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V. PRETORIUS ET AL  
METHOD AND APPARATUS FOR THE INTRODUCTION OF SAMPLES  
INTO CHROMATOGRAPHIC SEPARATING SYSTEMS  
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3,582,475

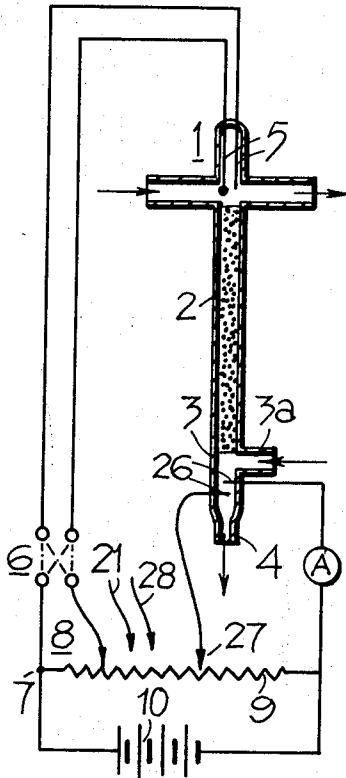


FIG. 1

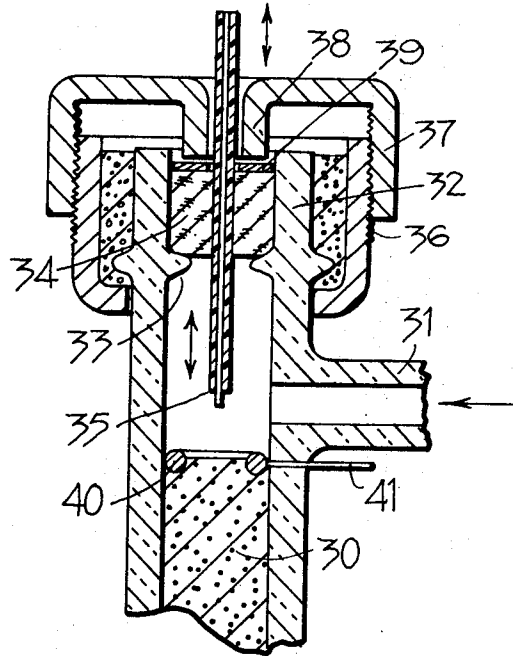


FIG. 3

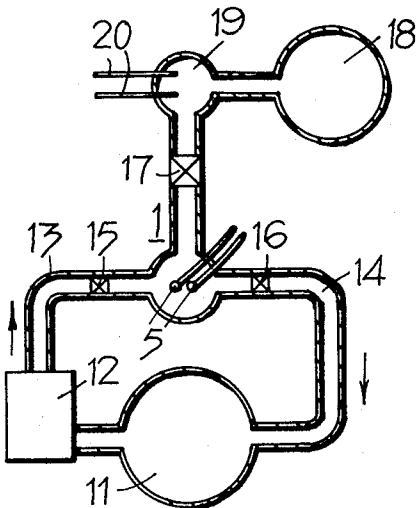


FIG. 2

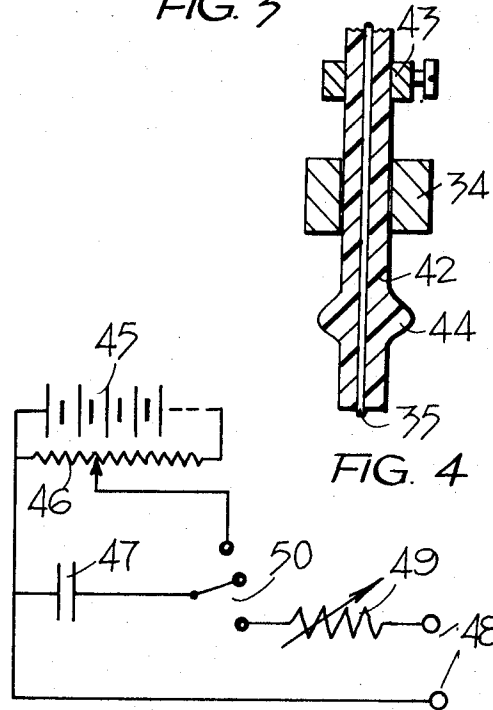


FIG. 4

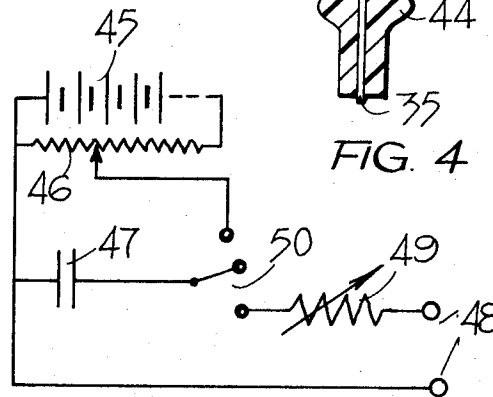


FIG. 5

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## METHOD AND APPARATUS FOR THE INTRODUCTION OF SAMPLES INTO CHROMATOGRAPHIC SEPARATING SYSTEMS

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Continuation-in-part of application Ser. No. 583,788, Oct. 3, 1966. This application Aug. 2, 1967, Ser. No. 657,814

Claims priority, application Republic of South Africa, Aug. 2, 1966, 66/4,568

Int. Cl. B01d 59/40; B01k 5/00

U.S. Cl. 204—1

21 Claims

### ABSTRACT OF THE DISCLOSURE

The solutes of a sample to be separated chromatographically are first plated onto an electrode. This electrode is then maintained in the inlet of a chromatographic system where by reversal of the current the sample is returned to solution. By controlling the size of the electrode and the time taken for the sample to return to solution, the plate height contribution of the inlet is limited. A preliminary partial separation of the sample by electrolytic means is also provided for.

### CROSS-REFERENCES TO RELATED APPLICATIONS

This is a continuation in part of application Ser. No. 583,788 filed Oct. 3, 1966. Also relevant is the disclosure of our pending applications entitled "Chromatographic Separation" and "Improvements Relating to Detection in Chromatography" (which will be filed approximately simultaneously).

### BACKGROUND OF THE INVENTION

The present invention relates to a method of introducing a sample of substances amenable to reversible electrodeposition into a chromatographic separating system prior to chromatographic separation of the sample. The invention furthermore provides an apparatus for liquid chromatography in combination with means for introducing the sample substantially by the said method.

Sample introduction in chromatography, whether for analytical or preparative purposes is a critical operation which can have a decisive influence on the eventual degree of separation attained. A convenient measure of the separating ability of a chromatographic system is its total plate height. This, in turn, is the sum total of the plate height contributions, namely, that of the separating system proper, i.e. the internal plate height contribution of the column in the case of chromatography carried out on a column, the contribution of the detector at the outlet end (which in accordance with the teachings of our pending application Ser. No. 583,788 filed Oct. 3, 1966, can be kept negligibly small) and last, but not least, on the plate height contribution of the inlet system. If a high degree of band spreading is inherent in the method of sample introduction it can nullify completely any efforts to keep low the remaining plate height contributions.

One of the most effective conventional methods of sample introduction is carried out by injecting the sample into the system with a syringe. Nevertheless, the best one can normally expect with that kind of sample introduction is an inlet contribution to the plate height of 0.5 cms. which is far too high when the internal plate height contribution of the separating system approaches a value of

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the order of 0.01 centimetre. This band spreading effect may be due to the following:

- (a) the local disturbing effect due to the injection e.g. local turbulence effects and irregular displacement of a corresponding volume of liquid already present in the apparatus;
- (b) the time delay between the beginning and end of the introduction into a system in which the forwarding phase (mobile phase) is flowing during the introduction;
- (c) the unavoidable size of the sample itself.

The invention has as one of its main objects the provision of an elegant and effective sample introduction applicable to liquid chromatography, allowing in particular the attainment of low plate height contributions due to sample introduction. More particularly, the invention is designed to overcome or mitigate where applicable all three of the aforementioned factors contributing to high plate heights during sample introduction and at the same time to eliminate the need for a separate concentration step even in extreme cases of dilute samples.

In the latter context it is pointed out that solutes in concentrations of less than  $10^{-5}$  M are not detectable by many detectors. In practice it may be necessary, however, to analyse solutions as dilute as  $10^{-10}$  M and the invention may be applied with particular advantage to such cases.

Further objects and advantages aimed at by the invention will be referred to in the course of the following description in the proper context.

### SUMMARY OF THE INVENTION

The method in accordance with the invention as generally outlined above comprises the steps of:

- (a) bringing a solution of said sample into intimate contact with an electrode;
- (b) electrolytically depositing said sample from the solution on said electrode;
- (c) bringing the electrode into intimate contact with at least one of the separating phases of the chromatographic system in the inlet zone thereof; and
- (d) electrolytically re-dissolving the sample off said electrode in said at least one separating phase.

The chromatographic system itself to which the method of sample introduction may be applied is open to numerous variations. It can comprise a conventional chromatographic column of any type suitable for the required separation, for example, packed with any suitable packing, e.g. with an organic or inorganic ion exchanger or a capillary or open-tube column. It can comprise a thin film chromatographic plate or means for paper chromatography, whether of the linear, one dimensional, two dimensional or radial type.

According to a preferred embodiment, however, the said inlet part leads into a system of electrochromatography i.e. a system in which a potential is placed on the separating medium so adapted to lead to a predetermined distribution co-efficient, thereby effecting a separation and causing the sample introduced to emerge from the system in a separated condition. Such a system is described and claimed in our pending application No. 657,815, filed Aug. 2, 1967, entitled "Chromatographic Separation" which by reference thereto forms part of the present disclosure.

The method offers a variety of useful modifications. Thus, by suitable selection of the potential at which the substances to be separated are initially precipitated on to the said electrode it is possible to achieve a preliminary selection of ions according to the principles of polarography. Thus, it is possible to remove from the solution certain substances of lower deposition electrolytically on

one electrode prior to collecting the actual sample in the manner described. Alternatively the sample collection may be carried out at a potential kept deliberately below the potential at which some components of the solution would precipitate, in order to achieve a preliminary selection of ions to be further separated. The two possibilities just described may also be combined.

Apart from or in addition to the foregoing the method can be used to particular advantage when some or all of the substances to be separated are present initially in extremely low concentrations, say, as low as about  $10^{-10}$  M. Under those conditions particularly the sample collection is carried out preferably with an electrode of very small surface area, e.g. a wire or pin-shaped electrode of which only the tip is exposed to the solution. For example, all but the tip of the electrode may be sheathed with insulation.

On the other hand the method need not be confined to microtechniques but may be scaled up for large scale operations as well, e.g. by the employment of a large number of "pinhead" electrodes in parallel or by the use of a wire gauze as the electrode.

In all cases where the time of sample introduction is significant the lower limit of the surface area of the electrode is determined by the amount of solute in the sample. The layer of sample deposit collected on the electrode must be sufficiently thin, whenever the sample is introduced into the chromatographic system whilst the moving phase is flowing so as to make it possible to limit the time taken for the sample to enter into solution to a value not exceeding the time in which the mobile phase travels a distance equal to the internal plate height contribution of the separating system by mere adjusting of the electric current for the electrolytic redissolving above a predetermined minimum value as a function of the flow rate of the mobile phase and the said plate height contribution.

A potential of 100 v. will satisfy this requirement almost without fail. The fastest chromatograms are usually those run in open capillaries and may require the return of the sample into solution in  $10^{-3}$  seconds in an extreme case.

It will also be readily appreciated that a large surface area of the electrode is not detrimental provided such surface area is limited to a narrow zone which, in the direction of flow of the moving phase, does not exceed the plate height contribution of the inlet aimed at. Where the sample introduction takes place whilst the moving phase is flowing, this zone width must furthermore be reduced by the distance travelled by the moving phase during the period of the return of the sample into solution.

The method may be carried out with the sample being collected in a separate vessel, from where the loaded electrode in the transferred to the inlet part of the chromatographic system. This is particularly advantageous in some high velocity chromatographic systems which require very high pressures for their operation. However, in a preferred embodiment of the invention the said inlet part is provided with means for passing therethrough, if desired circulatorily, the solution from which the sample is to be collected, the said sample collection then taking place right in the inlet system where by mere reversal of the current the sample is subsequently introduced into the chromatographic or like separatory system.

A particular embodiment comprises depositing the sample on said electrode whilst the latter is out of physical contact with the retarding phase of the chromatographic system, after said depositing bringing the electrode with the sample deposited thereon into physical contact with the retarding phase and, whilst in such contact, electrolytically re-dissolving the sample.

The apparatus in accordance with the invention for liquid chromatography comprises in combination with a chromatographic separating system having an inlet portion:

(a) a sample introduction electrode, a second electrode

and means for applying a controlled voltage across said electrodes,

(b) an electro-deposition vessel,

(c) means for holding the sample introduction electrode in said inlet portion; and

(d) means for reversing the voltage applied to the sample introduction electrode relative to a reference electrode.

In one embodiment the plating vessel coincides with the said inlet portion and is then preferably connected to means for circulating a sample solution through said inlet portion.

In such embodiments in which the inlet portion precedes a packing including the retarding phase comprising the retarding phase of the separating system, the apparatus may advantageously comprise holding means for the sample introduction electrode, holding the electrode in a manner adapted for movement into and out of contact with said packing.

Various suitable electrode materials will be apparent to those skilled in the art, which will satisfy the requirement of not contaminating the sample by not dissolving during the electrolytic redissolving of the sample. We have found it particularly advantageous to employ electrodes of platinum, including amalgamated platinum, graphite or of glassy carbon. An electrode material preferred by us because of its lack of porosity is glassy carbon.

In accordance with a particular method and apparatus provision is further made, for the introduction of the sample into the chromatographic system progressively, namely, by a reversed polarographic return of the sample into solution to achieve, if so desired, a preliminary separation during sample introduction prior to a more complete separation in the subsequent chromatographic stage, and/or to permit analytical deductions to be made by appropriate measurements. The means for achieving the voltage sweeping can operate in substantially the same manner as the voltage sweeping devices employed in polarographs, but in reverse. For a rapid voltage sweep use may be made of a condenser discharge or of more sophisticated electronic means which will be apparent to those skilled in the art.

The invention is particularly advantageously combined with the feature of the invention described and claimed in our aforesaid pending application Ser. No. 583,788 (Oct. 3, 1966) in which case the sample introduction electrode may form an electrode pair with a detector electrode. Moreover, as described in the said pending application, provision is made for a detector at the outlet end to be used in conjunction with a matching compensating cell which could be at the inlet end of the system, in which case the inlet system in accordance with the invention could, after the sample introduction, be used to perform the function of the compensatory cell.

The introduction electrode may also form an electrode pair with the wall of the apparatus, of which wall at least part is conductive or with a conductive packing comprising the retarding phase of the separating system.

#### BRIEF DESCRIPTION OF THE DRAWINGS

In the following the invention will be further explained by way of example with reference to the accompanying drawings, in which:

FIG. 1 represents a diagrammatic vertical elevation of a simple embodiment of a chromatographic apparatus in accordance with the invention;

FIG. 2 represents a diagrammatic plan view of the apparatus;

FIG. 3 represents a detail in sections of a holder arrangement for a sample introduction electrode;

FIG. 4 represents a detail of a movable sample introduction electrode; and

FIG. 5 represents a simplified wiring diagram of one form of voltage sweeping device for an apparatus in accordance with the invention.

## 5 DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring to FIG. 1 the inlet system of the chromatographic apparatus is generally indicated as 1. It is followed in downward direction by a chromatographic column 2, a detector cell 3 and an outlet 4. The inlet system comprises a pair of electrodes 5 connected through a current reversal switch 6 to the fixed terminal 7 and the sliding contact 8 of a potentiometer 9 connected across the terminals of a battery 10. Referring also to FIG. 2 a sample solution stored in vessel 11 is circulated by pump 12 via inlet pipe 13 and outlet pipe 14 through the inlet system 1 where a predetermined potential is maintained across electrodes 5 to plate the sample on to one of the electrodes. After this has been achieved to the desired extent the pump is stopped and valve 15 is closed. If it is considered necessary to rinse the inlet system first before the chromatographic separation of the sample, such is possible by now opening valve 17 and flushing the inlet system with some eluent derived from storage vessel 18 and flowing from the inlet through valve 16 and pipe 14 into sample vessel 11.

For the actual separation valve 16 is closed so that the eluent is now forced through column 2. The potential across electrodes 5 is now reversed in a predetermined manner, either so as to return the entire sample rapidly into solution or to do so in stages to achieve a preliminary separation before the chromatographic separation proper. In either case, provided the sample is returned with a voltage sweeping action, it is possible by means known per se to record the change of current to obtain some polarographic data which can subsequently be correlated with the chromatographic data.

The chromatographic separation takes place on column 2 by elution with the eluent which is first freed of oxygen in a manner known per se and then, on its way to the column, passes through an electro-purifier 19 across the electrode terminals 20 of which a predetermined potential is applied (tapped off from the potentiometer 9 by contact 21) to remove all impurities which might interfere with the chromatogram or the readings taken by detector 3. A suitable purifier is described in detail in our aforesaid patent application Ser. No. 583,788 filed Oct. 3, 1966.

Elution with the purified eluent causes the components to wander through the column 2 at different rates, and their arrival at the outlet end of the column is detected by the detector 3. The latter comprises a pair of electrodes 26 across which a potential is applied, tapped off from potentiometer 9 by means of the slidable contact 27. Any change in composition of the eluate passing through the detector becomes apparent by a change in the current measured by the amperemeter or equivalent current measuring device A. If necessary inlet 3a may be employed to introduce a supporting electrolyte.

A fourth movable contact 28 is provided for in the apparatus for use when the column 2 is replaced by an electrochromatographic column, as described in our aforesaid co-pending application No. 657,815 entitled "Chromatographic Separation." In that case the column packing 2 will itself be conductive and may be used as the reference electrode during sample introduction.

For very short columns that one of electrodes 5 serving as a reference electrode may be dispensed with, one of detector electrodes 26 performing that function instead. Furthermore the walls of the entire apparatus can be made partly or wholly of metal, e.g. platinum in the case of a microcolumn, which may then serve as the second electrode.

Electrodes 5, after the sample introduction has been performed may also be re-connected as a compensatory cell to the detector cell 3, 26.

Except when using a conductive column packing as

reference electrode, it is normally preferred to move the sample introduction electrode into contact with the packing for the final sample introduction into the system. This applies to the embodiment in accordance with FIGS. 1 and 2, where the sample introduction electrode is out of contact with the packing of column 2 during the sample deposition on the electrode. It also applies to the alternative case where the sample is electro-deposited on the sample introduction electrode in a separate plating vessel (which requires no detailed description). In either case the arrangement in accordance with FIG. 3 may be used for holding the electrode in a manner permitting movement when such movement is desired.

In FIG. 3 the column packing is represented by 30. The top of the column walls includes an eluent inlet 31 and a neck 32 having an internal flange 33 to act as a stop for an elastomer stopper 34, through which passes the plastics-sheathed sample introduction electrode 35. To the outside of the neck 32 a screw threaded collar 36 is fitted which is engaged by a screw cap 37 having a central projection 38 which via a washer 39 compresses the stopper 34 when the cap 37 is screwed home. When the cap is loose the electrode 35 can be moved up and down with ease. However, it is locked firmly when the stopper is compressed. In this particular example the second electrode is a ring 40 embedded in the top of the column packing 30 and having a terminal 41.

In FIG. 4 the electrode 35 just protrudes from a polyvinylchloride sheathing 42, around the upper end of which a metal ring 43 is clamped acting as a stop to limit the downward movement of the electrode relative to the stopper 34 or an extraneous abutment means (not shown). If the electrode is intended to be removed for sample deposition in an external plating vessel, the lower end of the sheathing will be smooth. If on the other end the deposition is to take place inside the inlet end of the column, a second stop 44, e.g. integral with the sheathing may be provided to limit the upward movement. (The terms "upward" and "downward" only refer to the positions shown in the drawing.)

Referring now to FIG. 5, a simplified version of a device for rapidly applying a voltage sweep to the sample introduction electrode is shown, comprising a battery 45 with a voltage divider 46, allowing a predetermined voltage to be applied to charge the condenser 47. The terminals 48 are connected to the sample introduction electrode and its reference electrode respectively. The total resistance composed of that of a variable resistor 49 and the internal resistance of the sample introduction cell will determine the rate of discharge through the cell when the two-way switch 50 closes the circuit. The ratio of the two resistances and the total voltage applied to the condenser 47 will determine the maximum potential applied to the sample introduction electrode. For gradual voltage sweeping effect one could employ the means known per se from polarography. More satisfactory means for applying short voltage pulses of predetermined shape, magnitude and duration are commercially available pulse generators.

### Example 1

A solution in 0.1 N HCl,  $10^{-9}$  M in respect of  $\text{Bi}^{\text{III}}$  and  $\text{Cu}^{\text{II}}$  is prepared. 100 ml. of this solution is circulated in contact with a pinhead sized platinum electrode, serving as the cathode and a calomel electrode of large surface area as reference. The voltage is adjusted to result in a current of approximately  $10^{-8}$  a. which is maintained for 30 minutes.

The current is then reversed to return the sample collected on the Pt electrode into solution in a matter of approximately  $\frac{1}{10}$  second, whilst an attempt is made to record the polarogram oscilloscopically. As is to be expected the polarogram shows only a single step at a voltage corresponding to that of a standard calomel electrode, since the oxidation potentials of Bi and Cu in 0.1

N HCl are very similar (+0.09 v. and -0.04 v. respectively measured against calomel).

The sample is fed into a column packed with Dowex 50 (1 micron particle) ion exchange resin. The eluent is also 0.1 N HCl which is first de-aerated by bubbling nitrogen through it at 80° C., whereafter the solution is cooled and passed through the eluent cleaner which is packed with tin particles and contains an Ag/AgCl anode. A voltage of 0.5 v. is maintained to clean the eluent.

The column has the following dimensions:

1 mm. internal diameter, 40 cm. long.

The linear flow rate is 1 mm./sec.

The detector is operated at a potential of 2 v. between anode and cathode.

Two well defined peaks are recorded.

#### Example 2

1 ml. of a solution of pHl (nitric acid) containing  $\text{Cu}^{++}$  in a concentration of  $2 \times 10^{-10}$  M and  $\text{Ag}^+$  in a concentration of  $5 \times 10^{-10}$  M is placed in a tiny cup of platinum serving as one electrode (anode) into which is dipped a platinum cathode serving as sample introduction electrode and consisting of polyvinyl chloride-sheathed wire having a diameter of 0.6 mm. and of which only the flat ground terminal surface is exposed (i.e. approximately 1 mm.<sup>2</sup>). The electrode is mounted on the end of a micro-agitator device so as to agitate the solution whilst electrodeposition proceeds.

A potential of 2.0 volts is applied and maintained for 30 minutes.

The introduction electrode is then introduced into the inlet end of a microcolumn packed as in the previous example and moved into contact with the ion exchanger. It is connected as an anode relative to a second platinum electrode spaced approximately 1 mm. from the introduction electrode in the direction of flow of the eluent. The eluent (0.1 N  $\text{HNO}_3$ ) is adjusted to 1 mm./sec. A single square half wave of 110 volt is applied to the introduction electrode for a duration of  $\frac{1}{400}$  sec. For all practical purposes the sample is stripped off the electrode instantaneously because the deposit on the electrode is only of the order of  $10^{-5}$  micron thick (average).

The chromatogram yields separate peaks due to Cu and Ag.

#### Example 3

The previous procedure is repeated with the same sample solution but containing in addition  $\text{Zn}^{++}$  in a concentration of  $5 \times 10^{-7}$  M. The voltage for the sample collection is maintained at 2.0 v. so that the deposition of the Zn is prevented. The chromatogram therefore again only shows the peaks for Cu and Ag.

#### Example 4

The procedure in accordance with Example 2 is repeated with a sample solution containing  $\text{Cu}^{++}$ ,  $\text{Ag}^+$ ,  $\text{Cd}^{++}$  and  $\text{Zn}^{++}$  each in a concentration of  $5 \times 10^{-10}$  M. The pH is again 1 (nitric acid). This time a potential of 2.8 v. is applied and maintained for 30 minutes.

The return of the sample into solution is carried out this time whilst the forwarding phase is at rest, by gradually lowering the applied voltage and simultaneously recording the reversed polarogram. This is done in two stages. In the first stage the voltage is lowered to approximately 1.8 volts during which the two plateaus due to Cd and Zn may be observed. These are then eluted first, yielding two peaks.

This is followed by further gradual lowering of the voltage down to zero, resulting in silver and copper being returned to solution as a single plateau. By proper elution the mixture is resolved, however, into two peaks.

Many modifications of the invention are possible without departing from the spirit thereof. For example, the sample introduction electrode may take the form of a porous conductive layer followed by an insulating layer,

followed in turn by the reference electrode, all of these preceding the inlet end of a column packing. The sample introduction may then take place with the sample solution being percolated slowly through the column itself.

What we claim is:

1. A liquid chromatography separating process which comprises introducing a sample of substances amenable to reversible electro-deposition into a chromatographic separating system by the steps of:

(a) bringing a solution of said sample into intimate contact with an electrode;

(b) electrolytically depositing said sample from the solution on said electrode by applying a current between the electrodes;

(c) bringing the electrode in the inlet zone of the chromatographic system into intimate contact with at least one of the mobile phase and stationary phase which constitute the separating phases of the system; and

(d) whilst the mobile phase of the separating system is flowing, electrolytically redissolving the sample off said electrode in said at least one separating phase and adjusting the electric current for the electrolytic redissolving as a function of the flow rate of the mobile phase and the plate height contribution of the separating system above a value predetermined to limit the time taken for the sample to enter into solution to a value not exceeding the time in which the mobile phase travelled a distance equal to said plate height contribution, the current for said redissolving being several times higher than the current for said depositing;

and subjecting the sample so introduced to liquid chromatographic separation.

2. A process as claimed in claim 1 which comprises controlling the electrolytical conditions during the combined steps of depositing and dissolving for achieving a partial separation of the sample prior to the chromatographic separation.

3. A process as claimed in claim 2 in which the redissolving is carried out with a progressively changing voltage applied to said electrode.

4. A process as claimed in claim 1 which comprises circulating the initial solution of the sample in contact with the electrode during the electrolytic deposition.

5. A process as claimed in claim 1 in which the sample is redissolved off said electrode in the mobile phase of a chromatographic system comprising a mobile and a stationary phase.

6. A process as claimed in claim 1 which comprises depositing the sample on said electrode whilst the latter is out of physical contact with the retarding phase of the chromatographic system, after said depositing bringing the electrode with the sample deposited thereon into physical contact with the retarding phase and, whilst in such contact, electrolytically re-dissolving the sample.

7. A process as claimed in claim 1 which comprises redissolving the sample from a zone of the electrode which in the direction of flow of the moving phase is narrower than that corresponding to the largest permissible plate height contribution ascribable to the sample introduction step.

8. A chromatographic separation apparatus for liquid chromatography comprising an inlet portion, an outlet portion and liquid chromatographic retarding means therebetween, said inlet portion communicating with and connected in advance of said chromatographic retarding means, said latter means being pervious to a mobile phase, and further comprising means for electrolytically depositing a sample on an electrode and for electrolytically redissolving the sample off the electrode, said means comprising a sample introduction electrode and at least one reference electrode forming an electrode pair with the sample introduction electrode, said electrode pair being disposed at said inlet portion, and means for applying a controlled voltage across said electrodes, having terminals

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electrically connected to said pair; and a switch for reversing the polarity of the sample introduction electrode and having terminals adapted to be electrically connected to the sample introduction electrode and a reference electrode, whilst together forming an electrode pair in said inlet portion.

9. Apparatus as defined in claim 8, further comprising an electro-deposition vessel having immersed therein said electrode pair whilst connected to the terminals of the means for applying a controlled voltage.

10. Apparatus as defined in claim 8, further comprising means for holding the sample introduction electrode in said inlet portion in a condition where a length of sample bearing electrode surface is in direct flowing contact with liquid mobile phase flowing through the inlet portion, said length measured in the direction of flow of mobile separating phase prescribed by the construction of the apparatus, being at the most as large as a length essentially equivalent to the plate height contribution inherent in the separating system.

11. Apparatus as defined by claim 8, said means for reversing being adapted for applying across its terminals a voltage substantially higher than the aforesaid controlled voltage and with said reversed polarity.

12. Apparatus as claimed in claim 8, wherein the electro-deposition vessel comprising the inside of said inlet portion, being in communication with said portion of the system and being connected by a pair of passages to means for circulating a sample solution through said inlet portion.

13. Apparatus as claimed in claim 12, wherein the retarding phase forms part of a packing of the system and wherein the inlet portion comprises holding means for the sample introduction electrode, holding the electrode in a manner adapted for movement into and out of contact with said packing.

14. Apparatus as claimed in claim 8 wherein at least part of the wall of the apparatus is electrically conductive and forms an electrode pair with the sample introduction electrode inside the inlet portion.

15. Apparatus as claimed in claim 8 wherein the sample introduction electrode inside the inlet portion also forms an electrode pair with an electrode forming part of a detector device connected following onto said portion of

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the system comprising a retarding phase and adapted for following the progress of separation.

16. Apparatus as claimed in claim 8 further comprising an electrode pair positioned at the outlet portion.

17. Apparatus as claimed in claim 8 in which the electrode material of the sample introduction electrode is platinum.

18. Apparatus as claimed in claim 8 in which the electrode material of the sample introduction electrode is essentially non-porous carbon.

19. Apparatus as claimed in claim 10 wherein the means for reversing the voltage include a voltage sweeping device.

20. Apparatus as defined by claim 8 further comprising: an inlet vessel constituting the inlet portion and comprising means for holding the sample introduction electrode therein whilst forming an electrolytic cell with the reference electrode in a manner adapted for movement of the sample introduction electrode into and out of contact, with said chromatographic retarding means; means connected to the inlet portion, for circulating a sample solution through the inlet portion in direct flowing contact with the sample introduction electrode.

21. Apparatus as defined by claim 8 further comprising: means for holding the sample introduction electrode in said inlet portion in direction flowing contact with a mobile liquid phase of the system; said chromatographic retarding phase comprising a conductive packing connected in communication with and following onto the inlet portion.

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HOWARD S. WILLIAMS, Primary Examiner

A. C. PRESCOTT, Assistant Examiner

U.S. CL. X.R.

204—180, 299

UNITED STATES PATENT OFFICE  
CERTIFICATE OF CORRECTION

Patent No. 3,582,475 Dated November 24, 1971

Inventor(s) V. Pretorius et al.

It is certified that error appears in the above-identified patent  
and that said Letters Patent are hereby corrected as shown below:

Column 10, line 26, change "direction" to --direct--.

Signed and sealed this 21st day of March 1972.

(SEAL)  
Attest:

EDWARD M. FLETCHER, JR.  
Attesting Officer

ROBERT GOTTSCHALK  
Commissioner of Patents