

[54] **METHOD AND APPARATUS FOR ANALYZING A LIQUID CONTAINING MACROMOLECULES THAT WOULD INTERFERE WITH THE ANALYSIS**

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[58] Field of Search 23/230, 253, 230 B; 210/25, 37, 38

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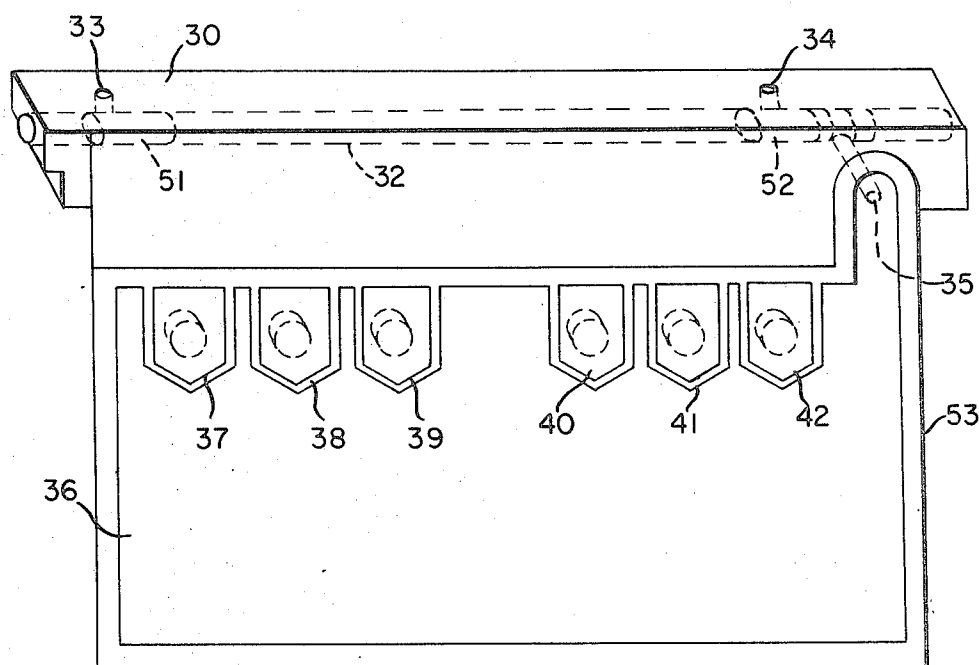
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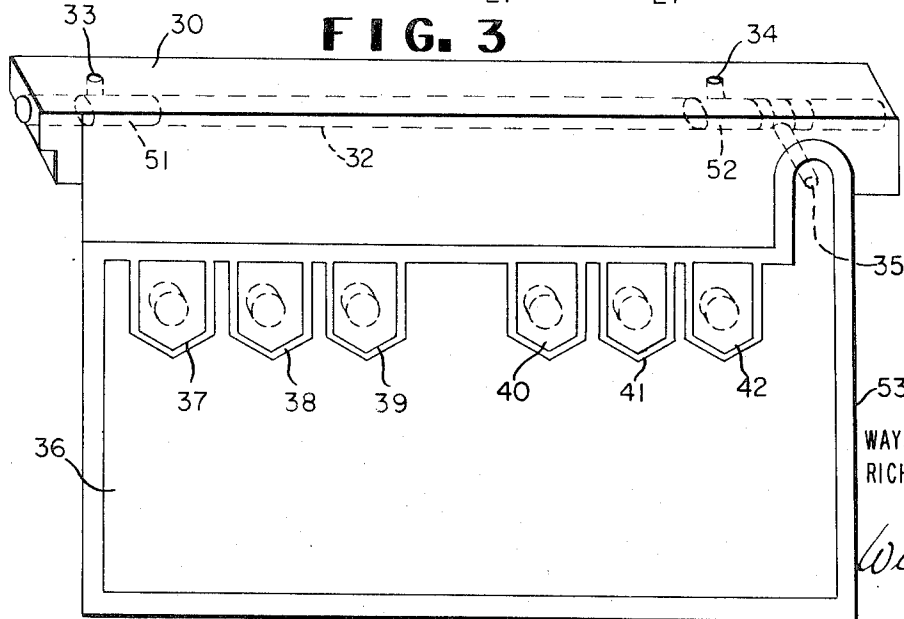
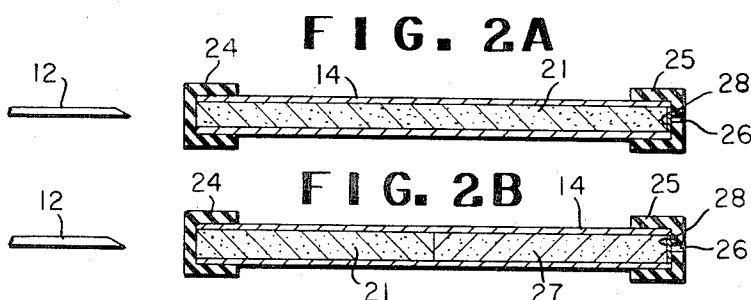
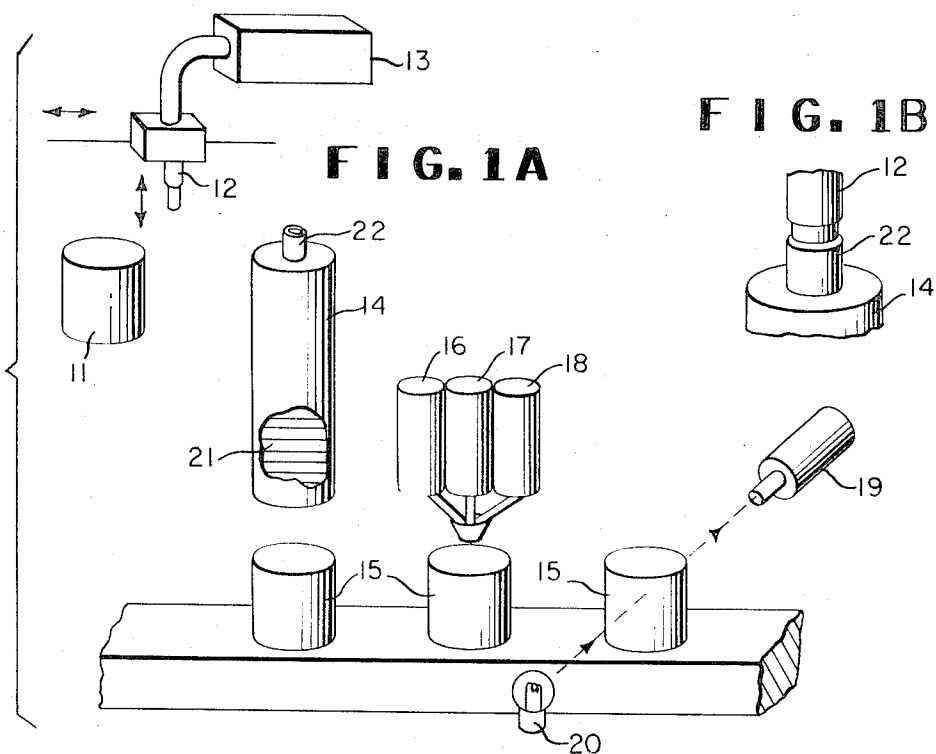
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[57] **ABSTRACT**

A method and apparatus for analyzing a liquid for a particular constituent when the liquid contains charged macromolecules that would interfere with the analysis. The method comprises forcing the liquid through an ion exchange medium into a reaction chamber in a manner such that substantially all of the liquid passes through the ion exchange medium and determining the presence of said particular constituent. The ion exchange medium is adapted to remove substantially all of at least one type of charged macromolecule from the liquid without removing substantially any of the particular constituent. Normally at least one reagent reactive with said particular constituent must be introduced into the liquid before the determination is made. Positively or negatively charged macromolecules, or both, can be removed from the liquid by using positive or negative ion exchange resins, or combinations thereof, as the ion exchange medium. The process is particularly effective for removal of dipolar ions, especially protein molecules, whose charge depends on the pH of the medium in which they are found. The charge on the macromolecule can then be adjusted so that the ion exchange medium can selectively remove the macromolecule without removing any of the particular constituent.

12 Claims, 5 Drawing Figures





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METHOD AND APPARATUS FOR ANALYZING A LIQUID CONTAINING MACROMOLECULES THAT WOULD INTERFERE WITH THE ANALYSIS

CROSS REFERENCE TO RELATED APPLICATIONS

This invention is particularly related to the subject matter of U.S. Pat. application Ser. No. 753,197 for an Automatic Clinical Analyzer, filed on Aug. 6, 1968 by E. P. Carter et al. in that the instrument disclosed therein can be adapted to perform the method of the present invention. It is also particularly related to U.S. Pat. application Ser. No. 753,199 for an Apparatus and Method for Fluid Handling and Sampling, filed on Aug. 16, 1968 by W. J. Ambrose et al. in that the device disclosed therein is useful as the liquid handling means of the present invention; and to U.S. Pat. application Ser. No. 545,494 for an Analytic Test Pack and Process for Analysis, filed on Apr. 26, 1966 by D. R. Johnson et al now U.S. Pat. No. 3,476,515. in that the analytic test pack disclosed therein can be adapted to contain some of the elements of the present invention. These cross references are merely illustrative and are not intended to restrict the scope and/or use of either the present invention or the inventions of the cross-referenced applications.

BACKGROUND OF THE INVENTION

Photometric analysis of liquid samples is a well known and important tool in chemical analysis, particularly in clinical areas where the need for the rapid assay of body fluids has led to the development of a number of automatic instruments designed to perform a series of analytic tests rapidly and accurately. Most of these instruments employ well-known biochemical tests for the presence or absence of a particular constituent in a liquid sample, in which a reagent, which will induce a reaction in the liquid sample when the particular constituent is present, is introduced into the liquid sample. The induced reaction affects the photometric density of the liquid sample and the change is detected by a photometer. Tests to determine the presence or the concentration of urea nitrogen or blood glucose, as set forth in the examples to U.S. Pat. application Ser. No. 545,494, are common examples of this type of analysis.

In some instances, however, these tests lose their effectiveness because the reagents used to produce the desired reaction react with macromolecules present in the liquid sample. These reactions interfere with the analysis, usually by producing a precipitant which clouds the liquid sample enough to make photometric analysis unreliable. The problem is particularly prevalent when the liquid sample is a body fluid, in which case the interfering macromolecule is usually a protein molecule. Consequently, many routine analytic tests require the chemical removal of macromolecules from the sample liquid prior to analysis. When the analysis is performed by hand, the removal of macromolecules can be accomplished by precipitation and centrifugation, but in the case of automatic instruments such a procedure is impractical. The need for some automatic method to remove macromolecules from sample liquids prior to analysis was recognized early in the development of automatic analysis instruments. L.T. Skeggs in U.S. Pat. No. 2,797,149 approached the problem by

incorporating a dialysis apparatus in his instrument which was designed to remove what he referred to as "crystalloid" constituents from a solution consisting of "crystalloid" and "non-crystalloid" (or colloidal) constituents, in proportion to the proportion of crystalloid substance in the liquid. This technique depends on a more or less continuous flow of the sample liquid and is, therefore, impractical for use in batch analysis. Other than this, the technique has several positive disadvantages. Since dialysis depends upon the diffusion of the crystalloid constituents through a semi-permeable membrane, it is a slow process in which more liquid sample than is necessary for the analysis must be introduced into the instrument so that a sufficient amount of the liquid sample will pass through the membrane to be analyzed in a reasonable length of time. In addition to this, dialysis operates by excluding the larger colloidal or macromolecular material from passing through the membrane and acts nonselectively to remove all macromolecules, regardless of type. Also, since the diffusion rate of the different macromolecules is different, the amount of each macromolecule removed is difficult to standardize. Finally, the membrane, which functions primarily as a filter suffers from the defects of a filter; it is a source of contamination which must be replaced periodically, and a small pinhole in it will make it inoperative.

Accordingly, it is an object of the present invention to provide an improved process for analyzing a liquid containing charged macromolecules which would interfere with the analysis if not removed.

It is a further object of the present invention to provide a process which can be used in an automatic instrument for the analysis of a liquid containing charged macromolecules, particularly protein molecules or other dipolar ions whose charge depends upon the medium in which they are found, that would interfere with the analysis if not removed.

It is a still further object of the present invention to provide a method and apparatus for such analysis which requires an extremely small amount of liquid sample and which can selectively filter out the unwanted type of charged macromolecule without removing any of the sample liquid or a substantial amount of the desirable macromolecule content.

It is a still further object of the present invention to provide an apparatus for use in such analysis which will not become contaminated by repeated tests requiring the removal of macromolecular material.

SUMMARY OF THE INVENTION

Accordingly, these objects are achieved by providing an ion exchange medium, to remove charged macromolecules from the liquid sample prior to analysis. The method comprises forcing substantially all of the liquid sample through a selective ion exchange medium which is adapted to remove substantially all of at least one type of charged macromolecule from the liquid sample, depositing the liquid sample from which the undesirable macromolecular content has been removed into a reaction chamber, and determining for the presence of the particular constituent. Usually prior to the determination, reagents which will initiate a reaction in the liquid sample must be introduced into the liquid. If the reaction affects the photometric density of the liquid sample when the particular constituent is present, then the determination can and usually is made photometri-

cally. Depending on the needs of the analysis, different charged macromolecules can be removed by choosing the ion exchange resin so that it will selectively, usually by charge, remove the desired macromolecule leaving the remainder of the liquid sample unaffected. In a preferred embodiment, useful when the macromolecule is a protein molecule or other dipolar ion (Zwitterion) whose charge is dependent on the pH of the medium in which it is found, the pH of the liquid sample is adjusted so that the macromolecule has the proper charge to be selectively removed from the liquid sample.

The apparatus comprises an ion exchange means containing a selective ion exchange medium adapted to remove substantially all of at least one type of charged macromolecule from the liquid sample; a sampling means adapted to remove the liquid sample from its original container and to force substantially all of it through the ion exchange medium into a reaction chamber; and means for analyzing the liquid sample. Reaction initiating means are also usually included. The ion exchange column can contain either positively or negatively charged ion exchange resins, or both, so that either positively or negatively charged macromolecules, or both, can be removed. Various types of ion exchange resins such as cellulose ion exchange resins or dextran ion exchange resins are contemplated. In one embodiment the ion exchange medium is saturated with a first quantity of diluent, and the liquid sample is forced through the ion exchange medium by introducing a second quantity of either the same or a different diluent into the ion exchange medium behind the liquid sample so that it displaces substantially all of the liquid sample and a quantity of the diluent equivalent to the second quantity of diluent, into the reaction chamber. Since substantially all of the liquid sample passes through the ion exchange medium to the analysis stage, not just some proportion of it, a smaller initial amount of liquid sample can be used. Since the ion exchange medium can be selective, either the positive or negative macromolecules can be removed, or both, depending on the selective needs of the analysis; and finally, since the ion exchange means can be in the form of a small inexpensive cartridge, the liquid sample can be introduced into the automatic instrument through a disposable cartridge which eliminates the problem of contamination.

The ability of certain ion exchange resins to adsorb macromolecules, particularly protein molecules, has been known for some time. This ability forms the basis for protein chromatography in which protein molecules are first removed from the carrier fluid by an ion exchange column, then separated within the column and finally selectively eluted from the column. In an article in the Technicon Symposium on "Automation in Analytic Chemistry," published by Mediad in 1966, D. L. Bettner described a related use for ion exchange resins, in the determination of serum thyroxine. The method consists of adsorbing amino acids, including thyroxine and thyronime on the resin, then eluting the protein molecules and other contaminants from the resin using a series of acetate solutions, and finally eluting the thyroxine and thyronime from the resin using an acetic acid wash. The final elution, containing the material to be analyzed, is collected and automatically analyzed for iodine content.

The teaching of the prior art, therefore, is characterized by the fact that the material to be analyzed is first

totally removed from a carrier liquid by adsorption on the resin, and then recovered from the resin by eluting. The carrier liquid is discarded, and when the material to be analyzed is finally released to analysis, it is carried by a liquid which is different from the original medium. The present invention is characterized by the fact that the material to be analyzed is actually the carrier liquid. The protein molecules or macromolecules are the unwanted constituent. They are removed by the resin and discarded.

The present method has an inherent advantage, especially if it is to be used in conjunction with an automatic instrument, because no intermediate removal and recovery steps are necessary. The liquid sample is passed directly through the ion exchange medium, which removes the undesired macromolecule, into the reaction chamber. Despite this advantage any process using an ion exchange medium to remove charged macromolecules from a liquid sample which is to be integrated into an automatic instrument, must deal with at least two problems that are not present if the removal is to be performed prior to the introduction of the liquid sample into the instrument. First of all, the method must remove substantially all of the unwanted macromolecules, and it must do so within the limits of space and time set by the instrument. The medium through which the liquid sample passes can only be a few inches long, if it is to be physically integrated into an instrument, and the time required for the liquid sample to pass through the medium cannot exceed a few minutes, without significantly decreasing the efficiency of the instrument. The second problem that the present invention must deal with is the the present invention must deal with problem of presenting the liquid sample to the reaction chamber in a form suitable for analysis by an automated procedure, which by its very nature must deal with precise predetermined volumes and concentrations of the sample to be analyzed and the reagents used in the analysis. Normally, the sample liquid is mixed with a diluent before the reagents are added, and if the means used to remove the interfering macromolecule alters the concentration of liquid sample in the mixture in an unpredictable way, then the analysis cannot proceed automatically with any accuracy. Prior to this time it was the opinion of those skilled in the art that substantial removal of macromolecules such as protein molecules from a liquid sample could not be accomplished, using ion exchange resins within the strictures of time and space imposed by automation without substantially altering the properties or concentration of the liquid sample.

By the practice of the present invention substantial removal of the interfering macromolecule is accomplished by using an ion exchange medium, adapted to act only on the desired macromolecule so that the remaining liquid sample can be passed through the ion exchange medium unaffected. A short column of the proper ion exchange medium is effective in removing substantially all of the macromolecular material, and it has been found that by forcing the liquid sample through the short ion exchange column under pressure, the liquid sample can be passed entirely through the column in less than two minutes. The liquid sample is usually forced through the column by using a diluent under pressure behind it, and in one embodiment the column is first saturated with the same or a different diluent than that used to force the liquid sample

through the column. If the proper amount of diluent is used, all the liquid sample and a known amount of the diluent is passed into the reaction chamber, so that there is no uncertainty in the concentration or properties of the mixture in the reaction chamber.

Other advantages, and the operation of the present invention can best be described by reference to the following figures in which:

FIG. 1A is a schematic diagram of a simple embodiment of an automatic analysis instrument which can be used in the practice of a present invention;

FIG. 1 is a schematic representation of how probe 12 mates with the ion exchange column;

FIG. 2A is a cross-sectional view of one ion exchange column and FIG. 2B is a cross-sectional view of another ion exchange column which can be used as the ion exchange means in an automatic analysis instrument, such as the one shown in FIG. 1A; and

FIG. 3 is a diagram of an analytic test pack, for use in an automatic analytic instrument, comprising an ion exchange column, a reaction chamber and reagents which can be used in the practice of a present invention.

DESCRIPTION OF THE DRAWINGS

In FIG. 1A the liquid to be analyzed is initially contained in sample container 11. A portion of the liquid sample is drawn up from this container into probe 12 under the action of pump 13, and discharged into ion exchange column 14 under sufficient pressure to cause it to pass entirely through the ion exchange medium 21 contained in the column, and within a short period of time to be discharged into reaction chamber 15. One way to insure that all of the liquid sample passes through the column is to have the pump draw in sufficient diluent prior to drawing in the liquid sample, so that the liquid sample will be forced through the column, by the force of the diluent entering behind it. If the ion exchange medium contained in the column is dry, there may be some difficulty in insuring that all of the liquid sample passes through the column, because some of it may be adsorbed by the medium in a manner which will make it difficult to dislodge. Presumably, if enough diluent is used, substantially all of the liquid sample will be recovered from the column, but there is a simpler way to achieve the same result using only a small amount of diluent. The column is first saturated with a first quantity of diluent. The pump 13 draws a second quantity of the same or a different diluent up into the probe and then draws the desired amount of liquid sample into the probe behind the diluent. When the contents of the probe is injected into the ion exchange column, the liquid sample is discharged into the column first, followed by the diluent. As the liquid in the probe is being introduced into the column, it displaces the liquid already in the column. If the second quantity of diluent drawn into the probe is greater than the first quantity of diluent originally contained in the column, then as the contents of the probe is injected into the column, the first quantity of diluent is first displaced from the column, then the liquid sample is displaced, followed by that portion of the second quantity of diluent which exceeds the saturation capacity of the column. At the end of the pumping action, substantially all of the liquid sample and a quantity of diluent equivalent to the second quantity of diluent is discharged into the reaction chamber, so that the precise amount of liq-

uid introduced into the reaction chamber and the concentration of sample in the liquid in the reaction chamber can be controlled. Various reagents from reagent chambers 16, 17 and 18 are then introduced into the reaction chamber to initiate a reaction, and after a set time the opacity of the liquid in the reaction chamber or the change in opacity over a set period of time, is measured photometrically, by irradiating the liquid with light source 20 and monitoring the intensity of the transmitted light with photometer 19.

FIG. 1A illustrates a simple automated analysis apparatus. The variations on this instrument are too numerous to discuss. They include variations on the method of transferring the liquid from the sample container to the reaction chamber, variation on the method of introducing the reagents, variations on the method of photometrically analyzing the liquid and enumerable intermediate conditioning steps that are not illustrated. Two variations on the above-described procedure are worth mentioning, however. In some instances, the change in the liquid that is monitored to determine the presence of the particular constituent may occur automatically and does not need to be induced. In this case the step of introducing a reagent into the reaction chamber is unnecessary. Also, while photometric analysis is the normal procedure for determining the pressure of the particular constituent, other analytic procedures, such as colorimetric analysis can be envisioned. I wish it to be known that I do not wish to be limited to reaction induced changes and photometric analysis. It is to be understood that the heart of the present invention is the removal of charged macromolecule from the liquid sample via an ion exchange means, such as ion exchange column, and that the particular means of introducing the sample into the ion exchange means and of analyzing the liquid after it has passed through the ion exchange means are mere collateral consideration for the present invention.

One instrument which is particularly well adapted for the methodology described above is described in U.S. Pat. application Ser. No. 753,197. A particular sample transfer device which is also particularly well adapted for the methodology described above is described in U.S. Pat. application Ser. No. 753,199.

Since the ion exchange means is to be used in an automatic instrument, the instrument must be designed so that the liquid sample passes through the ion exchange medium and substantially all the desired macromolecules are removed in a time period short enough to be consistent with the other operative steps of the instrument. Since most analytic instruments are designed to perform their analysis in time ranging from 10 to 20 minutes, the present invention must be designed so that the liquid sample spends no more than a few minutes, and preferably less than a minute, in the ion exchange medium. To lengthen this time period would substantially impair the time saving features of the automated instrument. In ion exchange chromatography the time required for the liquid to pass through the column usually exceeds 2 hours. To reduce this period, the column used in the present invention has been materially shortened, and the liquid sample is forced through the column under pressure, preferably in the range of 1 to 100 psi. This means that a pump must be used to force the liquid directly into the ion exchange column, and the ion exchange column must be designed so that the path from the pump to the discharge port and into the reac-

tion chamber is substantially leak tight, so that the thrust of the pressure developed by the pump is expended in forcing the liquid through the column and is not dissipated by leaks in the system. As illustrated in FIG. 1, the ion exchange column is a tube with an entrance port 22 which is designed to mate in some leak-tight manner with probe 12. A preferred way to accomplish this is discussed below.

The ion exchange means used in this invention can be in any form which is suitable for use with a particular instrument design. As illustrated in the figures it is an ion exchange column 14 which is filled with an ion exchange medium 21. The ion exchange medium can be any ion exchange resin which has the capacity to remove the desired charged macromolecule. This generally means an ion exchange resin with a charge opposite to that of the macromolecule to be removed. Under some circumstances, however, the macromolecule has a charge which is the same as that of some constituent of the sample which cannot be removed. In this case the process is no longer useful. For this reason, the process is particularly useful in the situation when the macromolecule to be removed is a dipolar ion or Zwitterion whose charge is dependent on the pH of the medium in which it is found. In this case, the pH of the liquid sample of the mixture of liquid sample and diluent can be altered to the point where the effective charge of the dipolar ion is different from the desirable constituents, so that an ion exchange medium that will remove the macromolecule without removing the desirable constituents can be found. Since body fluids generally contain protein molecules, which are a type of dipolar ion, the process finds its most useful application for use in removing protein molecules from the liquid sample prior to analysis. The ion exchange resin is then chosen for its capacity to remove protein molecules from the liquid. Not all ion exchange resins will do this, or at least do it to an extent which makes them efficient enough for use in the automated procedure of this invention. To eliminate the problem of the interaction of the protein molecules with the reagents used in the analysis substantially all of the interfering protein molecules must be removed from the liquid sample. In some applications removal of 80 percent of the protein molecules is sufficient, in others all of the interfering protein molecules must be removed.

The following ion exchange resins have been found to be most effective in the removal of positively charged protein molecules:

1. Carboxymethyl cellulose (sold under the designation: Whatman CM 23 by the Reeves Angle Co., and Cellex CM by Bio-Rad Laboratories);
2. Carboxymethyl dextran (sold under the designation Sephadex C-50 [CM] by Pharmacia Fine Chemicals, Inc.);
3. Sulfoethyl cellulose (sold under the designation Cellex SE by Bio-Rad Laboratories); and
4. Styrene-divinyl benzene sulphonic acid (sold under the designation CG 120 by the Rohm and Hass Co.).

The following ion exchange resins have been found to be most effective in the removal of negatively charged protein molecules:

1. Diethyl aminoethyl cellulose (sold under the designation Whatman DE 23 by the Reeves Angle Co. and Cellex D by Bio-Rad Laboratories); and

2. Diethyl aminoethyl dextran (sold under designation of Sephadex A-50 [DEAE] by Pharmacia Fine Chemicals Inc.).

The following ion exchange resins have not been tested but are expected to be effective in the removal of protein molecules:

1. Cellulose phosphate;
2. Ecteola cellulose; and
3. Sulfoethyl dextran (sold under the designation Sephadex C-50 [SE] by Pharmacia Fine Chemicals, Inc.).

It is to be noted that these are only examples of the types of ion exchange resins which will effectively remove protein molecules and are not intended to be the only effective materials. Most of the above ion exchange resins can be generically described as cellulosic ion exchange resins or dextran ion exchange resins. It is to be expected that all such resins will remove protein molecules to some degree, but that some, among which are those tested, will be more effective than others.

In some cases both positive and negative protein molecules must be removed from the liquid sample. In this case a two component column, one end of which contains positive ion exchange resins and the other end of which contains negative ion exchange resins, can be used. The following combination has been found to be effective:

$\frac{2}{3}$ Cellex SE and $\frac{1}{3}$ CG 120.

It is also conceivable that integral mixtures of the two resin types rather than separate regions would also be effective.

FIG. 2A illustrates a simple ion exchange column which can be used in the practice of the present invention. In FIG. 2A the ion exchange column 14 contains a single ion exchange resin 21. In FIG. 2B the ion exchange column 14 contains a positive ion exchange resin 21 and a negative ion exchange resin 27. In both cases, tube 14 is capped by two rubber caps 24 and 25, one of which has an exit port 26 in it. The probe 12 as illustrated in this configuration is a hypodermic needle which can be inserted through rubber dam 24 to form a leak-tight connection between the probe and the column. Liquid forced through the probe is forced directly through the column and out exit 26 into the reaction chamber.

FIG. 3 illustrates an analytic test pack in which an ion exchange column, a reaction chamber and the reagents to be introduced into the reaction chamber are all contained in the same package. The pack is described in U.S. Pat. application Ser. No. 545,494 now U.S. Pat. No. 3,476,515. It consists of a rigid header 30 to which a pouch 31 made from a transparent plastic material is attached. The header contains an ion exchange column 32 with an entrance port 33 located at one end and an exit port 35 located at the other end. The ion exchange column is capped by rubber caps 51 and 52 so that a hypodermic needle can be inserted in port 33, and the operation of the column is similar to that described in conjunction with FIG. 2. In this case, however, the exit port 35 empties into a reaction chamber 36 which is formed in the transparent plastic pouch by a seal 53 extending around the perimeter of the pouch, as shown. The reagents, in the form of solids or liquids, are contained in the pouches which are formed in the reaction chamber by rupturable seals 37 and 42. The

reaction is initiated by squeezing the reaction chamber 36 to the point where the liquid sample contained in it is forced against the rupturable seals causing them to rupture and to discharge their contents into the reaction chamber. By alternatively protecting certain of the rupturable seals and replacing others, the proper reagents can be introduced into the reaction chamber at the proper time. Once the reaction has been initiated the contents of the reaction chamber can be analyzed in accordance with the method set forth in U.S. Pat. application Ser. No. 753,200. The system illustrated in FIG. 3 is a preferred system in many respects, in that a separate ion exchange column is associated with each test sample and in that the whole system including the ion exchange column can be disposed of after the analysis is complete.

The following example is of an analytic determination which is impossible without the removal of certain macromolecular constituents, particularly protein molecules, will serve to illustrate the usefulness of the present invention.

SERUM PHOSPHATE ASSAY

A $\frac{1}{4}$ by 3 inch tube was filled three quarters full of Cellex SE which has first been water equilibrated and from which the fines have been decanted. The remaining third of the tube is then filled with CG 120 II which has also been water equilibrated and has had the fines removed, and which has been washed in a 10 percent solution of H_2SO_4 . The acid wash converts the CG 120 II from a sodium salt to a hydrogen ion so that this portion of the tube is highly acidic, having a pH of 0.55.

A 5 λ sample of a reference serum (Versatol) containing a known concentration of phosphate was titrated through the column using 2.0 ml. of H_2O as the eluent. A pressure of about 25 psi was used and 2.0 ml. of sample liquid was passed through the column in 0.7 minutes. The test reaction is a well known analysis in which 0.8 ml. of the sample liquid was then combined with the following reagents:

0.8 ml. of 25 percent solution of trichloroacetic acid
200 λ ascorbic acid
0.5 ml. Ammonium molybdate, and
1.0 ml. arsenite-citrate.

The absorbence of the sample liquid at 600 A was then measured. When the absorbence of a blank (K_2O) which has been passed through a similar column is subtracted from the sample liquid value, the result is close to the value of absorbence recorded on a control sample containing the same amount of phosphate as shown below. The absorbence has not been converted to absolute concentrations.

	pH	Absorbence at 600 A
Control	6.38	0.10
Sample	1.26	0.113
Blank	1.20	0.017

Without the protein removal the test could not be run.

What is claimed is:

1. A method for automatically analyzing a liquid sample for a particular constituent when said liquid sample contains a plurality of different charged macromole-

cules which would interfere with the analysis comprising the steps of:

- providing an ion exchange region containing an ion exchange medium with a pH adjusted to the point where said ion exchange medium will retard passage of all charged macromolecules in said liquid sample of at least one charge type, said ion exchange medium being saturated with a first quantity of a diluent;
- introducing a quantity of said liquid sample into said ion exchange medium;
- forcing a second quantity of diluent through said ion exchange medium, behind said liquid sample under a given pressure to displace said liquid sample in said ion exchange medium, the quantity of said liquid sample and the pressure with which it is forced through said ion exchange region being chosen so that substantially all of said particular constituent and substantially none of the charged macromolecule which would interfere with the analysis of said particular constituent pass through said ion exchange medium; and
- determining the presence of said particular constituent.

2. The method of claim 1 wherein the determination of the presence of said particular constituent is accomplished photometrically.

3. The method of claim 1 wherein said liquid sample is forced through said ion exchange region into a reaction chamber and wherein the method further comprising the step of introducing at least one reagent into said reaction chamber, said reagent being reacted with said particular constituent in a manner such as to alter the physical properties of said liquid sample.

4. The method of claim 1 wherein said liquid sample is forced through said ion exchange region into a reaction compartment and wherein said method further comprising the step of introducing at least one reagent into said reaction chamber, said reagent being reacted with said particular constituent in a manner such as to alter the photometric density of said liquid sample, and wherein the determination of the presence of said particular constituent is accomplished photometrically.

5. The process of claim 1 wherein said charged macromolecule is a dipolar ion whose effective charge depends on the pH of the medium in which it is found and wherein the process further comprises initially adjusting the pH of said liquid sample to a level at which the effective charge of said dipolar ion assumes a value such that said ion exchange medium will selectively remove substantially all of said dipolar ion without substantially removing any of said particular constituent.

6. The process of claim 5 wherein said charged macromolecule is a protein molecule.

7. The method of claim 1 wherein said liquid sample is forced through said ion exchange medium under a pressure of between 1 and 100 psi, whereby substantially all of said liquid sample passes through said ion exchange medium into said reaction chamber in less than two minutes.

8. The method of claim 1 wherein said ion exchange region contains a positively charged ion exchange medium adapted to remove substantially all negatively charged macromolecules from said liquid sample.

9. The method of claim 1 wherein said ion exchange region contains a negatively charged ion exchange me-

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dium adapted to remove substantially all positively charged macromolecules from said liquid sample.

10. The method of claim 1 wherein said ion exchange region contains both positive and negative ion exchange media and wherein said ion exchange medium is adapted to remove substantially all charged macromolecules from said liquid sample.

11. The method of claim 1 wherein said liquid sample contains protein molecules and said ion exchange region contains an ion exchange cellulose or an ion ex-

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change dextran.

12. The method of claim 1 wherein said liquid sample contains protein molecules and said ion exchange region contains an ion exchange medium selected from the group consisting of: carboxymethyl cellulose; carboxymethyl dextran; sulfoethyl cellulose; sulfoethyl dextran; sulfate cellulose; diethyl aminoethyl cellulose; diethyl aminoethyl dextran; cellulose phosphate; and ecteola cellulose.

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