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(54) Title: ANALYTICAL ELEMENT WHICH AVOIDS PREMATURE REACTION

(57) Abstract

An analytical element is disclosed which is useful in determining an analyte. The element contains a reagent matrix which in turn contains an integral array of an indicator system and a reactive system. These are disposed so as to prevent interaction therebetween until and unless a solvent is applied in which both are soluble. At least one of the systems is disposed in an interaction preventing substance. Also disclosed is a reagent which is used to prepare the element.

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ANALYTICAL ELEMENT WHICH AVOIDS PREMATURE REACTION

FIELD OF THE INVENTION

This invention relates to an analytical element useful in determining an analyte or analytes in a sample, as well as apparatus containing this element. It also relates to the process of making said elements and apparatus, as well as methods of using these.

BACKGROUND AND PRIOR ART

"Dry chemistry" is a field which has expanded dramatically in recent years. Broadly stated, the field relates to the use of dry reagents, such as test strips, film strips, and so forth, for the analysis of liquid samples.

The use of test strips frequently involves the detection of a reaction between the analyte, or substance being determined, with some material which has been incorporated into the dry reagent or test strip, or the detection of a further reaction which can occur only when the first one occurs. Examples of the former include "diffusion assays", such as those taught by Deutsch et al., U.S. Patent No. 4,235,601, where one determines formation of a reaction complex by its slower movement through a test strip, as compared to unreacted materials. Such tests are not as common as the latter type, however, which include such systems as "IEMA" assays, displacement or competition assays, and so forth. In these systems, a complex may form, but it is not the complex formation that is determined; rather the complex which forms is between the analyte being determined and a binding partner, which either carries a reactive label or causes the release of a reactive label. This label then reacts with its own reaction partner to form a detectable substance, such as a

color. Exemplary of such tests are Liotta, U.S. Patent No. 4,446,232.

One problem encountered in dry chemistry test strips is that of stability. This does not refer to the carrier material or test paper itself, but rather, to keeping the reagents which are necessary to accomplish the test from reacting with each other unless and until the test sample is brought in contact with the dry chemistry elements and hence the reagents. For example, in Jones, U.S. Patent No. 4,689,309, a reactant system is described which contains a cured, polymerizable silicon containing compound. This silicon-containing compound is a carrier matrix which immobilizes the reactant system and maintains component permeable. Talmage, U.S. Patent No. 4,673,654 acknowledges the problem of premature contact of indicator and reactive substance. His solution, however, is to prepare two separate compositions, which are mingled into a dry powder. The reason why there is not premature reaction is that one powder is coated with a film. This type of reagent is appropriate for capsules and the like, but is inappropriate for test strips.

It is an object of this invention to provide a stable composition useful in test strips and other dry chemistry apparatus, which contains both an indicator system and a reactive system which either form a detectable signal when they react with each other or form a product which, upon reaction with another component of the test system, forms a detectable system.

It is a further object of this invention to provide an apparatus useful in analysis of samples which contains this composition.

It is yet a further object of this invention to provide a process for making both the composition or the test apparatus described herein.

Yet another object of this invention is to provide a method for using the apparatus.

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

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

It has now been found that a composition containing an integral array of an indicator system and a reactive system can be prepared, which does not undergo reaction prior to contact with a sample contained in a carrier solvent in which both the reactive system and the indicator system are soluble. The composition is prepared by incorporating one of the two systems, generally, but not exclusively the reactive system, into a carrier in which the system is insoluble and which prevents interaction between these systems. This mixture, slurry, or other formulation is combined with the indicator system. Any liquid carrier in the mixture is drawn off, by evaporation, or other means, to produce an integral array containing both the indicator system and the reactive system. The insoluble carrier may remain a part of the integral array. If it does so, it is chosen so that it is inert with respect to the different systems.

By "indicator system" is meant any of the various reagents known to the art which are progenitors of substances which give detectable signals when reacted with other components of a test system. Generally, these are enzyme substrates which, when cleaved by an enzyme, produce a color. Other types of indicators may include, e.g., fluorescent systems. The indicator system should not be thought to be limited to substrates alone; by system is meant one or more components which are involved in a reaction. A first component may be, e.g., a substrate, and a second component, an enzyme. The substrate, in unreacted form, may not react with the enzyme in the indicator system, but require reaction with

some other substance to form a substrate which can react with the enzyme. One such example of this type of system is elaborated upon infra.

The reactive system comprises a reagent or reagents which act in concert with the indicator system to form either a detectable substance or some product which is an intermediate prior to formation of the detectable signal. In a very simple embodiment, the reactive system can be nothing more than an enzyme which reacts with a substrate in an indicator system. It can also be, however, a substance which is necessary to activate one or more components of the indicator system, or a necessary reagent for activation of the reagent system. For example, if the indicator system contains a portion of an enzyme, the reactive system may contain the other portion. When the two are brought together, a complete, active enzyme forms which can then react with a substrate molecule. Another example of this is where the reactive system contains a substance which is necessary to complete a color forming reagent. For example, the indicator system may contain a colorless enzyme substrate system which, after reaction, forms a second substrate which is also colorless. The second substrate, however, may react with the reactive component to form a colored product where the first one cannot. Where the reactive component requires an enzyme to carry out the color forming step of the reaction, one has an ideal opportunity to incorporate the test system into an analytical apparatus. If an enzyme is required to carry out the reaction of reactive system and indicator system product, then one can incorporate the second enzyme, in the form of a labeled analyte binding partner in a test apparatus which also contains a test element as described supra. When sample analyte is added, some of the label reacts therewith, and this reacted complex can be complexed out of solution, e.g. In another position, some of the label can be displaced by the presence of analyte. In any event, when the label contacts the test

system, the label, indicator system and reactive system are brought into contact and a set of mutually dependent reactions takes place leading to formation of the detectable substance.

The manner in which the analytical element is formed calls for an integral disposition or array of both reactive and indicator systems. "Integral array" simply means that the components are disposed in a manner such that they are contacted at the same time by the sample being analyzed. Thus, the systems can be in a single layer uniformly distributed throughout, or in, apparently separable layers which are in physical contact with each other. The actual form of the integral array is not an essential feature of the invention. What is important is that they be configured such that reaction between the two does not take place until desired, i.e., by contact with the sample. As pointed out, supra, this is accomplished by incorporating one of the systems into a carrier in which it is insoluble. Preferably, this carrier substance is water soluble. The insoluble carrier precludes any reaction taking place, because both reaction system and indicator system must be in solution for the reaction to take place. This does not happen until the sample contacts the integral array.

The analytical element integral array can include other materials. For example, it may include a soluble matrix, such as a soluble polymer, which facilitates entry into the solution phase once the mutual solvent sample reaches the element. A particularly desirable embodiment also includes an inert material, such as titanium dioxide. The TiO_2 does not react with any of the components of the element or test apparatus, but alters the optical properties of the matrix in which it is incorporated, thus rendering the signal more easily observable.

Preferably, the reactive system contains a first component which reacts with a second component which is either a part of the indicator system or some other part

of the analytical element. These two components form a reaction product which then reacts with a third component, which is part of the reactive system, leading to formation of a detectable signal. One such system involves formation of a peroxidase substrate as the reaction product, where the reactive system contains a peroxidase and a peroxide source.

The manner of producing the element will vary, and there is nothing critical about the manner in which it is formed. One possibility is to impregnate a carrier, such as a fleece, filter paper, or film, with one of the systems, such as the indicator systems, and to wait until this is dry. The second system, such as the reactive system, is then applied in its insoluble carrier. Any liquid vehicle is then removed, such as by evaporation, and an analytical element containing the integral array which is desired remains. Additionally, one can combine two solutions of the systems onto a carrier, such as those listed supra, or can even combine the two systems into one "master solution" which is then applied to a carrier. Other means of doing this will be evident to the skilled artisan.

Once the analytical element is prepared, it can be sized, cut, and incorporated into a test strip in the same way as any other component is incorporated into a test strip. Those skilled in the art are familiar with such procedures, and they will not be repeated here.

The following example is exemplary of the invention described herein, but is not to be seen as limitative in any way. Other systems will be seen to be as workable as that specifically illustrated.

Example

One preferred system for use in test systems involves the indicator system 1-naphthol-β-galactopyranoside and β-galactosidase, and the reactive system peroxidase and sodium perborate. The naphthol galactoside is colorless,

and, upon reaction with β-galactosidase, releases 1-naphthol, which is also colorless. This product, however, reacts with an oxidant, e.g., $NaBO_3$, catalyzed by a peroxidase, to form a blue colored product. The problem results from the instability of peroxidase when co-impregnated with the oxidant, so that they cannot both be impregnated into reagent paper. The following composition overcomes this problem.

A mixture of sodium perborate and TiO_2 was combined in a pulverizer for 10 minutes. 50 mg of $NaBO_3$ was used for each 7.5 gm of TiO_2. Following pulverization and mixing, a 2.5 g aliquot of the mixture was added to 8 ml of a 5% solution of Klucel® in acetone. Klucel is a registered trademark (Hercules) for a cellulose ether polymer. A substrate paper was prepared by impregnating a piece of Schleicher & Schuell 3512 paper with 10 mg/ml of 1-naphthol-β-galactopyranoside in methanol, and allowed to dry. This paper was then impregnated with 100 µg/ml of horseradish peroxidase in 10 mmol/L Na_3BO_3 and allowed to dry. The Klucel mixture was applied to the substrate paper and allowed to dry.

The element was tested for reaction by applying 25 ul of a 1 µg/mL solution of β-galactosidase. The formation of a blue color indicated the reaction of the substrate with the β-galactosidase to release 1-naphthol, which subsequently reacted with peroxidase and $NaBO_3$ to produce a blue dye.

Test strips were prepared containing the element described supra. Briefly, the test strip contained, in this embodiment, a first part, which was a wick of Whatman 54 filter paper (0.6 by 2.0 cm) for introduction of sample. This wick contacted a conjugate pad (0.6 x 0.6 cm of filter paper) containing 0.16 U of a monoclonal antibody to phenobarbital conjugated to B-galactosidase. The conjugate zone contacted a matrix (0.6 x 2.0 cm of filter paper) which contained 24 µg of a phenobarbital-

protein conjugate immobilized to the paper. This matrix contacted the analytical element described supra.

To run the test, standards of phenobarbital were prepared in urine at concentration of 0 and 1 µg/mL. 25 µl of the sample was applied to the sample application zone, and the test strip placed in a tube containing 0.5 mL of PBS. The test was allowed to run for 10 minutes and results were evaluated by observing the appearance of the substrate-containing analytical element. The analytical elements appeared blue when samples did contain phenobarbital, and white when samples did not contain phenobarbital. The blue color is the reaction product of 1-naphthol with Peroxidase/$NaBO_3$, indicating the generation of 1-Naphthol by β-galactosidase, and its subsequent reaction with POD/$NaBO_3$.

It will be clear, of course, that other enzymes, such as oxidase, hydrolase, amylase, alkaline phosphatase, and catalase can work in this system. Additional indicators may be used as well.

While there have been described what are at present considered to be the preferred embodiments of this invention, it will be obvious to one skilled in the art that various changes and modifications may be made therein without departing from the invention, and it is, therefore, aimed to cover all such changes and modifications as fall within the true spirit and scope of the invention.

WE CLAIM:

1. Analytical element for determining an analyte, comprising a reagent matrix containing an integral array of an indicator system and a reactive system, wherein at least part of said reactive system or indicator system is disposed in a substance which prevents interaction between said reactive system and indicator system in the absence of a solvent in which both systems are soluble.

2. Analytical element of claim 1, wherein said matrix is a single layer.

3. Analytical element of claim 1, wherein said substance is water soluble.

4. Analytical element of claim 1, wherein said reactive system and said indicator system are soluble in water.

5. Analytical element of claim 1, wherein at least one of said systems contains an enzyme.

6. Analytical element of claim 1, wherein said indicator system contains an enzyme.

7. Analytical element of claim 1, wherein said indicator system contains a color forming substrate.

8. Analytical element of claim 1, wherein said reactive system contains an enzyme.

9. Analytical element of claim 1, wherein said reactive system contains a first component which reacts with a second component to form a reaction product which reacts with at least one further component contained in said reactive system to give a detectable signal.

10. Analytical element of claim 1, wherein said matrix contains substance which alters optical properties of said matrix.

11. Analytical element of claim 6, wherein said enzyme is beta galactosidase.

12. Analytical element of claim 6, wherein said enzyme is an oxidase, a hydrolase, an amylase, an alkaline phosphatase, a catalase, or a peroxidase.

13. Analytical element of claim 6, wherein said enzyme is a galactosidase and said indicator system further comprises a galactosidase substrate.

14. Analytical element of claim 9, wherein said reaction product is a peroxidase substrate and said reactive system comprises a peroxidase and a peroxide source.

15. Analytical element of claim 14, wherein said reaction product is 1 naphthol or a substituted 1-naphthol.

16. Analytical element of claim 9, further comprising a separate zone which contain a substance which reacts with said detectable moiety to provide a second detectable moiety.

17. Analytical element of claim 16, wherein said separate zone contains beta-galactosidase.

18. Analytical element of claim 16, wherein said separate zone contains beta galactosidase, said matrix comprises a reactive substance containing a peroxide source, and said indicator substance contains a peroxidase and a peroxidase substrate.

19. Reagent for preparing an analytical element, comprising a first sample of a reactive component, and a second sample of an indicator component which reacts with said reactive component, wherein at least one of said reactive system and said indicator system is disposed in a carrier in which it is insoluble, said carrier preventing reaction between said indicator system and said reactive system.

20. Reagent of claim 19, wherein said reactive system and said indicator system are disposed in separate containers.

21. Reagent of claim 19, wherein said reactive system is disposed in an insoluble carrier.

22. Reagent of claim 19, further comprising a further system which reacts with a product of reaction between said indicator system and said reactive system.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00588

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):C12N 9/96; C12Q 1/ 00,26,28,30,32,34,54 G01N 31/22
U.S. CL.: 422/55,56,57; 435/4,7,14,18,25,26,27,28.188

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	422/55,56,57 436/810 435/4, 7, 14, 18, 25-28, 188, 805

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

CAS and APS 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, lines 8-26; column 5, lines 16-28; column 5, line 48 - column 8, line 11)	1,2,4-7 10,12,19, 21 3,8,9,11, 13-18,20, 22
X Y	US, A, 4,447,529 (GREENQUIST) 08 May 1984 (08.05.84) (Note: Abstract; column 4, line 49- column 5, line 6; column 8, lines 49-56; column 12, lines 10-40; column 13, lines 26-35).	1-6,8,10, 19,21 7,9,11-18 20, 22
Y	US, A, 4,235,601 (DEUTSCH) 25 November 1980 (25.11.80) (Note: column 2, line 64 - column 3, line 28).	1-22
Y	US, A, 4,446,232 (LIOTTA) 01 May 1984 (01.05.84) (Note: column 1, line 56 - column 2, line 20)	1-22

* 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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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 April 1989	06 JUN 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	CAROL A. SPIEGEL

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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, 4,673,654 (TALMAGE) 16 June 1987 (16.06.87) (Note: column 3, lines 18-59; column 4, line 41 - column 5, line 3).	1-22
Y	US, A, 4,555,484 (LA ROSSA) 26 November 1985 (26.11.85) (Note: column 2, lines 39-47; column 4, lines 46-48; column 5, lines 5-19).	1-8,10, 12,19-21
Y	US, A, 4,629,690 (WENG) 16 December 1986 (16.12.86) (Note: Abstract; column 3, lines 30-50; column 7, lines 5-13, Table I).	9,14-18, 22
Y,P	US, A, 4,732,736 (KOBAYASHI) 22 March 1988 (22.03.88) (Note: Abstract; column 1, lines 61-65; column 6, lines 10-29; column 7, lines 32-40).	1-7,10, 19-21
Y	GB, A, 2,113,839 (FARMITALIA CARLO ERBA SPA) 10 August 1983 (10.08.83) (Note: page 2, lines 32-49).	11,13,17 18