United States Patent

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[33] **Great Britain**
[31] **48,912/68;**
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[54] **PREPARATIVE SEPARATION OF FLUID SAMPLES**
5 Claims, 6 Drawing Figs.

[52] **U.S. Cl.** **210/31,** 210/198
[51] **Int. Cl.** **B01d 15/08**
[50] **Field of Search** 210/31, 198; 55/67, 197, 386

[56] **References Cited**

UNITED STATES PATENTS

3,078,647	2/1963	Mosier	55/197
3,113,103	12/1963	Lowery	210/198
3,194,400	7/1965	Herndon	210/198 X
3,395,093	7/1968	Liberti	210/198 X

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ABSTRACT: A method and apparatus for preparative separation of the components of a fluid sample as a result of differential migration through a porous medium in which the porous medium is in the form of an annulus to the inner surface of which both the fluid sample and a fluid carrier for the sample are supplied continuously and the separated components are collected continuously from the outer surface of the annulus as the result of relative rotation preferably produced by causing the annulus to rotate at a steady speed while keeping the point of application of the sample and a collection arrangement at the outer surface of the annulus stationary.

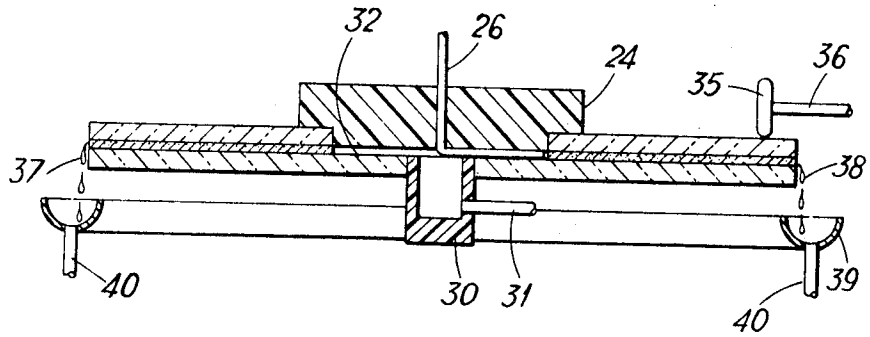
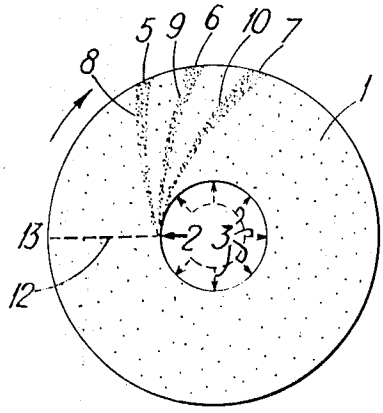


Fig. 1.



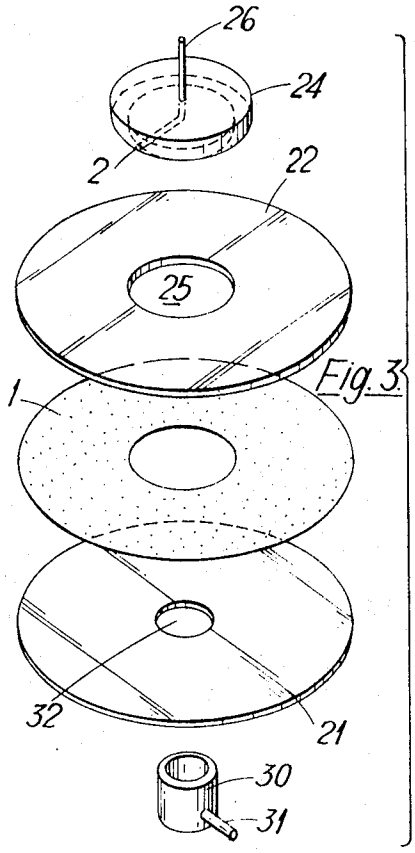
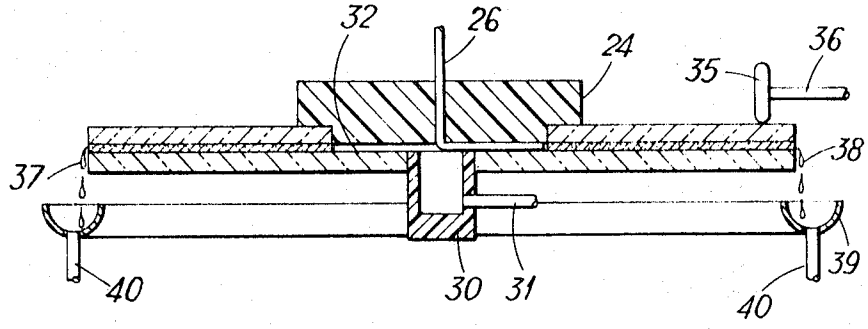


Fig. 3.

Fig. 2.



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Fig. 4.

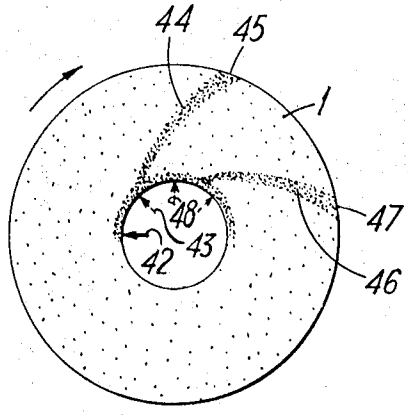


Fig. 6.

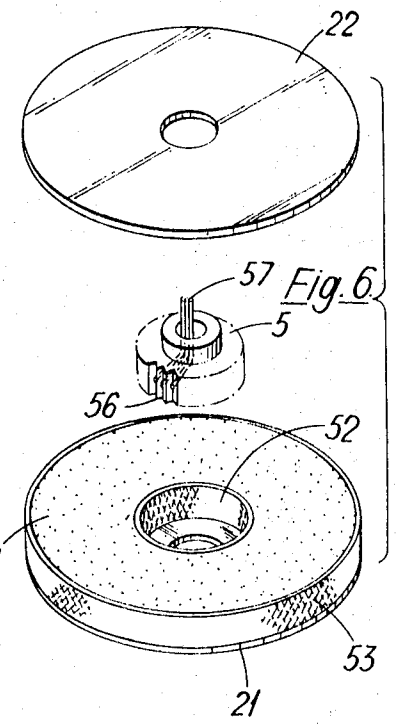
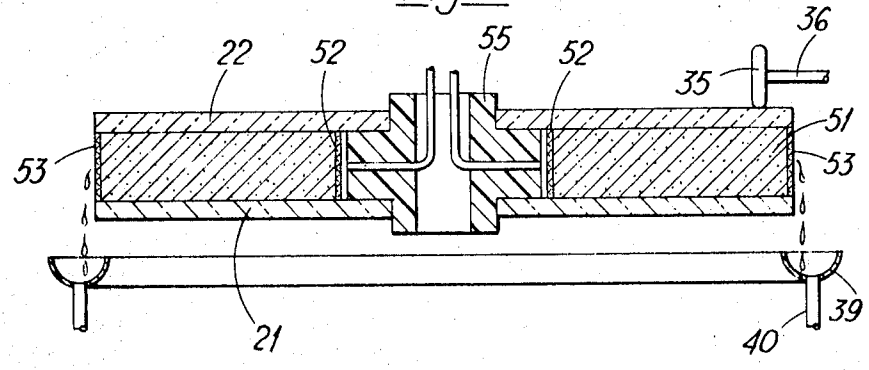


Fig. 5.



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PREPARATIVE SEPARATION OF FLUID SAMPLES

This invention relates to the preparative separation of the components of a fluid sample as a result of differential migration through a porous medium. Such separation involves broadly processes normally referred to as chromatography but also includes some specific processes falling outside this definition. For purposes of explanation the invention will be described in connection with its application to chromatography but it is to be understood that it is not so limited. The description will moreover be given in terms of what is commonly known as "paper chromatography" but this term is used broadly so as to cover the use of paper or of any equivalent porous medium. The invention, however, also includes gas chromatography in which there is a similar mechanism of differential migration through a porous medium.

Paper chromatography as such is normally carried out discontinuously. In other words a discrete quantity of a sample to be separated is applied to the surface of the paper and under the influence of a solvent is allowed to disperse in the usual way. When a further sample is to be separated this must be carried out as an entirely separate operation. Attempts have been made to carry out such a process on a continuous basis particularly for preparative purposes and one well-known form of apparatus for this purpose comprises a hollow cylinder of filter paper or similar material mounted to rotate about a vertical axis and supplied with solvent from its interior. The sample is supplied continuously to the upper edge of this cylinder and diffuses downwardly under the assistance of gravity. Owing to the differing rates of diffusion of the different components of a sample mixture these different components reach the bottom of the cylinder at different points in the rotation and may thus be collected in receptacles arranged at the appropriate points. It is impossible to carry out such separation in anything other than very small quantities and moreover it is difficult to support the wet cylinder of filter paper which owing to its vertical arrangement becomes quite unstable.

According to the present invention the porous medium through which differential migration occurs is in the form of an annulus to the inner surface of which both the fluid sample and a fluid carrier for the sample are supplied continuously and the separated components are collected continuously from the outer surface of the annulus as the result of relative rotation between the annulus on the one hand and the point of application of the sample and a collection arrangement on the other hand. Preferably the point of application of the sample to the inner surface of the annulus and also the collection assembly are stationary and the annulus is caused to rotate at a steady speed.

In order most easily to understand the outcome of such a process the following description will be made in terms of paper chromatography using a liquid sample and a solvent as previously mentioned. As a result of the application of the sample and the solvent to the inner surface of the annulus both tend to diffuse radially outwardly through the porous medium. The components of the sample will diffuse at differing rates and will thus reach the outer edge of the annulus at different points in time. Since the annulus is continuously rotating each component of the sample will thus reach the edge of the annulus at a different point in space and may thus be collected and separated from the other components. By the use of an appropriate number of receptacles all the constituents of a mixture can be collected. These receptacles may, for example, be defined by compartments in an annular trough each having a pipe for leading away the component in question.

In carrying out the process the supply of both sample and solvent is maintained continuously and since the rate of diffusion of each component is also substantially constant each of the components will reach the edge of the annulus at a fixed point in space and can thus be collected on a continuous basis. When the annulus has completed one revolution the sample will have cleared from that portion of the annulus previously used and the annulus may thus be reused on a continuous basis. The speed of rotation must, of course, be controlled to ensure that this occurs, i.e. to ensure that the portion of the annulus is clear before fresh sample is added and this will also depend on the nature of the sample to be separated. In practice this may be anything between a few minutes and a few hours, e.g. between 5 minutes and 2 hours.

From the point of view of the overall result the use of a rotary annulus is somewhat similar to the use of the rotary cylinder previously proposed except that there is no direct assistance from gravity during migration. From a practical point of view, however, the use of an annulus is much more convenient since it may be mounted without difficulty on a rotary support, for example, in the form of a plate of glass or plastic and it is an extremely simple matter to remove one annulus and to replace it by another. The quantity of sample which may be separated will depend largely on the thickness of the annulus. For small quantities paper or a stack of annuli may be used but for greater thicknesses a paid of absorbent may be cast in the form of an annulus. The rate of flow of the components may also be increased by supplying them to the annulus under pressure so that the effect of this pressure assists the normal diffusion, in which case the annulus needs to be enclosed between upper and lower impermeable supports. Gravity may thus be used indirectly to assist the flow of a liquid, i.e. by maintaining the supply pressure by means of a gravity head.

It will be understood that the description just given applies equally to other forms of chromatography and indeed to other forms of differential migration processes. Thus the migration may be assisted in the case of liquids by means of electric potentials applied as in normal electrophoretic processes and similar principles may also be used for gas chromatography. In this case the sample may be either a gas or vapor which is caused to diffuse by a carrier gas rather than by a solvent as in the case of a liquid. Throughout this specification the term "carrier" is used to include either a solvent for liquids or a carrier gas for a sample in the form of a gas or vapor. The essential characteristic is that there should be differential migration of the components of a fluid sample through a porous medium.

The process can also be applied where the mechanism is not strictly chromatographic in the generally accepted use of the term. For example some liquid mixtures may be separated more conveniently by means of a process in which one or more of the components are completely adsorbed on an appropriate porous medium. Any free components can be removed by withdrawal of the liquid phase containing them, after which the other component or components can be desorbed in succession and thus separated. For this purpose the sample in an appropriate solvent is applied to the inner surface of the annulus as previously described and, if only one component is adsorbed, at a subsequent point in the relative rotary movement of the annulus a second solvent is supplied which will desorb the adsorbed component. The initial solvent is then reintroduced after the completion of desorption. As a result the component which is initially adsorbed will not diffuse outwardly over the first stage but the other component or components will diffuse in the normal way.

In other words the first component may be regarded as being held back while the other component or components migrate. When the second solvent is supplied to desorb the first component this then diffuses outwardly over a region separate from that of the other components and is then separately collected. The desorbing solvent is continued until all the first component is desorbed after which the initial solvent is introduced so that at the completion of a revolution of relative motion the medium is ready for the reception of a further sample and continuation of the process. If more than one component is initially adsorbed, the adsorbed components may be released in succession and thus separately collected. In this way it is possible to collect, on a continuous basis, one or more components of a sample which could not con-

veniently be separated as the result of a normal chromatographic separation.

Apparatus for carrying out a process in accordance with the invention comprises basically a support for an annular body of porous medium, connections for the supply of a fluid sample and a fluid carrier to the inner surface of an annular body of the support, collectors for sample components separated by differential migration at the outer surface of the body and mechanism for producing continuous relative rotation between the supply connections and the collectors on the one hand and the support on the other hand. Generally speaking the supply connections and the collectors will be stationary and the support will be caused to rotate. It will be understood that the body of porous medium itself does not form part of the basic apparatus in that it is interchangeable and different materials, grades and thicknesses may be used with the same basic apparatus according to the nature of the sample to be separated, the scale of separation required and so forth. If a fluid sample and a single solvent or other carrier are to be supplied the supply connection for the sample may lead directly to the inner surface of the annular body so as to supply sample over a narrow angle and the supply connection for the carrier may lead to a chamber bounded by the inner surface of the annular body so as to supply carrier over the whole of the inner surface except for the narrow angle to which the sample is supplied. If, however, a porous medium is used which is capable of adsorbing one or more of the components of the sample as mentioned above, the supply connections need to lead to a distributor for engaging the inner surface of the body and which is formed with axially extending recesses each corresponding to one of the connections and each spanning a relatively small angle of the inner surface. Sample is supplied to one of these recesses and differing solvents are then supplied to successive recesses over the required angles.

Forms of apparatus and processes in accordance with the invention will now be described by way of example with reference to the accompanying drawings in which:

FIG. 1 is a plan view of an annular body illustrating the effects of differential migration in accordance with the invention;

FIG. 2 is a sectional elevation to an enlarged scale of apparatus including the body shown in FIG. 1;

FIG. 3 is an exploded perspective view illustrating the components of the apparatus of FIG. 2;

FIG. 4 is a view similar to FIG. 1 illustrating a modified form of process;

FIG. 5 is a sectional view similar to FIG. 2 illustrating a form of apparatus for use with the modified process; and,

FIG. 6 is an exploded view showing the components of the apparatus of FIG. 5.

The apparatus illustrated in FIGS. 1 to 3 is intended primarily for liquid chromatography but can be adapted without difficulty to other forms of process such, for example as gas chromatography. The principle of operation is best illustrated from FIG. 1 which shows the effects of differential migration. An annular body 1 comprising any paper chromatographic material such as a standard chromatographic paper has a sample which is to be separated applied to it at a point on its inner surface indicated as 2. Solvent is applied to all the remainder of the inner surface as indicated by arrows 3. Under the influence of the solvent the components of the sample migrate outwardly at differing speeds and in relation to the body 1 follow a generally radial path. The body 1 is caused to rotate in a clockwise direction, however, as indicated by the arrow and as a result the different components of the sample reach the outer surface at different points in space indicated as 5, 6 and 7 for three of these components. It will be seen that there are corresponding bands 8, 9 and 10 leading to the respective points 5, 6 and 7 and these represent the paths followed in space (rather than in the body 1) followed by the different components of the sample. The band 8 represents the path followed by the component which migrates fastest. In the absence of rotation of the body 1 the path would be along a dotted line 12 and would reach the outer surface at a point 13. Owing to the rotation of the body, however, during the time in which the component in question is migrating to the outer surface the point 13 has moved to the point 5 and this component of the sample therefore leaves the body 1 at the point 5 where it can be collected.

The band 9 represents the path in space of the component having the next fastest migration rate. Again, but for the rotation of the body 1, this second component would also reach the point 13 but later than the first component. Owing to the longer time taken for migration the point 13 will have travelled further to reach the point 6 by the time the second component has reached the outer edge and this second component may therefore be collected at the point 6. Similarly a third component with a slower rate of migration may be collected at the point 7 and if other components are present then they may be collected at corresponding points round the outer edge.

The foregoing description assumes that the point of application of the sample at the point 2 is stationary and that the body 1 is rotating thus enabling the different components to be collected at fixed points in space. In fact, what is important is the relative rotation between the body 1 on one hand and the point of application of the sample and the points of collection of the components on the other hand. It would therefore be possible to invert the arrangement and to keep the body 1 stationary but causing the point 2 and the points 5, 6 and 7 to move round the outer surface of the body 1 at a corresponding speed. From a practical point of view, however, it is much more convenient to rotate the body 1 and the following description will assume that this is done.

The collection points 5, 6 and 7 are all grouped together over a relatively small arc of the circumference thus allowing ample space for the separation of further components if they are present. With only the three components illustrated a greater angular separation can be obtained by an increase in the speed of revolution of the body 1 and in any particular circumstances this is adjusted to suit the rates of migration of the different components. Obviously, however, the speed of rotation must not be too great since it is essential that the slowest component should have completely cleared from the body 1 before the sample application point is again reached.

Details of the apparatus are shown in FIGS. 2 and 3. As best seen from FIG. 3 the body 1 is sandwiched between a lower annular plate 21 and a corresponding upper annular plate 22. These two plates between them constitute a support for the body 1 and, if made of an impermeable material such as glass, permit the supply of fluids under pressure which increases the rate of diffusion. It will be understood that the plates 21 and 22 are permanent parts of the equipment whereas the body 1 is removed and is replaced by a body of whatever material is appropriate for the separation which is to be carried out. An upper plug 24 fits into the central opening 25 in the plate 22 and enables sample to be supplied to the body 1 at the point 2 by way of a pipe 26 passing through the thickness of the plug 24. Solvent is supplied from below through a cup-shaped member 30 fitted with a connection 31 and which is located in the central space 32 in the plate 21. The solvent fills the interior of the cup 30 and then flows out into a space 32 best seen in FIG. 2 so that it is applied to the whole of the inner surface of the body 1 apart from the point 2 where the sample itself is supplied. The plug 24 and the cup 30 remain stationary but the plates 21 and 22 together with the annular body 1 caused to rotate as described with reference to FIG. 1. Various methods of driving are possible but as illustrated rotation is obtained by engagement of a small rubber-covered driving roller 35 which is mounted on a shaft 36 and engages the upper surface of the plate 22 close to its edge. As a result of the differential migration already described components of the sample reach the edge of the body 1 at different points and drops of different components are illustrated at 37 and 38. These fall into an annular trough 39 divided into compartments from which individual pipes 40 conduct the different

components away. In this way it is possible to separate a sample into its components for preparative purposes on a continuous basis. The rate of separation, that is to say, the volume of sample which can be separated in unit time depends on the capacity of the body 1 which in its turn is largely dictated by its thickness. It will be understood that the apparatus illustrated is capable of accepting a wide range of thicknesses of annular body.

In a particular construction of the apparatus the plates 21 and 22 are made of glass one-fourth inch thick and 10 inches diameter, the opening 25 in the plate 22 has a diameter of 3 inches and the opening 32 has a diameter of 1 inch. The annular body 1 is a Whatman No. 17 chromatography paper of which the edge may be serrated with a pair of pinking shears in order to assist flow from the edge of the paper. To ensure that the assembly of plates 21 and 22 and the body 1 rotate as a unit they are held together by means of four screw clamps (not shown in the drawings). In a particular experiment using indicator dies of methyl red and bromocresal green and a solvent of 3 percent aqueous sodium chloride the assembly was caused to rotate at a speed of one revolution in 40 minutes.

The apparatus just described can readily be modified either for use with volatile solvents or for use with gas chromatography by completely enclosing the apparatus and choosing appropriate material for the body 1. In order to collect the emerging vapor or gas the trough 39 needs to be replaced by a manifold forming a seal fitting with the edge of the rotary assembly.

If instead of chromatographic paper the body 1 is made as a cake of porous material it can be used, for example, for molecular sieving. This may be used for various purposes, for example, for the desalting of a protein. As a particular example the body may be made up from a cross-linked polydextran gel available under the trade name Sephadex G.25 and the sample to be separated may be a solution of bovine serum albumen together with sodium chloride. Elution is carried out with distilled water which moves the protein faster than the sodium chloride due to the greater retardation by the gel of the small molecule of sodium chloride. Consequently the proteins will precede the salt to the edge of the body where they may be collected free from salt.

The apparatus illustrated in FIGS. 4 to 6 is similar in principle to that of FIGS. 1 to 3 but enables a number of different solvents to be supplied so as to achieve the separation of components of a sample which are not conveniently separated by the basic apparatus of FIGS. 1 to 3. FIG. 4 illustrates the principle of this modified process. Here the annular body is again shown as 1 and is constituted by a material capable of adsorbing all the components of the sample in the solvent in which it is initially supplied. Between the point 42 and 43 therefore the sample remains adsorbed and no migration occurs. At the point 43 a different solvent is supplied which desorbs the first components of the sample and allows it to migrate outwardly along the path shown as 44 so as to reach the edge at a point 45. By the time a subsequent point 48 is reached all the first component has been removed and a third solvent is then added which desorbs a second component of the sample. This in its turn migrates outwardly along a path 46 to reach the outer edge at 47. The same process can be continued with any number of components which can then be collected at the edge of the annular body in a manner similar to that described with the apparatus of FIGS. 1 to 3.

Upper and lower plates 21 and 22 are similar to those previously described but the annular body is shown as being considerably thicker and is shown in FIG. 5 as 51. In order to support this material the lower plate 21 is provided with inner and outer thin porous walls 52 and 53 respectively. These walls may consist of thin porous metal or plastic, for example, sintered stainless steel or sintered polyethylene are suitable for this purpose. Since there are a number of application points firstly for the sample and then for differing solvents this is achieved by means of a distributor 55 which replaces the plug 24 and the cup 30 shown in FIG. 2. As best seen in FIG. 6 this has a generally cylindrical body formed with spaced circumferential grooves 56 of which only three are shown but which extend around the whole circumference. A connection 57 extends to each of these grooves so that by passing sample or solvent down the appropriate connection it can be supplied to the inner surface of the annular body 51. The distributor 55 fits tightly between the upper and lower plates 22 and 21.

In assembling the apparatus the body 51 is formed in situ either by pouring it into the space illustrated in the form of a slurry or by building it up in layers such as with filter paper. Operation proceeds as already described in relation to FIG. 4 and the separated components are collected in the compartments of a trough 39 fitted with individual connections 40 as shown in FIG. 2.

In particular example the requirement is to separate insulin from a mixture of other materials including other proteins. For this purpose the annular body 51 is made up from carboxymethyl cellulose which has the property of adsorbing insulin from a mixture containing insulin at pH 3. The mixture is therefore brought to this pH and is applied to the inner face of the body 51 over one of the grooves 56. Immediately thereafter hydrochloric acid of pH 3 is applied as eluent thus permitting outward diffusion of the constituents of the mixture other than the insulin. At the end of this arc of relative movement representing sufficient time for the diffusion of the other constituents of the mixture the eluent is changed to hydrochloric acid of pH 2. This has the property of desorbing the insulin which thus starts to diffuse outwardly in its turn and the supply of this eluent is maintained over a further arc sufficient to produce complete desorption of the insulin. Over the final arc needed to complete one revolution of relative motion the pH of the eluent is brought back to its initial value in readiness for the reception of further sample. As a result of this the insulin reaches the outer edge of the body 51 over a range of angular positions separate from those of the other constituents and may thus be collected in a pure form free of contaminants.

I claim:

1. A method for the continuous separation from a liquid which is a mixture of components, at least one component of which is capable of complete adsorption by a porous body comprising the following steps continuously performed:

applying said mixture from a first source to a region of a first surface of a porous body whereby said one component is completely adsorbed and other components diffuse through said body to a second surface thereof;

collecting said other components at said second surface;

applying from a second source to said region of said first surface a solvent capable of desorbing said adsorbed component and permitting it to diffuse to said second surface;

collecting said one component at said second surface; and

simultaneously maintaining relative movement between said porous body and said first and second sources, said sources being spaced from each other in the direction of said relative movement.

2. A method as defined by claim 1 in which said porous body is an annulus in which said first and second surfaces are the inner and outer surfaces, respectively, of said annulus.

3. A method as defined by claim 2 in which said sources are stationary and said annulus is rotated continuously with respect thereto.

4. A method as defined by claim 2 in which said annulus is enclosed between upper and lower impermeable supports and said liquid and solvent are supplied to said annulus under positive pressure.

5. A method as defined by claim 3 in which said liquid contains a plurality of components which are adsorbable by said porous body and in which a plurality of solvents are sequentially applied to said region of said first surface to sequentially desorb said components and permit separate collection thereof at said second surface.

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