



US005174959A

# United States Patent [19]

[11] Patent Number: **5,174,959**

Kundu et al.

[45] Date of Patent: **Dec. 29, 1992**

[54] **BREATH COMPONENT MONITORING DEVICE**

[58] Field of Search ..... 422/58, 59, 84, 85, 422/61; 436/128, 132, 167, 181

[75] Inventors: **Samar K. Kundu; Richard W. George; Steven C. March**, all of Libertyville; **Sangvorn Rutnarak**, Long Grove, all of Ill.

[56] **References Cited**

**U.S. PATENT DOCUMENTS**

3,196,689 7/1965 Forrester et al. .... 422/85 X

[73] Assignee: **Abbott Laboratories**, Abbott Park, Ill.

*Primary Examiner*—Jill A. Johnston  
*Attorney, Agent, or Firm*—Frank S. Ungemach; Jeffrey Sharp

[21] Appl. No.: **685,317**

[57] **ABSTRACT**

[22] Filed: **Apr. 15, 1991**

The present invention relates to methods and materials for the detection of ketone and aldehyde analytes in fluid samples by means of reacting analyte containing samples with a first solid matrix material to which a nitroprusside salt is coupled and a second solid matrix material to which an amine is covalently coupled. Methods and devices are also provided for ascertaining the fat catabolism effects of a weight loss dietary regimen comprising determining the breath acetone concentration of the subject.

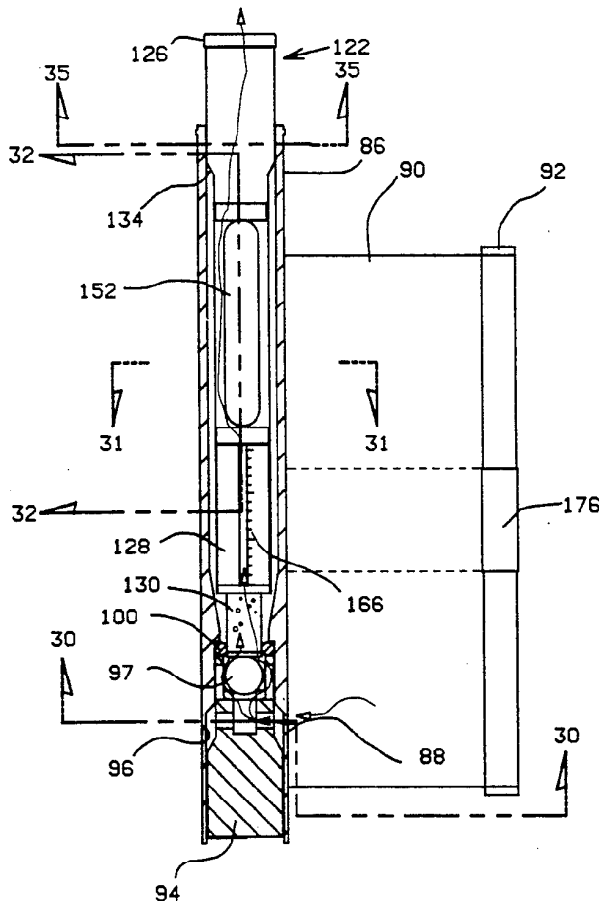
**Related U.S. Application Data**

[60] Division of Ser. No. 131,811, Dec. 17, 1987, Pat. No. 5,071,769, which is a continuation-in-part of Ser. No. 944,083, Dec. 22, 1986, Pat. No. 4,970,172.

[51] Int. Cl.<sup>5</sup> ..... **G01N 31/22**

[52] U.S. Cl. .... **422/59; 422/58; 422/61; 422/84; 422/85; 436/128; 436/132; 436/167; 436/181**

**15 Claims, 21 Drawing Sheets**



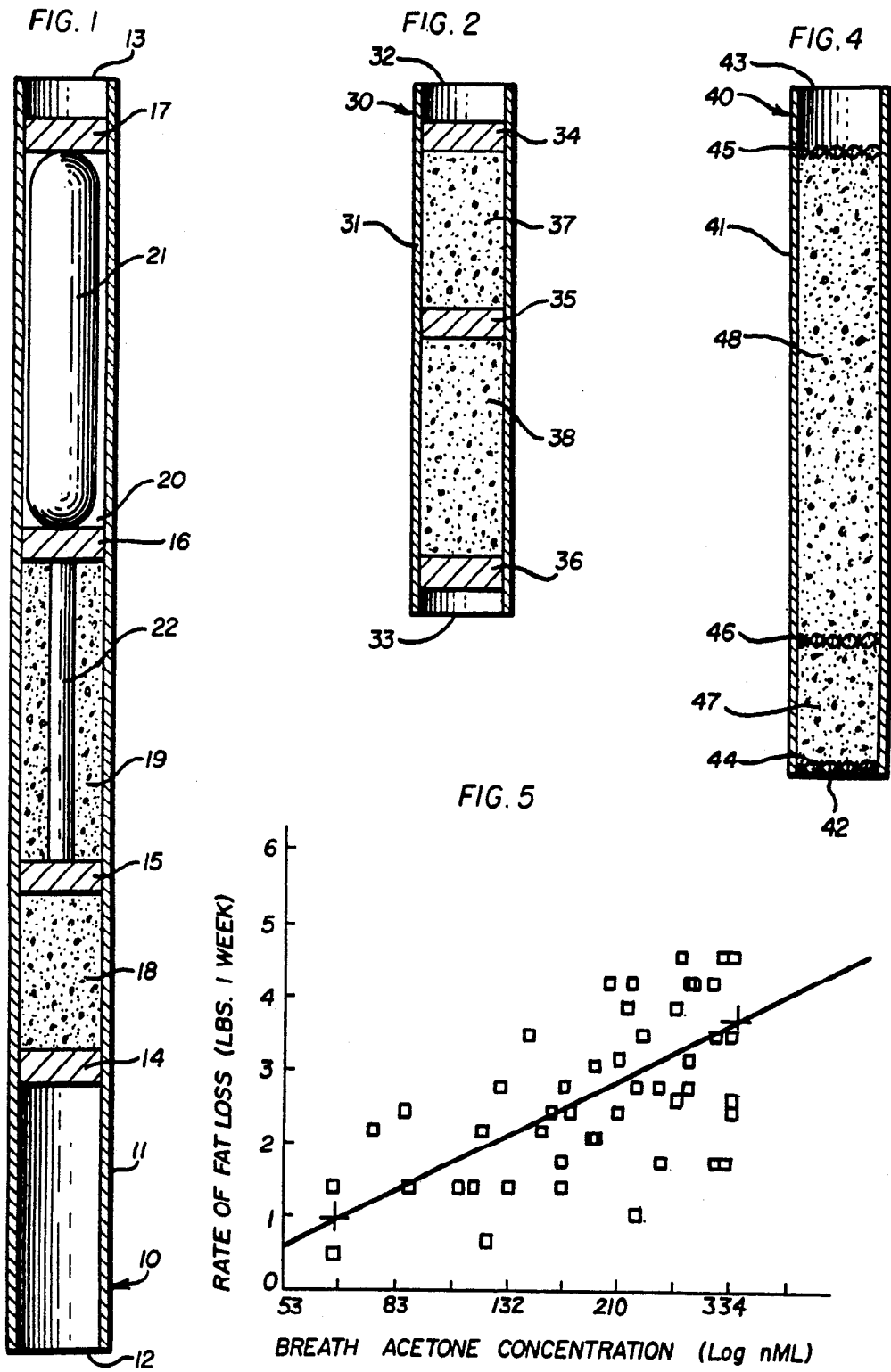


FIG. 3

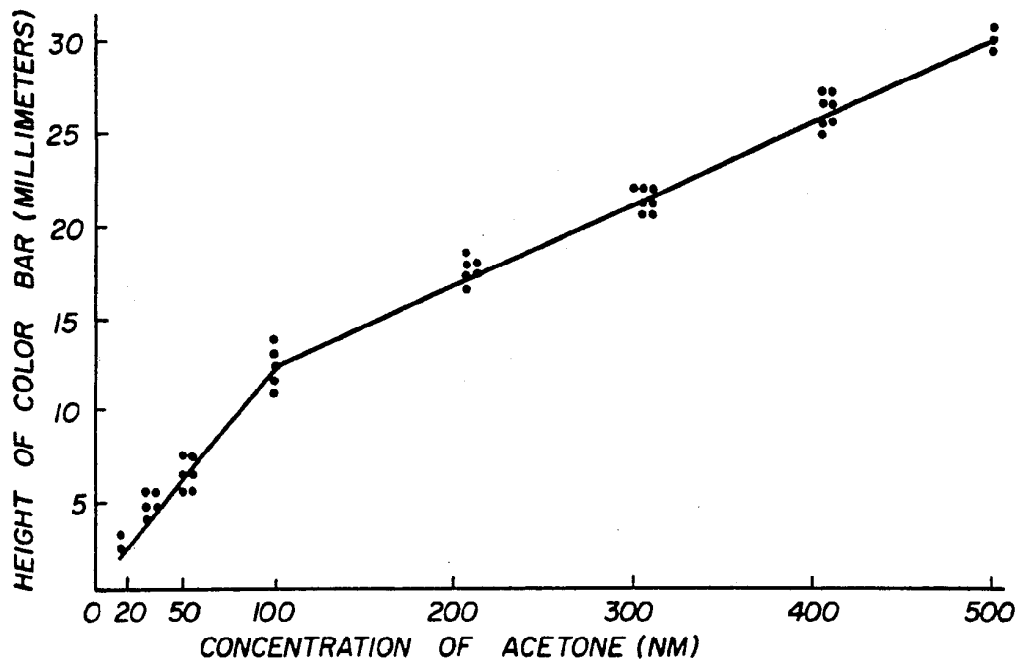


FIG. 6

WEIGHT, WATER AND FAT LOSS PROFILE OF 0-10 LBS. OVERWEIGHT DIETERS

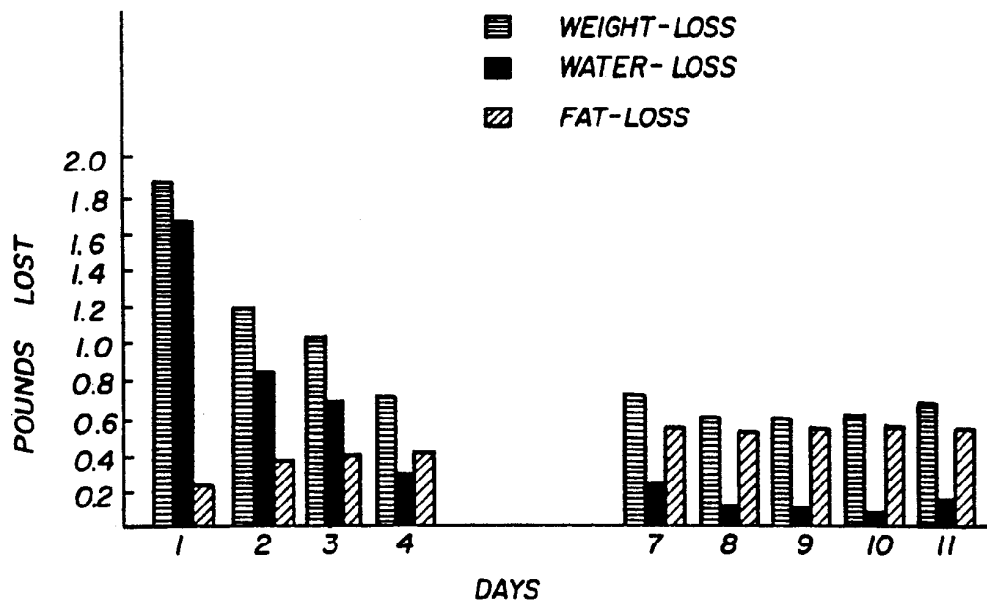


FIG. 7

WEIGHT, WATER AND FAT LOSS PROFILE OF  
10-20 LBS. OVERWEIGHT DIETERS

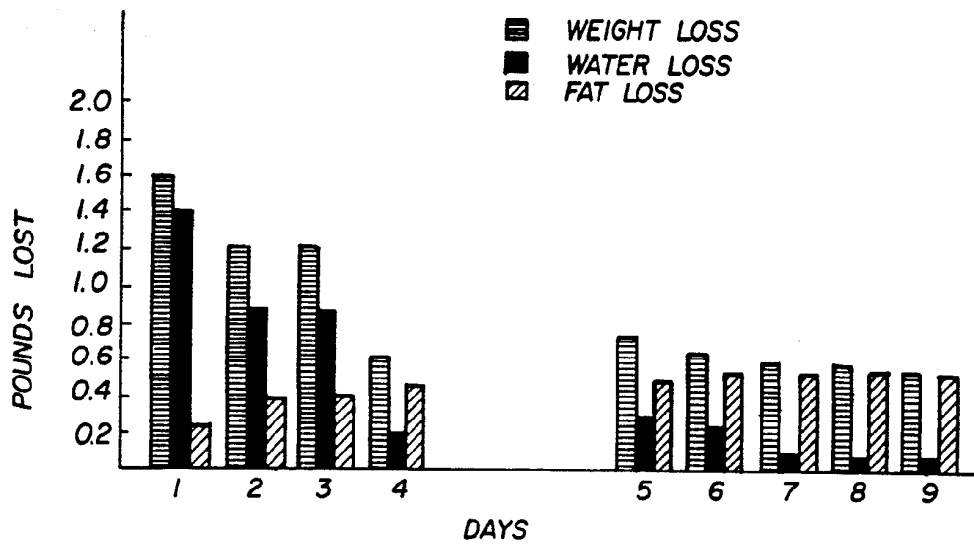


FIG. 8

WEIGHT, WATER AND FAT LOSS PROFILE OF  
20-40 LBS. OVERWEIGHT DIETERS

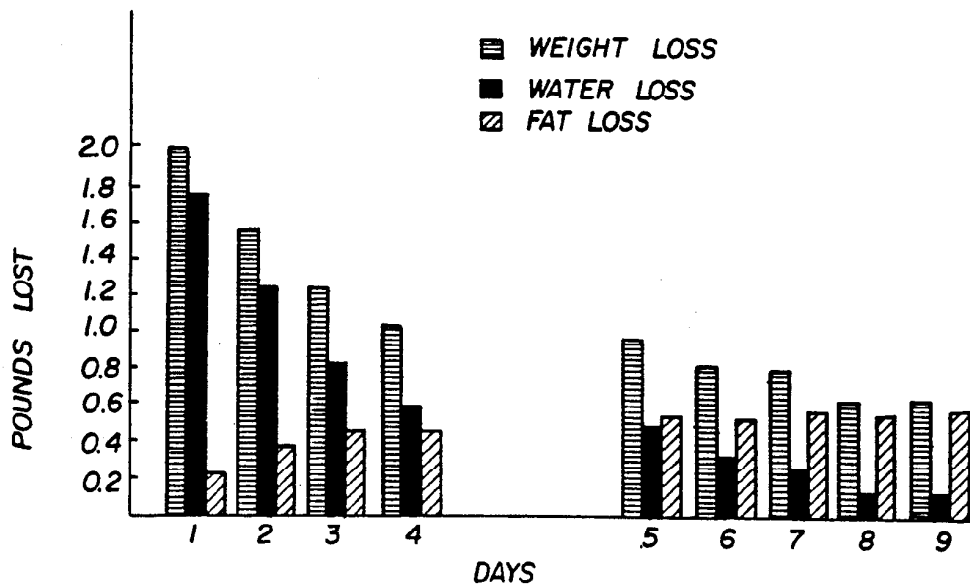
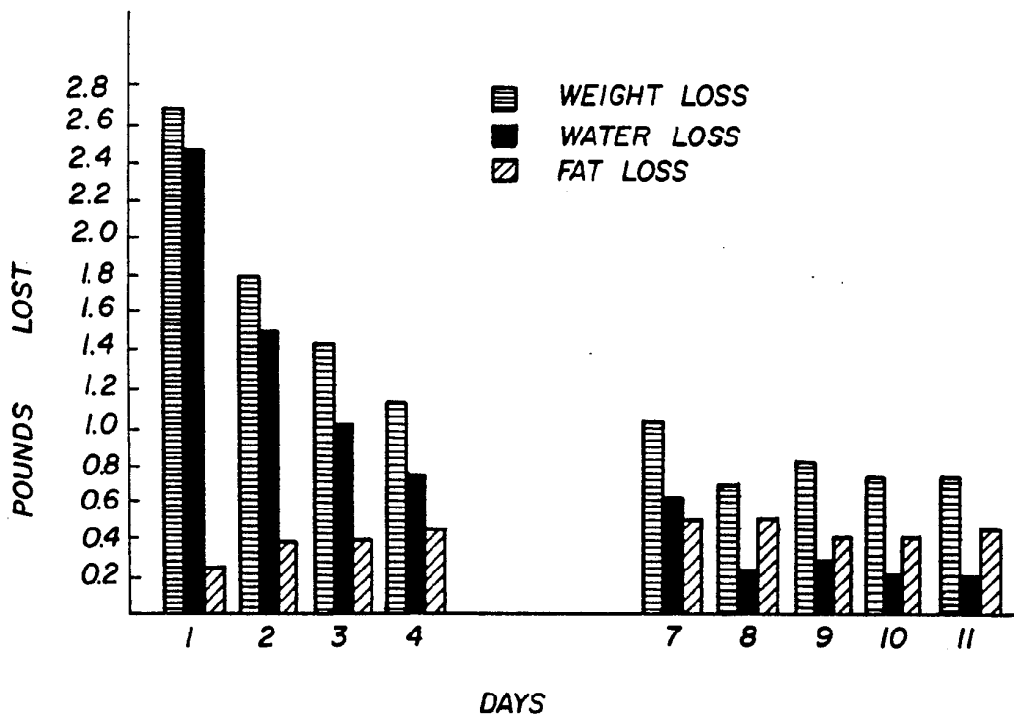


FIG. 9

WEIGHT, WATER AND FAT LOSS PROFILE OF  
40-100 LBS. OVERWEIGHT DIETERS



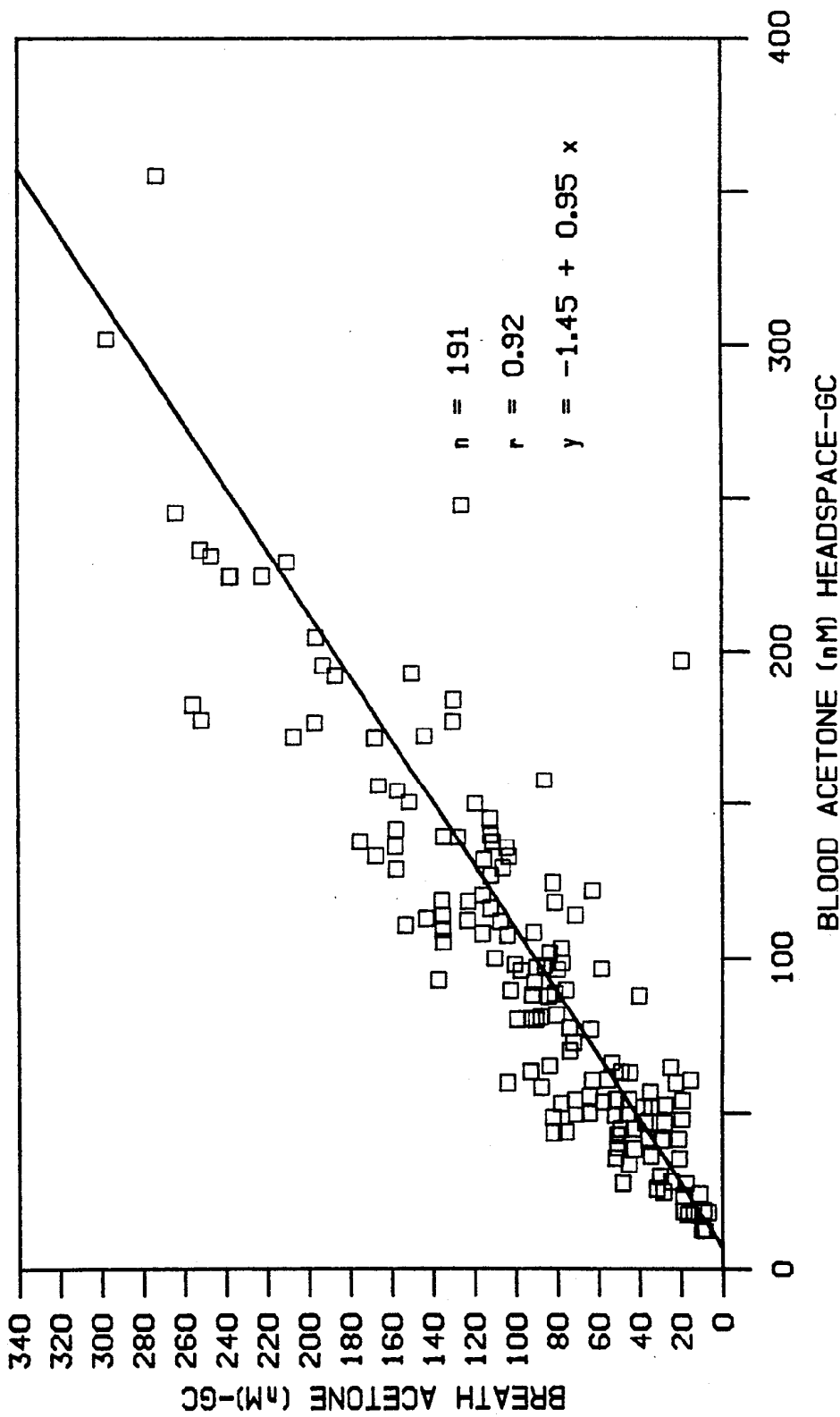


FIG. 10

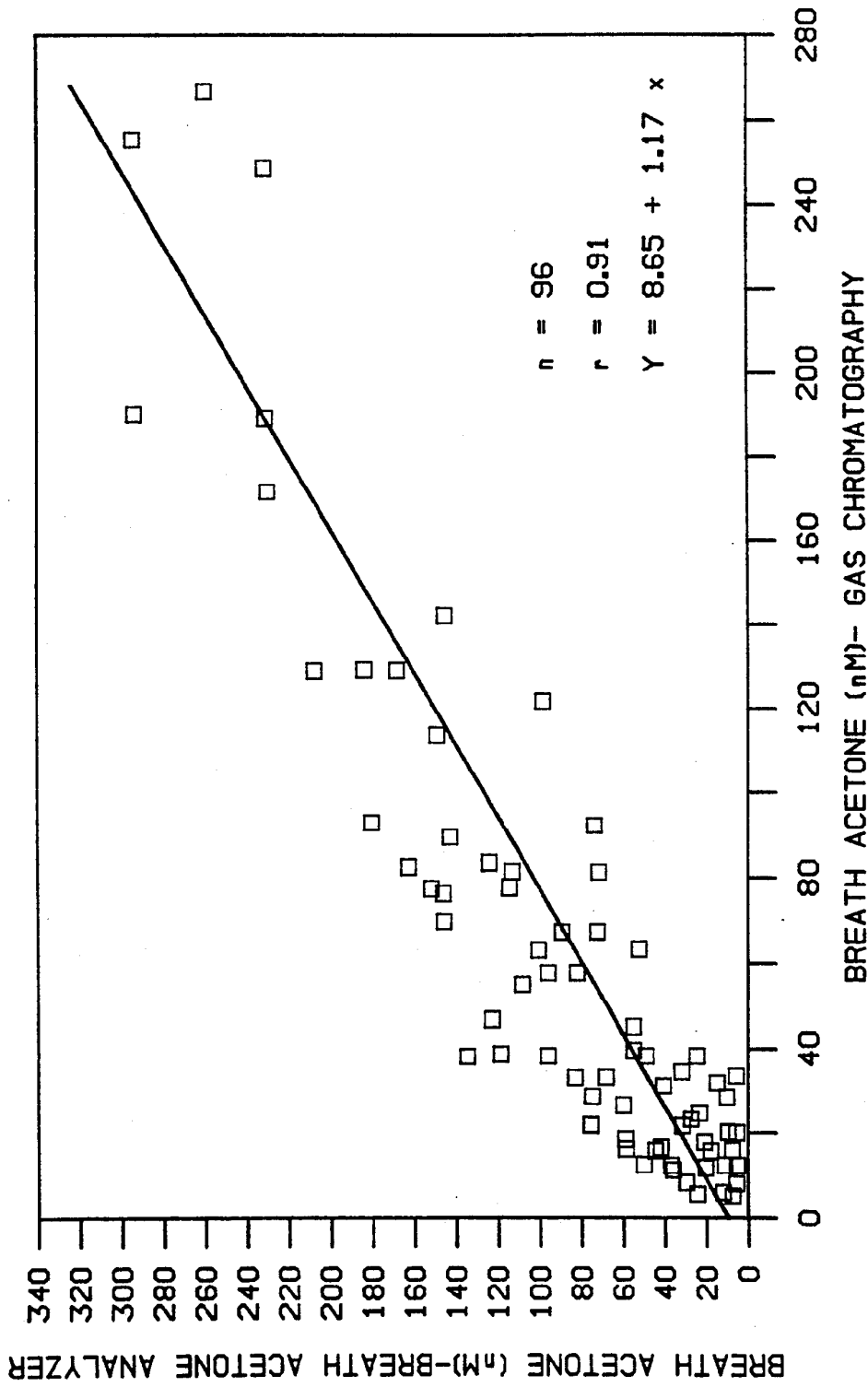
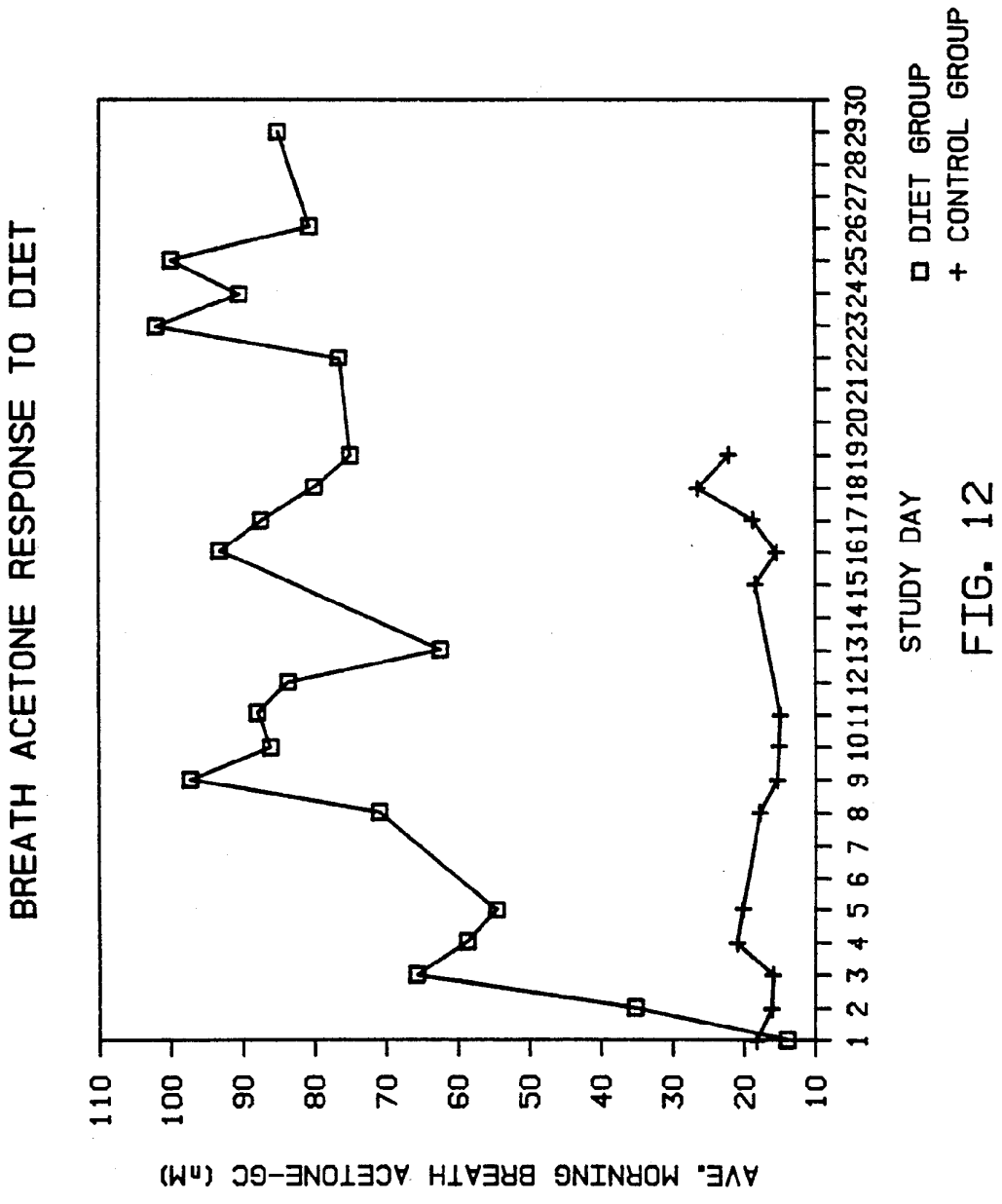


FIG. 11





AVERAGE COLOR BAR HEIGHT  
DIET AND NON-DIET GROUPS

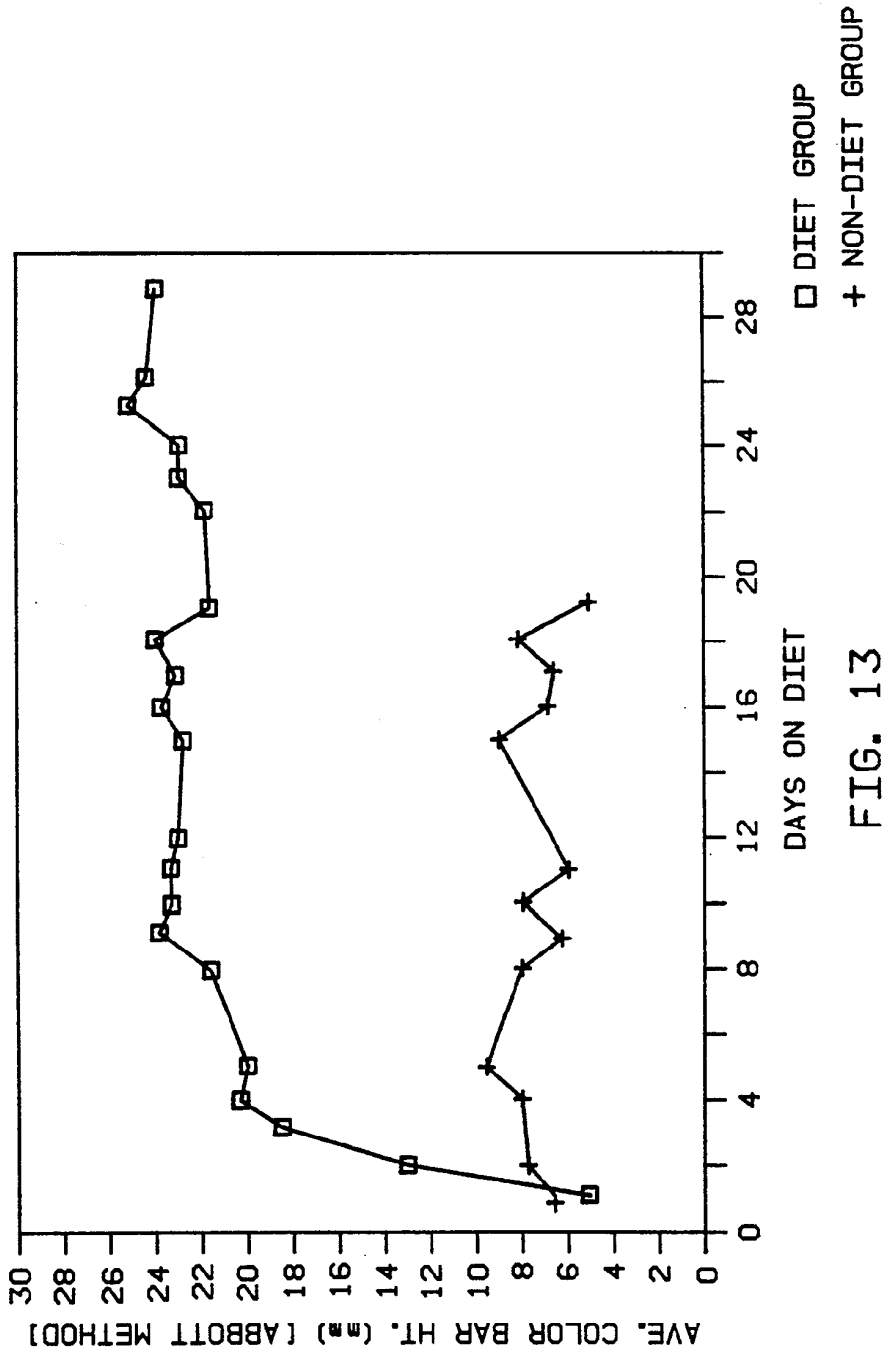


FIG. 13

DAILY CHANGES IN FAT LOSS TO DIET  
FOR INDIVIDUALS ON DIET & CONTROL GROUP

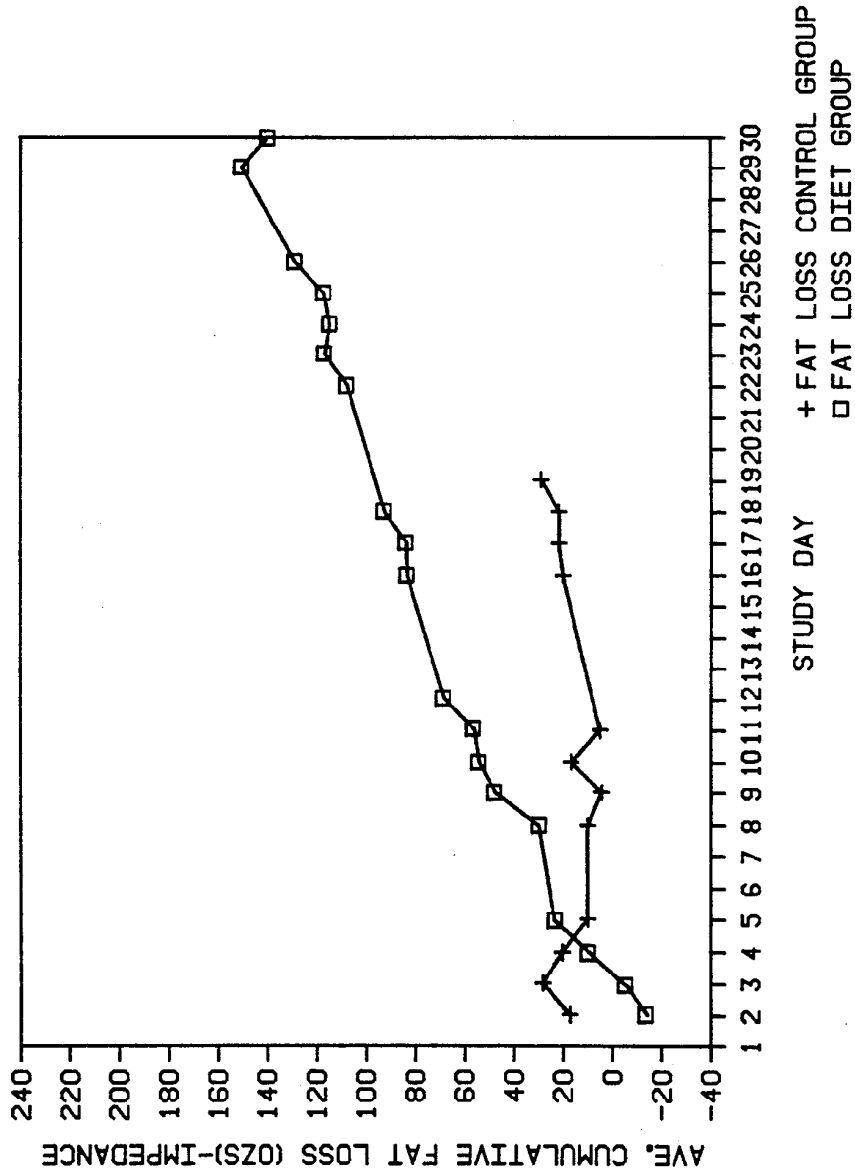


FIG. 14

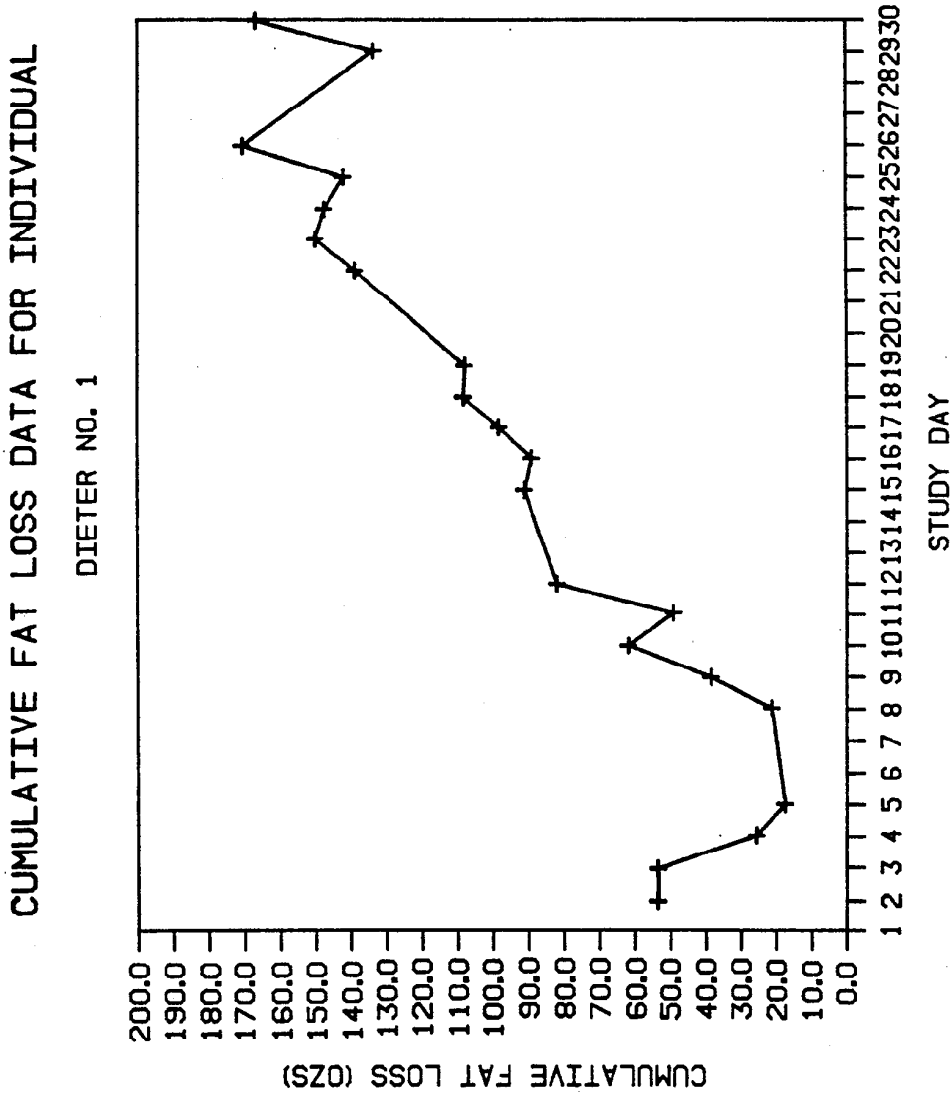


FIG. 15

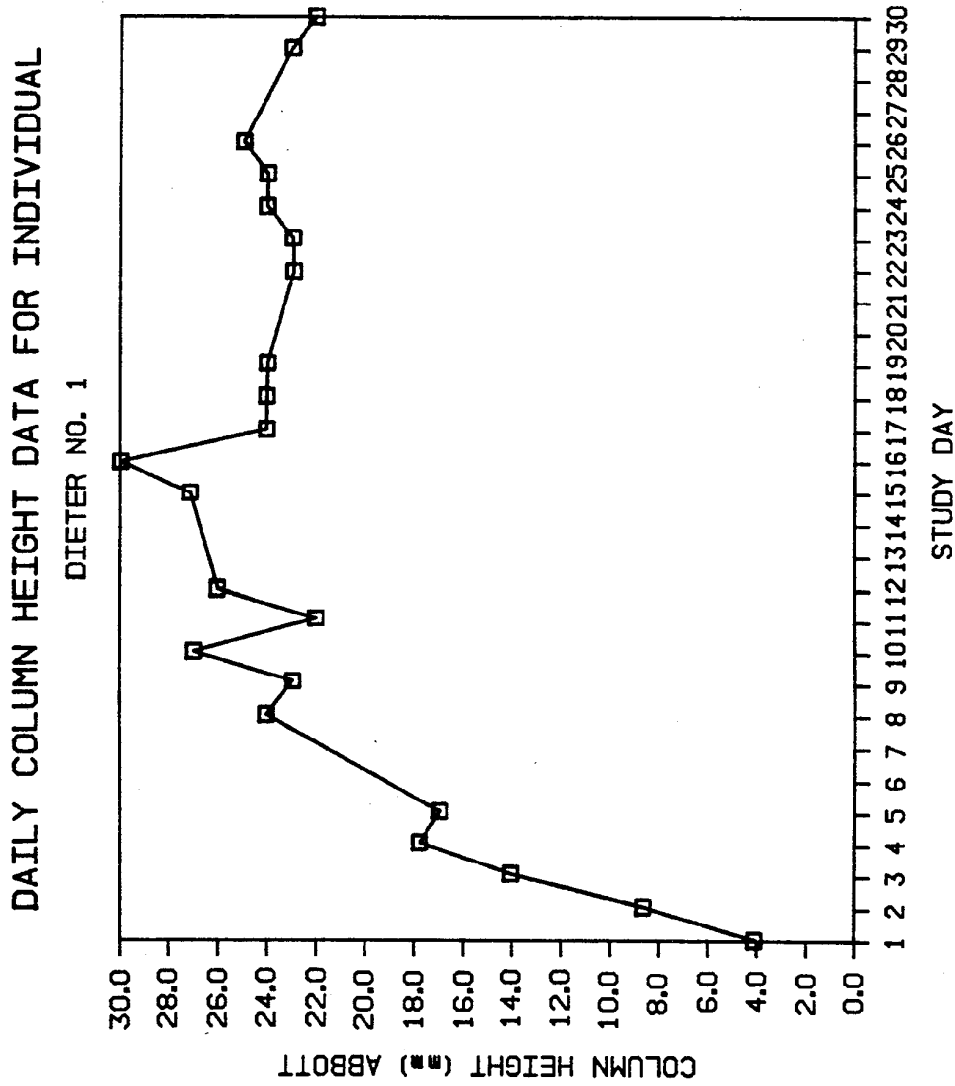


FIG. 16

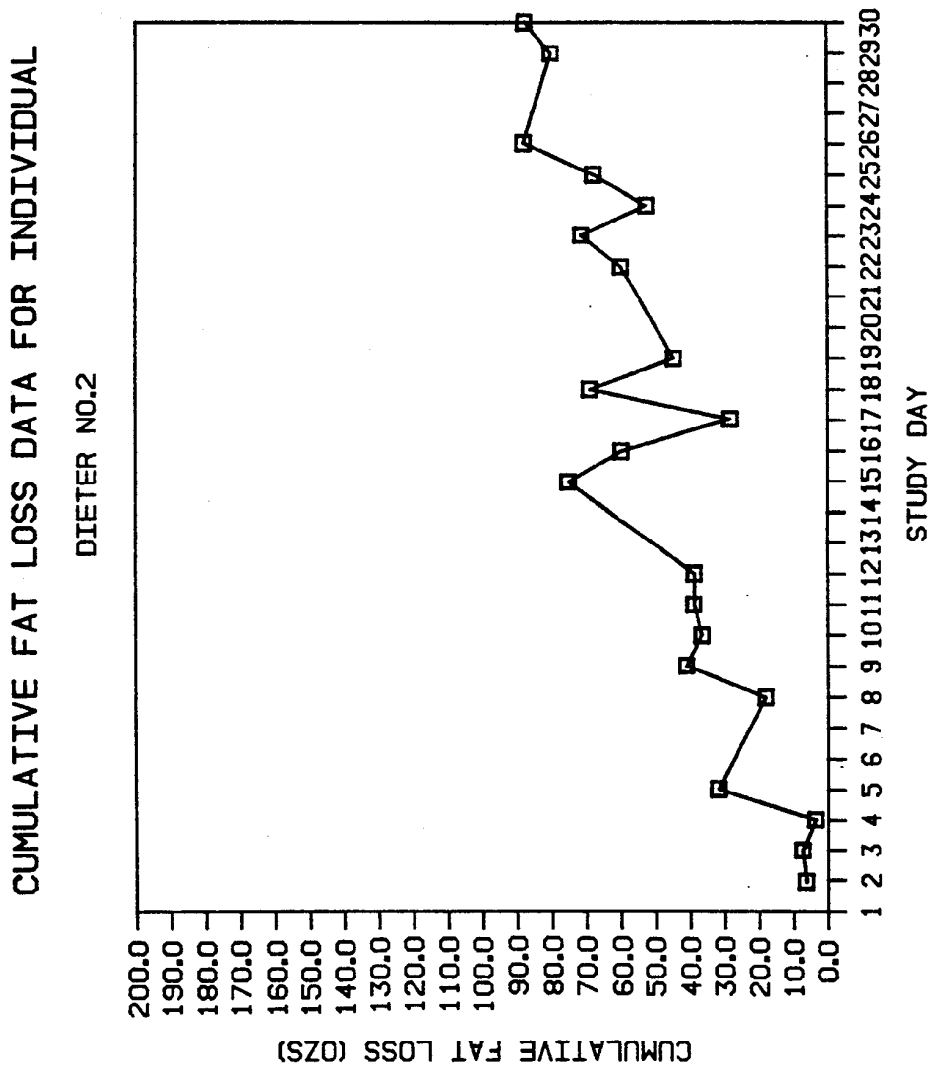


FIG. 17

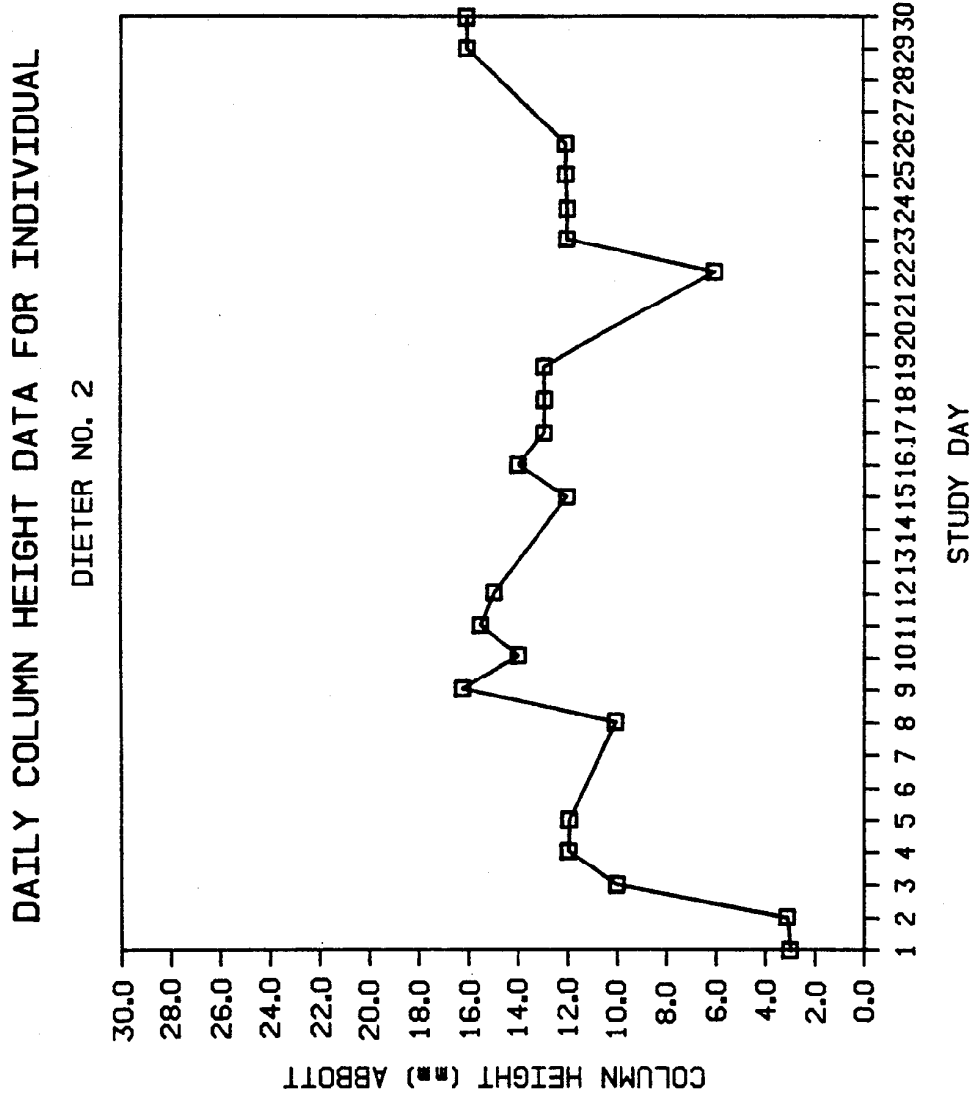


FIG. 18

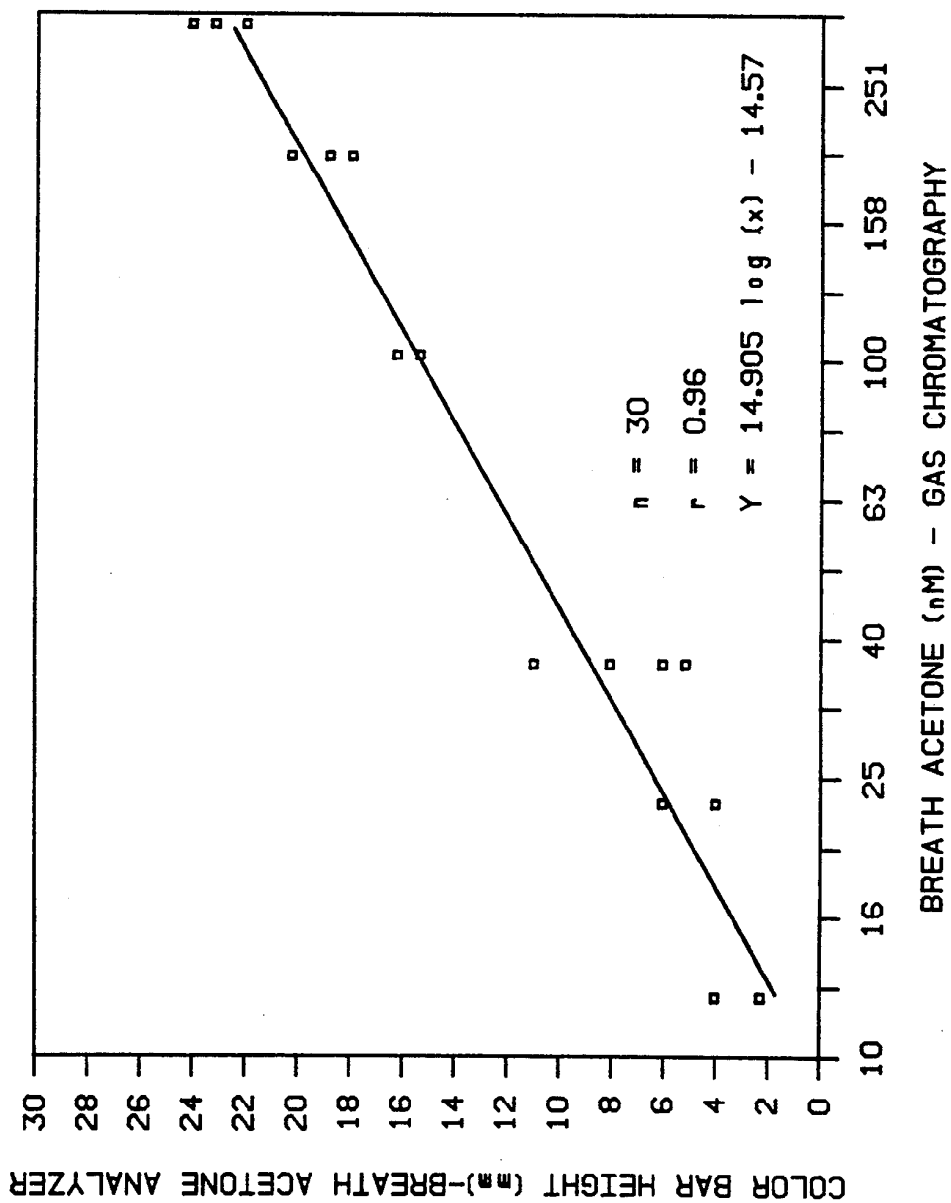


FIG. 19

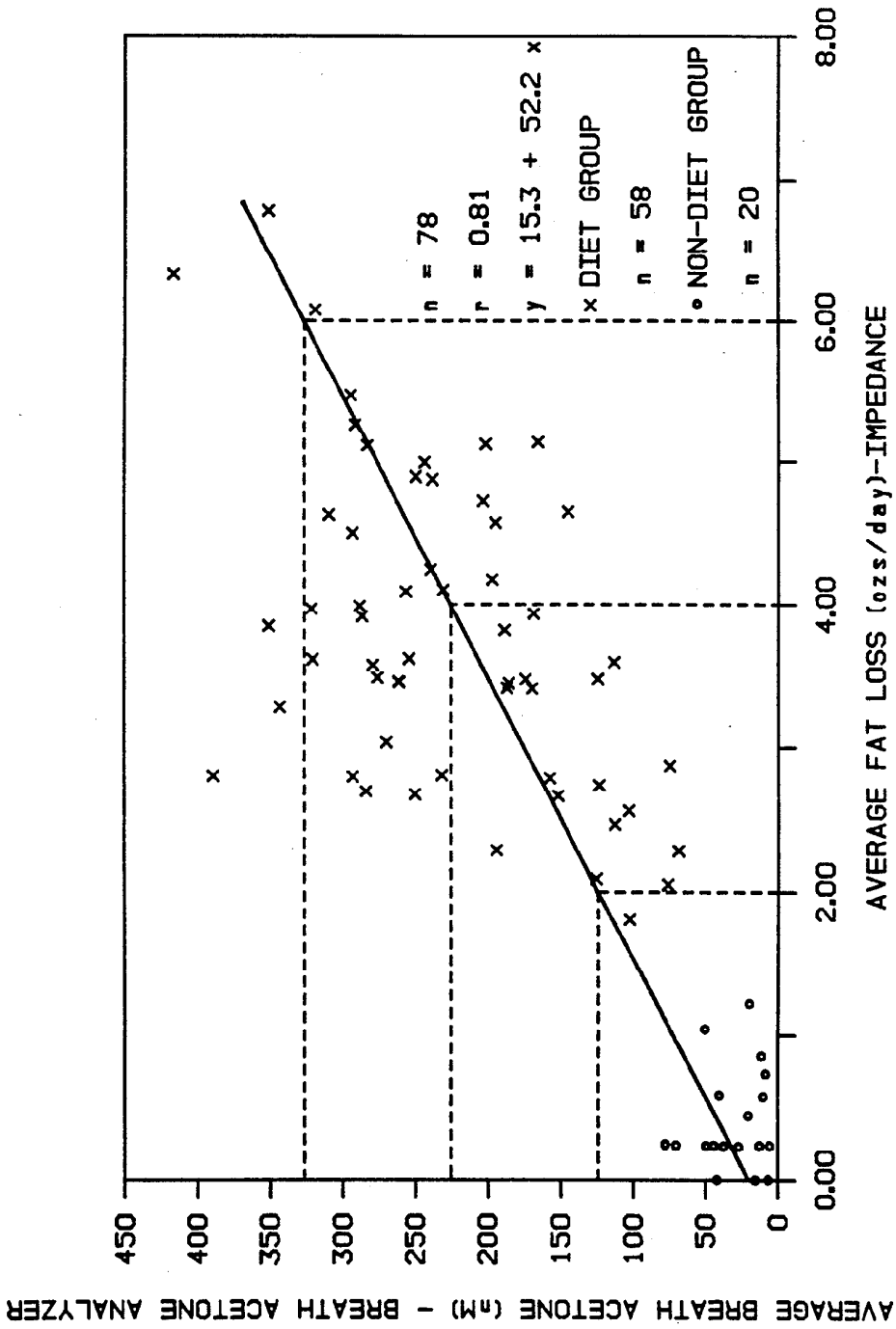
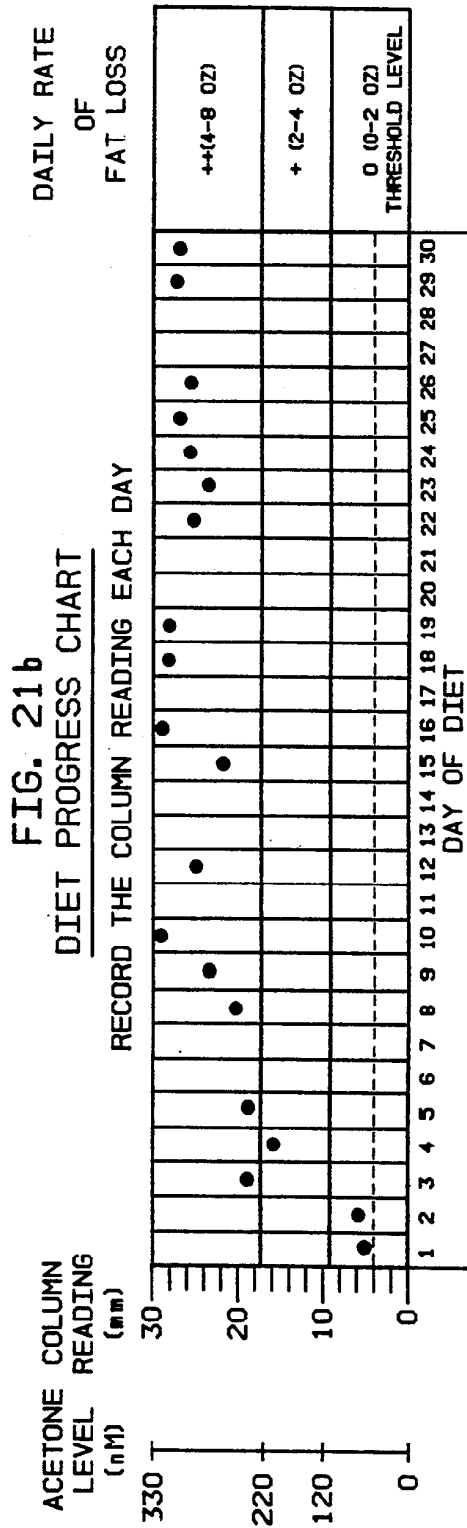
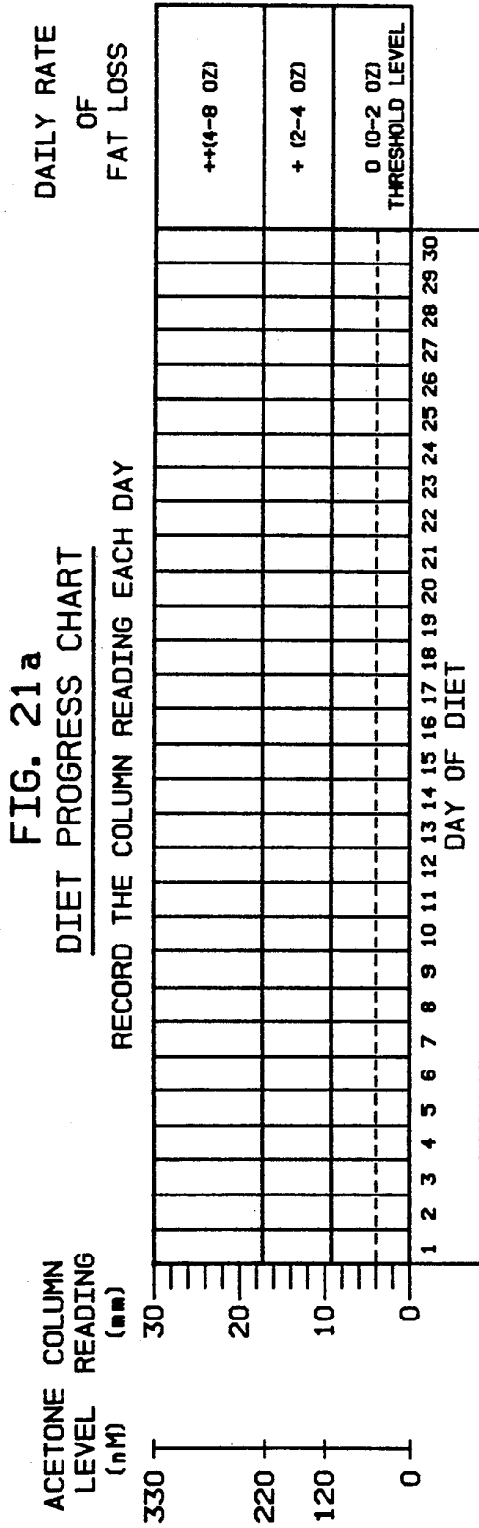


FIG. 20





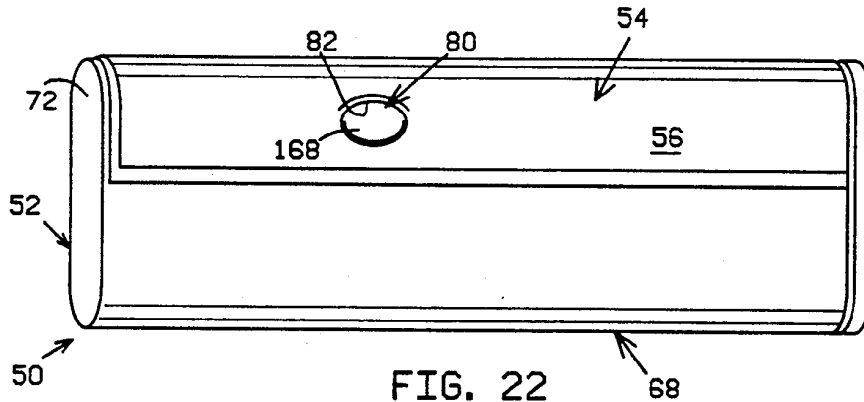


FIG. 22

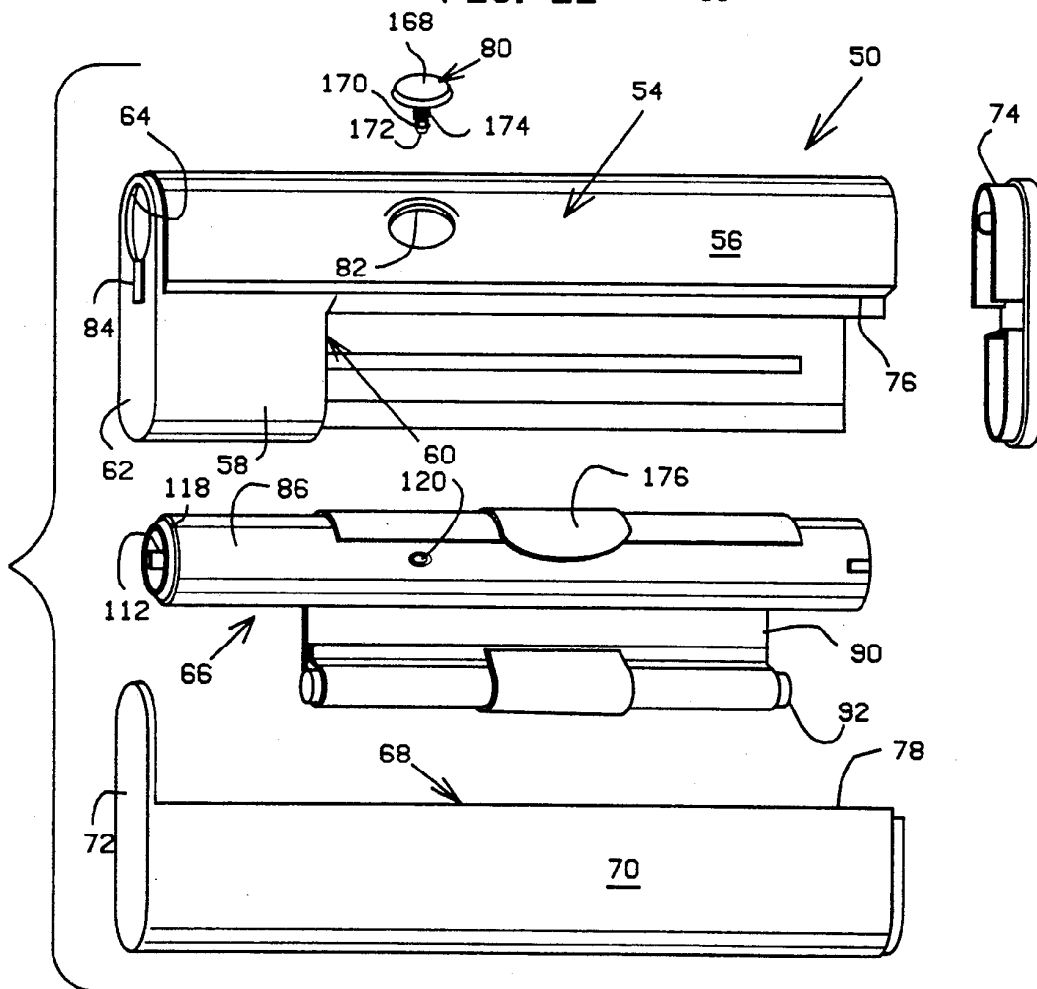
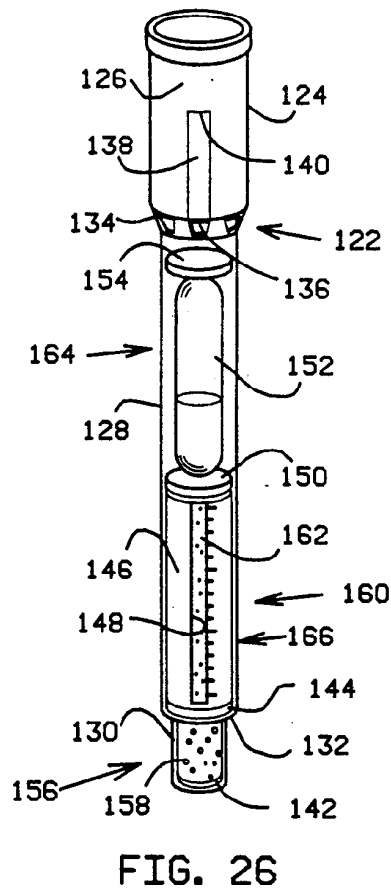
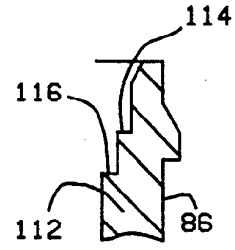
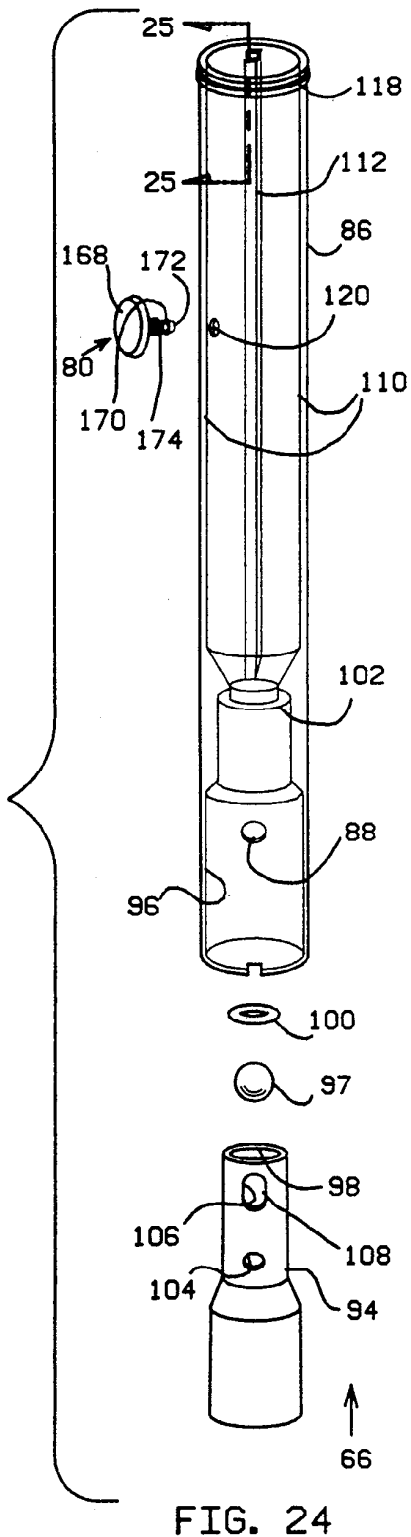


FIG. 23



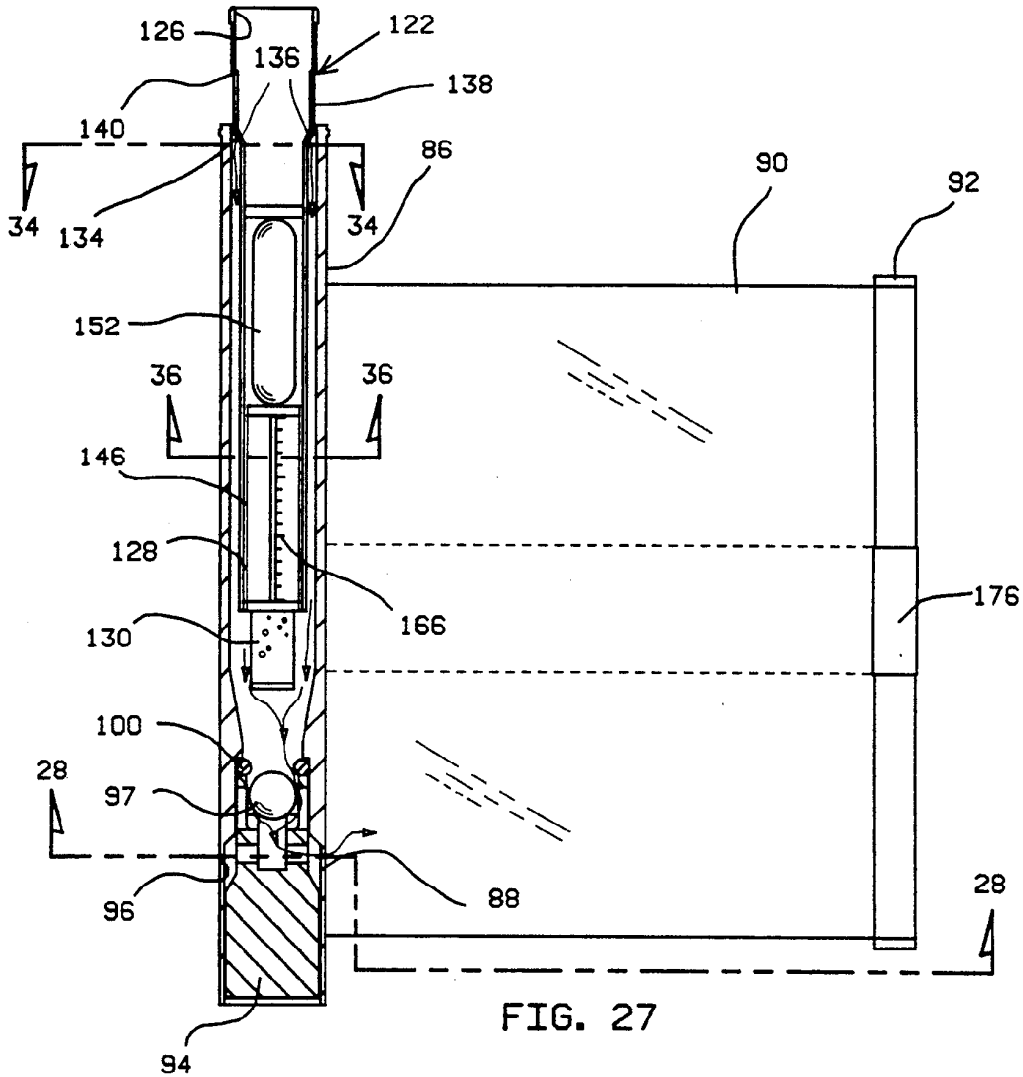


FIG. 27

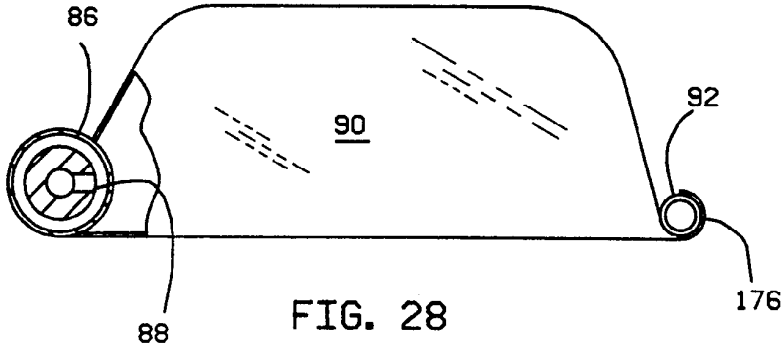


FIG. 28

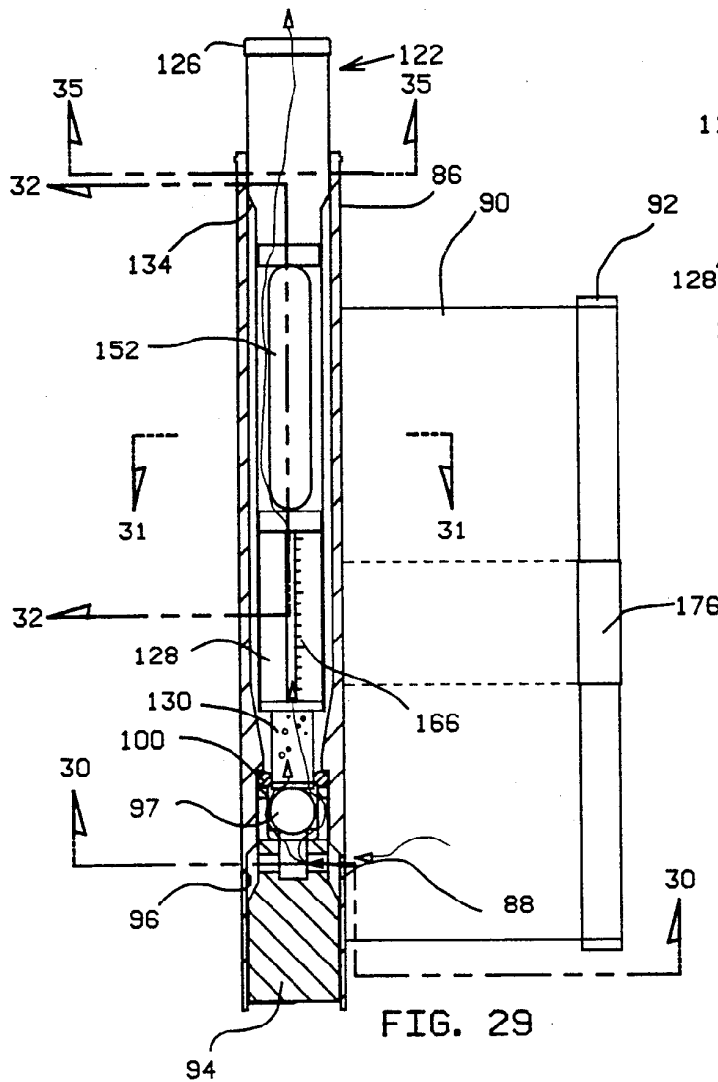


FIG. 29

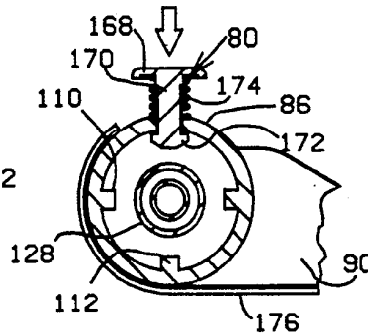


FIG. 31

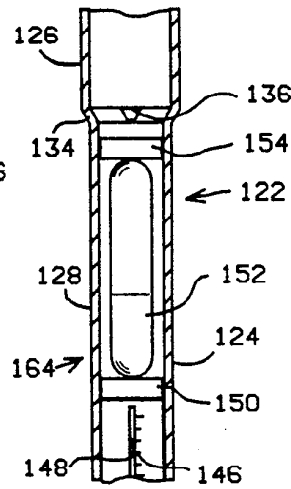


FIG. 32

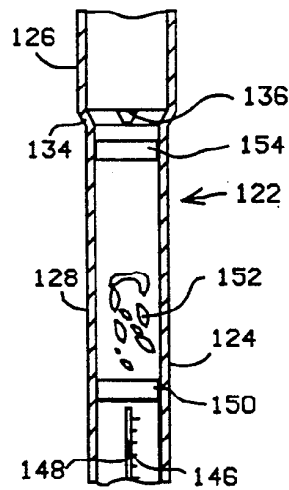


FIG. 33

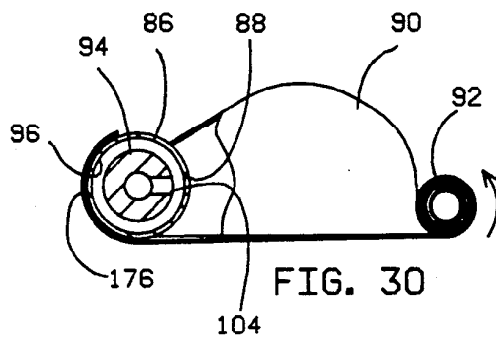


FIG. 30

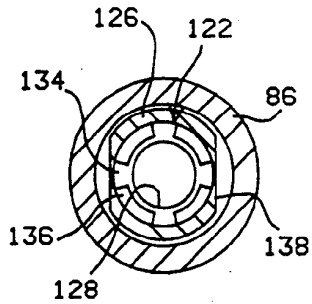


FIG. 34

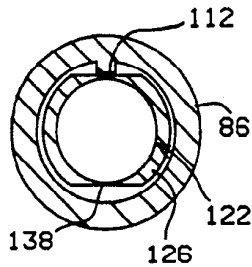


FIG. 35

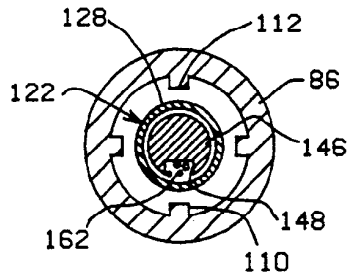


FIG. 36

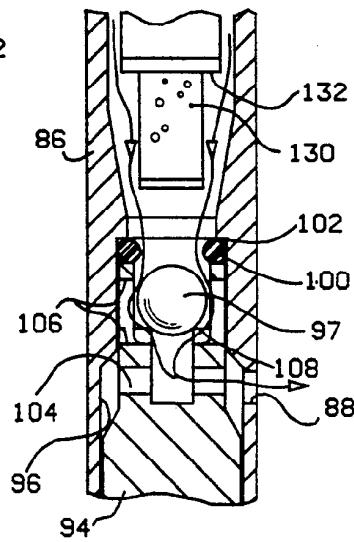


FIG. 37

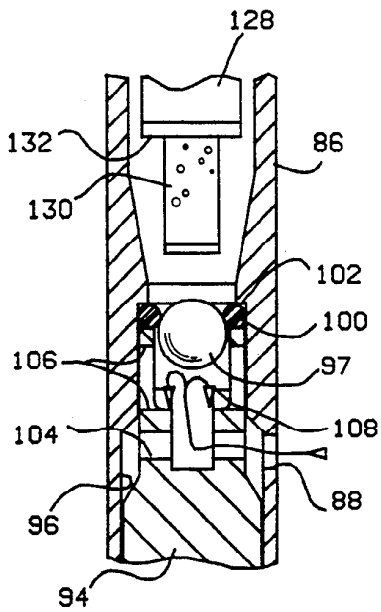


FIG. 38

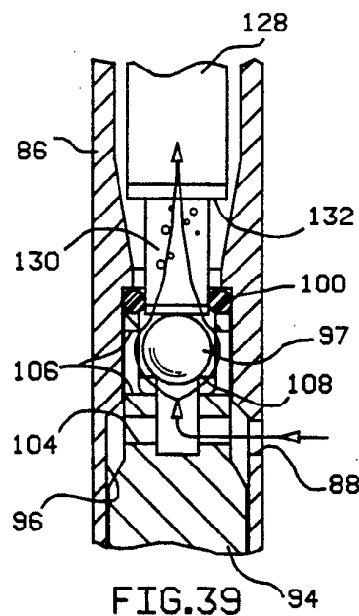


FIG. 39

**BREATH COMPONENT MONITORING DEVICE**

This is a divisional of U.S. patent application Ser. No. 07/131,811, filed Dec. 17, 1987, now U.S. Pat. No. 5,071,769, issued Dec. 10, 1991 which is a continuation-in-part of U.S. patent application Ser. No. 06/944,083, filed on Dec. 22, 1986, now U.S. Pat. No. 4,970,172, issued Nov. 13, 1990.

**BACKGROUND**

The present invention relates generally to methods and materials for the detection of ketones and aldehydes in fluid (liquid or vapor) samples. The invention is particularly directed to the quantitative determination of ketone and aldehyde concentrations in physiological fluids including blood, urine and breath samples. The invention further relates to methods and materials for monitoring the effects of diet, exercise and diabetic conditions through the quantitative measurement of breath acetone levels.

It is known that "ketone bodies" by which term is generally meant acetone, acetoacetic acid and  $\beta$ -hydroxybutyric acid, tend to accumulate in the blood stream during periods of relative or absolute carbohydrate deprivation due to the breakdown of storage triglycerides. The process through which overproduction of ketone bodies occurs is not well defined but is related to increased oxidation of long chain fatty acids by the liver. Specifically, acetoacetic acid and  $\beta$ -hydroxybutyric acid are formed by the liver as intermediates during the oxidation of fatty acid molecules by acetoacetyl coenzyme A. Acetone is formed from the spontaneous decarboxylation of acetoacetic acid. Under normal conditions the intermediate products are further degraded to carbon dioxide and water and the ketone products do not appear at significant concentrations in the bloodstream. Nevertheless, certain metabolic and disease states interfere with the normal degradation of these intermediates which then accumulate in the bloodstream as a result.

The quantitative measurement of ketone concentrations in blood serum is important because of the relationship between elevated serum ketone levels and clinical conditions such as diabetes, disorders of the digestive organs, renal insufficiency, uremia and malignant carcinoma. In the course of these disorders, ketone bodies pass into the blood stream and a state of metabolic acidosis (ketosis) occurs. Monitoring for the onset of ketosis is of particular importance in the maintenance of diabetics because the occurrence of ketosis may indicate the need for modification of insulin dosage or other disease management.

The concentration and identity of various ketone and aldehyde components present in the serum may be determined by direct chemical or chromatographic analysis. While such direct analysis provides the most accurate determination of serum ketone and aldehyde concentrations it suffers from numerous deficiencies including the requirement that blood be drawn to provide serum for analysis. Moreover, the analysis must be carried out promptly due to decomposition of acetoacetic acid to acetone during storage. In addition, the analysis of blood serum for ketones and aldehydes by chemical means requires the use of various reagents and procedures which can be complex and inconvenient for consumer use. Further, the use of certain chromatographic

techniques such as gas chromatography is often impractical for consumer and many types of professional use.

As a consequence of the limitations of measuring serum ketone levels directly, a large body of art has developed directed to the testing of urine for the presence of ketone bodies. It is known that the concentration of ketone bodies in urine bears an imperfect relationship to serum ketone concentrations. While urine ketone concentrations depend on numerous factors and are not always directly proportional to serum ketone concentrations, testing of urine for ketones is a simple and relatively inexpensive means of monitoring serum ketone concentrations. Such methods are in widespread use by diabetics in both home and clinical settings.

A number of test devices and methods for the determination of urine ketone concentrations are known to the art. Some assays utilize the reaction of acetone with salicylaldehyde in alkaline solution to give the deeply colored orange to red compound salicylalacetone. Any acetoacetic acid in such solutions is converted by the alkali to acetone which further contributes to the color reaction.

Kamlet, U.S. Pat. No. 2,283,262 discloses compositions for the detection of acetone and acetoacetic acid in solutions such as urine. The materials comprise a dry mixture of a member of the group consisting of the alkali metal and alkali-earth metal bisulfite addition products of salicylaldehyde and a member of the group consisting of the alkali metal and alkali-earth metal oxides and hydroxides.

Many assays take advantage of the "Legal" method which utilizes the reaction of a carbonyl group containing compound such as a ketone or an aldehyde with a nitroprusside (nitroferricyanide) salt in the presence of an amine to form a colored complex. While acetone will react, albeit slowly, with nitroprusside under aqueous conditions, the reaction of acetoacetic acid is some 100 to 200 times faster with the result that "Legal" reactions under aqueous conditions whether detecting "acetone," "acetone bodies" or "ketone bodies" primarily detect acetoacetic acid. The color reaction is believed to occur as a result of a coupling reaction through the nitroso group of the nitroprusside with the analyte to form an intermediate which then complexes with the amine to produce a color characteristic of the specific amine. In forming the complex, the trivalent iron of the nitroprusside is reduced to its divalent state. The color complex, however, is unstable because nitroprusside decomposes rapidly in alkaline solutions. Further, nitroprusside salts are subject to decomposition in the presence of moisture and high pH. Frequently during storage, a brown decomposition product is formed which can interfere with sensitive detection during assays. These limitations have led to numerous attempts to stabilize the color complex by utilizing mixtures of nitroprussides and amines or amino acids in combination with a variety of buffers, metal salts, organic salts, organic stabilizers and polymers. Numerous combinations of reagents have been shown to be suitable for detection of a variety of ketone bodies in liquid samples although the analyte predominantly detected in physiological fluids is acetoacetic acid.

Fortune, U.S. Pat. No. 2,186,902 discloses the use of soluble nitroprusside chromogens in the presence of ammonia and soluble carbonates for the detection of what was termed "acetone" (actually acetoacetic acid) in urine samples. Varying colorations are observable for the quantitative determination of "acetone" levels.

Galat, U.S. Pat. No. 2,362,478 discloses a solid reagent for the detection of "acetone" (actually acetoacetic acid) in liquid samples. The reagent comprises a dry mixture of a powdered anhydrous soluble nitroprusside, granular anhydrous soluble nitroprusside and granular anhydrous ammonium sulfate. The reagent signals the presence of "acetone" by producing a color reaction when a drop of sample is added thereto.

Free, U.S. Pat. No. 2,509,140 discloses improvements on the materials of Fortune comprising solid dry formulations which may be in the form of tablets for the detection of "acetone bodies" or "ketone bodies" in liquids. The materials comprise a nitroprusside salt, glycine and an alkaline salt.

Nicholls, et al., U.S. Pat. No. 2,577,978 discloses improvements on the dry formulations of Free for the detection of "acetone bodies" or "ketone bodies" in bodily fluids. Such compositions comprise alkali metal nitroprussides and alkali metal glycinates combined with sugars such as lactose, dextrose and sucrose.

While many assay devices of the prior art utilize dry tablets or powders in performing an assay, other assay devices utilize adsorbant carriers upon which some or all of the reagents have been dried. The adsorbant carriers may be in the form of strips which can be immersed in a sample of the liquid to be analyzed with the color reaction taking place in solution on the carrier. These assay devices, like those utilizing tablets or powders, suffer from decomposition of the nitroprusside indicator. In addition, indicator materials which are merely adsorbed onto the adsorbant carriers tend to suffer from diffusion of reagents away from the strip which affects the strength of the color signals. Further, the strips exhibit a certain amount of "bleeding" of color product in the aqueous environment which limits the stability of the color indicator signal of the reacted device.

Magers, et al., U.S. Pat. No. 4,147,514 discloses test strips for the detection of ketone bodies such as acetoacetic acid in bodily fluids utilizing a solution comprising nitroprusside in combination with at least one inorganic metal salt where the metal is selected from the group of magnesium and calcium. The solution optionally comprises at least one primary amine combined therewith. Test strips are dipped in the solution and are dried. They may be immersed in fluid samples and the occurrence of a color reaction observed.

U.K. Patent No. 1,012,542 discloses methods for the detection of ketone bodies in bodily fluids wherein alkaline components, in an aqueous solution are impregnated onto a carrier to which, sodium nitroprusside salt in an organic carrier also containing large amounts of an organic film-forming polymer is later applied. The carrier material is said to be very stable and is used for the detection of ketone bodies (acetoacetic acid) in liquid samples.

U.K. Patent No. 1,369,138 discloses improved methods for the detection of ketones in bodily fluids wherein an adsorbant carrier is first impregnated with a solution consisting of an amino acid, tetrasodium ethylenediamine-tetraacetate buffer and water which is then dried. The carrier is then impregnated with a solution of sodium nitroprusside in dimethyl formaldehyde and optionally an alcohol containing one to four carbons and is dried.

Smeby, U.S. Pat. No. 2,990,253 discloses a device for the detection of ketone bodies in fluid samples comprising a bibulous carrier onto which nitroprusside is first applied in an aqueous acidic media and to which is

subsequently applied a non-aqueous solution of organic bases such as amines or amino alcohols to achieve the alkalinity necessary for the assay reaction.

Mast, et al., U.S. Pat. No. 3,212,855 discloses an improved method for the production of a "dipstick" device for the detection of ketone bodies in fluids in which a bibulous carrier is first impregnated with an aqueous solution comprising an alkaline buffer and a water soluble amino acid. The carrier is then dried and impregnated with a solution in an organic solvent comprising an alkali metal nitroprusside and an organic film producing polymer.

Takasaka, Japanese Patent Application No. 1980-45270 discloses methods for the detection of ketones in body fluids utilizing test strips impregnated with alkali metal salts of nitroprusside and yttrium metal salts. The strips indicate a color reaction in acidic pHs in the presence of acetoacetic acid.

Federal Republic of Germany Patent No. 3,029,865 discloses improved test strips for the detection of ketones in bodily fluids comprising absorbant carriers impregnated with sodium nitroprusside, a water-soluble amino acid, an alkaline buffer compound and phosphoric acid trimorpholide as a stabilizer.

Kikuchi, Japanese Patent Application No. 1982-10208 discloses test strips for the detection of ketones in bodily fluids which are produced by immersion of absorbant carrier material in a solution comprising an amino acid, sodium triphosphate and sodium hydroxide and distilled water. The carrier strips are then dried and are immersed in a solution comprising a nitroprusside salt dissolved in dimethylformamide. They are then dried again and are ready for use.

Hirsch, U.S. Pat. No. 4,097,240 discloses a process for the production of dipstick devices for the detection of ketones in fluids such as urine. The process comprises the impregnation of an adsorbant carrier with sodium nitroprusside, an alkaline buffer substance and a water soluble amino acid. The carrier is first impregnated with an aqueous solution of amino acid and tetrasodium ethylenediamine tetraacetate buffer and dried. It is then impregnated with a solution of sodium nitroprusside in a solvent mixture consisting of methanol and an organic solvent miscible with methanol such as a linear or branched aliphatic alcohol with two to six carbon atoms.

Habenstein, U.S. Pat. No. 4,184,850 discloses a dipstick device for the detection of ketone bodies in fluids comprising an adsorbant carrier medium impregnated with sodium nitroprusside, a water-soluble lower amino acid, an alkaline buffer substance, and at least one organic acid which serves to form a stabilizing environment around the nitroprusside salt.

Kohl, U.S. Pat. No. 4,405,721 discloses devices for the detection of ketone bodies in bodily fluids comprising a carrier impregnated with a buffer, an amino acid, sodium nitroprusside and a heterocyclic stabilizing compound.

Tabb, et al., U.S. Pat. No. 4,440,724 discloses devices for the detection of ketone bodies in bodily fluids and methods for their preparation. The devices may be constructed according to steps comprising; impregnating a carrier with an aqueous solution of a soluble nitroprusside chromogen, drying the carrier, impregnating the carrier with an aqueous solution including a metal salt, a primary amine, TAPS (N-Tris (hydroxymethyl) 3-aminopropane sulfonic acid) and TRIS (tris-hydrox-



ymethyl aminomethane) and drying the carrier, the pH of the finished test device being no greater than 7.0.

Of interest to the present application is the disclosure of Ogawa, et al., U.S. Pat. No. 3,880,590 which discloses a dipstick device for the semiquantitative detection of acetoacetic acid in liquids such as urine. The Ogawa, et al. strip is said to be incapable of detecting other ketone bodies, such as acetone and 8-hydroxybutyric acid. The device comprises an absorbant material, a nitroprusside salt and a heavy metal salt such as nickel or ferric chloride. The absorbant materials include silica gel paper, diethylaminoethyl (DEAE) cellulose paper and amino ethyl cellulose paper with which the nitroprusside salt is associated. The absorbant strips are impregnated with a solution of a nitroprusside salt and a heavy metal salt in water or organic solvents including dimethyl formamide, dimethyl sulfonate methanol and ethanol or mixtures thereof. Solvents disclosed to be useful in forming the devices include dimethylformamide, dimethylsulfoxide, methanol and ethanol and mixtures thereof. According to one example, dimethyl formamide solution is used to impregnate DEAE cellulose paper along with nickel chloride and sodium nitroprusside. The strips were dried and later used to detect the presence of acetoacetic acid in urine. It is disclosed that the impregnating solution itself may be useful for the detection of ketone bodies but that the dried test strips are preferred in view of preservation, stability and handling considerations.

While references variously refer to the use of nitroprusside and amine compositions for the detection of "acetone", "acetone bodies" and "ketone bodies", the assays primarily detect acetoacetic acid and are generally incapable of distinguishing between reaction products formed from reaction of acetone and reaction products formed from reaction of other ketone bodies including acetoacetic acid. Other assays, such as those of Ogawa, et al. are disclosed to be incapable of detecting acetone at all. While numerous advances have been made with respect to "Legal" assays for the detection of ketones and aldehydes, such assays are still limited by the instability of nitroprusside at pHs greater than 7. Finally, such assays still measure only the concentrations of ketone bodies in urine and fail to necessarily provide accurate measurements of ketone bodies present in the blood serum.

It is well known in the art that breath samples may be assayed for the presence of acetone in order to determine serum acetone levels. Acetone is a relatively volatile compound having a partition coefficient of approximately 330. It readily diffuses from the blood into the alveolar air of the lungs according to an equilibrium relationship. As a consequence of this equilibrium state, concentrations of acetone in alveolar air are directly proportional to those in the blood and measurements of acetone in alveolar air can be used to determine the concentration of acetone in the serum. Crofford, et al., *Trans. Amer. Clin. Climatol. Assoc.* 88, 128 (1977). Crofford, et al. also discloses the use of head space analysis to determine the ketone concentration of liquid samples.

Current methods for the measurement of breath acetone levels include the use of gas chromatography. Rooth, et al., *The Lancet*, 1102 (1966) discloses the use of a gas chromatograph capable of detecting acetone at concentrations of 2 to 4 nM of air with 18 nM being the concentration for breath of normal individuals. Subjects breathe directly into the device and the acetone peak is

read after 40 seconds. Reichard, et al., *J. Clin. Invest.* 63, 619 (1979) discloses gas chromatographic techniques for the determination of breath acetone concentrations wherein breath samples are collected through the use of a calibrated suction flask into which the test subject breathes through a glass inlet tube. These methods and the instruments required for their use are complicated and expensive and tend to be impractical for use by consumers.

Other methods for the measurement of breath acetone levels involve the use of mass spectrographic equipment. Krotosynski, *J. Chrom. Sci.*, 15, 239 (1977) discloses the use of mass spectrographic equipment in evaluating the ketone content of alveolar air. Twelve ketone components of breath were identified with acetone comprising the major component. Mass spectrographic methods suffer from the same limitations, however, as relate to gas chromatographic techniques.

Methods utilizing color reactions for the detection of acetone in liquid or air have also been reported in the art. Greenberg, et al., *J. Biol. Chem.* Vol. 154-155, 177 (1944) discloses methods for the determination of acetone levels apart from those of other ketone bodies in solution. The methods involve reaction of acetone and other ketones with 2,4-dinitrophenylhydrazine, to form hydrazone products which may then be separated and isolated owing to differing solubilities.

Peden, *J. Lab. Clin. Med.* 63,332 (1964) discloses improvements over the methods of Greenberg, et al. utilizing salicylaldehyde as a coloring reagent. According to this method,  $\beta$ -hydroxybutyric acid is converted to acetone by oxidation with the amount of acetone formed measured by reaction with salicylaldehyde. Preformed acetone and acetoacetic acid are removed prior to the conversion of the  $\beta$ -hydroxybutyric acid by heating in the presence of acid. While these methods are useful for the determination of acetone concentrations apart from those of other ketone bodies they are complex and time consuming.

These various colorimetric methods known for detection of acetone in biological fluids are complex, time consuming and necessitate the use of a spectrophotometer or color charts. Moreover, the methods often require the use of high concentrations of alkali or acids. Alternative methods for the detection of acetone often require the use of complex and expensive apparatus. There thus continues to exist a need for methods for the quantitative determination of fluid acetone concentrations which are simple, accurate, inexpensive and do not require the use of high concentrations of hazardous reagents.

There exists a desire for methods for the measurement of the rate of fat catabolism. It is a particular problem that many individuals undergoing diets are unable to determine their rate of fat-loss because of daily variation in their body fluid content. Significantly, it is known that early in a diet individuals lose high proportions of fluid as compared to fat. Later in their diets, when individuals may still be catabolizing fat at a constant rate they may cease to lose fluids at the previous high rate or may, if only temporarily, regain fluid weight. The experience of hitting a plateau in weight loss or even regaining weight is psychologically damaging and weakens the subject's resolve to continue with the diet often with the effect that the subject discontinues the diet.

Recently, a method has been disclosed for the determination of daily rate of fat loss. Wynn, et al., *Lancet*,

482 (1985) discloses that daily fat-loss may be calculated by subtracting daily fluid and protein mass changes from daily weight changes. Changes in body water are estimated from the sum of external sodium and potassium balances and protein mass changes are calculated from nitrogen balances. Such a method is complex and time consuming thus making it inconvenient for the consumer.

One set of methods for measuring body fat is by quantitating total body water (TBW). A number of methods are available for determining TBW. These include isotopic dilution procedures using deuteriated water, tritiated water and  $^{18}\text{O}$ -labelled water. Urine, blood serum or saliva samples are collected after a 2 to 4 hour equilibration. The fluid samples are then vacuum sublimed and the concentration of tracer in the sublimate is determined by mass spectrometer, gas chromatography, or infrared or nuclear magnetic resonance spectroscopy. Body composition can also be measured by a bioelectrical impedance method using a body composition analyzer. These methods are well known in the literature and are readily performed by those of skill in the art. Equipment for performing such measurements is available commercially from medical instrument manufacturers such as RJL Systems, Inc. (Detroit, Mich.).

Hydrostatic weighing method is a well known method wherein the subject is completely submerged in a tank of water and the body fat is calculated by taking into account the average density of fat and the amount of water displaced. This method is inconvenient and is still not completely accurate because assumptions must be made relating to nonfat density, lung capacity and other factors. Another method for calculating the percentage of body fat utilizes skin calipers to measure the thickness of fat deposited directly beneath the skin. Pincers are used to measure the thickness of folds of skin and fat at various locations on the body. The results of these measurements are compared with standardized tables to arrive at a figure for percentage of body fat. This method, while more convenient than the use of hydrostatic weighing is less accurate. All methods for determination of body fat content suffer from the fact that they do not reveal the rate of fat loss but only the fat content of the body at a particular time. Because means for determining body fat content are of limited accuracy, means for the determination of the rate of fat loss are similarly limited. Nevertheless it is desired that a simple and convenient method be developed for the determination of the rate of fat-loss wherein such a method is capable of distinguishing weight loss due to loss of fat as opposed to weight loss from the elimination of bodily fluids.

Of interest to the present invention are observations that ketosis occurs in non-diabetic individuals undergoing weight loss through diet, fasting or exercise. Freund, *Metabolism* 14, 985-990 (1965) observes that breath acetone concentration increases on "fasting." It is disclosed that breath acetone concentrations increased gradually from the end of the first day of the fast to approximately 50 hours into the fast at which time the concentration rose sharply in a linear fashion and reached a plateau on the fourth day. The acetone concentration of the plateau was approximately 300  $\mu\text{g}/\text{liter}$  (5,000 nM) a hundred-fold increase over the normal value of 3  $\mu\text{g}/\text{liter}$  (50 nM). When, instead of fasting, the subject was placed on a "ketogenic" diet with a minimum of 92% of calories derived from fat, the subject suffered a lesser degree of ketosis wherein the

plateau had an acetone concentration of approximately 150  $\mu\text{g}/\text{liter}$  (2,500 nM).

Rooth, et al., *The Lancet*, 1102-1105 (1966) discloses studies relating to the breath acetone concentrations of a number of obese and diabetic subjects. When the caloric intake of three non-diabetic obese subjects was reduced, their breath acetone concentrations as measured by a gas chromatograph increased approximately three-fold. On fasting, the subjects' breath acetone concentrations increased to one hundred times normal. Within 16 hours after a heavy meal the subjects' breath acetone concentrations dropped almost to normal. In a study of obese diabetic patients, the authors disclosed evidence that those obese patients who had lost weight in the last three months had higher breath acetone concentrations than those patients who had gained weight.

Walther, et al., *Acta Biol. Med. Germ.* 22, 117-121 (1969) discloses the results of a study on the effects of continued exercise of a well-trained cyclist. The authors disclose that breath acetone concentration, increases prior to, during and after the cessation of the physical load and reached a maximum 15 to 20 minutes after cessation of the physical load. Breath acetone concentrations approach a normal level one to two hours after the cessation of the physical load. It is suggested that the increased production of acetone is due to the increased utilization of plasma free fatty acids in liver and reduced utilization in peripheral tissue.

More recent studies have shown a correlation between fasting in normal and obese patients and increased blood acetone levels. Rooth, et al., *Acta Med. Scand.* 187, 455-463 (1970); Goschke, et al., *Res. Exp. Med.* 165, 233-244 (1975); and Reichard et al., *J. Clin. Invest.* 63, 619-626 (1979) all show the development of ketosis in both overweight and normal individuals during fasting. Rooth, et al., (1970) suggests the use of breath ketone measurements as a motivational tool to enforce against dietary cheating. The studies disclose that development of ketosis is slower in overweight than in normal weight individuals. Reichard, et al., discloses that there is a better correlation between breath acetone and plasma ketone concentrations than between urine ketone and plasma ketone concentrations. In addition, Rooth, et al., (1970) discloses that certain urine ketone tests which detect the presence of acetoacetic acid are not entirely reliable because some individuals do not excrete acetoacetic acid in the urine despite increased blood serum concentrations.

Crofford, et al., (1977) discloses the use of breath acetone monitoring for monitoring of diabetic conditions and as a motivational tool in following patients on long-term weight reduction programs. Such monitoring is said to be particularly effective as normalization of the breath acetone is disclosed to occur upon significant dietary indiscretion. Patients' breath samples were monitored using a gas chromatograph and it is suggested that patients be instructed to restrict their caloric input to that which will maintain breath acetone concentrations of approximately 500 nM. It is further suggested that if breath acetone is controlled at this level and the proper balance of carbohydrate, protein and fat are maintained in the diet that weight loss will occur at a rate of approximately one-half pound per week.

#### SUMMARY OF THE INVENTION

The present invention relates to methods and materials for the detection of ketones and aldehydes in fluid (liquid or vapor) samples. The invention is particularly

directed to the quantitative determination of ketone and aldehyde concentrations in physiological fluids including serum, urine and breath samples. The invention is particularly suited for the determination of acetone concentrations. According to one aspect of the invention, methods are disclosed for the quantitative determination of serum acetone concentrations through the measurement of breath acetone concentrations. The method of breath acetone measurement utilizing the methods and materials of this invention is also adoptable for monitoring the insulin dose requirement for Type 1 insulin-dependent diabetic patients and to distinguish between Type 1 (ketotic) and Type 2 (non-ketotic) diabetic patients. Alternatively, concentrations of acetone or other ketones or aldehydes in serum, urine or other liquids may be determined by head space analysis of vapors in equilibrium with a liquid sample. According to further aspects of the present invention, liquid samples may be analyzed quantitatively in a liquid phase reaction for the presence of aldehydes or ketone bodies such as acetoacetic acid. According to still further aspects of the invention, methods are disclosed for ascertaining the fat catabolism effects of a weight loss dietary regimen comprising diet, fasting or exercise through the quantitative determination of serum or alveolar air (breath) acetone concentrations. Preferred methods for determination of the rate of fat catabolism comprise measurement of breath acetone concentrations and may be readily determined by utilizing the devices of the invention. The present invention also provides kits for the determination of fluid ketone and aldehyde concentrations and for the determination of the rate of fat catabolism.

The present invention comprises methods and materials for the determination of fluid ketone and aldehyde analyte concentrations through the reaction of analytes present in the sample fluid with a nitroprusside compound in the presence of an amine and a solvent to produce a colored reaction product. Devices according to the invention comprise a first solid matrix material to which a nitroprusside salt such as sodium nitroprusside is coupled. The devices further comprise a second solid matrix material to which is covalently bound an amine. According to one aspect of the invention, the nitroprusside salt and the amine may be coupled with and covalently bound, respectively, to the same solid matrix material. Preferably, however, the first and second solid matrix materials are in the form of discrete particles which are treated accordingly with a nitroprusside salt or an amine and are intermixed so as to place nitroprusside and amine moieties in intimate contact with one another. The solid matrix materials may be selected from a variety of materials including cellulose and silica gel which present suitable coupling moieties or are susceptible to reaction with suitable coupling moieties.

While the various methods of the present invention vary according to their specifics, they share the common aspect wherein ketone or aldehyde analytes present in a sample are contacted in the presence of a solvent with a nitroprusside salt coupled to a first solid matrix material and an amine covalently bound to a second solid matrix material. These materials together react to form detectable reaction products of characteristic colors which may then be observed for a qualitative or quantitative determination of the presence of ketone or aldehyde analytes.

Specific methods and configurations of devices for carrying out those methods are known according to the

identity of the analyte of interest and the nature of the sample material to be assayed. When the sample material is a vapor, a fixed quantity of the vapor may be collected by suitable means and the ketone or aldehyde analyte component preconcentrated on a preadsorbant material. The adsorbant for preconcentration of such analytes may be a material such as Tenax TA (a 2, 6-diphenyl-p-phenylene oxide polymer) or activated silica which may be maintained in the device in a preadsorbant zone separate from the first and second solid matrix materials associated with the nitroprusside salt and amine which are located in a reaction zone. After preconcentration, the ketone or aldehyde analytes are desorbed from the adsorbant by means of a solvent and contacted with the first and second solid matrix materials for reaction with the nitroprusside and amine reagents. According to a preferred embodiment, ketone and aldehyde components present in a vapor sample may be preconcentrated on the first and second solid matrix materials themselves. The analytes may then be solubilized by addition of a solvent to react with the nitroprusside and amine moieties present on the solid matrix materials. Where the analyte of interest is acetone methods for the assay of vapor samples, particularly breath samples, preferably utilize a desiccating bed for the preadsorption of water which can interfere with the quantitative detection of acetone.

In vapor sample devices wherein ketones and aldehydes are preconcentrated by adsorption onto the first and second solid matrix materials, a "linear reading system" for determination of analyte concentration may be utilized. The system provides a visual indication, in the form of a color bar of colored reaction products, indicating the quantity of ketone or aldehyde analytes adsorbed onto the first and second solid matrix materials. Because the adsorption sites on the matrix materials are limited, the extent to which ketone and aldehyde analytes will be adsorbed is dependent upon the quantity of analytes in the vapor sample. Analyte vapors will initially be adsorbed onto the solid matrix materials at the first portion of the reaction zone. As adsorption sites on those materials are saturated analyte vapors are adsorbed at more distant points within the reaction zone. Where the volume of the vapor sample is fixed, the distance from the first end of the reaction zone at which the analyte vapors are finally adsorbed is dependent upon the concentration of the analytes in the vapor sample. The extent to which the analytes are adsorbed, and hence the ketone or aldehyde concentration of the sample, is indicated by the extent of formation of colored reaction products.

When the sample material is a liquid such as urine or serum, head-space vapor assays may be carried out by analysis of vapor in equilibrium with the liquid for the presence of acetone and other volatile ketone and aldehyde components. After collection of a known volume of vapor in equilibrium with the liquid sample, the vapor is analyzed in the same way as breath and other vapor samples. Such head-space analysis is particularly suitable for analysis of the more volatile ketone and aldehyde fractions of samples as "lighter" analytes such as acetone will be present in the head space vapor in higher proportions than other less volatile "heavy" analyte components.

Quantitative liquid phase colorimetric assays may also be conducted on samples such as serum or urine according to the methods of the present invention. Liquid assays are useful for detection of most ketones and

aldehydes but are particularly useful for quantitative detection of less volatile analytes such as acetoacetic acid. According to such methods, liquid samples are applied to microcolumns packed with or dipsticks coated with the solid matrix materials of the invention. The presence of ketones or aldehydes in such samples produces a color reaction. Quantitative results can be obtained through use of ascending chromatography methods in microcolumns comprising the solid matrices of the invention. The concentration of ketone and aldehyde analytes present in the sample may be determined by the height of color bar produced in the tube. Where a dipstick coated with the solid matrix materials of the invention is used, analyte concentrations may be determined by visual or spectrophotometric evaluation of the color signal.

These liquid phase methods for analysis of liquid samples are not particularly suitable for detection of acetone in aqueous solutions such as bodily fluids, however, because the presence of water in these solutions retards the reaction rate of acetone to less than one one-hundredth the reaction rate of acetoacetic acid. Nevertheless, if the concentration of acetone in an aqueous solution is sufficiently high, the liquid phase methods may be adopted.

One preferred device of the present invention utilizes a breath collection device into which a subject breaths and which can collect a selected amount of alveolar air. The breath sample is then passed through the analyzer device wherein an anhydrous calcium chloride desiccant bed removes water vapor from the breath sample. The sample is then passed through a bed filled with a mixture of first and second solid matrix materials comprising nitroprusside-DEAE silica gel and aminopropyl silica gel where acetone contained within the breath is adsorbed into the matrix. The distance to which the acetone is adsorbed is dependent upon the total amount of acetone present in the sample. A solvent mixture containing either methanol or methanol and dimethyl sulfoxide (DMSO) is then added to the matrix to activate the color reaction and form a blue color bar. The length of the color bar is proportional to the concentration of acetone in the fixed breath sample volume and may be compared with a table or calibration marks on the side of the matrix bed to determine the breath and serum acetone concentrations.

The methods and materials of the present invention may be utilized to monitor diabetic patients, to analyze for various metabolic abnormalities or may be utilized according to one aspect of the present invention for the monitoring of the rate of fat catabolism. It has been found that serum acetone concentrations and hence breath acetone concentrations which can be measured by the methods and devices of the present invention may be correlated directly with the rate of fat catabolism and fat-loss experienced by a subject undergoing a weight loss dietary regimen comprising fasting, dieting, exercise or a combination of the three. Serum and breath acetone concentrations may be determined by a variety of means and the rate of fat-loss calculated therefrom according to the invention. The methods and devices of the invention, however, are extremely convenient, are accurate within about 10% in determining serum acetone levels and are therefore particularly suitable for measuring fat catabolism and the rate of actual fat-loss as opposed to determining weight loss which is variable and often reflects variations in fluid losses. By measurement of breath acetone levels, a sub-

ject will be able to estimate with a high degree of accuracy his rate of fat-loss, the water-loss/fat-loss ratio and be able to adjust his diet and amount of exercise according to his desired weight loss goals.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a view of a vapor test device of the present invention.

FIG. 2 is a view of an alternative vapor test device of the present invention.

FIG. 3 is a graph illustrating the relationship between the height of the color bar in a vapor test device of the present invention and the concentration of acetone present in a vapor sample.

FIG. 4 is a view of a liquid test device of the present invention.

FIG. 5 is a graph illustrating the relationship between breath acetone concentrations and the rate of fat loss corresponding thereto.

FIG. 6 is a graph illustrating the degree of water and fat loss for dieters 0 to 10 pounds overweight over a period of days.

FIG. 7 is a graph illustrating the degree of water and fat loss for dieters 10 to 20 pounds overweight over a period of days.

FIG. 8 is a graph illustrating the degree of water and fat loss for dieters 20 to 40 pounds overweight over a period of days.

FIG. 9 is a graph illustrating the degree of water and fat loss for dieters 40 to 100 pounds overweight over a period of days.

FIG. 10 is a graph illustrating the relationship between breath acetone concentrations and blood head-space acetone concentrations measured by gas chromatograph.

FIG. 11 is a graph illustrating the relationship between breath acetone concentrations as measured by a gas chromatograph and by devices according to the invention.

FIG. 12 is a graph illustrating the average morning breath acetone concentration for a dieting and a non-dieting population.

FIG. 13 is a graph illustrating the average height of an indicator color bar in breath acetone measurement devices according to the invention for a dieting and for a non-dieting population.

FIG. 14 is a graph illustrating the average cumulative fat loss for a dieting and for a non-dieting population.

FIG. 15 is a graph illustrating the cumulative fat loss for a first individual dieter.

FIG. 16 is a graph illustrating the daily height of an indicator color bar in breath acetone measurement devices according to the invention for the first individual dieter monitored in FIG. 15.

FIG. 17 is a graph illustrating the cumulative fat loss for a second individual dieter.

FIG. 18 is a graph illustrating the daily height of an indicator color bar in breath acetone measuring devices according to the invention for the second individual dieter monitored in FIG. 17.

FIG. 19 is a graph illustrating the relationship between the concentration of breath acetone and the height of an indicator color bar in breath acetone measuring devices according to the invention.

FIG. 20 is a graph illustrating the relationship between breath acetone concentrations and the rate of fat loss.

FIG. 21a depicts a diet progress chart for use in monitoring a diet program in conjunction with the present invention. FIG. 21b depicts the diet progress chart of FIG. 21a filled out to monitor a dietary weight loss program.

FIG. 22 is a perspective view of a breath-sampling kit of the present invention.

FIG. 23 is an exploded perspective view of the kit shown in FIG. 22.

FIG. 24 is an exploded perspective view of the outer tubular member of blow tube of the kit shown in FIGS. 22 and 23.

FIG. 25 is an enlarged fragmentary longitudinal sectional view taken generally along line 25—25 of FIG. 24.

FIG. 26 is a perspective view of a disposable analyzer column which is usable with the breath-sampling kit of FIGS. 22 and 23.

FIG. 27 is a side elevational view of the breath-sampling assembly of the present invention with the housing portions of the kit removed and showing the position of the parts and the flow of air when a user is blowing into the mouth piece to expand the inflatable bag and provide a known-volume of breath to be analyzed.

FIG. 28 is an end elevational view, partially in transverse section, of the assembly taken generally along the line 28—28 of FIG. 27.

FIG. 29 is a side elevational view of the assembly of FIG. 27 showing the position of the parts and the flow of air when the collected sample of air is being discharged from the bag through the analyzer column.

FIG. 30 is an end elevational view, partially in transverse section, of the assembly taken generally along the line 30—30 of FIG. 29.

FIG. 31 is a transverse sectional view taken generally along line 31—31 of FIG. 29.

FIG. 32 is a fragmentary longitudinal sectional view taken generally along line 32—32 of FIG. 29 showing the unbroken ampule of reactant in the disposable analyzer column.

FIG. 33 is a fragmentary longitudinal sectional view similar to FIG. 32 but showing the ampule of reactant after same has been broken.

FIG. 34 is a transverse sectional view taken generally along line 34—34 of FIG. 27 with the analyzer column in its first rotary and axial position (breath-receiving mode) in the blow tube.

FIG. 35 is a transverse sectional view taken generally along line 35—35 of FIG. 29 with the analyzer column in its second rotary and axial position (breath-discharging mode) in the blow tube.

FIG. 36 is a transverse sectional view taken generally along line 36—36 of FIG. 27.

FIG. 37 is an enlarged fragmentary longitudinal sectional view showing the valve operation of the breath-sampling assembly when a user is blowing into the mouth piece as shown in FIG. 27.

FIG. 38 is an enlarged fragmentary longitudinal sectional view similar to FIG. 37 that is showing the valve operation after the user has completely filled the breath collection bag, a static condition during which the breath sample is retained in the collection bag.

FIG. 39 is an enlarged fragmentary longitudinal sectional view similar to FIGS. 37 and 38 but showing the valve operation after the analyzer column has been rotated 90 degrees from its initial position and then pushed further inwardly into the blow tube to the position shown in FIGS. 29 and 39 whereby the collected

sample of breath is forced through the analyzer column and out through the mouth piece as a result of biased deflation of the collection bag.

#### DETAILED DESCRIPTION

The present invention comprises methods and materials for the determination of fluid ketone and aldehyde concentrations through the reaction of such carbonyl group containing compounds with a nitroprusside compound in the presence of an amine and a suitable solvent to produce a color reaction. Devices according to the invention comprise a first solid matrix material to which a nitroprusside salt is coupled and a second solid matrix material to which an amine is covalently bound. The addition of magnesium or calcium salts in the test composition promotes chelate formation thus stabilizing the color product and enhancing the kinetics of the reaction between the carbonyl compound, the amine and the nitroprusside.

Specifically, the first solid matrix material may be coupled to the nitroprusside salt by means of a suitable secondary or tertiary amine compound. The secondary or tertiary amine compound is itself coupled either directly to the first solid matrix material or to a coupling agent or coupling moiety which is attached to the first solid matrix material. Such coupling moieties include silane epoxides such as 3'-glycidoxypropyltrimethoxysilane having a first functionality reactive with materials such as silica gel and a second epoxide functionality reactive with an aspect of a secondary or tertiary amine compound. Matrix materials presenting suitable coupling moieties include gels, ion exchange resins, glasses and cellulosic materials which may be obtained commercially. Such matrix materials include diethylaminoethyl (DEAE) silica gels, DEAE cellulose, diethylamino (DEA) silica gel, aminoethyl (AE) silica gel, quarternary aminoethyl (QAE) silica gel as well as other weakly or strongly basic ion exchange materials.

Matrix materials comprising suitable coupling moieties for coupling of nitroprusside salts need not be obtained commercially, but may be produced according to known procedures in the art. In Kundu, et al., *J. Lipid Res.* 19, pp 390-394 (1978) the disclosure of which is hereby incorporated by reference applicant discloses methods for the preparation of DEAE-silica gel. In Kundu, et al., *J. Chrom.* 170, pp. 65-72 (1979) the disclosure of which is hereby incorporated by reference applicant discloses methods for the preparation of DEAE-silica gel as well as DEAE-controlled porous glass.

The second solid matrix material is covalently bound to an amine by means of a coupling moiety which may be initially coupled to either the solid matrix material or to the amine. Illustrative of suitable chemistry is the reaction between 3'-aminopropyltrimethoxysilane and silica gel to produce aminopropyl silica gel. Lower alkyl amine silica gels such as aminopropyl silica gel are available commercially but may readily be produced according to methods known to the art. In Kundu, et al., *J Lipid Res.*, 20, pp. 825-833 (1979) hereby incorporated by reference, applicant discloses suitable methods for the preparation of aminopropyl silica gel.

#### Nitroprusside Salts

Nitroprusside salts suitable for coupling with the first solid matrix material of the present invention include those salts capable of reacting with ketone and aldehyde analytes in the presence of an amine and a solvent to

produce a detectable color complex. Suitable nitroprusside salts include elemental metals and preferably alkali metal and alkali earth metal salts of nitroprusside. Preferred alkali metal salts of nitroprusside include sodium nitroprusside, while preferred alkali earth metal salts include salts of magnesium and calcium.

#### Secondary and Tertiary Amines

Secondary and tertiary amines suitable for coupling the nitroprusside salt to the first solid matrix material include those amines capable of forming an ionic complex with the nitroprusside salt and immobilizing it on the first solid matrix material. A preferred material is N,N-diethylethanolamine the hydroxy group of which can react with the epoxide moiety of a silane epoxide such as 3'-glycidoxypolytrimethoxysilane to form diethylaminoethyl substituted materials such as DEAE silica gel and DEAE cellulose.

#### Coupling Agents

Coupling agents suitable for use with the present invention include those agents having a first group reactive to form a bond with the first matrix material and a second group reactive to form a bond to a secondary or tertiary amine compound. Particularly preferred is the use of silane coupling agents having an alkoxy silane group. Preferred coupling agents include those such as  $\alpha$ -aminopropyltriethoxy silane, N- $\beta$ -(aminoethyl)- $\gamma$ -aminopropyl-trimethoxy silane and  $\alpha$ -chloropropyl triethoxy silane. Particularly preferred are silane coupling agents such as 3'-glycidoxypropyl trimethoxy silane having a first alkoxy silane group and a second epoxide group

#### Amines

Amines suitable for covalent binding to the second solid matrix materials of the present invention include those amines capable of reacting with ketones or aldehydes and nitroprusside materials in the presence of a solvent to produce a detectable color complex. Suitable amines include primary and secondary polyamines and primary and secondary lower alkyl amines with from 1 to 10 carbons. Primary amines are preferred although secondary amines are also suitable for methods and procedures of the present invention. Amines are coupled to the second solid matrix materials of the invention by means of coupling moieties. Typically the matrix materials are reacted with silane substituted amine-coupling agent conjugates such as 3'-aminopropyltrimethoxysilane. This material will react with a suitable matrix material such as silica gel or cellulose to produce aminopropyl silica gel or aminopropyl cellulose although the invention is not limited to aminopropyl moieties and other materials are equally suitable.

#### Solid Matrix Materials

Suitable solid matrix materials for coupling with nitroprusside salts and for covalent binding to amines include high surface area materials such as silica gels, glass materials such as controlled porous glass, granular cellulosic or agarose based materials, cross-linked dextran polymers, inorganic or organic ion exchanger materials, kieselsur and other silicate materials. Preferred first and second solid matrix materials for the vapor phase devices of the present invention are the high surface area gel materials such as silica gels which are characterized by their high surface area, high flow properties and exceptional dimensional stability. While

silica gels of varying sizes and porosities may be used, materials with pore diameters between about 60 and about 1000 angstroms and particle sizes between about 40 and about 400 microns are preferred. Particularly preferred are silica gel particles with pore diameters between about 100 and about 200 angstroms and particle sizes ranging between about 200 and about 400 microns. Most preferred for use as the first solid matrix materials for coupling with nitroprusside salts are diethyl amino (DEA) silica gel particles obtained from Diagnostic Specialties/Separation Industries, (Metuchen, N.J.). The particles are characterized by having particle sizes ranging from about 250 to about 400 microns, mean pore diameters of 130 angstroms; mean surface area of 194 m<sup>2</sup>/g; mean settle volumes of 1.9 cc/g; and an elemental composition comprising 10.90% C, 0.85% N, and 2.17% H. Other suitable first solid matrix materials include diethylaminoethyl (DEAE), aminoethyl (AE), quaternary aminoethyl (QAE) and other weakly or strongly basic ion exchangers on different organic or inorganic supports

First and second solid matrix materials suitable for the liquid phase detection devices of the present invention include those gel materials generally suitable for the vapor phase detection devices, although preferably with smaller diameters. A preferred DEA-silica gel for use as the first and second matrix materials in the liquid phase assays according to the invention may be obtained from Diagnostic Specialties/Separation Industries (Metuchen, N.J.). The material is characterized by having particle sizes ranging from about 40 to about 60 microns in diameter, mean pore diameters of 200 angstroms, mean surface area of 180 m<sup>2</sup>/g, mean settle volume of 1.8 cc/g; and an elemental composition of 10.57% C, 0.82% N and 2.10% H. Suitable materials additionally include a number of materials less suited for the vapor phase devices of the present invention such as cellulosic materials. Preferred cellulosic materials to be coupled with a nitroprusside salt include diethylaminoethyl (DEAE) cellulose and diethylamino (DEA) cellulose. A preferred material for the second matrix material is aminopropyl cellulose.

Numerous other appropriately substituted materials are suitable as the matrix materials of the present invention. These include:

(A) Natural polymeric carbohydrates and their synthetically modified, cross-linked or substituted derivatives, such as agar, agarose and cross-linked dextran polymers

(B) Synthetic polymers which can be prepared with suitably porous structures, such as (a) vinyl polymers, such as polyethylene, polypropylene, polystyrene, polyvinylchloride, polyvinylacetate and its partially hydrolysed derivatives, polyacrylates, polyacrylamides, polymethacrylates; (b) copolymers and terpolymers of the above vinyl monomers among themselves and with other monomers; (c) polycondensates, such as polyesters, polyamides and (d) addition polymers, such as polyurethanes or polyepoxides.

(C) Inorganic materials which can be prepared in a suitably porous form, such as sulfates or carbonates of alkaline earth metals and magnesium, e.g., barium sulfate, calcium sulfate, calcium carbonate, magnesium carbonate, or silicates of alkali and alkaline earth metals and/or aluminum and/or magnesium, and aluminum or silicon oxides or hydrates, such as clays, alumina, talc, kaolin, zeolite, silica gels and glass such as controlled

porous glass. These materials can be used as such or as fillers in one of the above polymeric materials.

(D) Mixtures or copolymers of the above classes, such as graft copolymers obtained by initiating polymerization of synthetic polymers on a pre-existing natural polymer.

The following examples disclose methods for the production of the solid matrix materials of the invention.

#### EXAMPLE 1

In this example, DEAE silica gel was prepared according to the procedure described by Kundu, et al., *J. Lipid Res.*, 19, 390-395 (1978). According to this procedure 100 grams of silica gel which was obtained from Diagnostic Specialties/Separation Industries (Metuchen, N.J.) was deaerated under vacuum for 30 min. and then heated at 45° C. for 20 hours with a mixture containing 1000 ml of 10% 3'-glycidoxypropyltrimethoxysilane (Polyscience, Inc., Warrington, Pa.) and 100 ml of N,N-diethanolamine (Aldrich Chemical Co., Milwaukee, Wis.). The reaction mixture was allowed to cool to room temperature. It was filtered through a coarse-porosity sintered glass funnel and washed with 2 liters of methanol to remove unbound reactants and by-products. The silica matrix was then converted to the chloride form by treatment with hydrochloric acid until the pH became 4.5.

According to an alternative procedure described by Roy and Kundu, *Anal. Biochem.*, 98, 238-241 (1979), 100 grams of silica gel is heated with 1000 ml of 10% 3'-glycidoxypropyltriethoxysilane in toluene at 60° C. for 15 hours. After cooling to room temperature, the reaction mixture is filtered and washed with 2 liter of acetone and dried under vacuum to yield epoxy silica gel. The epoxy silica gel (100 g) is heated with 1000 ml of 10% diethylamine (Sigma Chemical Company, St. Louis, Mo.) in toluene at 50° C. for 20 hours. Diethylamino (DEA) silica gel thus obtained is processed to the chloride form as described above for DEAE-silica gel).

The DEA-silica or DEAE-silica gel prepared according to the above procedure is then treated with sodium nitroprusside alone or sodium nitroprusside mixed with magnesium or calcium sulfate to form nitroprusside DEA or DEAE-silica. According to one procedure, one hundred gram aliquots of DEAE silica gel were then taken in dark bottles and each mixed with one liter of aqueous solution of sodium nitroprusside at concentrations of 2 g/liter, 4 g/liter, 5 g liter, 6 g liter, 8 g/liter and 10 g/liter. The mixtures were rotated in the dark for 5 minutes, filtered on coarse-porosity sintered glass funnels and dried thoroughly under vacuum. Alternatively, the DEA or DEAE-silica materials were additionally treated with nitroprusside at concentrations of 2 g/liter, 4 g/liter, 5 g/liter, 6 g/liter, 8 g/liter and 10 g/liter mixed with equimolar amounts of magnesium sulfate. The mixtures were then rotated in the dark for 5 minutes, filtered on coarse-porosity sintered glass funnels and dried thoroughly under vacuum.

The total binding capacity of this preferred DEA silica gel matrix was 100 mg nitroprusside per gram of matrix. The binding efficiency of sodium nitroprusside alone or when mixed with equimolar amounts of magnesium sulfate was 100% for materials treated with nitroprusside at concentrations of 2 to 5 g/liter, 98% for materials treated with 6 g/liter, 96% for 8 g/liter and 90% for 10 g/liter. Because the nitroprusside-DEA or DEAE silica gel matrix is sensitive to light these opera-

tions were performed so as to avoid direct exposure to light. Nevertheless, the matrix is stable at room temperature for extended periods if protected from the light.

#### EXAMPLE 2

In this example, aminopropyl silica gel was prepared according to the procedure described by Kundu, et al., *J. Lipid Res.*, 20, 825-833 (1979). In this method, 100 grams of silica gel was deaerated under vacuum for 30 min. and then shaken at 50° C. for 20 hours with 600 ml of a solution comprising 10% (by weight) 3'-aminopropyltriethoxysilane (Polyscience, Inc., Warrington, Pa.) in toluene. The reaction mixture was allowed to cool to room temperature and filtered through a coarse-porosity sintered glass funnel. The gel was washed with 2 liters of methanol to remove unreacted materials and other by-products and then with water. The material was then vacuum dried and stored at room temperature. The aminopropyl silica in the basic form is stable for an extended periods at room temperature. Similar procedures may be carried with other silanes containing an amino function. It could be a short chain (C<sub>1</sub>-C<sub>10</sub>) or polymeric type amine containing a silane function.

The aminopropyl silica matrix most preferred for use as the second solid matrix material for vapor phase detection devices according to the invention may be obtained from Diagnostic Specialties/Separation Industries (Metuchen, N.J.) and is characterized by having a mean particle size ranging from about 250 to about 400 microns; mean pore diameter of 130 angstroms; mean surface area of 194 m<sup>2</sup>/g; mean pore volume of 0.63 m<sup>3</sup>/g; and an elemental composition comprising 6.67% C, 2.42% N and 1.64% H.

#### EXAMPLE 3

In this example, aminobutyl silica gel was prepared utilizing epoxy silica gel prepared according to Example 2. One hundred grams of epoxy silica gel was deaerated under vacuum for 30 min. and then shaken at 50° C. for 20 hours with 600 ml of a solution comprising 10% (by weight) 1,4-diaminobutane (Aldrich Chemical Company, Milwaukee, Wis.) in toluene. The reaction mixture was allowed to cool to room temperature, was filtered through a coarse-porosity sintered glass funnel and washed successively with 1 liter of toluene, 2 liters of methanol and 2 liters of water. The mixture was then vacuum dried and was stored at room temperature.

Similar procedures comprising opening of an epoxy silica matrix with diamines may be carried out with any short chain (C<sub>1</sub>-C<sub>10</sub>) or polymeric amines. The opening of epoxy silica can also be extended by ammoniacal toluene or aqueous ammonia to generate a primary amine. In addition, the epoxy silica gel may be used as an intermediate to form secondary amine function with any short chain or polymeric secondary amine. Similarly, short chain or polymeric compounds containing tertiary amine functionalities may be used to produce a silica matrix with a tertiary amine structure.

#### EXAMPLE 4

In this example, test matrices were formed by mixing varying amounts of nitroprusside-DEAE silica produced according to the methods of Example 1 with aminopropyl silica produced according to the methods of Example 2. The matrices comprised varying amounts of nitroprusside ranging from 20 to 90 mg nitroprusside per gram of matrix. The matrices were prepared using DEA-silica, characterized by particle sizes ranging