

[54] REAGENT AND METHOD FOR ALBUMIN DETERMINATION

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[56] References Cited

UNITED STATES PATENTS

3,533,749 10/1970 Kleinman 23/230 B

OTHER PUBLICATIONS

Bigat, et al., "Some Methodological Modifications of the Technicon SMA 12/60 Auto Analyzer System," Clinical Chemistry, Vol. 18, No. 7, 1972, p. 633.

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[57] ABSTRACT

A reagent for albumin determination comprising an aqueous solution of 0.1 – 0.2 grams of bromcresol green, 4 – 6 grams of sodium salt of EDTA and 4.5 – 6 ml. of polyoxyethylene fatty alcohol ether per liter of said solution buffered to pH 4 – 4.2.

4 Claims, No Drawings

REAGENT AND METHOD FOR ALBUMIN DETERMINATION

This invention relates to a reagent and method for the determination of albumin. More particularly, this invention relates to an improved reagent and method for the colorimetric determination of serum albumin with the dye-binding agent bromocresol green (BCG).

Serum albumin quantitation based on the protein-error of dye-binding indicators has been known for some time. This quantitation is based on the phenomenon that the absorbance of dilute solutions of the dye will be selectively diminished at its peak wavelength due to the affinity of the dye for albumin. The total albumin content of the sample is then determined by the difference of absorbance with a dilute solution of the dye.

Various indicators heretofore used for such dye-binding colorimetric determination of albumin are azo dyes such as methyl orange and 2-(4'-hydroxybenzeneazo) benzoic acid and the phthalein dyes such as bromocresol purple and bromocresol green (BCG) described in U.S. Pat. No. 3,558,278 and references cited therein.

The BCG method of serum albumin determination finds wide use in clinical laboratories in manual procedures and has also been adapted to automated procedures such as the SMA 12/60 (Trademark of Technicon Instruments Corporation, Tarrytown, N.Y.); Westgard, et al., *Clin. Chem* 18, pp. 647-53 (1972). This method has undergone various modifications since its original introduction by Rodkey, *Clin. Chem.* 11, pp. 478-87 (1965). A recent modification described by Doumas, et al., changes the operative pH from 7 to 14 and includes a nonionic surfactant (Brij - 35, Trademark, of Atlas Powder Company), in the reagent, *Clin. Chem. Acta* 31, pp. 87-96 (1971). This modification is said to provide greater linearity in the absorbance-concentration relationship and to prevent turbidity. Similar results at this acid pH level and with other nonionic surfactants are described by Miyada, et al., *Clin. Chem.* 18, pp. 51-55 (1972) and in British Pat. No. 1,267,186.

Notwithstanding the improvements ascribed to the BCG reagent which contains nonionic surfactant and is buffered to an acid pH of about 4, it has been found by the present inventors that when said reagent is employed in automated instruments such as the Technicon SMA 12/60, turbidity occurs within a relatively short period of time, for example, within about one half to one hour of continued usage. The walls of the reagent tubing and the flow cell became coated with protein precipitate which undesirably affects the precision of the albumin determination.

Another modification of the BCG method of serum albumin determination incorporates a small amount of tetrasodium ethylenediamine tetraacetate (Na_4EDTA) in the BCG reagent, Bigat, et al., *Clin. Chem.* 18, pp. 630-42 (1972). This modification employs 2.65 grams of Na_4EDTA and 30 ml. of Brij - 35 with 0.168 grams of BCG in one liter of reagent. However, it has now been found that these proportions of components in the BCG reagent do not provide the desired formulation of the reagent for use with automated instruments such as the Technicon SMA 12/60. The Brij - 35 is a relatively heavy component which does not mix well and tends to settle out of the mixture in use. Moreover, when the

aforesaid previously disclosed proportions of Na_4EDTA and Brij - 35 are used, an undesirable baseline drift occurs. That is, the baseline, namely the maintenance of a constant absorbance while running only the BCG reagent without added protein in the instrument, gradually changes during operation of the instrument over extended periods of time.

In accordance with the present invention, an improved reagent and method for the determination of albumin is provided which employs the dye-binding agent bromocresol green (BCG) in aqueous solution. It has been found that the tendency to coat the walls of the reagent tubing and the flow cell can be significantly and substantially reduced without causing settling of material in the BCG reagent and without causing baseline drift by employing certain unique proportions of nonionic surfactant and sequestering agent in the BCG reagent. By employing this improved reagent in the Technicon SMA 12/60, the walls of the reagent tubing and the flow cell have been kept clear for more than four hours during continuous usage for albumin determination without settling of any of the reagent components and without baseline drift.

In general, the BCG reagent of this invention will comprise bromocresol green together with a small amount of the nonionic surfactant, a small amount of the sequestering agent, and buffer in an amount sufficient to provide a pH of from about 4 to about 4.2.

The nonionic surfactants which are used in the BCG reagent are the polyoxyethylene fatty alcohol ethers such as polyoxyethylene lauryl ether, polyoxyethylene stearyl ether, polyoxyethylene cetyl ether and polyoxyethylene oleyl ether (available under the trademark "Brij") and similar such polyethylene fatty alcohol ethers having from about 12 to about 30 carbon atoms in the fatty alcohol moiety and from about 2 to about 23 ethylene oxides in the molecule.

The sequestering agent employed in the BCG reagent is a substance capable of sequestering the protein, preferably a calcium sequestering agent such as disodium ethylenediamine tetraacetate (Na_2EDTA), or its tetrasodium analog (Na_4EDTA).

Examples of buffers which can be used to provide the desired acid pH are succinic acid-sodium succinate, citric acid-sodium citrate, and lactic acid-sodium lactate. These buffers can be prepared by conventional methods such as, for example, suitable admixture of the acid and sodium hydroxide base in distilled water in proportions appropriate for providing a pH of from about 4 to about 4.2.

The preferred nonionic surfactant is polyoxyethylene (23) lauryl ether (available under the trademark "Brij - 35"); and the preferred sequestering agent is Na_2EDTA .

In accordance with the present invention it is critical to use the foregoing materials in proportions of from about 4 to about 6 grams of the sequestering agent and from about 15 to about 25 ml. of the nonionic surfactant (on a 30% by volume concentration in aqueous solution basis) per liter of the BCG reagent containing about 0.1 gram of BCG. Brij - 35 is a waxy solid and is therefore supplied by the manufacturer in a 30% aqueous solution. Accordingly, the above amounts are equivalent to about 4.5 to 6 ml. of the surfactant itself.

The following example will further illustrate the invention although the invention is not limited to this specific example.

EXAMPLE

Bromcresol green reagent solution having a pH of 4.2 is prepared by admixing the following reagents in distilled water and diluting to a volume of one liter:

Component	Amount
Bromcresol green WS	
water soluble indicator	0.108 gram
Sodium hydroxide	2.0 grams
Succinic Acid	8.85 grams
Sodium azide preservative	0.50 gram
"Brij - 35" nonionic surfactant,	
30% aqueous solution	17.5 ml.
Disodium EDTA (Na ₂ EDTA)	5.3 grams

The above BCG reagent is used for the determination of albumin at 630 m in the Technicon SMA 12/60 as follows:

The unknown sample is pre-diluted, resampled and then introduced into a stream of the BCG reagent and measured at 630 m.

The walls of the reagent tubing and the flow cell remained clear without any coating of protein precipitate during four hours of continuous sampling. The absorbance-concentration relationship remained linear to a concentration of up to 6 grams percent albumin and at a rate of 80 samples per hour. By way of comparison, when another bromcresol green reagent which was similar to the foregoing reagent except that it did not contain the disodium EDTA was used in the identical procedure, coating of the walls of the reagent tubing and the flow cell occurred within one half hour of continuous sampling and at a rate of only 60 samples per hour. So also, when another bromcresol green reagent which was similar to the foregoing reagent except that the amount of Na₂EDTA was 2.65 grams and the amount of Brij - 35 was 30 ml. was used in the identical procedure, undesirable settling of the Brij - 35 occurred during the sampling and, gradual baseline drift occurred during running of the BCG reagent itself through the instrument during a one to one and one-half hour period.

Various other examples will be apparent to the person skilled in the art after reading the foregoing description without departing from the spirit and scope of the invention. All such further examples are included within the scope of the appended claims.

What is claimed is:

1. A reagent for use in the determination of albumin consisting essentially of an aqueous solution of from about 0.1 to about 0.2 gram of bromcresol green, from about 4 to about 6 grams of sequestering agent selected from the group consisting of disodium and tetrasodium salts of ethylenediamine tetraacetic acid, from about 4.5 to about 6 ml. of nonionic surfactant selected from the group consisting of polyoxyethylene fatty alcohol ethers having from about 12 to about 30 carbon atoms in the alcohol moiety and from about 2 to about 23 ethylene oxides in the molecule, per liter of said aqueous solution and buffered to a pH of from about 4 to about 4.2.

2. The reagent of claim 1 in which the sequestering agent is disodium ethylenediamine tetraacetate and the nonionic surfactant is polyoxyethylene (23) lauryl ether.

3. In the method of determining serum albumin by the use of bromcresol green, together with sequestering agent in aqueous solution buffered to a pH of 4-4.2, the improvement comprising employing from about 4 to about 6 grams of sequestering agent selected from the group consisting of disodium and tetrasodium salts of ethylenediamine tetraacetic acid, and from about 4.5 to about 6 ml. of nonionic surfactant selected from the group consisting of polyoxyethylene fatty alcohol ethers having from about 12 to about 30 carbon atoms in the alcohol moiety and from about 2 to about 23 ethylene oxides in the molecule, per liter of said aqueous solution.

4. The method of claim 3 in which the sequestering agent is disodium ethylenediamine tetraacetate and the nonionic surfactant is polyoxyethylene (23) lauryl ether.

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