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(54) Title: DRY POWDER DIAGNOSTIC REAGENT AND APPLICATIONS THEREOF

(57) Abstract

A dry powder reagent comprising powdered enzyme and powdered indicator for use in evaluating an analyte in various fluid samples is disclosed. Also disclosed is a method for preparing the dry powder reagent and a test apparatus comprising an adhesive surface to which the dry powder reagent is adhered, for example, by simply sprinkling it onto the adhesive surface.

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DRY POWDER DIAGNOSTIC REAGENT AND APPLICATIONS THEREOF

FIELD OF THE INVENTION

This invention relates to a dry reagent useful in analytical chemistry, apparatus which employ this reagent, processes for preparing both the reagent and the apparatus, and methods for performing analysis using these.

BACKGROUND AND PRIOR ART

The field of analytical and diagnostic chemistry has grown tremendously in recent years. The field relates to analysis of body fluids, such as blood, serum, urine and the like, food substances, such as milk, drinking water, and other fluid substances. Applications of the field include medical diagnosis, purity testing, forensic science, etc.

The apparatus employed in analytical chemistry of the type discussed herein is generally a device referred to at times as a test strip, a dip stick, or other terms which will be familiar to those skilled in the art. The device contains various substances incorporated therein, which react with a particular analyte, or substance to be determined, with formation of a determinable or detectable signal. Frequently, but not always, this is a color. Color formation, or change in color, permits one to give a "yes-no" answer to the question of whether a substance or analyte is present in a test sample. Degree of color formed, or the actual color, can be used as an indication of how much analyte is present.

In practice, the sample may be applied to the test device, or the device may itself be dipped in or otherwise contacted to the test sample.

One very common test which is presented as exemplary of the type of assay performable with test devices and dry chemistry is a determination of blood glucose level. This, of course, is important in many situations, such as monitoring of diabetic and hypoglycemic patients. A test strip is prepared which contains the enzymes glucose oxidase and a peroxidase, as well as the indicator 3,3',5,5'-tetramethylbenzidine, also referred to as "TMB". If glucose is present in the sample, it reacts with oxygen in the presence of glucose oxidase to form gluconic acid and hydrogen peroxide. The peroxide, in turn, reacts with the TMB in the presence of the peroxidase, oxidizing the TMB. TMB, in unoxidized form, is colorless. When oxidized, it is blue, red or purple, depending upon the change transfer complex formed. Thus, one can determine if glucose is present, and how much, by observing formation of color. The system is analyte specific, because hydrogen peroxide is not a normal component of the sample, and only forms when the glucose specific enzyme glucose oxidase acts on its substrate.

The system described supra, is, of course, only one example of the various assays which can be performed using dry chemistry techniques. By modifying the analyte specific enzyme and indicator, one can, of course, determine different analytes, using the system outlined.

Typically, a test device of the type used in the analysis under consideration contains three "layers". The first, which plays no actual part in the assay, is a base or carrier layer. This layer is inert, and is generally constructed from materials such as thermoplastic films. A second layer, which will be referred to as the "reflective layer", is employed to aid in reading the detectable signal when it forms. This is especially helpful in systems which use a reflected light detector, as discussed supra. This layer contains some material which reflects light not absorbed by the detectable signal generator. Generally this layer takes the form of a coating, foam,

membrane, paper, or metal foil which is reflective. Substance useful for this layer include TiO_2 and ZrO_2. The third layer, generally referred to as the reagent layer contains the ingredients which cause formation of the detectable signal, and it is this layer which is actually "read" to determine the analyte. Various enzymes, substrates, receptors, and binding partners can be incorporated in this layer, such as labeled antibodies, indicator molecules, fluorescent agents, capping or quenching agents, and so forth. This layer is generally constructed of paper or other fibrous materials, non-fibrous materials such as films of polymeric substances, as well as hybrid layers containing both.

While the type of apparatus described herein can be used for analysis by the naked eye, this is not necessarily preferred. The system can also be used via deployment of a visible light detecting system. In this system, the test device is placed in a "reader", in which incident light is directed to the reagent layer. Some will be absorbed by the signal generator, and some will be reflected, forming the "signal". This signal is reflected light, which is read by a detector. Reading the wavelength and intensity of the light at the detector is how the sample is analyzed.

The interest in this field is exemplified by the amount of literature and patents which have resulted. Examples of this literature include Spayd, et al., Clin. Chem. 24(8): 1343-1350 (1978), who teach analysis of blood components using dry, multilayer analytical elements. Curme, et al., Clin. Chem. 24(8): 1335-1342 (1987) also teaches general principles of the art as applied to film based test strips. Thirtle, ChemTech 25-35 (January 1979) shows the applications of this field to color photography. Ohkubo, et al., Clin. Chem. 27: 1287-1290 (1981) teach that film based test strips can be used to analyze glucose concentration in whole blood. Shirey, "Development of a Layered Coating Technology for Clinical Chemistry" (1982)

gives a general overview of the state of the art of layered chemistry as applied to chemical analysis in 1982. Thomas, et al., Ann. Clin. Biochem. 19: 214-223 (1982), show that bilirubin, uric acid, lactose dehydrogenase, and other components can also be analyzed using dry chemistry type devices. Walter, Anal. Chem. 55(4): 499A-514A (1983), is yet another overview of the art. Free, et al., Lab. Med. 15(9): 595-601 (1984), provides yet another review of the art. Free, et al., Clin. Chem. 30(6): 829-838 (1984) gives a teaching of a very important aspect of the field, that of self testing. Because the dry chemical test systems involve formation of visibly determinable systems, it is possible for one who is not a technician or physician to monitor the concentration of components in body fluids, based on applying the fluid to the device, followed by formation of a signal, usually a color, with comparison to a "master-guide". In more sophisticated self-test systems, the individual uses a measuring device (meter) such as the type described supra, which gives a precise concentration reading.

Genshaw, Color Res. & App. 10: 235-245 (1985), is a discussion of the quantitative analyses which can be done using the so-called "ΔE" values, or extinction changes. These provide a way to optimize for color formation and change. Finally, in the technical literature, Mayer, et al., Lab. Man. 43-50 (April 1986), give yet another overview of the test strip art.

All of these references teach to background information, and constitute what is generally known to the art. They are all specifically incorporated by reference herein.

What is clear from a consideration of this art is that a particular method is followed for preparing test strips for analysis. This involves mixing a solution or suspension of reagents and applying the mix to a test strip, followed by evaporation of the liquid. A water solution, or a solution containing a dispersed polymeric

binder is employed. The solution (usually water) based systems are problematic for several very important reasons. First, many important substances, including TMB, referred to supra, are insoluble in water. This, taken with other problems of hydrolytic stability and compatibility renders many substance unsuitable for use in test strip devices. Also, if enzymes and substrates, e.g., are mixed in a solution, they will of course react prior to application of the analyte, with resulting failure of the system. As was pointed out, the liquid phase, such as water, has to be removed from these systems. This must be done at elevated temperatures to remove all moisture. Generally, the parameters are 55°C, for 20-30 minutes. Few enzymatic systems can withstand these conditions without some damage. Additionally, frequently the test strip itself is damaged by this treatment. Indicative of the state of the art with respect to applications of liquid carriers are Japanese Patent Application 61247967, which teaches an "ink composition" of glucose oxidase (GO), peroxidase (POD), indicators, water absorbing powder, and binder. The key feature of this invention is that one of the indicators is present in a non-aqueous dispersion. Soviet Union patent application 1155944, teaches the preparation of a dispersion of a cation exchange cellulose, and an alcoholic solution of o-tolidine, prior to combination with enzymes. The o-tolidine is protected from the enzymes because of its incorporation into the cellulose. U.S. Patent No. 4,673,654 teaches separation of dry reagents necessary to form a test. It must be noted that the system described in this patent also excludes the presence of an enzyme in the reagent. This is also the case with U.S. Patent No. 4,615,972, which teaches a stabilized indicator powder, which lacks the enzymes with which it reacts.

It will thus be seen that none of the prior art teaches or suggests a dry reagent which contains both an indicator and an enzyme homogeneously distributed, which can be used for determination of an analyte in a sample.

It is thus an object of the invention to teach such a dry reagent, as well as apparatus containing the reagent which can be used for dry chemical analysis, as well as the method of making these and using them.

How these and other aspects of the invention are accomplished will be seen from the disclosure which follows:

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The practice of this invention involves preparing a mixture of powdered enzyme and powdered indicator to form a homogeneous reactive powder reagent composition containing both. Powdered indicators and enzymes are both readily available materials, purchasable from any number of suppliers. Additional materials may be added to the uniform powdered composition, as desired. Most desirably, a surfactant may be added. Surfactants, in powder form, are useful for several reasons. While they help to bring together the test sample and the indicator/enzyme powder mix, some possess additional useful properties. The surfactant sodium dodecyl-benzene sulfonate, for example, helps to stabilize the color forming species following reaction of the indicator and enzyme to form a signal. Some surfactants also help to remove red blood cells from samples under analysis. Red blood cells frequently interfere with the assay, because of their strong color. If a suitable surfactant is chosen, red blood cells do not tend to stick thereto and do not interfere with the reaction being studied. Additionally, rupture of these red blood cells can be mitigated by choosing surfactants which impede hemolysis. Among the preferred surfactants, in addition to sodium dodecyl-benzene sulfonate, are

non-ionic surfactants including, but not limited to, ethylene oxide/propylene oxide block copolymers; ethyleneoxy ethanol; ethylene oxide adducts; alkoxylated lauryl alcohol; nonylphenoxypolyethanol; alkylphenoxypoly-oxyethylene ethanol; ethoxylated fatty alcohols; ethoxylated alcohol; and ethoxylated octylphenol. Useful amphoteric surfactants include the sulfobetaines. Other anionic surfactants include sodium dodecylsulfate, triethanol amine alkylbenzene sulfonate and sodium alkylbenzene sulfonate.

Polymers are also useful in the powder reagent. These act as fillers, rather than binders, which is the normal role of polymers in the art. The polymer or polymers chosen, if chosen should be hydrophilic and not contain significant amounts of groups which interfere with enzyme activity, such as carboxylic acid groups. Examples of suitable polymers include polyvinyl alcohol; polyvinyl acetate; polyvinyl propionate; vinyl acetate/vinyl chloride copolymers; acrylic resins (acrylate and methacrylate); lightly hydroxylated acrylics; ethylene oxide/propylene oxide block copolymers; polyvinyl chloride; styrene acryonitrile resins; polyvinyl-pyrrolidone; hydroxyethyl cellulose; cellulose acetate; cellulose acetate propionate; alginates; xanthans; crosslinked polyvinyl alcohol, and polymerized and copolymerized diallyl phthalate. Other useful materials will be evident to the skilled artisan. Additional useful materials include fluorinated substances, as they are useful in repelling red blood cells. Useful fluorinated powders include polyvinylidene fluoride, especially of a particle size of 3-5 μ; vinylidene fluoride/hexafluoro propylene copolymers
(3-5 μ); and teflon.

Cationic polymers may be used as well as anionic polymers, as may mixes of these. This is unusual, because, in solutions of ingredients, mixtures of anionic and cationic materials lead to undesirable flocculation.

Such is not the case in this invention. The anionic substances are useful in repelling negatively charged red blood cells. Exemplary of such useful anionic materials include anionic compounds, surfactants, and polymers.

Buffers may optionally be used, for their known effect.

A typical dry powder, in accordance with the invention, and the broad ranges over which the various components may extend, is as follows:

	Typical	Range
GO	0.3	0.25-0.35
POD	0.8	0.70-0.90
Crotein	4.8	4.50-5.00
Phenylsemicarbazide	0.4	0.30-0.50
TMB	2.5	2.50-2.30
Polymer	86.1	90.75-40.95
Surfactant	5.1	1.00-10.00
Fluorinated Compound/ Polymer	0.0	0.00-10.00
Positively Charged Compound/Polymer	0.0	0.00-10.00
Silica	0.0	0.00-20.00

It will be understood that the ranges given are in weight percent. It must be understood as well that the only essential ingredients in the reagent are the enzyme or enzymes (in this case GO and POD: glucose oxidase and peroxidase, respectively), and the indicator (in this case TMB, 3,3',5,5'-tetramethylbenzidine). It will be noted by the skilled artisan that a reagent of this composition could not be prepared in aqueous form. TMB is not very soluble in water.

Once the reagent powder is prepared, it is applied to an adhesive surface to which it can stick or otherwise become affixed. In one embodiment, the adhesive surface is a film of polycarbonate or another thermoplastic to

which has been applied a pressure sensitive emulsion. This emulsion gives the film "tack" or, in other terms, makes it sticky. The emulsion, should it contain water, can be treated at temperatures of up to 80-90°C to drive the water off. Such treatment, of course, is not possible if the enzyme has already been applied to the carrier, as this temperature would deactivate the enzyme. Pressure sensitive emulsions are well known to the art. Examples include "Flexbond 149", and "Flexbond 150" of Air Products, "Aroset" produces of Ashland Chemicals, and so forth. These pressure sensitive emulsions are generally polymers and resins. "Flexbond 149", for example, is a vinyl acetate copolymer, and Flexbond 150 is a saturated vinyl acetate polymer. One common form of pressure sensitive emulsion will be recognized as "scotch tape", and other forms of sticky tape will be known to the skilled artisan as well. 21 C.F.R. §175.105, "adhesives", which is incorporated by reference, lists many adhesives which are usable in the invention. The choice of adhesive will, of course vary, depending on availability and the particular system being used.

The carrier on which the adhesive is applied and to which the dry composition adhered may be a polycarbonate. Typically, this is about 140 μm in thickness, but the thickness of the carrier and adhesive emulsion may cover a broad range of thickness. Typically, a range of from about 90 μm to about 250 μm is preferred. Other materials may also be used. Examples of these include caprolactam polymers ("nylon 6", and "nylon 12"); condensation polymers of 1,6-hexamethylene diamine and dodecanoic acid (known, as "nylon 612"); polyesters, such as polyethylene terephthalate, and polymethylene terephthalate, acetal homo- and co-polymers; nylon block co-polymers; polyester polycarbonates; styrene acrylonitrile and polystyrenes; acrylics; polysulfone; polyether sulfones; polyetherether ketones; and cellulose and cellulose derivatives, such as acetate propionate, acetate butyrate, and triacetate all

of which are useful carrier films. Other are polyesters, polyvinyl-chlorides, polypropylene-ethylene-vinylacetate blend. These may include a reflective agent, such as TiO_2, but this is not required.

As pointed out supra the pressure sensitive emulsion is applied to the carrier, by any conventional method. Once this is done, any water is removed by, e.g., evaporation under ambient or reduced pressure conditions. Once this is accomplished, the reagent powder is applied. Various, well known techniques can be utilized to apply the reagent powder. For example, the carrier containing the pressure sensitive emulsion can be pulled via, e.g., a conveyor belt past a hopper which distributes the powdered reagent uniformly thereover. Excess powder is brushed away, and recycled. Simple sprinkling, dry powder nozzle dispensing, and fluidized bed application are all possibilities, as are electrostatic applications, such as with an electrostatic gun.

One can control the actual product by varying, e.g., the type of adhesive used, or its thickness. Particle size of the reagent powder can be controlled by various sizing methods, e.g., a ball mill. Varying the applicator rate will result in varying the concentration of the powder.

Once this element is prepared in which the reagent is incorporated into the carrier, it can be used by itself, or it may also be cut, shaped, or otherwise styled to be part of different test strip embodiments. Once the element or the test strip is prepared, it may then be used to evaluate analytes in various fluid samples.

Different enzymes and indicators may be used. Among these enzymes which are used in test systems of this type and which will be recognized by the skilled artisan are amylase, beta galactosidase, alkaline phosphatase, catalase, uricase, and so forth. Different substrates include CPRG, all galactopyranoside derivatives, resorufin, ABTS, 4-methoxy naphthol galactopyranoside,

MBTS, AAP (4-amino antipyrine), TOOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine, TMB derivatives, such as TMB dihydrochloride, 3,3',5,5'-tetraethylbenzidine and its hydrochloride, 4AAP/MAOS (3,5-dimethyl-N-ethyl-N-(2-hydroxy-3-sulfopropyl)-aniline), 4AAP/ADOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-anisidine), 4AAP/ALPS (N-ethyl-N-sulfopropylaniline), 4-AAP/ADPS (N-ethyl-N-sulfopropyl-m-anisidine) and others. The following examples are presented to show particular embodiments of the invention, but should not be viewed as limiting it in any way.

Examples 1-6

Six reagent powders were prepared using powdered forms of the component ingredients.

	Formula 1	Formula 2
Crotein C	1.400g	1.400g
GO	0.100g	0.100g
POD	0.230g	0.230g
Phenylsemicarbazide	0.132g	0.132g
TMB	0.740g	0.740g
Sodium dodecyl benzene sulfonate	1.500g	1.500g
Polyvinylpyrrolidone	0.000g	10.00g

	Formula 3	Formula 4
Crotein C	1.400g	1.400g
GO	0.100g	0.100g
POD	0.230g	0.230g
Phenylsemicarbazide	0.132g	0.132g
AAP	0.203g	0.203g
TOOS	0.295g	0.295g
Nonionic surfactant	1.500g	1.500g
Polyvinylalcohol	0.000g	10.00g

	Formula 5	Formula 6
Crotein C	1.400g	1.400g
GO	0.100g	0.100g
POD	0.230g	0.230g
Phenylsemicarbazide	0.132g	0.132g
TMB	0.740g	0.740g
Sodium alkylbenzene sulfonate	1.500g	1.500g
Lightly hydroxylated acrylic ester resin	0.000g	10.00g

Each of these formulations was applied to a previously prepared polycarbonate film. The film had applied thereto the polymeric adhesive Flexbond 149, at thickness of 200 mμ and 40 mμ. These were allowed to dry overnight under a hood. Following removal of additional water, a tacky film resulted to which the formulations were applied. The dry films were about 100 mμ and 20 mμ thick when dry. The powdered reagents were then salted onto the films.

Once the films had the powder adhering thereto, they were tested against controls containing 60, 200, and 600 mg/dl glucose, in saline solutions as well as glucose spiked blood. The solutions were applied to the elements in suitable configurations, and allowed to develop. The results obtained compared to those with strips made from equivalent, waterborne formulations, as is now explained.

Equivalents of formulas 1, 3 and 5 when made in waterborne form were not homogeneous. When applied to polycarbonate and dried, they were found to have limited integrity due to absence of any resin binder. When tested with saline, glucose and whole blood, the color development was not homogeneous. Furthermore, drying at elevated temperatures also reduced their enzymatic activity. On the other hand, strips prepared from exemplified, dry formulas 1, 3 and 5 using dry powder techniques were homogeneous and coherent. They gave uniform color. Testing done on a reflectance visible

spectrometer gave rapid end-point color reaction with very good sensitivity. Formulas 2, 4 and 6 applied by dry form technique were found to maintain their enzymatic activity after strip preparation whereas waterborne formulations partially lost their activity during the drying process. When tests were run with saline, glucose and whole blood, the dry powder formulas gave more intense color (percent reflectance vs. wavelength and glucose concentration plots) with no or minimal adhesion of the red blood cells to the test strip surface. Also, the color reaction was rapid, reached end-point quickly and the sensitivity was excellent. Thus, results obtained with dry technique strips were better than obtained with other test systems.

It will be understood that the specification and examples are illustrative but not limitative of the present invention and that other embodiments within the spirit and scope of the invention will suggest themselves to those skilled in the art.

WE CLAIM:

1. Reagent useful in determining an analyte in a sample, said reagent comprising:

(a) an adhesive carrier which is inert with respect to said analyte, and;

(b) a reactive powder adhering to said adhesive carrier, said reactive powder containing at least one indicator powder and at least one enzyme powder, said indicator and enzyme selected to react with said analyte to provide a detectable signal and distributed homogeneously in said powder.

2. Reagent of claim 1, wherein said reactive powder further comprises an inert hydrophilic polymer powder.

3. Reagent of claim 1, wherein said reactive powder further comprises at least one surfactant.

4. Reagent of claim 1, wherein said reactive powder further comprises a buffer.

5. Reagent of claim 2, wherein said polymer is nonionic.

6. Reagent of claim 2, wherein said polymer powder contains anionic and nonionic polymers.

7. Reagent of claim 2, wherein said polymer powder contains cationic and nonionic polymers.

8. Reagent of claim 1, wherein said reactive powder comprises from about 1.0 to about 1.5 percent by weight of said enzyme powder.

9. Reagent of claim 1, wherein said reactive powder comprises from about 2.0 to about 3.0 percent by weight of indicator.

10. Reagent of claim 2, wherein said polymer powder comprises from about 40 to about 95 percent by weight of said reactive powder.

11. Reagent of claim 3, wherein said surfactant comprises from about 1.0 to 10.00 percent by weight of said reactive powder.

12. Reagent of claim 1, wherein said enzyme is glucose oxidase, peroxidase, an isoenzyme of alkaline phosphatase, an esterase, or a reductase.

13. Reagent of claim 1, wherein said indicator is 3,3',5,5'-tetramethylbenzidine, 3,3',5,5'-tetramethylbenzidine dihydrochloride, 3,3',5,5'-tetraethylbenzidine, 3,3',5,5'-tetraethylbenzidine dihydrochloride, diarylimidazole, 4-AAP/MAOS, 4-AAP/ADOS, 4-AAP/ALPS, or 4-AAP/ADPS.

14. Reagent of claim 1, wherein said adhesive carrier comprises a polyester, a polyvinyl chloride, a polypropylene/ethylene/vinylacetate blend, a nylon block copolymer, an acrylic, a polystyrene, a polycarbonate, a polysulfone, a polyester, a polyetheretherketone, a polyethersulfone, an acetal homopolymer, an acetal copolymer, a cellulose acetate propionate, a cellulose, a cellulose triacetate, or a cellulose acetate butyrate, wherein said carrier contains an adhesive, pressure sensitive emulsion applied thereto.

15. Reagent of claim 14, wherein said adhesive carrier is from about 90 μm to about 250 μm in thickness and said reactive powder is applied to a layer of from about 3 μm to about 75 μm in thickness.

16. Reagent of claim 14, wherein said emulsion is an acrylic or vinyl acetate emulsion.

17. Reagent of claim 1, wherein said enzyme is selected from the group consisting of glucose oxidase, peroxidase, beta galactosidase, catalase, amylase, and uricase.

18. Apparatus useful in determining an analyte in a sample, comprising:

(a) an inert carrier,

(b) a reflective carrier in at least partial physical contact with said inert carrier, and

(c) a reagent layer comprising an inert adhesive carrier which is inert with respect to said sample and analyte and a reactive powder adhering to said inert adhesive carrier, said reactive powder containing an indicator powder and an enzyme powder, said indicator and said enzyme selected to react with said analyte to provide a detectable signal and distributed homogeneously in said powder.

19. Process for preparing a reagent useful in determining an analyte in a sample, comprising applying to an adhesive carrier material a powdered enzyme and a powdered indicator so as to homogeneously distribute said enzyme and indicator on said adhesive carrier material.

20. Process of claim 17, wherein said powdered enzyme and said powdered indicator are applied simultaneously.

21. Process of claim 17, wherein said powdered enzyme is applied prior to said powdered indicator.

22. Process of claim 17, wherein said powdered indicator is applied prior to said powdered enzyme.

23. Process of claim 18, wherein said powdered enzyme and powdered indicator are applied in the form of a homogeneous powder which further comprises at least one of powdered inert polymer and surfactant.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US88/04698

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (4): C12Q 1/54, 26, 28

US Cl.: 422/56, 57; 427/2; 435/4, 14, 19, 20, 25, 28

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	435/4, 14, 19, 20, 25, 28, 805 436/904 . 422/55, 56, 57 . 427/2

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS, CAS, BIOSIS DATABASES

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	US, A, 4,438,067 (SIDDIQI) 20 March 1984 (20.03.84) (Note: column 3, line 8-column 4, line 37; column 6, lines 10-26; Examples 1-4)	1-7, 12, 14, 17-23 8-11, 13, 15, 16
X Y	US, A, 3,993,451 (VERBECK) 23 November 1976 (23.11.76) (Note: figure 1; column 1, line 54-column 2, line 4; column 2, lines 21-26 and 52-64; column 4, lines 15-20; column 7, lines 20-38)	1-7, 12, 14, 17-23 8-11, 13, 15, 16
Y	US, A, 4,258,001 (PIERCE ET AL) 24 March 1981 (24.03.81) (Note: column 13, line 65-column 16, line 54; column 25, line 55-column 26, line 5; column 36, Examples 4 and 5)	8-11, 13, 15, 16
Y	US, A, 3,963,442 (BULLAR ET AL) 15 June 1976 (15.06.76) (Note: Abstract; column 5, lines 3-4; column 6, lines 35-43; column 7, lines 17-21; column 8, lines 1-14)	8-11, 13, 15, 16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

07 March 1989 (07.03.89)

Date of Mailing of this International Search Report

[illegible]

International Searching Authority

ISA/US

Signature of Authorized Officer

Carol A. Spiegel

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	US, A, 4,421,719 (BURLEIGH) 20 December 1983 (20.12.83) (Note: column 5, lines 19-27)	1-23
Y	GB, A, 0,808,742 (MILES LABORATORIES, INC.) 11 February 1959 (11.02.59) (Note: column 2, line 46-column 3, line 21)	1-23
A	US, A, 4,106,991 (MARKUSSEN ET AL) 15 August 1978 (15.08.78) (Note: column 1, line 9-column 2, line 46)	1-23
A	US, A, 4,548,906 (SEKIKAWA ET AL) 22 October 1985 (22.10.85) (Note: column 3, lines 48-65)	14-16
A	US, A, 4,615,972 (GALLACHER) 07 October 1986 (07.10.86) (Note: Abstract)	1-23