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(54) **Method of preparing a bare, fused silica, capillary for capillary electrophoresis**

Verfahren zur Vorbereitung einer unbeschichteten Quarzglas-Kapillare für Kapillarelektrophorese

Procédé de préparation d'un capillaire nu en verre de silice pour électrophorèse capillaire

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Description

Background of the Invention

[0001] The use of capillary electrophoresis (CE) has greatly improved DNA sequencing rates compared to conventional slab gel electrophoresis. Part of the improvement in speed, however, has been offset by the loss of the ability (inherent in slab gels) to accommodate multiple lanes in a single run. Highly multiplexed capillary electrophoresis, by making possible hundreds or even thousands of parallel sequencing runs, represents an attractive approach to overcoming the current throughput limitations of existing DNA sequencing instrumentation.

[0002] A separate but related problem is the internal coating of the capillary tubes. Typically, the fused-silica capillaries used in DNA sequencing by CE have been pretreated with a bonded coating. These are mostly variations of a bonded polyacrylamide layer. The reason for the coating is to reduce or eliminate the electroosmotic flow (EOF) that exists in bare fused-silica capillaries. EOF can actually expel the sieving matrix from the capillary. Even when EOF is low, the fact that it is opposite to the migration direction of DNA fragments means long separation times. Since the net motion is dictated by (μ_{DNA} - μ_{FOF}), representing the corresponding difference in mobilities ($\Delta\mu$), the large fragments are affected much more severely than the short fragments-Where EOF is present, variability in migration times makes it difficult to analyze samples containing larger DNA fragments. Unfortunately, the coating designed to reduce EOF degrades with use. This is not surprising since polyacrylamide, when used as the sieving medium, also breaks down with time on interaction with the typical buffers used for DNA sequencing. There is definitely a need for better surface treatment procedures for the capillary columns to retain their integrity over many runs.

[0003] United States Patent No. 5,213,669 discloses flushing a fused silica capillary with a deactivation solution such as 1M HCl after approximately every analytical 10 runs. The capillary is then re-filled with a dynamically cross-linked composition for the next set of analyses.

Summary of the Invention

[0004] The present invention provides a method of preparing a bare, fused silica, capillary for capillary electrophoresis as defined in claim 1.

Brief Description of the Figures

[0005] Figure 1 shows electrophoretic separation of PGEM/U DNA fragments from the Sanger DNA sequencing reaction from base 28 to base 108.

[0006] Figure 2 shows electrophoretic separation of PGEM/U DNA fragments from the Sanger DNA sequencing reaction from base 420 upwards.

[0007] In capillary electrophoresis, a buffer-filled capillary is suspended between two reservoirs filled with buffer. An electric field is applied across the two ends of the capillary. The electrical potential that generates the electric field is in the range of kilovolts. Samples containing one or more components or species are typically introduced at the high potential end and under the influence of the electrical field. Alternatively, the sample is injected using pressure or vacuum. The same sample can be introduced into many capillaries, or a different sample can be introduced into each capillary. Typically, an array of capillaries is held in a guide and the intake ends of the capillaries are dipped into vials that contain samples. After the samples are taken in by the capillaries, the ends of the capillaries are removed from the sample vials and submerged in a buffer which can be in a common container or in separate vials. The samples migrate toward the low potential end. During the migration, components of the sample are electrophoretically separated. After separation, the components are detected by a detector. Detection may be effected while the samples are still in the capillaries or after they have exited the capillaries.

[0008] The channel length for capillary electrophoresis is selected such that it is effective for achieving proper separation of species. Generally, the longer the channel, the greater the time a sample will take in migrating through the capillary. Thus, the species may be separated from one another with greater distances. However, longer channels contribute to the band broadening and lead to excessive separation time. Generally, for capillary electrophoresis, the capillaries are about 10 cm to about 5 meters long, and preferably about 20 cm to about 200 cm long. In capillary gel electrophoresis, where typically a polymer separation matrix is used, the more preferred channel length is about 10 cm to about 100 cm long.

[0009] The internal diameter (i.e., bore size) of the capillaries is not critical, although small bore capillaries are more useful in highly multiplexed applications. The invention extends to a wide range of capillary sizes. In general, capillaries can range from about 5-300 micrometers in internal diameter, with about 20-100 micrometers preferred. The length of the capillary can generally range from about 100-3000 mm, with about 300-1000 mm preferred.

[0010] A suitable capillary is constructed of material that is sturdy and durable so that it can maintain its physical integrity through repeated use under normal conditions for capillary electrophoresis. It is typically constructed of nonconductive material so that high voltages can be applied across the capillary without generating excessive heat.

[0011] According to the present invention, fused silica is used to make capillaries.

[0012] It is routine in the art to apply an internal polymer coating, such as γ-methacryloxypropyltrimethoxysilane and polyacrylamide (S. Hjerten, J. Chromatogr., 347, 191 (1985)), to capillaries prior to use in capillary electrophoresis in order to protect the internal walls from being adversely affected by a high pH buffer environment used

in experiments such as DNA sequencing. However, after several runs the protective coating gradually degrades, causing unwanted variations in electroosmotic flow that interfere with the interpretation of result. It was found, however, that the use of a protective internal coating is not the only way to address problems associated with electroosmotic flow. Specifically, good performance and resolution was observed whenever the silanol groups on the internal capillary walls of a capillary were in a protonated state prior to an electrophoretic separation.

[0013] Accordingly, the present invention provides a method for detecting a target species in a sample during capillary electrophoresis using a bare capillary that has been treated with acid to protonate the silanol groups on its internal wall. Specifically, a bare capillary having an uncoated fused silica internal wall is brought into contact with acid, preferably 0.01-0.5 N hydrochloric acid, more preferably about 0.1 N HCl, for a time effective to protonate the silanol groups on the capillary internal wall, typically by flushing for about 2 hours. A sample containing a target species, preferably a fluorescent target species, more preferably a fluorescent DNA fragment, is introduced into the intake end of the capillary such that it migrates through the capillary toward the outflow end. Preferably, the sample is introduced by pressure injection. The sample is then detected by any convenient means, preferably by fluorescence or mass spectrometry.

[0014] A polymer matrix, preferably poly(ethyleneoxide), may be placed in the bare capillary immediately prior to introducing the sample, preferably by pressure injection using pressure of about 100-400 psi (5000-20000 torr). Preferably, the polymer matrix solution contains a polymer with a viscosity of less than 5,000 centipoise, more preferably less than about 2,000 centipoise, measured as disclosed above. To maintain a high level of performance, the bare capillaries should be regenerated (reprotonated) often, preferably after every run, whether or not a polymer matrix is used.

[0015] Thus, the used poly(ethyleneoxide) matrix is removed from bare capillary, and the capillary is flushed with acid to reprotonate the silanol groups, as described above. A fresh solution of poly(ethyleneoxide) matrix in then injected into the bare capillary, and another capillary electrophoresis experiment is performed. These steps can be repeated indefinitely, and multiple experiments can be performed using the same capillary, since the capillary is always regenerated in between runs using the acid wash. This protocol is particularly well suited to DNA sequencing experiments.

[0016] This method provides a capillary electrophoresis system that includes at least one bare capillary having an uncoated bare fused silica wall containing protonated silanol groups. The protonated capillary can be used in both capillary gel electrophoresis and capillary zone electrophoresis. If capillary zone electrophoresis is employed, the use of a dilute solution of polymer, preferably PEO, to dynamically coat and isolate the capillary walls from the high pH environment (about pH 8-9) of the buffer solution, is recommended to keep the silanol groups protonated as long as possible.

EXAMPLE

[0017] Capillary and reagent. Capillaries (Polymicro Technologies, Phoenix, AZ) with 75 μm i.d. (inside diameter) and 365 μm o.d. (outside diameter) were used.

[0018] All chemicals for preparing buffer solutions and for coating capillaries were purchased from ICN Biochemicals (Irvine, CA), except that acrylamide and formamide were from Sigma Chemical (St. Louis, MO) and poly(ethyleneoxide) was obtained from Aldrich Chemical (Milwaukee, WI). Fuming hydrochloric acid was obtained from Fisher (Fairlawn, NJ). Polyacrylamide solution (10% solution in water, 700,000 to 1,000,000 M_n) was obtained from Polysciences (Warrington, PA). A calibration standard of PGEM/U DNA, prepared by cycle sequencing using commercial four-color terminators and Taq polymerase was obtained from Nucleic Acid Facilities (Iowa State University, Ames, IA).

[0019] Gel and buffer preparations. The 1x buffer solution was prepared by dissolving tris(hydroxymethyl) aminomethane (THAM), boric acid, ethylenediaminetetraacetic acid (EDTA) and urea in deionized water, producing a solution containing 89 mM THAM, 89 mM boric acid, 2 mM EDTA, and 3.5 M urea, pH adjusted to 8.2.

[0020] The sieving matrix was prepared by gradually adding 1.5 g 8,000,000 M_n poly(ethyleneoxide) (PEO) and 1.4 g 600,000 M_n PEO in 100 ml buffer solution at 50-60°C. During the addition of PEO, a magnetic stirring rod was used at a high setting to enhance the dissolution of the polymer powder. After the addition was complete, the solution was stirred for another 30 minutes. Then, the solution was degassed in an ultrasonic bath for 30 minutes.

[0021] Capillary wall treatment. A bare fused silica capillary (i.e., a capillary without any added internal coating), typically 45 cm total length (35 cm effective length) was flushed with methanol for 10 minutes and then 0.1 N HCl for 2 hours, then filled with a very low-viscosity polymer solution (e.g., 0.5% PEO), then filled with the polymer matrix with a syringe. The filled capillary was equilibrated at the running voltage (12 kV) for 10 minutes before sample injection. The DNA sample was denatured by heating in a denaturing solution (5:1 formamide-50 mM aqueous EDTA solution) at 95°C for 2.5 minutes, and the injection was performed at 6 kV for 12 seconds. Between runs, the used polymer matrix was flushed out of the capillary with high pressure (400 psi, 20 x 10^3 torr, 3 minutes), and rinsed with 0.1 N HCl for 15-30 minutes before filling with new polymer matrix.

[0022] Base calling. Nucleotide identification in DNA sequencing experiments, i.e., "base calling", was performed using the ratio of emission intensities recorded through two different optical filters as described in Example V. Independent confirmation was accomplished by comparison with data obtained on a commercial DNA

sequencer (Applied Biosystems, Inc., Foster City, CA).

[0023] Novel sieving medium. A direct comparison between PEO and 6% T non-crosslinked polyacrylamide was made. (In nomenclature commonly used in the art, T represents the percentage of total acrylamide, and C represents the percentage of cross-linker.) The test sample was a DNA (PGEM/U) fragment ladder prepared by the Iowa State University Nucleic Acid Facility using the standard dye-labeled terminators (Applied Biosystems, Inc., Foster City, CA) and Taq polymerase. The sample preparation procedure was not altered in any way from that used to produce samples for the commercial DNA sequencing instrument (Applied Biosystems, Inc., Foster City, CA). The injected sample was identical in concentration and composition to those suggested for loading into the commercial instrument. The matrix used for DNA sequencing consisted of 1.4% PEO 600,000 M_n, 1.5% PEO 8,000,000 M_n, 1x TBE (pH 8.2), 3.5 M urea. Intermediate M_n polymers (see Example I) were not needed; apparently the polymers at the two extremes of the size range can entangle in such a way to form the intermediate pore sizes as well. This binary matrix provided very similar performance to the 0.7% multiple polymer matrix (see Example I) but had even lower viscosity (1,200 centipoise at room temperature, measured in a capillary at 1 atm, 25°C, using the Pouiselle equation) than the 0.7% mixture. All experiments in this example were performed using this particular binary matrix.

[0024] Commercial non-crosslinked polyacrylamide (10% T solution, 700,000 to 1,000,000 M_n) was diluted to form a 6% T, 1x TBE, 3.5 M urea matrix. This solution had a measured viscosity of 4,900 centipoise at room temperature (measured as above). Otherwise, identical conditions were used throughout. Excitation by a He-Ne laser at 543 nm and 2 RG610 long-pass filters selected primarily the cytosine (C) and thymidine (T) fragments from the Sanger DNA sequencing reaction. Lyophilized DNA samples were denatured by heating in a 5:1 formamide-50 mM aqueous EDTA solution at 95°C for 2 minutes. Electrokinetic injection was performed at 6 kV for 12 seconds and the separation was run at 13 kV. The results are shown in Figures 1 and 2 for the regions of 24-108 bp and greater than 420 bp DNA fragments, respectively. The time span of the abscissa is different in each of the panels. In Figure 1 (covering DNA fragments between 24-108 base pair in length), panel A shows a PEO matrix in a coated capillary (14-19 minutes); panel B shows a polyacrylamide matrix in a coated capillary (37-55 minutes); panel C shows a PEO matrix in a fresh bare capillary (15-20.3 minutes); panel D shows a PEO matrix in an HCl-reconditioned bare capillary (6th run, 9.5-13 minutes); and panel E shows a polyacrylamide matrix in a bare capillary (2nd run, 14.5-26.5 minutes). In Figure 2 (covering DNA fragments longer than 420 base pair in length), panel A shows a PEO matrix in coated capillary (39-52 minutes); panel B shows a polyacrylamide matrix in coated capillary (111-129 minutes); panel C shows a PEO matrix in fresh bare capillary (40-52 minutes); panel D shows a PEO matrix in an HCl-reconditioned bare capillary (6th run, 26-33 minutes); and panel E shows a polyacrylamide matrix in a bare capillary (2nd run, 60-69.5 minutes). The ordinate of each electropherogram was adjusted to roughly match the others and to emphasize the small peaks, as those cause the most problems in base calling due to overlap and inadequate signal to noise ratio (S/N). All peaks were on scale; they were merely truncated in the figures to allow plotting one on top of another. The abscissa of each electropherogram has also been adjusted to plot the same base-pair region in each case.

[0025] Comparison of panels A and B of Figure 1 shows that for the short fragments, PEO provided a resolving power quite close to that of polyacrylamide. The major difference was separation speed. The PEO plot (panel A) was from 14 to 19 minutes while the polyacrylamide plot (panel B) was from 37 to 55 minutes. This is due to the higher viscosity of the polyacrylamide matrix. Very striking was the difference in separation for the large fragments, Figure 2, panels A and B. The PEO matrix (panel A) clearly provided better resolution and may even be extending the convergence limit to longer fragments. In the middle range (108-420 bp, data not shown), there was a one-to-one correspondence between the resolution of DNA fragments in PEO compared to that in polyacrylamide, although some degradation was already evident in the polyacrylamide runs for DNA fragments longer than 320 bp.

[0026] A reasonable explanation for the differences in performance is that the maximum length of the polyacrylamide polymer is not sufficient to form dynamic pore sizes large enough for the large DNA fragments. In fact, a PEO molecule of the same M_n should be longer than polyacrylamide because of the specific atomic arrangement along the backbone. The same is true when PEO is compared with any other polymer that has been used for CE sieving. A polymeric material related to PEO is poly(ethyleneglycol) (PEG). Structurally, PEG is almost identical to PEO; but the starting monomer and the polymerization process are different. The latter is probably the main reason why commercial PEG preparations are not available out to the millions of daltons at which PEO can be purchased, and why PEG is not a suitable non-crosslinked matrix for capillary electrophoresis.

[0027] Column treatment protocol. Even when the sieving medium is replaced after every run to allow repeated usage of the capillaries, the protective coating (S. Hjertén, J. Chromatogr., 347,191-198 (1985)) on the internal wall of the capillary gradually degrades. Attempts have been made to regenerate the coating after several runs by repolymerization of polyacrylamide *in situ* (S. Hjertén. J. Chromatogr., 347, 191-198 (1985)). However, original performance was not reproducibly restored in this manner. Other attempts have involved omitting the polyacrylamide coating in the first place (H. Swerdlow et al., Electrophoresis, 13, 475-483 (1992), M. Starita-Geribaldi et al., Electrophoresis, 14, 773-781 (1993)). For example,

the silanol groups on the fused-silica wall can be irreversibly covalently modified by treating the capillary with 3-methacryloxypropyltrimethoxysilane (silanization) and the capillaries can be used without further polymerization with polyacrylamide. The electroosmotic flow was indeed substantially reduced using such capillaries, as judged by the migration times of the primer peak and the high MW convergence peak. However, the separation efficiency was compromised to the extent that sequencing of DNA fragments longer than 200 bp was not possible.

[0028] Because the main purpose of coating the capillary column is to eliminate electroosmotic flow (EOF), alternative approaches directed toward elimination of EOF were considered. To determine if DNA sequencing could be performed on a bare fused-silica column, i.e., whether the sieving polymer alone would effectively coat and isolate the capillary walls, commercial bare fused silica capillary columns were therefore washed with methanol and immediately thereafter, the polymer matrix was introduced into the bare capillaries and a DNA sequencing run was initiated.

[0029] DNA separations in bare capillary columns are shown in Figures 1, panel C (DNA fragments of 24-108 bp in length) and Figure 2, panel C (greater than 420 bp in length) for the PEO mixed-polymer matrix. Comparison of panels A (coated capillaries) and panels C (uncoated capillaries) in Figures 1 and 2 reveal that there is practically no difference in the electropherograms with or without a coating on the capillary wall. Even the actual migration times are almost identical. This is the first demonstration of DNA sequencing in CE without a bonded coating on the column wall. Unfortunately, while these results can be reproduced for a new capillary column, the resolution invariably starts to degrade after one or two runs. The migration times became progressively longer and the peaks associated with the longest fragments became unrecognizable. Replacing the capillary array after every run is of course not a viable option for high-throughput DNA sequencing.

[0030] Thus, the column was flushed with deionized water, methanol, buffer solution, or 1 M NaOH, plus combinations of these in an attempt to regenerate the surface characteristics of the column. In no case was the original performance restored, however. It was discovered, however, that the original column surface characteristics could be restored by flushing the column in between runs with acid (0.1 N HCl).

[0031] The performance of a bare fused-silica CE column in separating DNA fragments after 6 cycles of PEO fill, DNA electrophoresis, pressure removal of PEO, and 0.1 N HCl conditioning is shown in panels D of Figures 1 and 2 (showing separation of DNA fragments of 24-108 bp in length and greater than 420 bp in length, respectively). There is no obvious difference in resolution between this electropherogram and those for coated column/PEO (panels A of Figures 1 and 2) or for a fresh bare column/PEO (panels C of Figures 1 and 2). There are slight random variations in migration times, but no systematic change over time. Because no bonded coating was used, there was nothing to degrade. Thus, the column should in principle last indefinitely. This is the first demonstration of extended usage of a CE capillary for DNA sequencing.

[0032] A surprising result is that the migration times observed in the HCl-treated capillaries are much shorter than those found for any polymer matrix/surface preparation, either disclosed here or in the literature. The unexpected advantage to the use of HCl-treated, uncoated columns is that DNA fragments having lengths of 28-420 bp eluted within a time span of only 16 minutes for an average rate of 25 bp/minutes. This is faster by a factor of 3-5 compared to reported results using non-crosslinked polyacrylamide in coated capillaries.

[0033] Finally, the performance of a non-crosslinked polyacrylamide matrix in a bare fused-silica capillary was evaluated (panels E of Figures 1 and 2). Resolution for the short fragments (panel E, Figure 1) is the best of all the systems studied here, but the resolution of fragments longer than 420 bp (panel E, Figure 2)) is the worst. This electropherogram is actually the second run on the bare column, indicating that degradation is slower than for the case of the PEO matrix on a bare column. There was still gradual degradation due to an increase in electroosmotic flow, as the first run started 0.5 minutes earlier and ended 10 minutes earlier. This is consistent with the fact that polyacrylamide is a more viscous matrix, so it takes longer for the ions in the bulk medium to titrate the surface silanol groups to the same extent. The HCl reconditioning procedure was not effective after a column was filled with polyacrylamide.

[0034] System integration. In anticipation of the need to flush, recondition, and refill the capillary columns after each of many runs and the need to inject multiple samples separately into the array, a pressure cell suitable for these operations was developed. The pressure needed to fill a capillary with this relatively low-viscosity sieving matrix is only about 100-400 psi (5×10^2 - 20×10^3 torr), depending on the time allowed for each operation. For 100 capillaries in a bundle, the exit end can be gathered together to form a close-packed group only 2 mm in diameter. Pressure injection assists in avoiding cross contamination of the sample from the electrodes, and assists in avoiding the need to make electrical contacts with each sample vial in a large array entirely.

Claims

1. A method of preparing a bare, fused silica, capillary for capillary electrophoresis, comprising:

   contacting the internal surface of a bare, unused, fused silica capillary electrophoresis capillary, prior to first use, with an acid for protonating the silanol groups on said internal surface prior to filling the bare, unused, fused silica cap-

illary electrophoresis capillary with a polymer matrix, to form an acid-washed, unused, capillary; and
filling the acid-washed, unused, capillary, for the first time, with a polymer matrix to form a filled, unused, capillary.

2. The method of claim 1, wherein the acid comprises hydrochloric acid.

3. The method of claim 1, wherein the acid comprises hydrochloric acid having a normality of from 0.01N to 0.5N.

4. The method of claim 1, wherein the acid comprises about 0.1N hydrochloric acid.

5. The method of claim 1, further comprising conducting an electrophoretic separation in the filled capillary.

6. The method of claim 1, further comprising flushing the bare, unused, capillary electrophoresis capillary with methanol prior to the contacting with an acid.

7. The method of claim 6, wherein the flushing method comprises flushing the bare, unused, capillary electrophoresis capillary with methanol for 10 minutes.

8. The method of claim 1, wherein the contacting with an acid comprises flushing the bare, unused, capillary electrophoresis capillary wath acid for about two hours.

Patentansprüche

1. Verfahren zum Herstellen einer bloßen Quarzglas-Kapillare für die Kapillarelektrophorese, wobei

die Innenfläche einer bloßen, unbenutzten Quarzglas-Elektrophorese-Kapillare vor der ersten Benutzung mit einer Säure zum Protonieren der Silanol-Gruppen auf der Innenfläche kontaktiert werden, bevor die bloße, unbenutzte Quarzglas-Elektrophorese-Kapillare mit einer Polymermatrix gefüllt wird, um eine Säure-gewaschene, unbenutzte Kapillare zu bilden; und die Säure-gewaschene, unbenutzte Kapillare zum ersten Mal mit einer Polymermatrix gefüllt wird, um eine gefüllte, unbenutzte Kapillare zu bilden.

2. Verfahren nach Anspruch 1, wobei die Säure Chlorwasserstoffsäure aufweist.

3. Verfahren nach Anspruch 1, wobei die Säure Chlorwasserstoffsäure mit einer Konzentration von 0,01 N bis 0,5 N aufweist.

4. Verfahren nach Anspruch 1, wobei die Säure etwa 0,1 N konzentrierte Chlorwasserstoffsäure aufweist.

5. Verfahren nach Anspruch 1, wobei ferner eine elektrophoresische Trennung in der gefüllten Kapillare durchgeführt wird.

6. Verfahren nach Anspruch 1, wobei die bloße, unbenutzte Elektrophorese-Kapillare vor der Kontaktierung mit der Säure mit Methanol gespült wird.

7. Verfahren nach Anspruch 6, wobei bei dem Spülvorgang die bloße, unbenutzte Elektrophorese-Kapillare für 10 Minuten mit Methanol gespült wird.

8. Verfahren nach Anspruch 1, wobei bei der Kontaktierung mit der Säure die bloße, unbenutzte Elektrophorese-Kapillare für etwa zwei Stunden mit Säure gespült wird.

Revendications

1. Procédé pour la préparation d'un capillaire nu en silice fondue pour électrophorèse capillaire, comprenant :

la mise en contact de la surface intérieure d'un capillaire d'électrophorèse consistant en un capillaire nu en silice fondue, non utilisé, avant la première utilisation, avec un acide pour la protonation des groupes silanol sur ladite surface intérieure, avant remplissage du capillaire pour électrophorèse consistant en un capillaire nu en silice fondue, non utilisé, avec une matrice polymère, pour former un capillaire lavé à l'acide, non utilisé ; et
le remplissage du capillaire lavé à l'acide, non utilisé, pour la première fois avec une matrice polymère pour former un capillaire rempli, non utilisé.

2. Procédé suivant la revendication 1, dans lequel l'acide comprend l'acide chlorhydrique.

3. Procédé suivant la revendication 1, dans lequel l'acide comprend de l'acide chlorhydrique ayant une normalité de 0,01 N à 0,5 N.

4. Procédé suivant la revendication 1, dans lequel l'acide comprend de l'acide chlorhydrique à environ 0,1 N.

5. Procédé suivant la revendication 1, comprenant en outre la mise en oeuvre d'une séparation électrophorétique dans le capillaire rempli.

6. Procédé suivant la revendication 1, comprenant en outre le balayage du capillaire pour électrophorèse consistant en un capillaire nu, non utilisé, avec du méthanol avant la mise en contact avec un acide.

7. Procédé suivant la revendication 6, dans lequel le procédé de balayage comprend le balayage du capillaire pour électrophorèse consistant en un capillaire nu, non utilisé, avec du méthanol pendant 10 minutes.

8. Procédé suivant la revendication 1, dans lequel la mise en contact avec un acide comprend le balayage du capillaire pour électrophorèse consistant en un capillaire nu, non utilisé, avec un acide pendant environ deux heures.

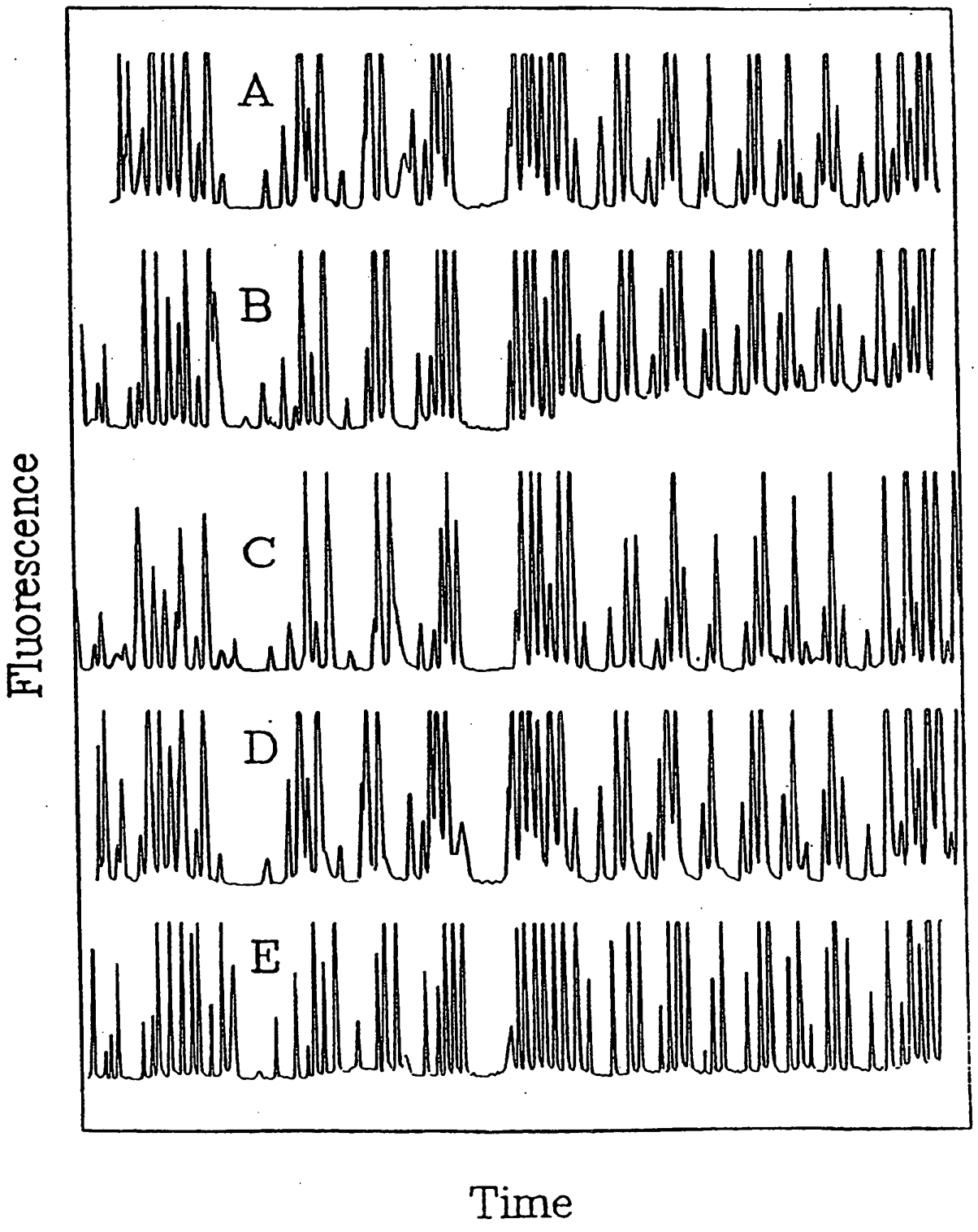

FIG. 1

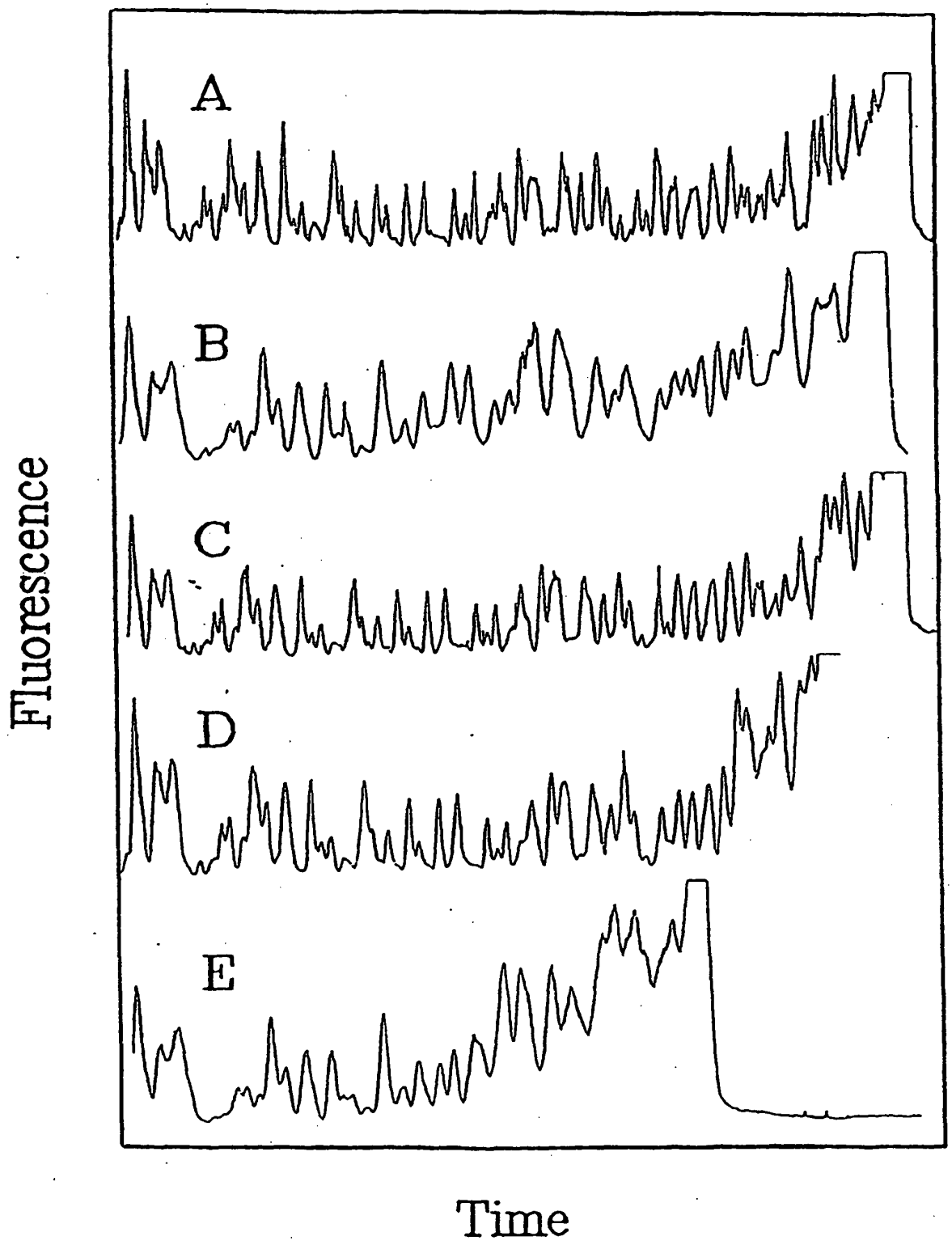

FIG. 12

REFERENCES CITED IN THE DESCRIPTION

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