PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 4:
G01N 15/10, 15/14, 21/11
G01N 21/51, 33/53, 33/536
G01N 33/543, 33/545, 33/546

(54) Title: AGGLUTINATION REACTION METHOD AND APPARATUS

(21) International Application Number: PCT/US87/02054
(22) International Filing Date: 19 August 1987 (19.08.87)
(31) Priority Application Number: 897,807
(32) Priority Date: 19 August 1986 (19.08.86)
(33) Priority Country: US
(71) Applicant: ANGENICS INC. [US/US], 100 Inmann Street, Cambridge, MA 02139 (US).
(11) International Publication Number: WO 88/01374
(43) International Publication Date: 25 February 1988 (25.02.88)
(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published

With international search report.

(57) Abstract

A method and apparatus for conducting agglutination reactions, preferably latex agglutination reactions. The method of the present invention utilizes a self-starting, continuous, capillary flow to assist in formation of aggregates. No externally applied kinetic energy is required, either to induce, or to continue producing the agglutination reaction of the present invention. The apparatus (10) of the present invention comprises one or more optically clear capillary tubes (18), reagent medium delivery means (12), and a reservoir (14) containing a sorbent (20), which will maintain a flow of reagent medium (16) through the capillary tube(s) over a specific period of time.
**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>Austria</td>
<td>FR</td>
<td>France</td>
<td>ML</td>
<td>Mali</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GA</td>
<td>Gabon</td>
<td>MR</td>
<td>Mauritania</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GB</td>
<td>United Kingdom</td>
<td>MW</td>
<td>Malawi</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>HU</td>
<td>Hungary</td>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>IT</td>
<td>Italy</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>JP</td>
<td>Japan</td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
<td>SD</td>
<td>Sudan</td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td>KR</td>
<td>Republic of Korea</td>
<td>SE</td>
<td>Sweden</td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>LI</td>
<td>Liechtenstein</td>
<td>SN</td>
<td>Senegal</td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>LK</td>
<td>Sri Lanka</td>
<td>SU</td>
<td>Soviet Union</td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>LU</td>
<td>Luxembourg</td>
<td>TD</td>
<td>Chad</td>
</tr>
<tr>
<td>DE</td>
<td>Germany, Federal Republic of</td>
<td>MC</td>
<td>Monaco</td>
<td>TG</td>
<td>Togo</td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>MG</td>
<td>Madagascar</td>
<td>US</td>
<td>United States of America</td>
</tr>
</tbody>
</table>
AGGLUTINATION REACTION METHOD AND APPARATUS

FIELD OF THE INVENTION

The present invention is directed to a method and apparatus for conducting agglutination reactions, preferably latex agglutination reactions.

The method of the present invention utilizes a self-starting, continuous, capillary flow to assist in formation of aggregates. No externally applied kinetic energy is required, either to induce, or to maintain, the agglutination reaction of the present invention.

The apparatus of the present invention comprises one or more optically clear capillary tubes, reagent medium delivery means, and a reservoir containing a sorbent, which will maintain a flow of reagent medium through the capillary tube(s) over a specific period of time.

More particularly, the invention is directed to antigen - antibody mediated latex agglutination reactions, driven by the continuous flow of a reaction mixture through a capillary column or tube, in which the contents of the tube mix, react, and form visual indicators, i.e., aggregates, signaling the presence or absence of a specific antigen or corresponding ligand.
BACKGROUND OF THE INVENTION

This invention relates to methods and products useful for the analysis of biological materials. More particularly, this invention is directed to providing a simplified test structure and method useful in assaying liquid samples for particular analytes of interest, wherein the presence or absence of an analyte in the sample is rapidly indicated by the formation of visible aggregates.

Various agglutination methods for the detection of analytes exist. Typically these methods involve the formation, and analysis of ligand-antiligand complexes, wherein the substance to be detected (analyte) is exposed to a substance which binds or complexes specifically to that analyte, and the resulting product of admixture either forms or fails to form an aggregate which indicates either the presence or the absence of the expected complex. The case where the presence of the analyte is detected by the formation of aggregates is known as a "direct test," whereas the failure to form aggregates as an indication of the presence of analyte is known as an "indirect test." The binding necessary for either the direct or indirect test takes place between a pair of materials (binding pair) one of which typically is the substance to be detected (analyte), and a receptor material.

As is known in the art, an assay of this type may utilize immunobinding techniques, or other known binding techniques. In immunoassays, the analyte may either be an antibody or a material to which an
antibody may attach (i.e. an antigen or a hapten.)

The analytes or ligands which can be analyzed with the present invention include all of the compounds or materials for which ligand-specific binding materials (antiligands) are available or can be prepared. These include most of the compounds and materials which are of interest in clinical chemistry, biology and medicine, including proteins and other polypeptides whether or not glycosylated, antigens, haptens, antibodies, allergens, hormones, enzymes, metabolites, pharmaceuticals, such as antibiotics, steroids, natural and synthetic toxins, viruses and parts thereof, bacteria and parts thereof, fungi and parts thereof, plant and animal cells and parts thereof, other organic molecules, such as carbohydrates, and wide varieties of other materials. Preferably the ligands tested for are antigens or haptens for which antibodies can be raised in mammals such as mice, rabbits, guinea pigs, and the like. Where the ligand of interest is not per se able to generate the formation of antibodies in such animals, it can often be treated as a hapten, and attached to another, typically larger, molecule which is then administered to a host animal or entity for antibody development. Specific examples include growth factors for types of cells, narcotics and other illegal drugs, antibiotics, hormones, nucleic acids and polynucleotides, immunoglobulins such as IgG, IgE, etc., from human, bovine, mouse, rabbit, or other species.

Aggregation apparatus typically involve flat, nonabsorbent surfaces. Such apparatus and their respective methods are usually characterized by the placement of liquid reagents on a glass slide or plate, plastic-coated cardboard, or a similar surface,
and a gentle rocking and swirling of the plate is employed to cause the liquid reagents to swirl around and form agglutinations. Without this swirling and/or rocking motion, the reagents do not generally form visible agglutinations, or the resulting agglutinations are not reproducible.

To obtain optimum performance, the rocking and/or swirling of the surface must be performed in an exact manner, for a precise period of time. At the end of the rocking and/or swirling period the results must be read immediately. If the swirling is performed either too quickly or too slowly, or if the rocking and swirling time is either too short or too long, the results will be incorrect. Similarly, if the results of the reaction are not observed at the end of the rocking time the reagents could continue to react, again giving incorrect results. If the reagents spill off the edges of the plate during rocking or other handling, inaccurate results will follow. Conventional agglutination reagents also evaporate in a matter of minutes thereby rapidly destroying the visual record of the particular reaction.

Improvements in conventional agglutination reactions are constantly being developed. For example, U.S. Patent No. 4,436,827 to Tamagawa describes a particle agglutination reaction with the aid of a reaction vessel of a flow cell type having an inlet, an outlet and an inclined passage communicating the inlet and outlet to each other. A test liquid containing particles is supplied into the passage via the inlet and is retained stationary therein for a given time. Particles descending upon an inclined bottom surface of the passage form a first stable base layer due to regular steps formed in the bottom
surface. When a particle agglutination reaction has occurred, a uniformly deposited particle pattern is formed on the inclined surface while in case of non-agglutination reaction, the particles descending upon the inclined bottom surface roll down along the base layer and are collected in the lowermost portion of the passage. By detecting the particle pattern formed on the inclined bottom surface, it is possible to detect the agglutination reaction.

U.S. Patent No. 4,398,894 to Yamamoto discloses a method for quantitatively determining the degree of agglutination of particles which comprises causing a liquid containing the particles to be agglutinated to slowly transfer to a small tube in the form of a vacuole within a fluid immiscible with said liquid. The particles in said liquid are allowed to agglutinate and to accumulate as agglutinated clots on the front part of said vacuole, thereby forming within said vacuole an accumulation layer of said agglutinated clots and a suspension layer of non-agglutinated particles, then measuring the degree of agglutination within said liquid by the use of optical means after the elapse of a predetermined time.

U.S. Patent No. 4,290,997 to Souvaniemi discloses an apparatus useful for automatic measurement of the results of agglutination test having at least one vertical side wall portion and a bottom portion and at least one recess for receiving the non-agglutinated particles, and an area for receiving the agglutinated particles such that said recess is out of the position of passage of a measurement beam which is adapted to measure the amount of agglutination which takes place within the reaction vessel.
Such methods can require complicated equipment for either conducting the agglutination reaction, or for determining the amount of agglutination which has occurred. In contrast, the present invention is directed to a simple apparatus, comprising one or more optically clear capillary tubes having two chambers, a reagent medium delivery chamber and a sorbent chamber. The presence or absence of analyte of interest is readily determined after a short reaction period, by the presence or absence of aggregates inside the capillary tube. The method may be conducted by any person briefly trained in its use, as it requires no technical skill to operate or interpret the results. The apparatus is designed to be economical and disposable after use.

SUMMARY OF THE INVENTION

The present invention is thus directed to a method and apparatus for conducting agglutination reactions, preferably latex agglutination reactions, which method utilizes a continuous capillary flow to assist in formation of aggregates.

The apparatus of the present invention comprises one or more capillary tubes, reagent delivery means, and a sorbent containing means, such as a reservoir, the combination of which will maintain a flow through the capillary tube(s).

More particularly, the present invention is directed to antibody-driven latex agglutination reactions, driven by flow of a reaction medium through
a capillary column or tube, causing the contents of the tube to mix, react, and form visual indicators, i.e., aggregates, indicating the presence of a specific antigen or like analyte.

BRIEF DESCRIPTION OF THE DRAWING

The Figure illustrates one embodiment of the capillary tube agglutination reaction apparatus of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention removes the need for imparting externally applied kinetic energy (e.g., shaking, rocking, etc.) to an agglutination apparatus by providing a self-starting, slow, continuous flow of the reaction medium from a reservoir through a capillary tube and into a bed of sorbent material.

The self-starting nature of the apparatus is governed by the phenomenon known as "capillary flow." Capillary flow occurs whenever a liquid comes into contact with a capillary tube via the molecular attraction (similar to surface tension) of the liquid and the inner surface of the tube. Due to the preferably horizontal, or nearly horizontal, operating posture of the apparatus of the present invention, and the sorbent for the liquid reaction medium at one end of the capillary tube, the flow is started by capillary action and the flow continues until the sorbent is saturated or the source of the reaction
medium runs dry. Flow may also stop due to accumulation of sufficient aggregates which block the flow of the reaction medium through the capillary tube. The speed of the flow may be either increased or decreased by changing the operating posture away from the preferred horizontal position.

The internal kinetic energy of the reaction medium moving from the reaction reservoir to the sorbent material gives rise to the aggregation reaction. Aggregates accumulate inside the capillary tube, and the occurrence of an agglutination reaction can be resolved visually. Once flow has stopped, the reaction is stable for a prolonged period of time (e.g., for approximately 1 hour).

In order to understand the preferred apparatus and manner in which the agglutination reaction of the present invention functions, reference will be made to the Figure accompanying this disclosure.

As illustrated, an agglutination test is conducted within an agglutination assay apparatus comprising a capillary tube agglutination reaction chamber (10); reaction medium delivery reservoir 12; and sorbent reservoir 14.

In preferred embodiments, a reaction mixture containing the test sample (potentially containing the analyte, e.g., antigen), anti-ligand reagent (e.g., antibody), and polystyrene latex reagent is pipetted into the reaction medium delivery reservoir 12.

Reagents and test sample 16 are drawn by capillary action through capillary tube 18 toward the sorbent bed 20. The length of time flow can be regulated by
the quantity of sorbent used.

In an indirect (i.e., inhibition) test, if the test sample does not contain the molecule of interest (analyte), the reagent sample begins to react immediately and form small agglutinations. In a direct test, if the test sample does contain the molecule of interest, aggregates slowly form between the analyte in the sample and its binding pair, located in the reaction medium.

In either event, as soon as two particles react to form an aggregate their combined velocity is reduced relative to the unreacted particles. This reactive process continues until the size of the aggregates increase to the extent that their velocity approaches zero, and they deposit on the bottom of the capillary tube at which point any unreacted particles either stick to the agglutination or continue on past the agglutinations in the liquid medium surrounding the agglutinated particles. This results in a high contrast visual display of the agglutinations when they occur in the test sample. If the flow of reagents is limited after a predetermined time interval (sufficient to generate a complete reaction) the readout (i.e., the appearance of particles within the reaction chamber) is then stable for a prolonged period of time (e.g., for approximately 1 hour).

A number of variables can have an effect on both the method and the apparatus of the present invention. These include:

(a) Physical characteristics of the capillary tube:
The size of the capillary tube - both diameter and length are important. The diameter should be chosen so as to support the flow of the reagent mixture therethrough at a rate sufficient to promote agglutination but not so rapid as to prohibit the deposit of agglutinated particles on the floor of the capillary tube. In addition, the lumen of the tube must be large enough to permit visual determination of the results. In cases where capillary tubes of very small diameter are desired, parallel arrays of tubes in close proximity to one another ("rafts") can be used to enhance the degree of visual resolution. In the selection of the length of the tube, the same considerations apply. The length of the tube must be sufficient to allow formation of aggregates which are large enough to be deposited on the floor of the tube.

Suitable dimensions of useful capillary tubes herein include diameters in the range from about 0.01 to 0.2 centimeters with a preferred diameter of about 0.03 to 0.05 centimeters, and lengths ranging from about 5 to 30 centimeters, with the preferred length depending on the diameter used. In the case of a 0.03 cm diameter tube, the preferred length is about 15 cm.

(b) Sorbent physical characteristics:

The sorbent material should be chosen so as to maintain a constant and prolonged flow of reagent through the capillary tube, while not occluding the end of the tube in such a way that the establishment of capillary flow is impeded. Suitable materials include, but are not limited to, finely pulverized sucrose and pulverized polyethylene glycol.

The volume of sorbent material should be chosen so
as to maintain the reaction medium flow for a sufficient period of time to allow the agglutination reaction to occur. After the sorbent is saturated with the aqueous reaction mixture, flow automatically stops.

(c) Position of the apparatus:

The angle of the capillary tube with respect to horizontal can be used to further regulate the rate of flow of the reactants. An upward incline can be used to decrease the flow rate while a downward incline can be used to increase the rate of flow of the reactants. Depending upon the specific reagents utilized in the apparatus of the present invention, any one or more of these variables may need to be adjusted to control the flow rate through the capillary chamber in such a way that the aggregation of particles is promoted while aggregated particles are allowed to be deposited on the floor of the capillary chamber.

Upon consideration of the preferred teachings of the present invention, the skilled artisan will readily be able to determine appropriate modifications necessary for the determination of any ligand–antiligand agglutination reaction. For example, while the preferred test is an indirect assay, direct assays are readily performed using appropriate reagents. In addition, while the preferred embodiment is directed to assaying milk for penicillin, other samples can readily be assayed, as well as other analytes, without departing from the general teachings of this invention.

The present invention will be further illustrated
with reference to the following example which is intended to aid in the understanding of the present invention, but which is not to be construed as limitations thereof. All percentages reported herein, unless otherwise specified, are percent by weight.

EXAMPLE

The method of the present invention was conducted as an indirect assay for penicillin in milk, using the apparatus substantially illustrated in the Figure, and reagents typically employed in the commercially available "SPOT" Assay for penicillin in milk (Angenics, Inc., Cambridge, MA).

Raw milk containing 5 ng/ml of penicillin G was treated and filtered according to the standard protocol for the commercially available SPOT test for the detection of penicillin in milk. The SPOT test is normally run as a 4 minute indirect agglutination assay using reagents mixed on a glass slide with kinetic energy provided by a rocker.

20 ul of the treated milk sample was mixed with 10 ul of antibody reagent from the SPOT test and 10 ul of penicillin-coated latex preparation from the SPOT test in a 500 ul plastic microcentrifuge tube, taking care that the reagent mixture remained in the bottom of the conical test tube. This microcentrifuge tube served as the reagent reservoir.

The capillary chamber consisted of a 10 microliter Dade disposable Accupette, which was gently inserted into a second 500 ul microcentrifuge tube containing 0.15 g of sucrose which had been previously pulverized
to a fine powder by several seconds of treatment in a blender.

When the free end of the capillary tube was inserted into the liquid in the reagent reservoir, capillary flow commenced immediately. As the reagent mixture came into contact with the sucrose of the adsorbent bed, the reagent mixture began to flow slowly into the adsorbent. The capillary chamber was observed visually at two minute intervals (aided by means of a lamp held 6 inches away from the chamber).

After 4 minutes had elapsed, samples which contained no penicillin were clearly beginning to agglutinate, while penicillin containing samples showed no agglutination.

At 6 minutes of elapsed flow time, the penicillin-containing samples still showed no signs of agglutination while the samples without penicillin showed complete agglutination (i.e., macroscopic particles of agglutinated latex were visible against the completely clear background). The results of this assay were stable for over one hour.

Clearly, the reaction method and apparatus of the present invention provide a simple and efficient agglutination reaction. In contrast to the results obtained herein, the commercially available SPOT test must be read within minutes after the complete result is obtained, not to mention the requirement of rocking equipment, more handling steps, etc.

The present invention has been described in
detail, including the preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the present disclosure, may make modifications and/or improvements on this invention and still be within the scope and spirit of this invention as set forth in the following claims.
WHAT IS CLAIMED IS:

1. An agglutination assay apparatus comprising:

   (a) one or more optically clear capillary tubes, the tube or tubes being in direct contact at one end thereof with;

   (b) container means which includes an aqueous reaction medium, and at the opposite end thereof with;

   (c) container means which includes at least one sorbent material;

   said sorbent material being able to support a flow of the reaction medium through the capillary tube or tubes in which an optically detectable agglutination reaction occurs; and

   said apparatus requiring no externally applied kinetic energy either to induce the reaction medium flow or to maintain the agglutination reaction.

2. The agglutination assay apparatus of claim 1, wherein the reaction medium comprises a reaction mixture containing a test sample mixed with an effective amount of reagents suitable for forming macroscopic aggregates.

3. The agglutination assay apparatus of claim 2, wherein the reagents suitable for forming macroscopic aggregates are particulate materials selected from the
group consisting of polymeric microspheres, blood
cells, bentonite particles, liposomes and charcoal.

4. The agglutination assay apparatus of claim 1, 2, or 3, wherein said sorbent material is selected
from sucrose and polyethylene glycol.

5. The agglutination assay apparatus of claim 4,
wherein the sorbent material is finely pulverized.

6. A method of conducting an agglutination assay
for the determination of the presence of an analyte in
a liquid sample comprising the steps of:

   (a) placing an effective quantity of a reaction
       mixture containing the sample to be analyzed and an
       effective quantity of reagents suitable for forming
       macroscopic aggregates into a delivery container
       means;

   (b) placing an effective quantity of a sorbent
       material into a container means;

   (c) inducing flow of reagents via capillary
       action by inserting a capillary tube which will
       support capillary flow between the sorbent container
       means and the delivery container means; and

   (d) allowing sufficient time to pass for the
       reaction mixture to traverse the capillary tube and
       for any agglutination reaction to occur;

said method requiring no externally applied kinetic
energy either to induce the reaction mixture flow or to maintain the agglutination reaction.

7. The agglutination assay test method of claim 6, wherein the reagents suitable for forming macroscopic aggregates are particulate materials selected from the group consisting of polymeric microspheres, blood cells, bentonite particles, liposomes and charcoal.

8. The agglutination assay test method of claim 6 or 7, wherein the sorbent material is selected from sucrose and polyethylene glycol.

9. The agglutination assay test method of claim 8, wherein the sorbent material is finely pulverized.

10. An agglutination assay apparatus useful for the indirect detection of penicillin in milk, comprising in combination:

(a) one or more optically clear capillary tubes capable of initiating and sustaining capillary flow;

(b) the tubes being in direct contact at one end with an aqueous reaction medium, and at the opposite end with at least one sorbent material which will support a flow of the medium through the capillary tubes; and

(c) a binding-pair molecule which will bind to penicillin and polystyrene latex reagent suitable for
forming latex agglutination particles contained within the medium container;

said apparatus requiring no externally applied kinetic energy either to induce or to continue producing the agglutination reaction.

11. The agglutination assay apparatus of claim 10, further comprising an aqueous test sample containing penicillin G.

12. The agglutination assay apparatus of claim 11, wherein the aqueous sample is milk.
### INTERNATIONAL SEARCH REPORT

#### I. CLASSIFICATION OF SUBJECT MATTER

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

- **IPC(4):** G01N 15/10, 14; 21/11; 51/33; 53; 536; 543; 545; 546
- **U.S.Cl.:** 422/55, 58, 59, 68, 74; 436/517, 518, 519 (See Attachment)

#### II. FIELDS SEARCHED

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Classification Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.</td>
<td>422/55, 58, 59, 68, 74</td>
</tr>
<tr>
<td></td>
<td>436/517, 518, 519, 520, 528,</td>
</tr>
<tr>
<td></td>
<td>531, 534, 436/536, 807, 909</td>
</tr>
</tbody>
</table>

Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in the Fields Searched

#### III. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to Claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US, A, 3,888,629 (BAGSHAWE) 10 June 1975, see Abstract, column 1, lines 48-67, column 2, lines 1-2 and 44-62.</td>
<td>1-12</td>
</tr>
<tr>
<td>Y</td>
<td>US, A, 4,419,453 (DORMAN) 6 December 1983, see Abstract, column 5, lines 15-19.</td>
<td>1-12</td>
</tr>
<tr>
<td>Y</td>
<td>US, A, 4,427,779 (RECKEL) 24 January 1984, see column 3, lines 64-68, column 4, lines 1-4, 18-24 and 58-62.</td>
<td>1-12</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:
  - "A" document defining the general state of the art - not considered to be of particular relevance
  - "B" earlier document published on or after the international filing date
  - "L" document which may throw doubts on priority claim or which is cited to establish the publication date of another document (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" 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.

*"F" document member of the same family

#### IV. CERTIFICATION

- **Date of the Actual Completion of the International Search:** 13 NOVEMBER 1987
- **Date of Mailing of this International Search Report:** 01 DEC 1987
- **International Searching Authority:** ISA/US
- **Signature of Authorized Officer:**

  [Signature]

  Jack Spiegel

Form PCT/ISA/210 (second sheet) (May 1986)
I. CLASSIFICATION OF SUBJECT MATTER (CONTINUED)

U.S.Cl.: 436/520, 528, 531, 534, 536, 807, 909
<table>
<thead>
<tr>
<th>Claim numbers</th>
<th>because they relate to subject matter not required to be searched by this Authority, namely:</th>
</tr>
</thead>
</table>

| Claim numbers | because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: |

| This International Searching Authority found multiple inventions in this international application as follows: |

| As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. |

| As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims: |

| No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers: |

| As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee. |

Remark on Protest

- The additional search fees were accompanied by applicant's protest.
- No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document.(^{1}) with indication, where appropriate, of the relevant passages. (^{2})</th>
<th>Relevant to Claim No. (^{1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>US, A, 4,398,894 (YAMAMOTO) 16 August 1983, see Abstract and column 2, lines 30-56.</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>US, A, 3,861,877 (MATHARANI) 21 January 1975, see column 2, lines 17-68.</td>
<td></td>
</tr>
</tbody>
</table>