

[54] CUVETTE

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220/82 R

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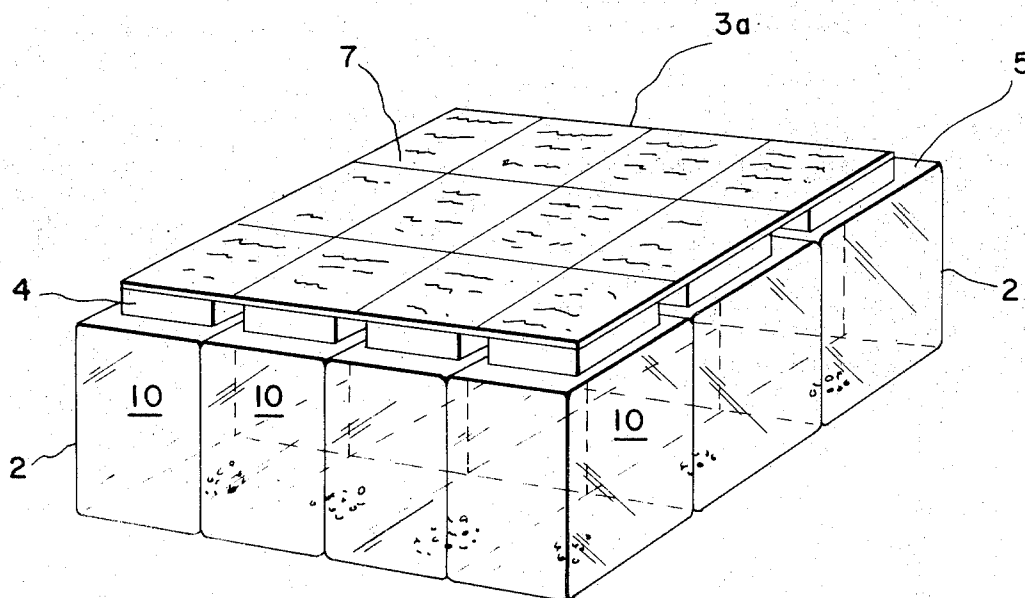
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[57]

ABSTRACT

Disposable cuvettes with square apertures which serve both as a container for reagents used in analytical determinations and for conducting photometric determinations therein are economically sealed by bonding a film or foil to the upper edges of the cuvettes which define their aperture and thereafter separating the sealed cuvettes thus joined together by severing the film or foil along lines defined by their side walls.

9 Claims, 3 Drawing Figures



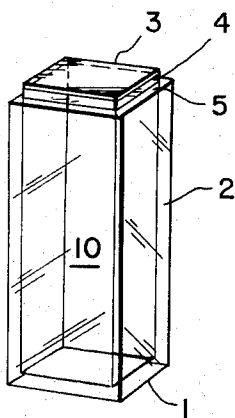


FIG. 1

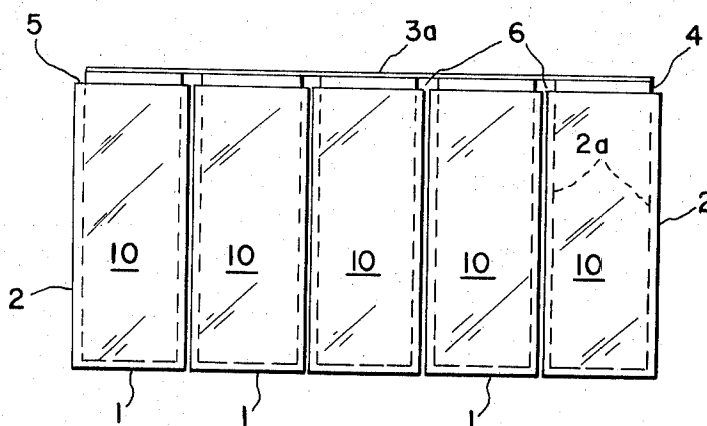


FIG. 2

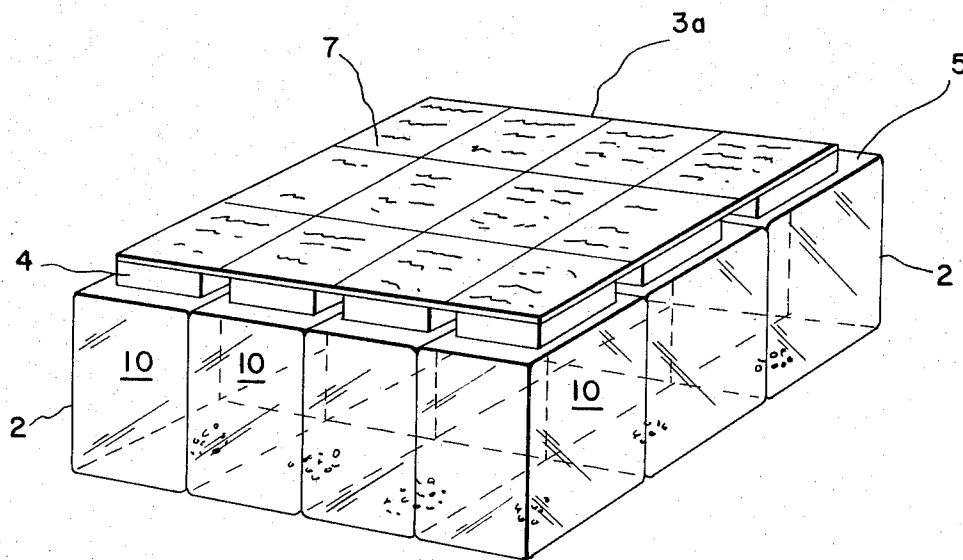


FIG. 3

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CUVETTE

BACKGROUND OF THE INVENTION

This invention relates to a cuvette for holding solids or liquids adaptable for the optical measurement of analytical samples.

The rapid growth of analytical chemistry favored two directions of development in recent years, viz., on the one hand, the manufacture of automatic analyzers which automatically conduct even the complicated working steps of specimen processing, and, on the other hand, the development of simple methods and techniques which enable even unskilled personnel to conduct reliable analyses. Particularly suitable for such developments are photometric measurements, wherein it is often merely necessary to mix a measured amount of sample with a measured volume of reagent, and introduce the mixture into the measuring cuvette of a photometer.

There has been a great number of attempts to simplify the operating steps during the photometric measuring procedure and to relieve the analyst of as much work as possible by mechanically produced, convenient forms of the reagents. For example, the reagent solution required for a single determination can be supplied in individual small bottles which are made available to the analyst ready for use. When the shelf life of the solution is too short, the reagent mixtures can be supplied in individual, single use small bottles or plastic capsules, either by introducing the solid substances directly, or by metering the solution into the individual containers and then freeze-drying the solution therein. In the last-mentioned form, such finished reagents have found wide application.

Particularly useful are cuvettes which can serve both as a storage vessel for the reagent as well as a container for measuring the analysis samples. Such cuvettes can be manufactured in good quality at very low prices from transparent and dimensionally stable material, especially plastics, so that it is economically feasible to use them only once. In addition to the fact that the analyst saves the expense of buying expensive glass or quartz cuvettes and the time required to fill them with the reagents, one-time usage offers the advantage that the cleaning and the drying (or the intermediate rinsing) of the cuvette after each measuring step are eliminated. For these reasons, disposable single-use plastic cuvettes are presently employed in most of the discontinuous automatic analyzers.

In the manual technique, the plastic cuvette has not received widespread acceptance heretofore, especially those which are adapted to serve both as the storage container for the reagents for a single determination and for the optical measurement or the visual evaluation of the analytical sample. The reason apparently is the lack of a simple closure device. Because of the measuring techniques employed, rectangular or square internal cross sections or basal surfaces are preferred. Because of the molding techniques employed in injection molding, the aperture cross section must not be smaller than the internal cross section, so that the inner surfaces of the cuvette form a truncated pyramid with an inclination of the side wall of 0°-5°. Consequently, rectangular or square apertures are produced which are difficult to seal. To solve this problem, it has been suggested, for example, to glue to the normal square opening of the cuvette a head portion of plastic having

a round aperture which latter is sealed after the introduction of the solid reagents or the reagent solution, e.g., by a rubber or plastic plug. However, the manufacture of such a cuvette is relatively complicated, so that production expenses are increased by a factor of approximately 5 to 6. It also has been suggested to employ rectangular plugs or rubber caps. However, difficulties are encountered in sealing the corners of this type of device. Furthermore, both of these sealing possibilities either require very complicated special machines to attach the plugs or caps, or they require manual labor to do so, which makes the sealing process even more expensive.

OBJECTS OF THE INVENTION

It is therefore an object of this invention to provide novel inexpensive disposable rectangular and square cuvettes. It is another object to provide such cuvettes adapted to be used as the container for an analytical sample during photometric analysis thereof. It is another object to provide such cuvettes also adapted to be used as a storage vessel for a reagent employed in such photometric analysis. It is another object to provide a method for sealing such cuvettes. It is still another object to provide a method for producing a plurality of such sealed cuvettes which are separably joined together. Other objects will be apparent to those skilled in the art to which this invention pertains.

SUMMARY OF THE INVENTION

According to this invention, the sealing of filled cuvettes having a rectangular or square aperture is accomplished in a simple and inexpensive manner by bonding, e.g., gluing, welding or sealing a film or foil to the upper edges of the side walls defining the aperture.

Although closing apertures of containers by gluing film or paper thereon has, for a long period of time, conventionally been employed, for example, in the packaging of foodstuffs, it was surprising such means could be employed to seal cuvettes having a square or rectangular aperture which would meet the high requirements of leakproofness, chemical stability, impermeability and pressure insensitivity, which must be satisfied by a cuvette seal despite the narrow bonding area provided by the thin upper edge of the vertical walls of the cuvette.

DRAWINGS

With reference to the drawings:

FIG. 1 is a perspective view of a single cuvette of this invention;

FIG. 2 is a side elevation of a plurality of cuvettes of this invention joined together by the sealing means; and

FIG. 3 is a perspective view of a plurality of cuvettes shown in FIG. 2 joined by a common sealing means.

DETAILED DISCUSSION

The preferred embodiment of the cuvettes 10 of this invention shown in the drawings is formed of a transparent and dimensionally stable material suitable for storage of solids and/or liquids as well as for the optical measurement of analytical samples and has a rectangular or square base 1 and flat side walls 2, the inner surfaces 2a of which form a truncated pyramid having a side wall inclination of 0°-5°. The side walls terminate in an aperture which is sealed, after the desired sub-

stance or substances have been added thereto, with a film or foil 3 by gluing, welding or sealing.

The outer surface of the upper end of each of the four side walls 2 of cuvette 10 have a recessed portion 4 of reduced thickness extending to the upper edge of the side wall and forming a shoulder 5 in the outer surface of the side wall. As shown in FIGS. 2 and 3, when the apertures of a plurality of cuvettes 2 in closely packed proximity with each other are sealed by a large common film or foil 3a, the indented portions 4 and shoulders 5 of adjacent cuvettes and the portion of the film or foil 3a above form a cavity 6 which facilitates separating the cuvettes by cutting the film or foil with a knife, pencil or other sharp or pointed utensil. Cutting of the common web 3a can be facilitated by optional score or printed guidelines 7. The upper surface of the film or foil 3a is printed with indicia identifying the contents of the cuvette.

The cuvettes of this invention can be formed of glass, quartz, or, preferably, plastic and are transparent, at least in the zone of the beam path, in the wavelength range to be measured, e.g., 200–800 nanometers or a partial range thereof.

Preferred materials are plastics which can be formed into cuvettes by injection molding, for example, polystyrene or polymethacrylates and, in particular, polymethacrylates formed from lower-alkyl methacrylates, e.g., polymethyl, ethyl, propyl, isopropyl, n-butyl or tert.-butyl methacrylate, or mixed polymeric esters of methacrylic acid with various, especially lower, alcohols. Polymethyl methacrylate and polyethyl methacrylate are especially suitable, since they have good transmissibility in the ultraviolet range and thus permit measurements in the UV range without large loss of energy. The novel cuvette can also be formed from cellulose esters, such as for example, cellulose or butyrate, or mixed esters of cellulose with various, especially lower, carboxylic acids, especially cellulose acetates/butyrate, for example, those sold under the tradename "Acetobutyrate." Also suitable are ionic carboxyl-group-containing ethylene copolymers which can optionally be cross-linked, for example, polymers of ethylene and other unsaturated monomers, e.g., acrylic acid derivatives, especially those polymers sold under the trademark "Surlyn A." Polymeric carbonic acid esters of aromatic bisphenols, e.g., those sold under the trademark "Makrolon," can also be used.

The plan form of the cuvette, i.e., its base cross section and aperture, is preferably square with an optical path length (= inner edge length of the plan form) of 10 mm. The cuvette preferably has a height of 30–60 mm. The outer walls of the cuvette are preferably plane-parallel. However, they can also be formed into other shapes suitable for optical measurement. For example, the outer surfaces of the side walls can form an angle with the vertical, for instance of the same order of magnitude as the inner surfaces thereof. The wall thickness of the cuvette is preferably 0.3–10 mm., especially 1–5 mm. The dimensions of the square or rectangular aperture preferably corresponds to the entire inner cross section of the cuvette.

To seal the cuvettes, a metal or metal alloy foil, preferably aluminum, or film (plastic or paper) can be employed. The foil or film can be rigid, self-supporting or non-self-supporting and can be of a thickness of 5–1,000 μ , preferably 20–50 μ . Laminated foils and films (which term includes sheets of paper) can also be em-

ployed. If the film or foil is not self-bonding, it can be provided with a layer of adhesive on one face thereof, preferably adhesives of the "hot glue" type which, at room temperature, establish a very tight bond between the cuvette and the foil and are inert at the non-bonded area. Suitable foils provided with a layer of hot glue are commercially available. Especially tight bonds between aluminum foils and polymethacrylate cuvettes are obtained, for example, by applying the commercial adhesive "V 274/6" (producer: Ardal-Klebstoffwerke, Mainz) to the foil, either in a molten condition or dissolved in a solvent. A uniform layer can be obtained by spreading the solution, applying it by a roller, or, best of all, by spraying. Thereafter, the solvent is allowed to evaporate, optionally at a higher temperature. Before or after applying the adhesive layer to the foil, the opposite face of the foils can be subjected to a continuous printing process in accordance with the conventional methods, suitably in such a manner that, later on, each cuvette seal bears at least once the text describing the contents. It is also possible to apply the layer of adhesive to the upper edge of the sides of the cuvette which define the aperture, rather than to the foil, or to both.

When using a foil or film formed of a material which, when heated, will bond itself to the upper edge of the side walls of the cuvette by softening the film or foil or the upper edge of the side walls, or both, the adhesive can be omitted.

The cuvette can be sealed by placing the surface of the film or foil coated with the adhesive layer in contact with the upper edges of the side walls 2 of the cuvette 10 and pressing it into place with a planar plate. When a hot glue is employed, the plate is heated to the temperature required to soften the glue, e.g., 150° C. For example, when a small number of cuvettes is involved, a normal household iron can be used as the heated plate. When a plurality of cuvettes are sealed simultaneously, the sealing step is conducted analogously to the sealing of a single cuvette, viz., with a heatable metallic plate. However, when a large number of cuvettes must be sealed simultaneously, they are preferably disposed on a planar surface. It is advantageous to employ a slightly resilient plate, or a plate covered with foam material, so that the cuvettes during sealing are resiliently pressed against the foil, and small differences in height can be compensated for. In order to be able to arrange the cuvettes readily in rows, they are advantageously placed in a wood, metal, or plastic frame. Because of the satisfactory alignment thereby attained, the film or foil 3a can later readily be severed to separate the cuvettes into single units, as shown in FIG. 1.

In a special embodiment of the method of making the cuvettes of this invention, the cuvette is first provided with a temporary seal. Such a seal is of interest when the reagent in the cuvette is to be freeze-dried and must be sealed, for preservation reasons, under a nitrogen atmosphere in the lyophilizing chamber. In this case, the film or foil can be attached to the plates disposed above the plates supporting the cuvettes. After the freeze-drying step, dry nitrogen is introduced into the chamber. Due to the sealing hydraulics, with the chamber closed, the foil is pressed so firmly against the cuvettes that a temporary seal is obtained. The permanent or final seal is achieved with the aid of a heated metal plate in the manner described above after the chamber has been opened.

The cuvette seals produced in this manner provide a very good seal. They can withstand an internal pressure of about 2 atmospheres gauge or more. Yet, later on, the sealing foil or film can very easily be removed during use by tearing off the film or foil. Alternatively, it is also possible to simply penetrate the foil, to put the cuvette to use, using the tip of the measuring pipette used to introduce the liquid sample into the cuvette.

The many advantages resulting from the use of the plastic cuvette of this invention, which considerably enhance the economical use thereof, could not be foreseen. These advantages become particularly apparent when large individual numbers (100 - 100,000, especially 1,000 - 10,000 per batch) must be sealed. It is not necessary, as with conventional closures, to apply the plugs individually. Instead, the foil to which the adhesive has been applied is simply placed, as described above, on the cuvettes which are disposed closely adjacent to one another in a frame. By pressing a planar metall plate thereon, all cuvettes are simultaneously glued with their upper edges to the foil. Only after the sealing step is the foil severed between the cuvettes, for example, by cutting.

As described above, the cuvettes are expediently provided on the outer upper edge, with a recessed portion 4 which acts as a guide for a blade during the cutting process which separates the cuvettes joined by a common film or foil 3a. The shoulder 5 advantageously has a width of about 0.2 - 1 mm. and the recessed portion 4 has a height of about 0.2 - 15 mm. However, the width of this recessed portion must be dimensioned so that the upper edges of the side walls 2 defining the aperture of the cuvette have a minimum thickness of about 0.2 mm, and preferably thickness of 0.8 - 1.2 mm. It is, of course, also possible to employ cuvettes having, in place of a recessed outer face, an outwardly beveled or an outwardly rounded upper edge to provide the cavity which guides the cutting means.

Since single-use cuvettes are advantageously sold packaged in a bundle, it is advantageous not to separate all the cuvettes which are sealed by a common foil or film 3a from each other, but instead first cut the cuvettes into bundles required for packaging reasons, as shown in FIG. 2. Thus, contrary to conventional processes, the sorting of individual cuvettes into packaged bundles is eliminated. A still further working step is omitted, viz., the labeling of each individual cuvette, since it is possible without difficulties to employ pre-printed foils for sealing the cuvettes. Furthermore, contrary to conventional processes, complicated machines for applying the plugs, for labeling, and for sorting of individual cuvettes into packaged bundles is likewise eliminated. FIG. 2 of the drawings shows such a packaged bundle in a lateral view.

The advantages of the novel single use "one-way cuvette" becomes abundantly apparent when it serves simultaneously as the supply and storage container for a measured amount of reagent, for example when the cuvette contains a substrate/buffer mixture for kinetic enzyme determinations. Examples of such substrate/buffer mixtures are:

- a. Potassium phosphate, monobasic; potassium phosphate, dibasic; sodium pyruvate; NADH₂ (dihyronicotinic acid amide adenine dinucleotide, which, when dissolved in water, is a reagent for lactate dehydrogenase determination).

- b. Potassium phosphate, monobasic; potassium phosphate, dibasic; DL-alanine; NADH₂; and lactate dehydrogenase (for determining glutamate-pyruvate transaminase); and

- c. Potassium phosphate, monobasic; potassium phosphate, dibasic; sodium-L-asparaginate; NADH₂; and malate dehydrogenase (for the determination of glutamate-oxalacetate transaminase).

After the solution to be tested has been added, the measurement can immediately be conducted, without danger of contamination or loss of reagent during refilling. When a mixture of several reagents are required which, as a mixture are stable for only a short period of time, the novel cuvette can be provided with a partition separating the lower portion of the cuvette into two chambers. The mutually incompatible auxiliary substances can be introduced in metered quantities into these chambers, e.g., in the form of solutions, with subsequent freeze-drying. After the measured amount of solution has been added, the reactants can be combined by shaking.

The cuvettes, or bundles of cuvettes, are preferably packed into a metal container or in a metallic foil, optionally in the form of packaged bundles, optionally with the addition of a drying agent.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

EXAMPLE 1

- a. Coating the Sealing Foil with a Layer of a Hot Glue

Two-hundred grams of "Ardal" V 274/6 contact cement is dissolved in 2 liters of chloroform, and applied by spraying to one side of a commercial aluminum foil of 30 μ thickness, the other side of which has been provided with a continuous imprint of the text of the identifying label. Thereafter, the foil is heated for about 10 minutes to 110° C.

- b. Preparation of the Cuvettes for Sealing

The plastic cuvettes, filled with reagent, are placed upright on a mat of foam material having a thickness of 5 mm., which mat is disposed on a planar surface. With the aid of a frame, the cuvettes are pushed together until they are packed closely adjacent one another, so that the indented portion 4 (grooves) of the cuvettes form continuous channels.

- c. Sealing Process and Cutting

The side of the foil coated with the layer of hot glue, is placed face down on the cuvettes and, to protect the labeling from being scratched, a second, uncoated aluminum foil is placed thereover. Then, pressure and heat are applied to the upper face of the foil for about 15 seconds with a household iron adjusted to 150° C. (wool). Thereafter, the foil is cut in the channels formed by the indented portion (grooves) of the cuvettes with a knife.

EXAMPLE 2

One kg. of Ardal V 274/6 contact cement is melted and mixed with 100 ml. of chloroform. The solution is introduced into a foil spreading machine wherein the solution runs, without the use of rolls, directly onto the aluminum foil disposed underneath the storage tank.

The foil moves at a speed of 10 cm./min. beneath the applicator blade, and is coated during this procedure with a thin film of adhesive. After passing a drying channel and cooling, the foil is again reeled up on a drum, suitably with the interposition of a separating paper (e.g., siliconized paper), to prevent the foil from sticking together.

The cuvettes are sealed as described in Examples 1(b) and 1(c).

EXAMPLE 3

Production of a Reagent Preparation for the Quantitative Determination of Glutamate-Oxalacetate Transaminase (GOT).

Approximately 6,000 plastic cuvettes are packed as densely as possible in three metallic frames and rigidly mounted thereon with setscrews. Then the cuvettes in the frames are rinsed. The cuvettes are allowed to drip-dry, and then each are filled with a filling machine at a rate of 50/minute with 1 ml. of a solution containing the following components per 500 ml.:

- 0.05 M of ethylenediaminetetraacetic acid
- 0.10 M of phosphate buffer ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$), pH 7.4
- 0.25 M of aspartic acid
- 0.01 M of ketoglutaric acid
- 2×10^{-4} M of dihydronicotinic acid amide adenine dinucleotide (NADH_2)
- 10 mg. of malate dehydrogenase (MDH)
- 10 mg. of lactate dehydrogenase (LDH)

For the subsequent freeze-drying step, a device is utilized having four temperature-controllable plates. The uppermost plate serves as radiation protection, and the lower three plates serve as supporting plates. The arrangement also contains a hydraulic unit with which the plates can later be pressed together.

The adhesive coated aluminum foil, produced as described in Example 2, is attached to the undersides of the upper three plates so that the printed side is oriented upwardly and the side having the coating of hot glue is directed downwardly. The three frames with the filled cuvettes are placed on the three lower plates, and a freeze-drying process is conducted in a conventional manner. After introducing a nitrogen atmosphere, but before opening the chamber, the plates and thus also the cuvettes positioned between the plates and the foil, are pressed one to the other with the aid of the hydraulic unit for a short period of time. When the frames with the cuvettes are withdrawn from the chamber, the foil adheres to the cuvettes so tightly that the nitrogen cannot escape. Thereafter, a plate heated to 150°C . is pressed for 30 seconds against the foil, which tightly seals the foil to the upper rim of the cuvette. The foil between the cuvettes is cut by guiding a blade along in the channels formed by the indented upper portion of adjacent cuvettes. Not all of the cuvettes are separated from one another. Instead, the foil is cut so as to leave, e.g., 25 cuvettes together in a bundle. After releasing the setscrews, the bundles of cuvettes, filled with reagent, sealed, and labeled, can be withdrawn from the frame and further packaged.

For use purposes, a single cuvette is separated from the bundle, simultaneously removing the foil closure. Two ml. of water and 0.5 ml. of the serum to be examined are added thereto, the components are mixed, and the reduction of extinction (ΔE per minute) is measured at 366 nanometers (1 cm. layer thickness). The

GOT concentration is calculated in accordance with the following formula:

$$\text{GOT Concentration} = \Delta E/\text{min.} \times 1.515 \text{ U/ml.}$$

(U = international enzyme unit)

Analogously, a reagent preparation for determining glutamate-pyruvate transaminase concentration is produced from a solution which, prior to the freeze-drying step, contains the following components (per 500 ml.):

- 0.1 M of phosphate buffer, pH 7.4
- 0.8 M of DL-alanine
- 0.01 M of α -ketoglutaric acid
- 2×10^{-4} M of NADH_2 and
- 10 mg. of LDH

To measure the LDH concentration, the solution contains 2×10^{-4} M of NADH_2 in 0.02 M of bicarbonate buffer, pH 9.5. In this case, the freeze-dried product is dissolved in a 6×10^{-4} M of pyruvate solution in 0.1 M of phosphate buffer, pH 7.4, rather than in water.

EXAMPLE 4

A commercial "hot melt" film (silicon paper with a coating of hot glue) is pressed, with the aid of an iron, heated roll or other suitable heated device, for a few seconds onto printed paper at about 180°C . After cooling, the silicon paper can be readily pulled from the printed paper, to which the hot glue adheres completely. The printed paper, provided with a layer of this hot glue, is employed for sealing the cuvettes in accordance with Examples 1(b) and 1(c).

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

What is claimed is:

1. A sealed cuvette having a rectangular or square basal surface and side walls whose inner surfaces form a truncated pyramid having a side wall inclination of 0° - 5° and whose upper edges form a rectangular or square aperture corresponding in dimensions to the inner cross section of the cuvette and whose side walls have a recessed outer face at their upper end which forms a shoulder in said side walls extending to their upper edge, formed of a transparent and dimensionally stable plastic which is light-permeable in at least a portion of the wavelength range from 200-800 nanometers and adapted for string solids and liquids and for the optical measurement of analytical samples, said aperture being sealed by a foil or film bonded to said upper edge of said side walls.
2. A cuvette according to claim 1, formed of polystyrene.
3. A cuvette according to claim 1, formed of polymethyl methacrylate.
4. A cuvette according to claim 1, whose aperture is sealed with aluminum foil.
5. A cuvette according to claim 1 wherein said foil or film bears identifying indicia on its outer surface.
6. A cuvette according to claim 5 whose aperture is sealed with aluminum foil.

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7. A plurality of cuvettes according to claim 1 whose side walls are in face-to-face contact and which are joined together by the film or foil sealing their aperture.

8. A cuvette according to claim 7 wherein said foil or

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film bears identifying indicia on its outer surface.

9. A cuvette according to claim 2 whose aperture is sealed with aluminum foil.

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