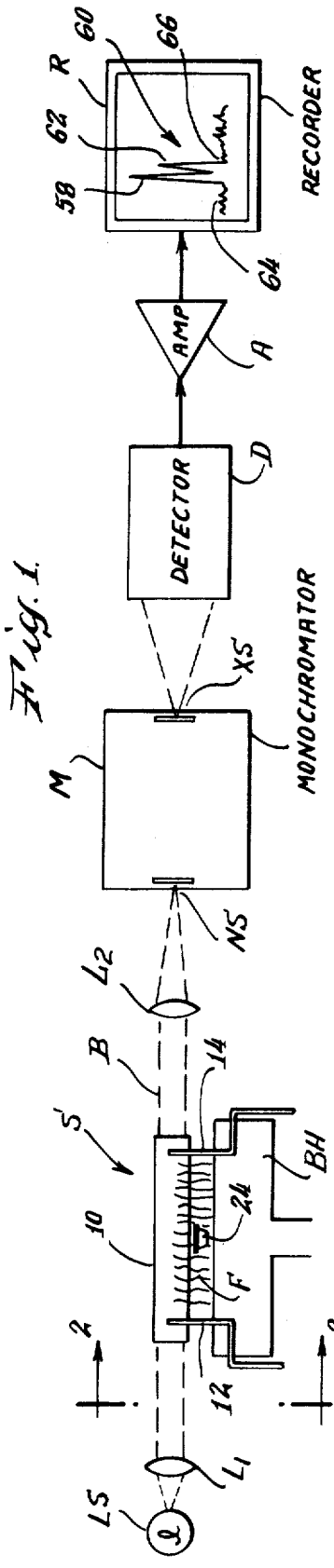


Fig. 1.

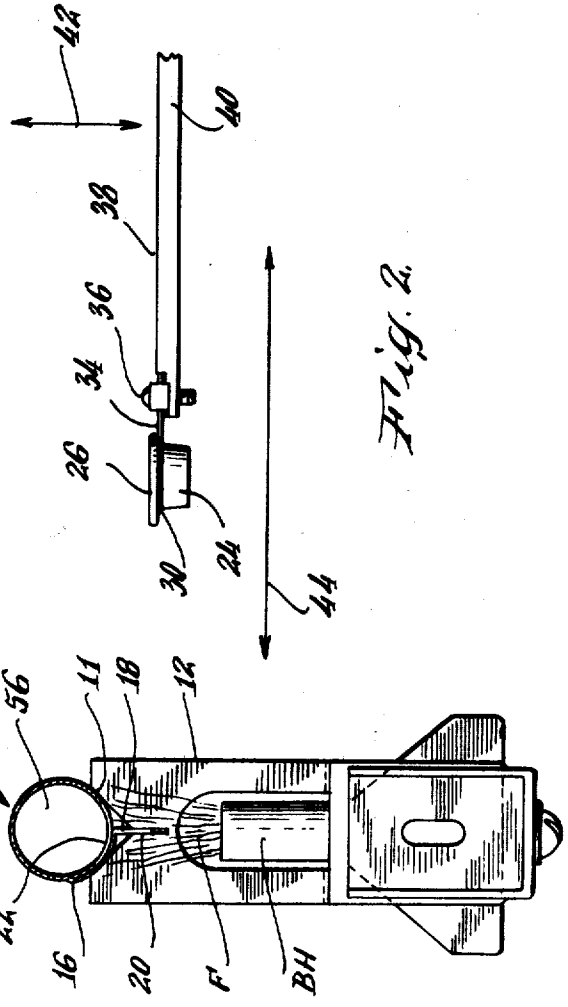


Fig. 2.

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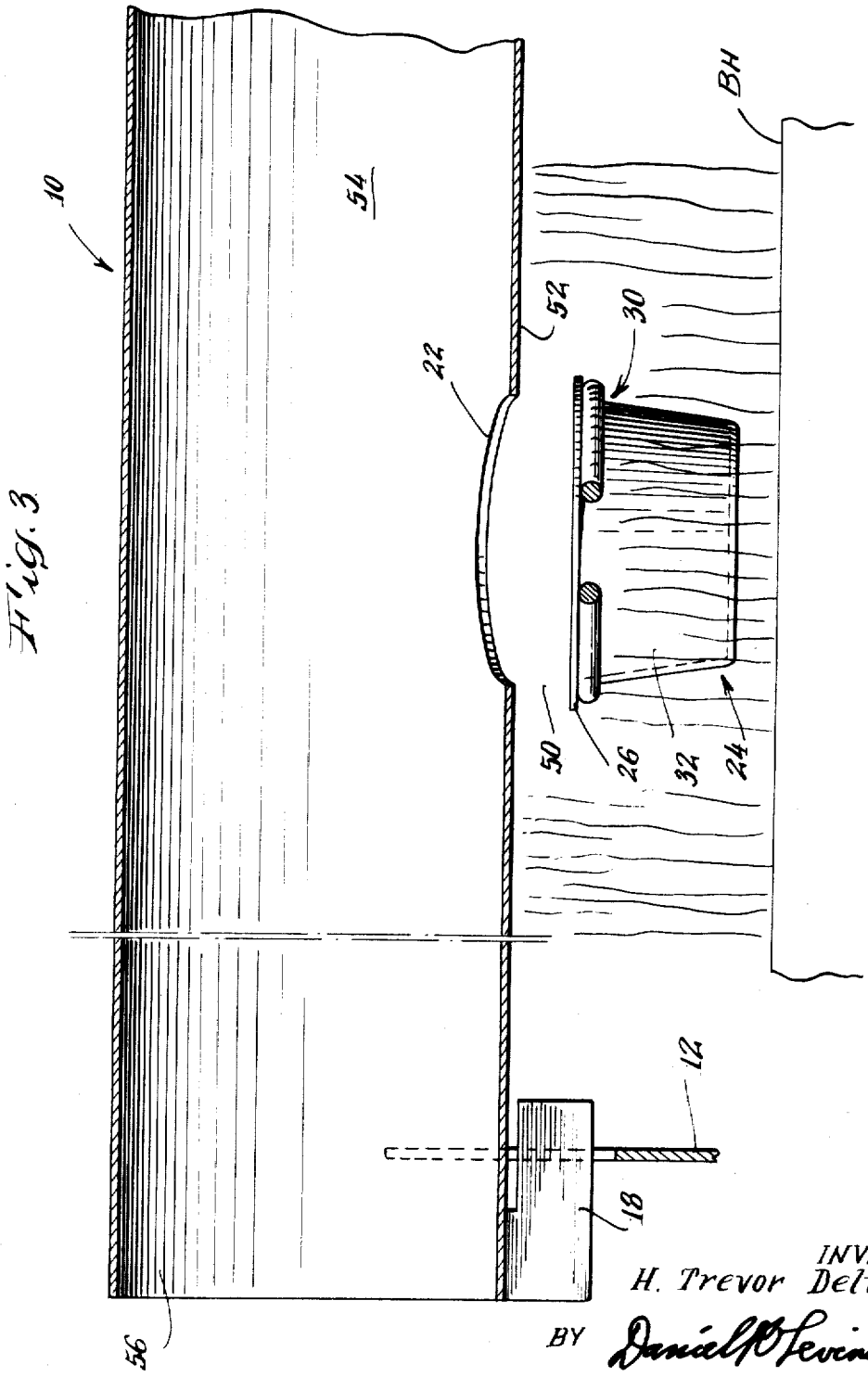


Fig. 3

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SAMPLING TECHNIQUE FOR ATOMIC ABSORPTION SPECTROSCOPY

GENERAL DESCRIPTION

This invention relates to an improved sampling technique and apparatus especially useful in atomic absorption spectroscopy. More particularly, the invention concerns analysis of relatively volatile metals (e.g., lead) of low concentration in a liquid containing a relatively high concentration of organic substances (e.g., blood).

The importance of determining relatively low concentrations of metals in human blood has recently been recognized, especially as to lead and mercury poisoning. Lead blood levels in adults of between approximately 0.2 to 0.6 μg/ml and in children of up to 0.36 μg/ml are considered normal; while as little as 0.8 μg/ml is considered dangerous for children. To determine relatively routinely whether the blood level of children is abnormally high, therefore, requires a technique that is both highly sensitive and relatively precise. Additionally, it is desirable that only a very small quantity of blood (such as may be obtained by the pricking of a finger) is required, and that the analysis may be performed rapidly and routinely in order to screen large numbers of children.

Most prior techniques require a relatively large blood sample (e.g., from ½ to 5 ml) or are time-consuming, or both. The atomic absorption sampling technique described in the HLKahn, GEPeterson and JESchallis article in Atomic Absorption Newsletter, Volume 7, page 35 (1968), and disclosed in U.S. Letters Pat. No. 3,565,538 issued on Feb. 23, 1971, which utilizes an open, generally boat- shaped sample holder, may be adopted to determining lead in blood as described in the HLKahn and JSSebestyen article in Atomic Absorption Newsletter, Volume 9, page 33 (1970). However, even this technique is neither as sensitive nor as precise (i.e., easily reproducible), nor allows as many determinations per hour as the present invention, since the results vary with positioning (including angular tilt) of the boat-shaped holder in the flame and with different holders, thereby requiring in practice that the same holder must be predried adjacent to the flame and then positioned in the flame for analyses so that an atomic absorption instrument is effectively limited to a single sample holder.

The present invention utilizes symmetrical smaller cup-shaped sample holders in conjunction with an absorption tube which just surrounds the radiation beam of the atomic absorption spectrometer. In particular, the present invention utilizes an absorption tube in the form of a cylinder open at both ends and a sample-receiving aperture in the middle of its lower surface. The sample holder is positioned in the flame just below this sample-receiving aperture in such manner that a small part of the flame enters the aperture so as to cause the vaporized sample components to enter the absorption tube but without allowing a large amount of the flame to enter, which would cause the vapors to leave the open ends of the tube before the few seconds required for determining the peak atomic absorption signal from the metal of interest (hereinafter assumed to be lead). Although the positioning of the sample holder relative to the sample-receiving aperture requires relatively high precision to obtain both optimum and reproducible results, the small symmetrical nature of the sample holder allows this to be obtained even though different sample holders are interchanged. This allows full utilization of the atomic absorption instrument in that the sample preparation steps are performed before the sample holder is positioned in the instrument. As will appear hereinafter, such sample preparation is itself relatively simple, requiring only pre-drying of the blood (for about 30 seconds at 140° C), addition of an oxidizing agent (e.g., 20 μl of 30 percent hydrogen peroxide for a 10 μl blood sample), and reheating to dryness (about 2 minutes at 140° C). Since a large number of samples may be simultaneously prepared by utilizing a hot plate, the number of actual analyses that may be accomplished with a single atomic absorption instrument may be at least as high as 50 per hour.

An object of the invention is the provision of an improved sampling technique, especially useful in atomic absorption spectroscopy, for determining low level concentrations of a metal of interest in a liquid which is inherently difficult to analyze directly by means of atomic absorption spectroscopy (e.g., blood).

A related object is the provision of an improved sampling technique as just described which is adapted to rapid repetitive measurements at relatively high sensitivity and precision.

Other objects, advantages and features of the invention will be obvious to one skilled in the art on reading the following specification and accompanying drawings, in which:

FIG. 1 is a partly schematic representation of a simplified atomic absorption spectrophotometer, showing exemplary sampling apparatus according to the invention;

FIG. 2 is an elevation of the essential parts of the improved sampling apparatus as seen from the direction 2—2 in FIG. 1, with the sample holder in its withdrawn position; and

FIG. 3 is an enlarged detail showing the relationship of the sample holder and the absorption tube, as seen generally from the right in FIG. 2, but with the sample holder in its operative position directly under the center of the absorption tube, which is shown in section.

SPECIFIC DESCRIPTION OF APPARATUS

A schematic illustration of a single-beam atomic absorption spectrophotometer is given in FIG. 1, merely for exemplary purposes, since this is the simplest type of instrument. Both single-beam and double-beam atomic absorption spectrometers are described, for example, in U.S. Letters Pat. No. 2,847,899 to A. Walsh, as well as being commercially available. The inventive sampling technique and apparatus may be utilized with any type of atomic absorption spectrophotometer, and in fact would preferably be used with a double-beam instrument (for example, the double-beam, ratio-recording atomic absorption spectrophotometer manufactured by The Perkin-Elmer Corporation, Norwalk, Connecticut, as the Model 303), for reasons inherent in such double-beam instruments and well known to those skilled in the art. The conventional, diagrammatically shown parts of the spectrophotometer have been labelled by the use of letters in FIG. 1, while the parts

more directly involved in the invention are subsequently described by the use of reference numerals.

In the exemplary single-beam instrument, a light source LS emits radiation including at least one spectral line which the tested-for metallic element absorbs in its atomic state. An optical system, schematically exemplified by lenses L_1 and L_2, directs a beam B of this radiation from the light source through a sample location, generally indicated at S, at which the sample material (in its atomized state) will intercept the beam. After passing through the sample location (and the second part of the optical system L_2) the beam will enter a monochromator M as at its entrance slit NS. The variable monochromator will be set so that only the desired narrow spectral interval intended to be measured will leave the exit slit XS. Thus, only the wavelength interval of the original beam which generally corresponds to a particular spectral line at which the tested-for sample absorbs will reach the detector D. The electrical output of the detector will be amplified (and in general rectified and often further processed) so as to provide a final electrical output which varies as the absorption (or the well-known logarithmic function, absorbance) of the sample material at the spectral line used in the analysis. This final electrical output may be fed to a, say, chart-type recorder R so as to yield a permanent record of the absorption or absorbance (the exemplary graph showing, say, the absorbance of the beam at the tested-for spectral line before, during and after the sample was present in the beam). Since the invention itself concerns solely the manner in which the sample is caused to be introduced into the beam, and the invention may be used with any atomic absorption spectrophotometer, the details and well-known operation of the exemplary spectrophotometer schematically shown are not further described (see the above-mentioned U. S. Pat. No. 2,847,899, for example). Therefore, the following will, for the most part, describe in detail only the structure and operation of those parts generally shown in the sample area S in FIG. **1**, and shown in more detail in FIGS. **2** and **3**.

The essential elements located at the sample area S comprise the conventional burner head BH having one or more elongated slots directly under the radiation beam B in the atomic absorption instrument. This burner head may be, for example, the three-slot burner head commercially available as part No. 303-0202 from The Perkin-Elmer Corporation, Main Avenue, Norwalk, Connecticut, attached to the premix type burner supplied with the Model 303 atomic absorption spectrophotometer of the same company. Mounted above the burner head BH is a substantially cylindrical absorption tube **10**, supported, by, for example, V-shaped notches (see FIG. **2** at **11**) in sheet metal brackets **12**, **14** attached near opposite ends of the burner head. The absorption tube may be formed from a sheet of preferably between 6 and 12 mils (that is, between 0.006 and 0.012 inches) foil of for example either substantially pure nickel or the commercially available alloy Iconel 601; in any case, the material should be substantially free of the metal of interest, hereinafter assumed to be lead. For example, if Iconel 601 is utilized it should contain less than 0.0003 percent (3 ppm) lead for lead analysis. The brackets **12** and **14** may be made from (substantially lead-free) sheet metal of, say, 20 mil thickness. In a successful embodiment of the invention, the length (i.e., the horizontal dimension in FIG. **1**) of the absorption tube **10** was 100 mm and its diameter approximately 12½ mm, so that the interior of the tube substantially encompasses the full width of the radiation beam of the particular atomic absorption spectrophotometer for most of its length in the sample area. Although the sheet metal or foil forming the absorption tube **10** is formed into an almost perfect cylinder by rolling and e.g. welding at **16** (see FIG. **2**), short end tabs such as **18** in FIGS. **2** and **3** are preferably provided to simplify supporting the tube in a non-rotatable manner as in slots in the brackets **12**, **14**, as indicated at **20** in FIG. **2**. The lower center of the absorption tube **10** is formed with an aperture **22**, as best seen in FIG. **3**. In a specific successful embodiment this aperture was circular and had a diameter of 9.5 mm (0.375 inches) in the sheet metal prior to its being formed into the tube **10**.

The previously dried and (in the case of organic samples such as blood, also previously at least partially oxidized) sample material is contained in a small, generally cup-shaped sample holder or crucible **24** as schematically shown in FIG. **1** but best seen in FIG. **3**. This small crucible may be made from metal foil from 5 to 9 mils in thickness. Nickel foil of 99.95 grade (i.e., 99.95 percent purity) has proved highly suitable for this purpose, as well as for the absorption tube **10**. The dimensions of the particular sample crucible utilized were 10 mm diameter at its widest upper lip portion **26** n FIG. **3** and a height of about 5 mm. Although the exact dimensions are not critical, the relationship between the diameter of the widest upper portion **26** of the crucible, the diameter of the opening **22** in the lower part of the absorption tube and their relative vertical spacing (see FIG. **3**) has been found to be important within a narrow range of relative values. The crucible is most conveniently supported by means of a wire loop **30**, which may be formed from a 0.5 mm diameter platinum or platinum-iridum wire. Loop **30** includes a substantially circular part of lesser diameter than the lip portion **26** of the crucible but slightly greater diameter than the remaining tapered part **32**, so that the sample crucibles may be readily removed by lifting them vertically. The ends **34** of the wire remote from the loop portion **30** are fastened as indicated at **36** to the end of a rod **38** (e.g., of stainless steel of approximately ⅛ of an inch in diameter). Alternatively the ends of the wire may be embedded, as by fusing, in the end of a similar diameter glass rod. The rod **38** is in turn adjustably supported (as by clamping its other end **40** to the movable platform part of a "sampling boat" support assembly, available as Part No 303-0296 from the above-mentioned The Perkin-Elmer Corporation). The arrangement of the parts for supporting the sample crucible **24**, loop **30** and rod **38** by such a commercially available assembly is shown in Applicant's published article entitled, " A Micro Sampling Method for the Rapid Determination of Lead in Blood by Atomic Absorption Spectrophotometry," in Analyst, Volume 95, pp. 431–438, especially in FIG. **1** thereof on page 432. As indicated in FIG. **2**, the important characteristics of any such supporting means for rod **38** is that its height (and therefore the height of the crucible **24** relative to

the aperture **22** in the bottom of the absorption tube **10**) is adjustable as indicated by the arrow **42** and that the crucible may be introduced into the flame F from the burner head BH directly under this sample-receiving opening **22**, as indicated by the arrow **44**.

As best seen in FIG. **3**, the crucible is adjusted so that when it is centered below aperture **22** the flame can enter this aperture only by means of a relatively small annular opening **50**. For the relative exemplary diameters of the upper part **26** (10 mm) of the crucible **24** and the diameter of the sample-receiving aperture **22** (9.5 mm) it has been found that the uppermost surface of the crucible should be between 1.5 and 2 mm below the lowermost surface **52** of tube **10**. This allowing a small part of the flame to cause sweeping of the sample material through aperture **22** into the interior **54** of the tube **10**, so that the sample is caused to substantially fill the tube within a reasonably short period of time (a very few seconds) after the crucible containing the dried sample is moved into the flame under the aperture **22**. If the relative dimensions of the upper part of the crucible **26**, the aperture **22**, and the vertical spacing therebetween are such that the annular space **50** approaches zero, the flame is precluded from sweeping the sample to be introduced into the interior of the tube in its atomic state and substantially no absorption signal is attained. On the other hand, if the relative diameter **26** of the crucible, the sample-receiving aperture **22** of the tube and their vertical spacing are such that a large quantity of the flame enters aperture **22**, the sample material is rapidly swept not only into the tube but out both opens ends (one of which is indicated at **56** in each of FIGS. **2** and **3**), so that at best the tube never contains the maximum amount of vaporized sample material and at worst the sample material leaves the tube so rapidly as to provide a very small (and generally inconsistent) absorption signal at the detector and therefore the recorder (see FIG. **1**).

EXAMPLES AND OPERATION

Blood samples of 10 μl each are placed in separate cup-shaped holders or crucibles of the type shown at **24**. Since the technique of the invention basically measures the absolute quantity of the metal of interest (lead), the amount of sample must be precisely known to measure original concentration, so that Eppendorf micro-pipettes or equivalent precision pipettes should be utilized. The nickel crucibles are then placed on a thermostated hot plate at about 140° C until the sample is dry (about 30 seconds). The crucibles are then removed from the hot plate, allowed to cool, and 20 μl of reagent grade 30 percent hydrogen peroxide solution is added to each crucible. The crucibles are then returned to the hot plate (still at 140° C) and left there until a dry creamy yellow residue is obtained, usually in slightly less than 2 minutes. During this second drying (and partial oxidation step) a white froth will form which may temporarily extend above the upper rim (**26**) of the crucible, but this froth will collapse back into the crucible without loss as the drying is completed. Failure to pre-dry the blood sample will usually cause this froth to spill out of the crucible, thereby destroying the ability to obtain quantitative results. Although hot plate temperatures below 140° C may be used, the evaporation becomes so slow as to require an undue amount of time. On the other hand, at temperatures of above about 150° C the solution boils so vigorously that sample loss is likely to occur, and at temperatures about 160° C or above spontaneous ignition occurs. For these reasons both the first and second drying steps should preferably be carried out within 5° C of 140° C.

One of the thus prepared crucibles is now placed within the wire loop **30** while the latter is out of the flame (see FIG. **2**), care being taken to avoid contamination when effecting such transfer. Assuming the height and lateral centering (i.e., the vertical and horizontal directions respectively in FIG. **3**) have been pre-adjusted, the crucible is now moved directly under the aperture **22** in tube **10**, as by the sliding movement indicated by arrow **44** in FIG. **2**. The speed with which the crucible is so inserted into the flame is not highly critical, but it should be sufficiently rapid to prevent precombustion of the sample before it has reached the position below the absorption tube aperture. The last step is of course done while the atomic absorption spectrophotometer is fully operating. For lead analysis the light source LS in FIG. **1** will be a lead hollow cathode lamp, operating, for example, at 6 ma current; the wavelength of the spectrophotometer will be set to an atomic lead absorption line, preferably 2833A, with a monochromator wavelength band pass of about 7A. Although an acetylene-air flame is preferred, the proportions of fuel and air are not at all critical. Satisfactory results have been obtained with acetylene flow rates of 4.0 liters per minute with an air flow rate of 22.8 liters per minute, as well as entirely different relative flow rates (e.g., acetylene at 13½ and air at 11¾ liters per minute). Although the particular atomic absorption spectrophotometer utilized is not critical, the response time of the detector and/or recorder should be relatively rapid, so that the lowest time constant of most commercially available instruments should be chosen. In general, the time constant should be less than about one second, which corresponds for example to a noise suppression setting of "1" on both the Perkin-Elmer Model 303 instrument as well as the similar Model 403 instrument and the single-beam Model 290B atomic absorption instrument, both of the same Corporation. In order to separate the nonspecific absorption signal caused by the combustion products of the partially oxidized blood sample (which occur first as indicated at **58** in the schematically shown recorder signal **60** in FIG. **1**) from the absorption signal caused at the resonance line of the atomic state lead (see peak **62** in FIG. **1**) a recorder chart speed of not less than 40 mm (preferably about 120 mm) per minute should be utilized. No "scale expansion" is ordinarily used, and the ordinate of the chart graph is preferably in absorbance units (the abscissa being of course time), with full scale set to about 0.500 absorbance.

After introduction of the crucible into the flame below the aperture in the absorption tube there is a short delay before the sample component is vaporized and swept into the tube by the flame (e.g., about 1 second) as indicated at **64** in FIG. **1**. The relatively narrow first signal (which in some cases may exhibit two peaks, rather than the single peak shown at **58**) which occurs first is always associated with the combustion products, which typically produces a visible cloud of

smoke. This nonspecific absorption may vary not only with different blood composition but even according to the drying characteristics; however, since the lead signal always follows, no particular interpretive problem is caused by this variation in the nonsignificant first absorption peak (or peaks). The second (or final) absorption signal **62** typically reaches its peak about 3 seconds after the (last) combustion product peak and requires about 4 seconds to complete its rise from almost baseline level to peak and back again to near baseline level as indicated at **66**. Assuming that the same procedures and placement of the crucible relating to the adsorption tube aperture are utilized, the height of this second peak **62** as measured in absorbance units, is substantially perfectly directly proportional to the amount of lead present in the sample.

This linearity is evident from the following exemplary standardization by the well-known technique of the so-called "method of additions." Since aqueous solutions containing lead yield different absorbances (approximately 40 percent lower) than the same lead concentrations in blood, the analysis cannot be directly calibrated by the use of standard aqueous lead solutions. Standardization is therefore preferably accomplished by forming blood "standards" by the method of additions, by adding known quantities of standard aqueous solutions of lead to a blood sample and analyzing the resulting component samples. In one such calibration, 10 μl of standard aqueous solutions containing, respectively, 0.3, 0.5, 0.8 and 1.0 μg of lead per ml were added to individual identical 10 μl samples of the same blood. To minimize errors, two identical mixtures of each concentration were prepared and analyzed, as well as two 10 μl samples of the blood without any addition (thus making five identical pairs of samples). Each sample was then prepared as described above, by predrying, partial oxidation, and redrying, etc., and each crucible measured in the atomic absorption instrument, in turn. The average analytical results of each identical pair were averaged and yielded the following results:

	Absorbance Units
10 μl blood	.042
10 μl blood + 10 μl 0.3 μg/ml Pb	.10
10 μl blood + 10 μl 0.5 μg/ml Pb	.14
10 μl blood + 10 μl 0.8 μg/ml Pb	.19
10 μl blood + 10 μl 1.0 μg/ml Pb	.24

Plotting of these points on a graph, having absorbance as the ordinate and added lead as the abscissa, yields an almost perfectly straight line which crosses the X axis (i.e., absorbance = zero) at −0.22 μg per ml. Thus, if we add this quantity of lead contained in the blood to the amount of lead added to each of the samples, we may tabulate the results as follows:

Actual Lead μg/ml	Absorbance Units
.22	0.42
.52	.10
.72	.14
1.02	.19
1.22	.24

As may be seen from this table, the results are almost perfectly linear with 1 μg/ml of lead yielding an absorbance of 0.19. Once the analysis has been calibrated by this "method of additions" technique, other unknown blood samples may be run under similar conditions, and the lead concentration in the sample computed from the simple formula: sample concentration = concentration (standard) X peak height (sample) peak height (standard).

Although some variation is caused using different crucibles which are nominally identical, the use of precision dies in making the crucibles appears capable of reducing this variation to less than about ± 10 percent. Greater precision may be obtained by "-matching" crucibles by analyzing identical samples in a large number of crucibles and grouping the crucibles into ones that yield substantially the same sensitivity.

It may be noted that utilization of 99.95 grade (or purer) nickel substantially eliminates the effect of any original lead contaminant in the crucible material. Lower grade nickel will initially add a spurious additional component to the absorbance signal caused by the small amount of lead contained in the foil, which component gradually decreases with use. By utilizing the high purity nickel, substantially no spurious lead component is obtained even with new or little-used crucibles. Nevertheless, all new crucibles should be conditioned for analytical use by inserting them into the flame several times (for a total of about ½ minute) before first analytical use. No cleaning or other treatment of the crucibles between uses is required, since the crucible is cleaned by exposure to the flame at the end of the previous analytical use. The same crucible may be used over and over again (say, forty times or more) before accumulative heat (and oxidation) damage becomes great enough to suggest replacement.

The sides of the crucible (at **32** in FIG. **3**) are preferably tapered (e.g., about 11° from being vertical) to both make insertion in the wire loop holder easier and to assist in "puddling" in the drying steps. As previously noted, for the particular exemplary relative diameters of the top of the crucible **26** and the sample-receiving aperture at **22** (see FIG. **3**) the crucible should be positioned approximately 1/16 inch (1.5 mm) below the aperture, although a variation in this distance by a factor of about 2 in either direction is of only minor significance. However, if the crucible is so close to the bottom of the absorption tube that the annular space **50** approaches zero, so that substantially none of the flame enters the aperture in the tube, the absorption signal is almost completely eliminated. If the crucible is positioned too far below the absorption tube (more than about ⅛ inch or 3 mm in the exemplary apparatus) a broadening of the lead absorption peak (**62** in FIG. **1**) will gradually occur and the sensitivity correspondingly decreases. Within the range of about 1 to 3 mm vertical spacing, the absorption tube increases the sensitivity by a factor of about 8 relative to use of the crucible without an absorption tube having the relative dimensions and spatial relationships previously described.

Because many of the cup-shaped crucibles can be dried, partially oxidized and redried together on a single hot plate, the preparation or treating time per sample will average substantially less than a minute if a batch of samples (e.g., 20) are handled together. To expedite such preparation of a group of samples, a basket-like holder accommodating, say, 20 samples may be advantageously utilized. This holder may com-

prise a handle and a simple plate in which a plurality of holes, each having a diameter preferably equal to the diameter of the crucible at some intermediate point along its tapered portion (say 32 in FIG. 3), but in any event larger than the smallest diameter at the bottom of the crucible and smaller than the external diameter of the lip 26 of the crucible. Such a basket-like holder allows a large group of crucibles to be simultaneously placed upon or removed from the hot plate during the sample preparation steps. Since each sample is in the atomic absorption instrument for less than about a minute, up to about 50 samples per hour may be tested by a single operator by means of the present apparatus and technique.

The absolute sensitivity of the present technique utilizing the cup-shaped holder and the apertured absorption tube is sufficient to yield a detectable absorption signal with as little as 6 times 10^{-10} grams (0.6 of a nanogram), corresponding for example to a 10 μl quantity of sample having 0.06 μg per ml lead. This is bout 5 times the absolute sensitivity of the previous technique using an open boat-shaped holder without an absorption tube (and about 8 times the sensitivity obtained with the present cup-shaped holders without an absorption tube). For normal blood levels of lead from 0.2 to 0.6 μg per ml the standard deviation is less than 10 percent with it being about 4 percent at the typical blood lead level of 0.3 μg per ml.

Although the previously mentioned materials for both the absorption tube and the crucibles appear highly satisfactory, the crucibles being useable more than 40 times without substantial deterioration, other materials may be utilized, especially for the tube. For example, quartz refractory ceramics or coated metal tubes (e.g., tantalum diboride or tantalum silicide on tantalum, or an aluminide on nickel) may be used instead. Although the invention has been described relative to the determination of lead in blood, it is adaptable for determining various other volatile elements (e.g., silver, bismuth, cadmium, copper, mercury, selenium, thallium and zinc) in small samples of various biological materials.

I claim:

1. In an atomic absorption spectroscopic analysis technique of a sample material of the type in which: a beam of radiation, including at least one spectral line corresponding to an absorption line of a metallic element of interest in its atomic state contained in said sample material, is traversed through a sample location at which a flame is located, and then is detected in such a way as to measure the amount of absorption occurring at said atomic spectral line, so as to yield an indication of the quantity of said element of interest in its atomic state, the improvement for analyzing a liquid sample containing both such metal of interest and a substantial quantity of at least one oxidizable organic component comprising:

a. placing a known quantity of said liquid sample in a small symmetrical crucible;

b. drying the original liquid sample in said crucible;

c. adding an excess quantity of an oxidizing agent in liquid form to said dried sample in said crucible;

d. heating said sample and liquid oxidizing agent mixture in said crucible at a temperature sufficiently high to cause both a substantial oxidization of at least some of the oxidizable components of the original liquid sample and subsequent drying of said mixture, said temperature, however, being below the ignition temperature of said components;

e. and positioning said symmetrical crucible into the flame at said sample location in such manner that the vaporized sample components, including said metallic element of interest in its atomic state are carried by the flame into the path of said beam of radiation while also causing said components to be held in said beam for a period of time substantially greater than the normal traversal time of said flame across said beam,

whereby the nonspecific radiation absorption of said organic components is reduced and the absorption of said metallic element of interest in its atomic state is separated in time from said nonspecific absorption, so as to facilitate separate measurement of said atomic state absorption.

2. In an atomic absorption spectrometer for determining the quantity of a metallic element of interest in a sample material of the type having: a source of radiant energy including at lease one spectral line corresponding to an absorption line of said element of interest in its atomic state; means for directing a beam of said radiant energy through a sample location at which a flame is located to a spectrometric system, said system including a detector for measuring the amount of absorption occurring at said atomic spectral line so as to yield an indication of the quantity of said metallic element of interest contained in said sample material, the improvement comprising:

an open-ended elongated tube of substantially constant interior cross-section positioned at said sample location, above and therefore in thermal contact with the flame, said tube having its longitudinal axis aligned with said beam of radiant energy so that said tube surrounds at least a substantial part of said beam;

said elongated tube comprising a symmetrical sample-receiving aperture in its bottom surface, substantially at the middle of its longitudinal length;

a symmetrical substantially cup-shaped sample holder positionable in the flame directly below said sample-receiving aperture;

said sample-receiving aperture and the upper part of said cup-shaped sample holder being of such relative diameter and being spaced vertically apart such a distance that an extremely small part of the flame enters said sample-receiving aperture along with the vaporized sample components,

whereby at least a substantial amount of said metallic element of interest is rapidly caused to enter said tube and therefore said beam of radiant energy in its atomic state, and said tube causes said metallic element of interest to remain in said radiant energy beam for at least a few seconds.

3. An improved atomic absorption spectrometer according to claim 2, in which:

the diameter of said upper part of said cup-shaped sample holder is slightly larger than the diameter of said sample-receiving aperture;

and said upper part of said sample holder is spaced below the effective plane of said sample-receiving

aperture a distance somewhat greater than the difference between said diameters,

whereby a small part of the flame can enter said sample-receiving aperture by travelling around the edge of said upper part of said sample holder.

4. An improved atomic absorption spectrometer according to claim 3, in which:

the relative diameters of said upper part of said cup-shaped sample holder and of said sample-receiving aperture are such that said sample holder upper part diameter is approximately ½ unit larger than said sample-receiving aperture diameter;

and said upper part of said sample holder is spaced below the effective plane of said sample-receiving aperture a distance of between about 1 and about 3 units.

5. An improved atomic absorption spectrometer according to claim 3, in which:

the longitudinal length of said elongated tube as measured between its open ends is about an order of magnitude greater than the diameter of said sample-receiving aperture,

whereby the vaporized sample components remain within said elongated tube for an appreciable time.

6. An improved atomic absorption spectrometer according to claim 5, in which:

the effective average interior diameter of said elongated tube is the same order of magnitude as said diameter of said sample-receiving aperture and therefore is about an order of magnitude less than said longitudinal length of said elongated tube,

whereby the vaporized sample components substantially fill the interior cross-section of said elongated tube well before they reach and therefore leave the open ends thereof.

7. An improved atomic absorption spectrometer according to claim 2, in which:

said open-ended elongated tube comprises at least about 99 percent nickel.

8. An improved atomic absorption spectrometer according to claim 2, in which:

said cup-shaped sample holder comprises at least 99 percent nickel.

9. In an atomic absorption spectroscopic analysis technique of a sample material of the type in which: a beam of radiation, including at least one spectral line corresponding to an absorption line of a metallic element of interest in its atomic state contained in said sample material, is traversed through a sample location at which a flame is located, and then is detected in such a way as to measure the amount of absorption occurring at said atomic spectral line, so as to yield an indication of the quantity of said element of interest in its atomic state, the improvement for analyzing a liquid sample containing both such metal of interest and a substantial quantity of at least one oxidizable organic component comprising:

a. placing a known quantity of said liquid sample in a small symmetrical crucible;

b. drying the original liquid sample in said crucible;

c. heating the dried sample in said crucible under such conditions and at a temperature sufficiently high to cause both a substantial oxidization of at least some of the oxidizable components of the original liquid sample and subsequent drying of said mixture, said temperature, however, being below the ignition temperature of said components;

d. and positioning said symmetrical crucible into the flame at said sample location in such manner that the vaporized sample components, including said metallic element of interest in its atomic state are carried by the flame into the path of said beam of radiation while also causing said components to be held in said beam for a period of time substantially greater than the normal traversal time of said flame across said beam, whereby the nonspecific radiation absorption of said organic components is reduced and the absorption of said metallic element of interest in its atomic state is separated in time from said nonspecific absorption, so as to facilitate separate measurement of said atomic state absorption.

10. An improved atomic absorption spectroscopic analysis technique according to claim 9 in which:

an oxidizing agent is added to the dried sample in said crucible prior to the heating step (c).

11. An improved atomic absorption spectroscopic analysis technique according to claim 9 in which:

said liquid sample is blood;

and a volume of 30 percent hydrogen peroxide solution approximately equal to twice the volume of said liquid blood sample is added following the initial drying step (b) and prior to the heating step (c).

12. An improved atomic absorption spectroscopic analysis technique according to claim 9 in which:

said heating step (c) is performed at approximately 140° C.

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