SAMPLE INJECTION METHOD AND APPARATUS

ABSTRACT: Injector cartridges having filter material are placed in receiving holes around the periphery of a rotating turntable after a sample has been centrifuged into the filter material and the sample is filtered during the centrifuging. The sample within the cartridge is preeluted and then the turntable is rotated sequentially positioning the cartridges beneath an extendable and retractable loader which engages the cartridge. Desired zones of the chromatographic spectrum of the preeluted sample are injected through a capillary outlet in the cartridge into a chromatographic system.
This invention relates to a new and improved method and apparatus for preparing and injecting samples into a chromatographic system particularly applicable for injecting samples of animal fluids for chromatographic analysis of amino acids.

According to present chromatographic methods, liquid samples are supplied directly to a chromatographic column by manual or automatic injection. Because the sample is introduced directly, the column must be regenerated between samples to remove impurities. In addition, high pressures accompanying the injection of the sample can compress the absorbent material used in the column and thereby distort the resulting chromatogram of the sample. Another problem encountered in practicing present techniques, is that in the sequential steps performed for injecting sequential samples air can be introduced into the operating column further distorting the chromatogram.

The object of the present invention is to provide a method and apparatus wherein a plurality of samples can be automatically and sequentially injected into a chromatographic system for analysis.

According to one aspect of the present invention, a sample injector is provided in which large molecules such as proteins first removed by centrifuging the sample through a membrane filter and into an injector cartridge containing chromatographic filter material. The sample is then preheated in the carriage so that predetermined zones of the chromatographic spectrum of the sample can be retained in the cartridge or injected into a chromatographic system. A sequence of samples can thus be continuously applied to a chromatographic column without the necessity of column regeneration between samples.

In accordance with another aspect of this invention the samples applied to the chromatography column are changed continuously and automatically with a positive displacement of all air so that air is not introduced into the operating column. The injector cartridge also prevents a pressure buildup in the sample and buffer which might be transmitted to the chromatographic column. The sample injector is particularly suitable for the chromatographic analysis of animal fluids, and predetermined amino acids or other molecules can be retained in the injector cartridge and only selected amino acids from the sample injected into the chromatographic column. The chromatographic column can thus be used continuously without regeneration and only the injector cartridges need be regenerated. The invention is also intended to provide means for automatically injecting consecutive samples into a chromatographic system.

In order to accomplish these results, the present invention contemplates providing a plurality of injector cartridges each having a channel therethrough containing a chromatographic filter material, an inlet opening at one end of the cartridge communicating with the channel for loading a sample, and a capillary outlet opening at the other end of the cartridge communicating with the channel for retaining a sample therein until it is ejected through the capillary outlet.

According to another aspect of the invention, a turntable is provided having a plurality of holes through the turntable from one side to the other around the periphery thereof adapted to receive and retain the injector cartridges therein, each hole including spring biasing means to bias an injector cartridge inserted therein in the direction of the side of the turntable from which the injector is inserted.

Samples to be analyzed in a chromatographic system are first centrifuged into respective injector cartridges through loading columns and membrane filters to remove protein. A buffer solution is added to the cartridge by centrifuging and each cartridge is then placed in the turntable. The sample in each injector cartridge is preheated for a predetermined period and positioned by the turntable beneath an extendable and retractable loader adapted to engage in sealing relationship the opening of the injector cartridge and extend in a direction against the biasing spring in the hole of the turntable in which the cartridge is seated. The injector cartridge is then pushed by the extending loader into an orifice in a receiving base positioned on the other side of the turntable from the loader for injection of predetermined zones of the chromatographic spectrum of the sample into a chromatographic system.

From the time that the sample is centrifuged into the injector cartridge and the buffer introduced over the sample, the sample is isolated from the air. Before the extendable loader engages an injector cartridge held in the turntable, a droplet of buffer falls on the inlet opening of the cartridge so that air is not introduced upon sealing engagement of the inlet opening by the extendable loader. Back pressure in the orifice of the receiving base from a chromatographic system also backs up the buffer level into the orifice to meet the capillary outlet from the injector cartridge. No air is thereby introduced into the system during the sample injection. The capillary outlet also serves to prevent premature flow-out of the sample and buffer.

The chromatographic filter material provided in the injector cartridge can be a plurality of cross-linked polymer resin beads mechanically supported within a channel through the injector cartridge. The resin beads can be mechanically supported by screens at either end of the cartridge which also serve to prevent pressure buildup on the resin beads.

Other objects, features and advantages of the present invention will become apparent in the following specification and accompanying drawings.

In the drawings:

FIG. 1 is a side cross-sectional view of an injector cartridge embodying the present invention.

FIGS. 1A and 1B are plan views of two types of caps provided over the outlet from the sample injector.

FIG. 2 is a plan view from the top of the injector cartridge illustrated in FIG. 1.

FIG. 3 is a side cross-sectional view of an injector cartridge and loading column in sealing engagement within a centrifuge tube.

FIG. 4 is a fragmentary plan view of an automatic sample injector embodying the present invention.

FIGS. 5 and 6 are detailed side views of the extendable and retractable loader for the automatic sample injector showing an injector cartridge in the turntable before and during injection of a sample respectively.

As broadly set forth above, the present invention provides a method and apparatus for automatically injecting a plurality of samples into a chromatographic apparatus wherein separate samples are injected into sample injectors containing a chromatographic filter material and these sample injectors sequentially inserted in the stream of the chromatographic apparatus.

In the embodiment of the present invention illustrated in FIG. 1 there is provided a sample injector 10 having an elongated housing 11 of plastic or other suitable material with a chamber or elongated channel formed centrally therein. The channel 11 is lined with a tubular support column 12 of glass or suitable plastic material for supporting the chromatographic absorptive filter material 13 such as microspherical resin beads. Microspherical resin beads for use as the filter material can be formed of the cross-linked copolymer such as, for example, a styrene and divinyl benzene copolymer at set forth in more detail in my U.S. Patent application Ser. No. 530,051, now Pat. No. 3,463,320, entitled "Microsphere Filter and Method of Filtration" filed on Feb. 25, 1966. The resin beads 13 are mechanically retained within the support column 12 at each end by screens 14 such as a 10 micron mesh screen. At one end of the sample injector cartridges there is provided an inlet opening 15 communicating with the central channel in which the chromatographic filter material is retained. Positioned at the inlet opening of the cartridge over the screen 14 is a membrane filter 16 sandwiched between...
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3 glass mat filters 17 which support the membrane filter and provide a prefilter for liquid samples entering the cartridge inlet. At the opposite end of the injector cartridge from the inlet 15, the cartridge is provided with a connector 10 having a conical projection 18 having a capillary outlet 19 formed therein communicating with the elongated channel 11 in the cartridge containing the chromatographic filter material 13. The capillary outlet 19 serves to retain fluid in the cartridge before injection. A cap 19a of teflon or other suitable material may be provided over the capillary outlet 19 and truncated conical projection 18, having an angled slit cut through the cap as illustrated in FIG. 1 and FIG. 1A to further retain fluid and release fluid from the cartridge only upon application of pressure on the fluid in the cartridge. Cross angled slits may also be provided in the cap as illustrated in the cap 19b of FIG. 1B. The injector cartridge 10 illustrated in FIG. 2 is circular in cross section and is provided with a flat surface 20 on one side thereof to facilitate use in the automatic sample injector as hereinafter described.

In accordance with one aspect of the present invention a liquid sample is introduced into the sample injector cartridge 10 by centrifuging the sample through the membrane filter 16 into the column of resin beads or other chromatographic filter material 13. To this end, a loading column 30 is provided as illustrated in FIG. 3. The loading column 30 is formed of an elongated tube of plastic or other suitable material having an inlet 31 for inserting the sample and an annular projection 32 at the opposite end supporting an O-ring seal 33 for engaging the inlet opening 15 of the injector cartridge 10 in sealing relationship. The samples may then be centrifuged through an outlet passageway 34 through the annular projection 33 of the loading column 30 into the injector cartridge 10.

According to the operation by one aspect of this invention, the injector cartridge 10 is first washed with a buffer solution such as a citrate buffer solution of pH 2.2, and 0.2N in sodium ion, by centrifuging the injector cartridge 10 alone in a centrifuge tube 35. The washings at the bottom of the centrifuge test tube 35 are discarded. The liquid sample such as an animal fluid or a serum with adjusted pH is then applied to the loading column 30 which has been inserted into the inlet opening 15 of the injector cartridge 10. The liquid sample is then centrifuged into the cartridge through the membrane filter 16 including the glass mat filters 17 and screen 14. Centrifuging is accomplished in a centrifuge test tube 35 as illustrated in FIG. 3. To insure that the liquid sample has been completely introduced to the cartridge 10, the buffer of the same pH as the sample may be introduced into the loading column 30 and washed through the system by centrifuging in the same manner. The screen 14 which mechanically support the resin beads during loading of the sample into the injector cartridge.

Sufficient buffer is applied during sample loading into the injector cartridge so that the liquid level after centrifuging does not fall below the level of the injector cartridge in the centrifuge tube.

The loaded injector cartridge and loading column are then removed from the centrifuge test tube and the loading column separated from the cartridge. After removing the loading column 30, the glass mats 17 and membrane filter 16 are also removed from the top of the injector cartridge leaving the top screen 14 in place. Proteins filtered by the membrane filter are thereby removed eliminating a source of contamination. The injector cartridge 10 is then inserted into the automatic sample injector assembly illustrated in FIGS. 4 and 5 for injecting the sample into a chromatographic system.

The automatic sample injector assembly includes a drum or turntable 40 having a plurality of holes 41 formed therethrough from one side to the other around the periphery of the turntable. A flat disc 42 is provided centrally on the turntable 40 so that it slightly overlaps the edge of each of the holes 41 formed around the periphery of the turntable 40. Each hole 41 is adapted to receive a sample injector cartridge 10 with the flat edge 20 of the cartridge facing the disc 42 so that it will fit into the hole 41. The cartridge 10 is then rotated so that it is retained in the hole 41 by the disc 42 until rotated with the flat edge 20 again facing the disc 42. Each hole 41 includes a biasing spring 38 and a post 39 which is formed and adapted to engage an annular projection around an injector cartridge to spring bias an inserted injector cartridge against the bottom of disc 42.

Means are provided for rotating the turntable 40 relative to an extendable and retractable loader 45 positioned at the edge of the turntable. The extendable and retractable loader 45 includes an extendable and retractable annular projection or piston 46 positioned over the turntable and in alignment with the inlet opening 15 of injector cartridges 10 as they are sequentially positioned beneath the loader 45 by rotation of the turntable 40. The piston 46 on loader 45 extends and retracts in a direction perpendicular to the face of the turntable 40 and supports an O-Ring seal. Upon extension of the piston 46, the piston enters and engages the inlet opening 15 of a cartridge 10 in sealing relationship and pushes the cartridge downward in a direction against the biasing spring 43. On the lower side of turntable 40, i.e., the side opposite the extendable and retractable piston 46, there is provided a receiving base 50 stationary relative to the rotating turntable 40. The receiving base 50 includes an O-Ring seal 51a adapted to receive the truncated conical extension 18 at the outlet end of an injector cartridge in sealing relationship as illustrated in FIG. 6. The orifice 51 terminates in a passageway 52 connected to a tube 53 which leads to a chromatographic system in which the sample is to be injected. Upon extension of the piston 46 from the loader 45 the capillary outlet end of the cartridge 10 is inserted into the orifice 51 so that a buffer under pressure in tube 54 may be transferred through the piston 46 and injector cartridge to inject the sample through the tube 53 into a chromatographic system.

After a loaded injector cartridge is placed in the turntable 40, the cartridge may be delivered to the stationary loader 45 according to a controlled timing sequence. The controlled timing sequence of delivery of cartridges by the turntable 40 may include a predetermined preflow cycle prior to positioning the cartridge into position beneath the loader 45 for injecting the sample into a chromatographic system. By preflow of the liquid sample in the cartridge, amino acids or other molecules may be removed from the sample to a controlled initial level in the chromatographic system. After the preflow of the liquid sample is complete the injection position beneath the loader 45 after a predetermined time interval so that amino acids or other molecules beyond a desired level in the chromatographic are retained in the cartridge. After injection of predetermined zones of the chromatographic spectrum of the sample into a chromatographic system, the cartridges are removed from the injection position by turntable 40 for regeneration of the filter column of resin beads contained in the cartridge in preparation for loading another liquid sample into the cartridge by centrifuging as hereinafter described.

Once a liquid sample and buffer have been centrifuged into the injector cartridge, the sample is maintained in buffer solution and isolated from air throughout the subsequent stages of sample delivery. When the sample injector is removed from the centrifuge test tube after centrifuging, flow-out from the cartridges is prevented by the capillary outlet 19 and cap 19a. The cartridge is then inserted in one of the holes in the periphery of the turntable 40. Before each cartridge is delivered to the loader 45 and extendable piston 46 for insertion of the end of the cartridge into the orifice 51 of the receiving base 50, back pressure from the chromatographic system through tube 53 causes the level of buffer to rise in the tapered orifice 51 to receive the capillary outlet 19 and tapered end 18 of the cartridge so that air is not introduced into the system. Furthermore, as a cartridge is delivered by the turntable 40 into alignment beneath the piston 46 on the loader 45, a droplet of buffer falls from the piston 46 onto the
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5 top of the cartridge in the inlet opening 15 so that when the piston 46 engages the opening of the injector cartridge forcing the tapered end 16 of the cartridge into the orifice 51, an excess of buffer occupies the space in the inlet so that again air is not introduced into the system. The excess buffer is forced out and collected in a waste tube as the piston 46 extends into the cartridge 10. After the sample is injected from a cartridge into a chromatographic system, the turntable rotates and the cartridge may then be processed through a regeneration cycle. The cartridge is then removed by rotating the cartridge until the flat edge 20 faces the disc 42 so that the biasing spring 43 ejects the cartridge for loading another sample by centrifuging.

Although only certain embodiments of the present invention have been shown and described, other adaptations and modifications would be apparent without departing from the true spirit and scope of the invention.

1. A sample injector assembly for automatically injecting samples into a chromatographic system comprising: a plurality of sample injector cartridges each having a channel therethrough containing a chromatographic filter material, an inlet opening at one end of said cartridge communicating with said channel for loading a sample therein, and a capillary outlet opening at the other end of said cartridge communicating with said channel ejecting a sample therethrough; a turntable having a plurality of holes therethrough from one side to the other around the periphery thereof adapted to receive said injector cartridges from one side, and retain said cartridges therein, each said hole including spring biasing means to bias an injector cartridge inserted therein in the direction of the side of the turntable from which said injector cartridge is inserted, means for rotating said turntable, means stationary relative to said rotating turntable positioned on one side at the periphery thereof comprising an extendable and retractable loader adapted to engage in sealing relationship the inlet opening of an injector cartridge received in a hole in the periphery of the turntable and extend in a direction against the biasing spring means in said hole; a receiving base stationary relative to said rotating turntable positioned on the side of the turntable opposite and in alignment with the extendable and retractable loader, said receiving base having an orifice therein adapted to receive the capillary outlet opening of an injector cartridge in sealing relationship upon engagement of a cartridge by said loader and extension of said loader against a biasing spring means.

2. A method of preparing a sample and injecting the sample into a chromatographic system comprising: centrifuging the sample into a chromatographic filter material contained in an injector cartridge, centrifuging a buffer solution through the membrane filter and into the chromatographic filter contained in the injector cartridge; precluding the sample in the chromatographic filter contained in the injector cartridge; positioning the injector cartridge for injecting the sample into a chromatographic system; and injecting desired zones of the chromatographic spectrum of the sample from the injector cartridge into the chromatographic system.

3. A method of preparing a sample and injecting the sample into a chromatographic system comprising: centrifuging the sample through a membrane filter into a chromatographic filter material contained in an injector cartridge; centrifuging a buffer solution through the membrane filter and into the chromatographic filter contained in the injector cartridge; precluding the sample in the chromatographic filter contained in the injector cartridge; positioning the injector cartridge for injecting the sample into a chromatographic system; injecting predetermined zones of the chromatographic spectrum of the sample from the injector cartridge into the chromatographic system.

4. A sample injector assembly for automatically injecting samples into a chromatographic system comprising: a fluid line arranged for directing fluid and sample material to said chromatographic system; a plurality of sample injector cartridges, each having a chamber therein, chromatographic filter material located in said chamber, means defining an inlet into said chamber for loading a sample therein, and means defining an outlet from said chamber for ejecting a sample therefrom; table means having a plurality of holes formed therethrough to receive the injector cartridges, each said hole including resilient biasing means to bias an injector cartridge inserted therein in a direction away from the table; said fluid line connected at one end to said chromatographic system and at its other end to a position adjacent said table means; means translating said table means relative to the fluid line for successively positioning said holes in alignment therewith; and means engaging an injector cartridge contained within a hole in the table in alignment with said fluid line to depress the injector cartridge against the resilient biasing means, thereby to engage the outlet from said injector cartridge and the end of the fluid passageway in sealing relationship.

5. A sample injector for a chromatographic system comprising: an elongated injector cartridge having a channel therethrough containing a chromatographic filter material, an inlet opening at one end of said cartridge communicating with said channel for loading a sample therein and a capillary outlet opening at the other end of said cartridge communicating with said channel for ejecting a sample therethrough, a membrane filter in the inlet opening end of said cartridge, and at least one screen placed in the inlet opening end of said injector cartridge to prevent pressure buildup in the injector cartridge on the chromatographic filter material.

6. A sample injector for a chromatographic system comprising: an elongated injector cartridge having a channel therethrough containing a chromatographic filter material, an inlet opening at one end of said cartridge communicating with said channel for loading a sample therein and a capillary outlet opening at the other end of said cartridge communicating with said channel for ejecting a sample therethrough, said chromatographic filter material comprising a plurality of microspherical cross-linked polymer resin beads, and a tubular support column coaxially mounted within the channel and fine mesh screening fixed at each end of the support column for containing said resin beads.