

DESCRIPTION

Title of Invention: CAPILLARY ELECTROPHORESIS DEVICE AND CAPILLARY ELECTROPHORESIS METHOD

Technical Field

[0001]

The present invention relates to a capillary electrophoresis device and a capillary electrophoresis method.

Background Art

[0002]

Instrumental analysis in analytical chemistry is analysis using equipment for performing nuclear magnetic resonance spectroscopy, absorption spectroscopy, Raman spectroscopy, fluorescence spectroscopy, mass spectrometry, a chromatography analysis, electrophoresis analysis, and the like.

[0003]

As shown in NPL 1 and NPL 2, in these instrumental analyses, an unknown concentration of an analysis target contained in a sample is often quantified using an external standard method (absolute calibration curve method) or an internal standard method.

[0004]

The external standard method is a method of creating a calibration curve, which is a relation between a concentration of an analysis target and a signal intensity, in advance using a standard sample including analysis targets of various known concentrations, and quantifying an unknown concentration of the analysis target contained in the sample. In general, it is assumed that the signal intensity is proportional to the concentration of the analysis target, that is, an increase in the signal intensity with respect to the concentration of the analysis target is linear, and an increase rate of the signal intensity with respect to the concentration of the analysis target is constant.

[0005]

However, when the increase in the signal intensity with respect to the concentration of the analysis target is non-linear, for example, when the increase rate of the signal intensity with respect to the concentration of the analysis target decreases together with the concentration of the analysis target, the external standard method can function. However, in the external standard method, the quantitative accuracy decreases due to an influence of a matrix effect of the sample and an influence of the variation in an injection amount of the sample into an analysis device.

[0006]

On the other hand, the internal standard method is a method of improving the quantitative accuracy by reducing these influences. The internal standard method is a method of preparing in advance a calibration curve, which is a relation between a concentration ratio of an analysis target to an internal standard and a signal intensity ratio of the analysis target to the internal standard by using a standard sample including analysis targets of various known concentrations and an internal standard of a known concentration, and quantifying an unknown concentration of an analysis target contained in the sample.

[0007]

Here, it is necessary to independently measure signal intensities of the internal standard and the analysis target. As shown in NPL 1 and NPL 2, in the internal standard method, it is necessary that the respective signal intensities are proportional to the concentrations of the analysis target and the internal standard, that is, the respective signal intensities increase linearly with respect to the concentrations of the analysis target and the internal standard.

[0008]

On the other hand, when the respective signal intensities increase nonlinearly with respect to the concentrations of the analysis target and the internal standard, the internal standard method does not function. That is, the quantitative accuracy cannot be improved by reducing the influence of the matrix effect of the sample and the influence of the variation in the injection amount of the sample into the analysis device. In addition, as pointed out in NPL 2, it is known that the quantitative accuracy rather decreases.

[0009]

The case of quantifying, by capillary electrophoresis analysis of laser-induced fluorescence measurement, a concentration $C(\text{tg})$ of fluorescently-labeled DNA fragments as analysis targets contained in a sample will be described more specifically. In the present specification, a subscript used for a coefficient or a variable used in a mathematical expression may be indicated in parentheses. For example, when a subscript tg is added to a variable C, the variable C may be expressed as $C(\text{tg})$ or may be expressed as C_{tg} .

[0010]

In FIG. 1 of PTL 1, a capillary electrophoresis device that performs four capillary electrophoresis analyses in parallel is used, and the electrophoresis analysis is performed using one capillary of the capillary electrophoresis device. The sample may contain fluorescently-labeled DNA fragments other than the fluorescently-labeled DNA fragments as analysis targets. Salts (ions) other than the DNA fragments contained in the sample are removed as much as possible in advance by ethanol precipitation or column purification.

[0011]

First, a part of the sample is injected from a sample-injection end of the capillary by electric field injection. In general, an amount of DNA fragments to be injected is proportional to an electric field intensity E and a time T of electric field injection, and the concentration $C(\text{tg})$ of DNA fragments in the sample.

[0012]

Next, the injected DNA fragments are separated depend on a base length while moving toward a sample-elution end of the capillary by electrophoresis. At this time, the DNA fragment passing through a measurement point on the capillary by electrophoresis is irradiated with a laser beam, and a fluorophore labeled on the DNA fragment emits fluorescence.

[0013]

The emitted fluorescence is sequentially measured by an image sensor, and the time series thereof gives an electropherogram. A peak corresponding to the DNA fragment as an analysis target is obtained on the electropherogram.

[0014]

A signal intensity $S(tg)$ of the DNA fragment as an analysis target is generally indicated by an area of the peak of the DNA fragment as an analysis target, or is indicated by a height of the peak of the DNA fragment as an analysis target when a width of the peak can be regarded as constant. Since the signal intensity $S(tg)$ of the DNA fragment as an analysis target is proportional to the amount of the DNA fragments as an analysis target injected into the capillary, when a proportionality coefficient is $K(tg)$, the signal intensity $S(tg)$ is represented by (Math. 1).
[Math. 1]

$$S_{tg} = K_{tg} \cdot E \cdot T \cdot C_{tg} \quad (\text{Math. 1})$$

Here, E represents an effective electric field intensity in a sample near a sample-injection end of a capillary at the time of electric field injection, and T represents a time of electric field injection.

[0015]

When E and T are fixed, (Math. 1) shows that the signal intensity $S(tg)$ of the DNA fragment as an analysis target is proportional to the concentration $C(tg)$ of the DNA fragment as an analysis target in the sample, which is a calibration curve of the external standard method. An unknown concentration $C(tg)$ of an analysis target contained in a sample can be quantified using (Math. 1) from the signal intensity $S(tg)$ of the DNA fragment as an analysis target to be measured.

[0016]

On the other hand, in the internal standard method, a DNA fragment, which is an internal standard of a known concentration, is mixed with a sample to perform capillary electrophoresis analysis. The DNA fragment of the internal standard is also fluorescently-labeled. The peak of the DNA fragment as an analysis target and a peak of the DNA fragment of the internal standard are independently observed on the electropherogram. Since the signal intensity $S(st)$ of the DNA fragments of the internal standard is proportional to the amount of the DNA fragments of the internal standard injected into the capillary, when a proportionality coefficient is $K(st)$, the signal intensity $S(tg)$ is represented by (Math. 2).

[Math. 2]

$$S_{st} = K_{st} \cdot E \cdot T \cdot C_{st} \quad (\text{Math. 2})$$

[0017]

(Math. 2) indicates that the signal intensity $S(st)$ of the DNA fragment of the internal standard is proportional to the concentration $C(st)$ of the DNA fragment of the internal standard in the sample. Since the electric field injection of the DNA fragment as an analysis target and the electric field injection of the DNA fragment of the internal standard in the sample are collectively performed, the electric field intensity E and the time T of the electric field injection in (Math. 1) and (Math. 2) are the same. Therefore, (Math. 3) is obtained by taking a ratio of (Math. 1) and (Math. 2).

[Math. 3]

$$\frac{S_{tg}}{S_{st}} = \frac{K_{tg}}{K_{st}} \cdot \frac{C_{tg}}{C_{st}} \quad (\text{Math. 3})$$

(Math. 3) shows that a signal intensity ratio $S(tg)/S(st)$ of the DNA fragment as an analysis target to the DNA fragment of the internal standard is proportional to a concentration ratio $C(tg)/C(st)$ of the DNA fragment as an analysis target to the DNA fragment of the internal standard in the sample, which is a calibration curve of the internal standard method.

[0018]

Using (Math. 3), the concentration ratio $C(tg)/C(st)$ of the DNA fragment as an analysis target to the DNA fragment of the internal standard in the sample can be quantified from the measured signal intensity ratio $S(tg)/S(st)$ of the DNA fragment as an analysis target to the DNA fragment of the internal standard. Since the concentration $C(st)$ of the DNA fragment of the internal standard in the sample is known, this is synonymous with quantifying the unknown concentration C_{tg} of the DNA fragment as an analysis target in the sample.

[0019]

In the external standard method according to (Math. 1), for example, if the electric field intensity E of the electric field injection varies by $\pm 10\%$, as is clear from (Math. 1), this variation directly leads to a decrease in the quantitative accuracy of the concentration of the DNA fragment as an analysis target. On the other hand, in the internal standard method according

to (Math. 3), since the electric field intensity E of the electric field injection is not included in (Math. 3), this variation does not influence the quantitative accuracy of the concentration of the DNA fragment as an analysis target. This is because the influence of the variation in the electric field intensity E in (Math. 1) and the influence of the variation in the electric field intensity E in (Math. 2) are the same, and the influence is canceled by taking a ratio of (Math. 1) and (Math. 2). The above is the reason why the quantitative accuracy of the analysis target by the internal standard method can be improved as compared with the external standard method.

Citation List

Patent Literature

[0020]

PTL 1: WO2023/007567

Non Patent Literature

[0021]

NPL 1: Harvey, David. Modern analytical chemistry. Vol. 1. New York: McGraw-Hill, 2000.

NPL 2: A. K. Hewavitharana (2009) Internal Standard-Friend or Foe?, Critical Reviews in Analytical Chemistry, 39:4, 272-275

Summary of Invention

Technical Problem

[0022]

The reason why the external standard method can function but the internal standard method does not function when the signal intensity is not proportional to the concentration of the analysis target will be considered.

[0023]

For example, assuming that a signal intensity of an analysis target is represented by a quadratic function of a concentration according to NPL 2, $L(tg)$ and $K(tg)$ are coefficients, and

(Math. 4) is obtained.

[Math. 4]

$$S_{tg} = L_{tg} \cdot (E \cdot T \cdot C_{tg})^2 + K_{tg} \cdot E \cdot T \cdot C_{tg} \quad (\text{Math. 4})$$

A constant term of the quadratic function was set to zero so that $S(tg) = 0$ when $C(tg) = 0$. In addition, when $L(tg) = 0$, (Math. 4) is the same as (Math. 1).

[0024]

In the external standard method, (Math. 4) is a calibration curve. As in the case of (Math. 1), an unknown concentration $C(tg)$ of an analysis target contained in a sample can be quantified using (Math. 4) from the signal intensity $S(tg)$ of the DNA fragment as an analysis target to be measured.

[0025]

On the other hand, similarly, assuming that a signal intensity of the internal standard is represented by a quadratic function of a concentration, $L(st)$ and $K(st)$ are coefficients, and (Math. 5) is obtained.

[Math. 5]

$$S_{st} = L_{st} \cdot (E \cdot T \cdot C_{st})^2 + K_{st} \cdot E \cdot T \cdot C_{st} \quad (\text{Math. 5})$$

A constant term of the quadratic function was set to zero so that $S(tg) = 0$ when $C(tg) = 0$. In addition, when $L(st) = 0$, (Math. 5) is the same as (Math. 2).

[0026]

At this time, unlike (Math. 3), even if a ratio of (Math. 4) and (Math. 5) is taken, the expression is not simplified, and the signal intensity ratio $S(tg)/S(st)$ of the DNA fragment as an analysis target to the DNA fragment of the internal standard cannot be represented by the concentration ratio $C(tg)/C(st)$ of the DNA fragment as an analysis target to the DNA fragment of the internal standard in the sample. Further, unlike (Math. 3), the electric field intensity E and the time T of the electric field injection remain, and thus an influence of the variation cannot be canceled. Therefore, the quantitative accuracy of the analysis target by the internal standard method is not improved as compared with the external standard method. In addition, the

quantitative accuracy is lower than that of the external standard method according to (Math. 4). As described in NPL 2, if the signal intensity is not proportional to the concentration of either the analysis target or the internal standard, the quantitative accuracy by the internal standard method decreases.

[0027]

Although an example in which the signal intensities of the analysis target and the internal standard are represented by the quadratic functions of the respective concentrations has been considered above, the same applies to a case where the signal intensities are represented by any function other than a linear function in which the constant term is zero. As described above, when the signal intensity is not proportional to the concentration of the analysis target, the quantitative accuracy cannot be improved using the internal standard method.

[0028]

On the other hand, PTL 1 proposes to improve a dynamic range of fluorescence measurement of a capillary electrophoresis device by optimizing binning conditions according to noise conditions of an image sensor. According to PTL 1, it is expected that a concentration of a DNA fragment in a sample can be quantified in a wider concentration range than that in the related art.

[0029]

However, as a result of actual trial, it was found that when the concentration of the DNA fragment as an analysis target contained in the sample was low, the signal intensity proportional to the concentration was obtained, and when the concentration of the DNA fragment as an analysis target contained in the sample was high, the signal intensity was saturated relative to the concentration. In addition, since the saturated signal intensity is lower than the saturated signal intensity of the image sensor, it has been found that the saturation of the image sensor is not a cause.

[0030]

This phenomenon is a new problem that has been clarified by using a capillary electrophoresis device having a dynamic range wider than the related art. When the concentration of the DNA fragment as an analysis target contained in the sample is low, the

signal intensity proportional to the concentration of the DNA fragment as an analysis target is obtained, so that the quantitative accuracy of the analysis target can be improved using the internal standard method. However, when the concentration of the DNA fragment as an analysis target contained in the sample is high, a signal intensity not proportional to the concentration of the DNA fragment as an analysis target is obtained, so that it is impossible to improve the quantitative accuracy of the analysis target using the internal standard method.

[0031]

The invention has been made to solve such a problem, and an object of the invention is to provide a capillary electrophoresis device and a capillary electrophoresis method capable of improving quantitative accuracy of an analysis target using an internal standard method.

Solution to Problem

[0032]

An example of a capillary electrophoresis device according to the invention includes a capillary electrophoresis device for injecting a sample containing a first component and a second component into a capillary, separating the injected first component and the injected second component by electrophoresis, and measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, in which

a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the first component is proportional to a concentration of the first component, a concentration range in which the signal intensity of the first component is lower than a saturation signal intensity of the detector and is not proportional to the concentration of the first component and reaches the saturation signal intensity, and

the capillary electrophoresis device quantifies a ratio of the concentration of the first component to a concentration of the second component in the sample based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

[0033]

An example of a capillary electrophoresis device according to the invention includes a capillary electrophoresis device for injecting a sample containing a first component and a second component into a capillary, separating the injected first component and the injected second component by electrophoresis, and measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, in which

a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the second component is constant with respect to a concentration of the first component, a concentration range in which the signal intensity of the second component decreases non-constantly with respect to the concentration of the first component, and

the capillary electrophoresis device quantifies a ratio of the concentration of the first component to a concentration of the second component in the sample based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

[0034]

An example of a capillary electrophoresis method according to the invention includes a capillary electrophoresis method including:

injecting a sample containing a first component and a second component into a capillary;

separating the injected first component and the injected second component by electrophoresis; and

measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, in which

a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the first component is proportional to a concentration of the first component, a concentration range in which the signal

intensity of the first component is lower than a saturation signal intensity of the detector and is not proportional to the concentration of the first component and reaches the saturation signal intensity, and

the capillary electrophoresis method further includes quantifying a ratio of the concentration of the first component to a concentration of the second component in the sample based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

[0035]

An example of a capillary electrophoresis method according to the invention includes a capillary electrophoresis method including:

injecting a sample containing a first component and a second component into a capillary;

separating the injected first component and the injected second component by electrophoresis; and

measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, in which

a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the second component is constant with respect to a concentration of the first component, a concentration range in which the signal intensity of the second component decreases non-constantly with respect to the concentration of the first component, and

the capillary electrophoresis method further includes quantifying a ratio of the concentration of the first component to a concentration of the second component in the sample based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

Advantageous Effects of Invention

[0036]

By using a novel internal standard method found in the invention, DNA fragments contained in a sample and having a wider concentration range than that in the related art can be quantified with high accuracy.

[0037]

Problems, configurations, and effects other than those described above will be clarified by the following description of embodiments.

Brief Description of Drawings

[0038]

[FIG. 1A] FIG. 1A shows a configuration of a capillary electrophoresis device according to an embodiment of the invention.

[FIG. 1B] FIG. 1B shows a configuration of a capillary electrophoresis method according to an embodiment of the invention.

[FIG. 2] FIG. 2 is a schematic diagram of electric field injection.

[FIG. 3] FIG. 3 is an electropherogram of samples of various concentrations.

[FIG. 4] FIG. 4 shows a relation between a concentration of a sample and a fluorescence intensity (part 1).

[FIG. 5] FIG. 5 shows a relation between the concentration of the sample and the fluorescence intensity (part 2).

[FIG. 6] FIG. 6 shows an electropherogram of a sample in which two types of size standards are mixed at different ratios (part 1)

[FIG. 7] FIG. 7 shows the electropherogram of the sample in which two types of size standards are mixed at different ratios (part 2)

[FIG. 8] FIG. 8 shows a relation between a concentration of a sample and fluorescence intensities of two types of DNA fragments and a fluorescence intensity ratio (part 1).

[FIG. 9] FIG. 9 shows a relation between a concentration of a sample and fluorescence intensities of three types of DNA fragments.

[FIG. 10] FIG. 10 shows the relation between the concentration of the sample and fluorescence intensities of two types of DNA fragments and the fluorescence intensity ratio (part

2).

[FIG. 11] FIG. 11 shows a relation between a concentration of a sample and a fluorescence intensity ratio of two types of DNA fragments.

Description of Embodiments

[0039]

[Principles]

In the capillary electrophoresis analysis described above, a cause of a phenomenon was considered in which a signal intensity proportional to a concentration was obtained when a concentration of a DNA fragment as an analysis target contained in a sample was low, and the signal intensity was saturated relative to the concentration when the concentration of the DNA fragment as an analysis target contained in the sample was high.

[0040]

If the saturation of an image sensor is not the cause, another conceivable cause is self-quenching of a fluorophore at a measurement point. In general, it is known that when a concentration of the fluorophore becomes very high, self-quenching of the fluorophore occurs, an increase rate of a fluorescence intensity decreases according to the concentration of the fluorophore, and the fluorescence intensity reaches saturation. It is also known that when the concentration of the fluorophore further increases, the fluorescence intensity may turn to decrease.

[0041]

In order to verify the presence or absence of self-quenching in the capillary electrophoresis analysis described above, a plurality of types of DNA fragments were analysis targets, and samples having different concentrations were analyzed by the capillary electrophoresis device described above. When a concentration of the DNA fragment in the sample was increased, a total concentration of the plurality of types of DNA fragments was increased while maintaining a concentration ratio of the plurality of types of DNA fragments in the sample.

[0042]

The plurality of types of DNA fragments were spatially separated by capillary electrophoresis and detected independently of each other by laser induced fluorescence measurement at the measurement point, and different peaks on an electropherogram were provided.

[0043]

A DNA fragment having a high concentration in the sample had a high intensity peak, and a DNA fragment having a low concentration in the sample had a low intensity peak. When the total concentration of the plurality of types of DNA fragments as analysis targets contained in the sample was low, a signal intensity proportional to the concentration of each DNA fragment was obtained. On the other hand, when the total concentration of the plurality of types of DNA fragments as analysis targets contained in the sample was high, a signal intensity not proportional to the concentration of each DNA fragment was obtained. That is, regardless of the magnitude of the intensity of the peak on the electropherogram corresponding to the concentration in the sample, a change from a state in which "the signal intensity is proportional to the concentration of the DNA fragment in the sample" to a state in which "the signal intensity is not proportional to the concentration of the DNA fragment in the sample" simultaneously occurred.

[0044]

A degree of self-quenching of the fluorophore should depend on the concentration of the fluorophore at the measurement point on the capillary. However, the above change occurs without depending on the concentration of the fluorophore at the measurement point on the capillary. From the above, it was found that the cause of this phenomenon was not self-quenching of the fluorophore. Therefore, in the capillary electrophoresis analysis described above, the concentration of the DNA fragment at the measurement point of the capillary and the concentration of the fluorophore labeled on the DNA fragment do not reach a concentration at which self-quenching occurs. Of course, since this phenomenon does not occur due to the saturation of the image sensor, the cause of this phenomenon is not due to the saturation of the image sensor. Therefore, in the capillary electrophoresis analysis described above, the concentration of the DNA fragment at the measurement point of the capillary and the

concentration of the fluorophore labeled on the DNA fragment do not reach a concentration at which the saturation of the image sensor occurs.

[0045]

Therefore, as described below, the cause of this phenomenon was independently clarified by the study according to the invention. This phenomenon was found for the first time in the study of the invention, and the cause thereof was also found for the first time in the study of the invention. Further, while this phenomenon is allowed, a unique new internal standard method is devised based on the found cause, and highly accurate quantitative analysis of an analysis target is enabled.

[0046]

[Embodiment 1]

FIG. 1A is a configuration diagram of a capillary electrophoresis device. The capillary electrophoresis device is widely used as an analysis device that performs DNA sequencing or DNA fragment analysis. Using four capillaries 1, different samples can be analyzed by each capillary 1.

[0047]

FIG. 1B shows a configuration of a capillary electrophoresis method. One capillary electrophoresis analysis is performed by capillary electrophoresis method including the steps of (1) to (8) in FIG. 1B. The capillary electrophoresis analysis can be performed a plurality of times by repeating the steps of (1) to (8).

[0048]

(1) First, sample-injection ends 2 of four capillaries 1 are immersed in a cathode-side-buffer solution 6, and sample-elution ends 3 are connected to an anode-side-buffer solution 7 via a polymer solution 8 in a pump block 10.

[0049]

(2) Next, when a valve 11 of the pump block 10 is closed and a piston of the syringe 12 connected to the pump block 10 is pushed down, pressure is applied to the polymer solution 8 inside, and the polymer solution 8 is filled into each capillary 1 from the sample-elution end 3 toward the sample-injection end 2.

[0050]

(3) Subsequently, when the valve 11 is opened, the sample-injection ends 2 of the four capillaries 1 are immersed in different samples 9, and a constant voltage is applied to a power supply 13 between a cathode 4 and an anode 5 for a constant time, whereby a part of the different samples 9 (including at least a first component and a second component) is subjected to electric field injection from the sample-injection ends 2 to the respective capillaries 1. Thus, the samples 9 each containing the first component and the second component are injected into the capillaries.

[0051]

(4) Thereafter, the sample-injection ends 2 of the four capillaries 1 are immersed in the cathode-side-buffer solution 6, and a higher voltage is applied to the power supply 13 between the cathode 4 and the anode 5 to start capillary electrophoresis. A DNA fragment labeled with a fluorophore is subjected to electrophoresis from the sample-injection end 2 toward the sample-elution end 3. Thus, the injected first component and second component are separated by electrophoresis.

[0052]

(5) At the same time, a position of each capillary 1 that has been subjected to electrophoresis by a certain distance from the sample-injection end 2 is defined as a measurement point 16, and laser beams 14 emitted from a laser-light source 15 are collectively irradiated onto each measurement point 16. Here, the coating of the capillaries 1 in the vicinity of the measurement points 16 is removed in advance, the capillaries 1 in the vicinity of the measurement points 16 are arranged on the same plane, and the laser beam 14 is narrowed and introduced along an arrangement plane from a side of the arrangement plane.

[0053]

(6) When the DNA fragment labeled with the fluorophore passes through each measurement point 16, the DNA fragment is excited by the irradiation of the laser beam 14 and fluorescence is emitted. That is, the intensities of the fluorescence emitted from the four measurement points 16 change from moment to moment with the electrophoresis.

[0054]

(7) Next, fluorescence emitted from each measurement point 16 is measured by a detector 17. That is, the fluorescence emitted from the first component and the fluorescence emitted from the second component, which are induced by irradiating the capillary 1 with the laser beam, are measured by the detector 17, and a signal intensity of the first component and a signal intensity of the second component are thereby acquired. An electropherogram, which is time-series data of these signal intensities, is acquired, and the samples 9 injected into the capillaries 1 are analyzed. The detector 17 includes a spectroscope and an image sensor (not shown), and can spectrally measure the emitted fluorescence from the four measurement points 16 simultaneously and independently. Therefore, it is possible to identify the emitted fluorescence of a plurality of types of fluorophores.

[0055]

(8) Finally, a ratio of a concentration of the first component to a concentration of the second component in the sample is quantified based on a ratio of the signal intensity of the first component to the signal intensity of the second component. This quantification can be performed by the following method based on the description of the above [Principle].

[0056]

FIG. 2 schematically shows electric field injection of the sample 9 in the capillary electrophoresis analysis. The sample-injection end 2 of one capillary 1 filled with the polymer solution 8 is immersed in the sample 9 in a solution state, and (a) of FIG. 2 shows a state before electric field injection and (b) of FIG. 2 shows a state after electric field injection. After (b) of FIG. 2, the sample-injection end 2 is immersed in the cathode-side-buffer solution to start electrophoresis.

[0057]

The sample 9 contains first DNA fragments 18 (first component) which are negative ions, second DNA fragments 19 (second component), and negative ions 20 other than the DNA fragments. The first DNA fragments 18 and the second DNA fragments 19 as analysis targets are both a DNA fragment labeled with a fluorophore. A base length of the first DNA fragment 18 is different from a base length of the second DNA fragment 19. The first DNA fragment 18 and the second DNA fragment 19 have different concentrations in the sample 9.

[0058]

As in a specific example to be described later, the sample 9 may contain a size standard, and the second DNA fragment 19 may be a DNA fragment contained in the size standard. The sample 9 may contain a PCR product, and the first DNA fragment 18 may be a DNA fragment which is a PCR product or a DNA fragment derived from a PCR product. In addition, the sample 9 may include a single base extension product, the first DNA fragment 18 may be a first DNA fragment which is a single base extension product, and the second DNA fragment 19 may be a second DNA fragment which is a single base extension product.

[0059]

In this example, as the simplest example of the plurality of types of DNA fragments, two types of DNA fragments are mainly handled, and it is needless to say that the same consideration can be made for any number of three or more types of DNA fragments.

[0060]

In FIG. 2, cations and cathodes are omitted. A solvent of the sample 9 is pure water or formamide. In the sample 9, negative ions (salts) other than DNA fragments are removed in advance by ethanol precipitation or the like as much as possible, but the negative ions cannot be reduced to zero.

[0061]

In the state shown in (a) of FIG. 2, the sample-injection end 2 of the capillary 1 is on a cathode side, the sample-elution end 3 is on an anode side, and electric field injection is performed by applying a voltage to both ends of the capillary 1 so that a value obtained by a voltage $\times$ a time becomes constant. For example, a constant voltage may be applied for a certain time. As a result, the state shown in (b) of FIG. 2 is obtained. As shown in (b) of FIG. 2, the first DNA fragments 18, the second DNA fragments 19, which are negative ions in the sample 9, and a part of the negative ions 20 other than the DNA fragments are injected into the capillary 1 from the sample-injection end 2 of the capillary 1. An injection amount of each negative ion at this time is estimated as follows.

[0062]

A current I flowing when a constant voltage V is applied between a cathode and an

anode is substantially determined by an electric resistance R of the capillary 1 filled with the polymer solution 8, and $I \doteq V/R$ is established. This is because an electric resistance $R(i)$ between the cathode 4 (FIG. 1A) and the sample-injection end 2 of the capillary 1 and an electric resistance $R(o)$ between the sample-elution end 3 (FIG. 1A) of the capillary and the anode 5 (FIG. 1A) are sufficiently smaller than R ($R \gg R(i), R(o)$). That is, a combined resistance $R(i) + R + R(o)$ between the cathode 4 and the anode 5 is approximately equal to R ($R(i) + R + R(o) \doteq R$).

[0063]

Therefore, regardless of the composition of the sample, for example, regardless of whether the sample is pure water or a high ion concentration solution, the current I flowing when the constant voltage V is applied between the cathode and the anode hardly changes. Therefore, "applying a constant voltage between the cathode and the anode" may be expressed as "applying a constant voltage to both ends of a capillary".

[0064]

The current I flowing between the cathode and the sample-injection end of the capillary, the current I flowing in the capillary, and the current I flowing between the sample-elution end of the capillary and the anode are equal to each other in terms of current continuity.

[0065]

Here, the current I flowing between the cathode and the sample-injection end of the capillary at the time of electric field injection, that is, the current I flowing in the sample is carried by negative ions injected into the capillary if positive ions are ignored for simplicity. Therefore, a total amount of negative ions injected into the capillary by electric field injection by applying a constant voltage to both ends of the capillary for a constant time is constant regardless of the composition of the sample.

[0066]

An effective electric field intensity in the sample near the sample-injection end of the capillary at the time of the electric field injection is denoted by E , an electric field injection time is denoted by T , and an inner cross-sectional area of the capillary is denoted by A . When the mobility of negative ions other than DNA fragments in the sample is denoted by $\mu(0)$ and the

concentration is denoted by $C(0)$, the number of injected molecules $J(0)$ of negative ions other than DNA fragments by electric field injection is represented by (Math. 6).

[Math. 6]

$$J_0 = E \cdot T \cdot A \cdot \mu_0 \cdot C_0 \quad (\text{Math. 6})$$

The mobility indicates a moving speed of each negative ion per unit electric field intensity.

[0067]

When a plurality of types of negative ions other than DNA fragments are present, the averages of the mobility and concentration thereof are denoted by $\mu(0)$ and $C(0)$. When an average charge amount per molecule of negative ions other than DNA fragments is denoted by $q(0)$, an injection charge amount $Q(0)$ of negative ions other than DNA fragments by electric field injection is represented by (Math. 7).

[Math. 7]

$$Q_0 = q_0 \cdot J_0 = E \cdot T \cdot A \cdot q_0 \cdot \mu_0 \cdot C_0 \quad (\text{Math. 7})$$

[0068]

When the mobility of the first DNA fragments and the second DNA fragments in the sample is μ , the concentration of the first DNA fragments is $C(1)$, and the concentration of the second DNA fragments is $C(2)$, the number of injected molecules $J(1)$ of the first DNA fragments and the number of injected molecules $J(2)$ of the second DNA fragments by electric field injection are represented by (Math. 8) and (Math. 9), respectively.

[Math. 8]

$$J_1 = E \cdot T \cdot A \cdot \mu \cdot C_1 \quad (\text{Math. 8})$$

[Math. 9]

$$J_2 = E \cdot T \cdot A \cdot \mu \cdot C_2 \quad (\text{Math. 9})$$

[0069]

Here, the reason why the first DNA fragments and the second DNA fragments have the same mobility is that the mobility of the DNA fragments in a solution having no molecular sieve

effect, that is, in a sample is constant regardless of the base length.

[0070]

When average charge amounts per molecule of the first DNA fragments and the second DNA fragments are denoted by $q(1)$ and $q(2)$, respectively, injection charge amounts $Q(1)$ and $Q(2)$ of the first DNA fragments and the second DNA fragments by electric field injection are represented by (Math. 10) and (Math. 11), respectively.

[Math. 10]

$$Q_1 = q_1 \cdot J_1 = E \cdot T \cdot A \cdot q_1 \cdot \mu \cdot C_1 \quad (\text{Math. 10})$$

[Math. 11]

$$Q_2 = q_2 \cdot J_2 = E \cdot T \cdot A \cdot q_2 \cdot \mu \cdot C_2 \quad (\text{Math. 11})$$

[0071]

When a total concentration of the first DNA fragments and the second DNA fragments in the sample is represented by $C = C(1) + C(2)$, the total number of injected molecules J of the first DNA fragments and the second DNA fragments by electric field injection is represented by (Math. 12).

[Math. 12]

$$J = J_1 + J_2 = E \cdot T \cdot A \cdot \mu \cdot C \quad (\text{Math. 12})$$

[0072]

Here, when the average charge amounts per molecule of the first DNA fragment and the second DNA fragment are equal to each other and an approximation of $q = q(1) = q(2)$ can be established, a total injection charge amount Q of the first DNA fragment and the second DNA fragment by the electric field injection is represented by (Math. 13).

[Math. 13]

$$Q = q \cdot J = E \cdot T \cdot A \cdot q \cdot \mu \cdot C \quad (\text{Math. 13})$$

Alternatively, when the ratios of the concentrations $C(1)$ and $C(2)$ of the first DNA fragment and the second DNA fragment to the total concentration C of the first DNA fragment and the

second DNA fragment in the sample are constant, (Math. 13) can also be obtained by defining $q = (q(1) \cdot C(1) + q(2) \cdot C(2)) / (C(1) + C(2))$.

[0073]

In (Math. 6) to (Math. 13), E , T , and A are the same value. Since a total amount of injection charge amount of negative ions per unit time at the time of the electric field injection is equal to the current I , (Math. 14), that is, (Math. 15) is established.

[Math. 14]

$$\frac{Q_0 + Q_1 + Q_2}{T} = I \quad (\text{Math. 14})$$

[Math. 15]

$$E \cdot A = \frac{I}{q_0 \cdot \mu_0 \cdot C_0 + q_1 \cdot \mu \cdot C_1 + q_2 \cdot \mu \cdot C_2} \quad (\text{Math. 15})$$

[0074]

(Math. 14) and (Math. 15) mean that, since I is constant as described above, electric field injection is a competitive process between a plurality of types of negative ions including DNA fragments contained in a sample, and an injection charge amount of each type of negative ions is distributed according to the product of charge amount, mobility, and concentration of each type of negative ions. When the contribution of the positive ions to the current is considered, the contribution ratio of the negative ions to the current may be shifted to the right side of (Math. 14).

[0075]

Using (Math. 15), (Math. 8) to (Math. 11) can be modified as follows.

[Math. 16]

$$J_1 = \frac{\frac{I \cdot T}{q_1}}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0 + q_2 \cdot \mu \cdot C_2}{q_1 \cdot \mu \cdot C_1}} \quad (\text{Math. 16})$$

[Math. 17]

$$J_2 = \frac{\frac{I \cdot T}{q_2}}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0 + q_1 \cdot \mu \cdot C_1}{q_2 \cdot \mu \cdot C_2}} \quad (\text{Math. 17})$$

[Math. 18]

$$Q_1 = \frac{I \cdot T}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0 + q_2 \cdot \mu \cdot C_2}{q_1 \cdot \mu \cdot C_1}} \quad (\text{Math. 18})$$

[Math. 19]

$$Q_2 = \frac{I \cdot T}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0 + q_1 \cdot \mu \cdot C_1}{q_2 \cdot \mu \cdot C_2}} \quad (\text{Math. 19})$$

[0076]

Assuming that an approximation of $q = q(1) = q(2)$ can be established as described above, (Math. 12) and (Math. 13) can be modified as follows.

[Math. 20]

$$J = \frac{\frac{I \cdot T}{q}}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0}{q \cdot \mu \cdot C}} \quad (\text{Math. 20})$$

[Math. 21]

$$Q = \frac{I \cdot T}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0}{q \cdot \mu \cdot C}} \quad (\text{Math. 21})$$

[0077]

Alternatively, when the ratios of the concentrations $C(1)$ and $C(2)$ of the first DNA fragment and the second DNA fragment to the total concentration C of the first DNA fragment

and the second DNA fragment in the sample are constant, (Math. 20) and (Math. 21) can also be obtained by defining $q = (q(1) \cdot C(1) + q(2) \cdot C(2))/(C(1) + C(2))$.

[0078]

On the other hand, the respective sensitivity coefficients of the signal intensities $S(1)$ and $S(2)$ of the peaks of the first DNA fragment and the second DNA fragment on an electropherogram obtained by capillary electrophoresis are denoted by $m(1)$ and $m(2)$, (Math. 22) and (Math. 23) are established from (Math. 8) and (Math. 9), and (Math. 24) and (Math. 25) are established when (Math. 15) is used.

[Math. 22]

$$S_1 = m_1 \cdot J_1 = E \cdot T \cdot A \cdot m_1 \cdot \mu \cdot C_1 \quad (\text{Math. 22})$$

[Math. 23]

$$S_2 = m_2 \cdot J_2 = E \cdot T \cdot A \cdot m_2 \cdot \mu \cdot C_2 \quad (\text{Math. 23})$$

[Math. 24]

$$S_1 = \frac{\frac{m_1 \cdot I \cdot T}{q_1}}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0 + q_2 \cdot \mu \cdot C_2}{q_1 \cdot \mu \cdot C_1}} \quad (\text{Math. 24})$$

[Math. 25]

$$S_2 = \frac{\frac{m_2 \cdot I \cdot T}{q_2}}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0 + q_1 \cdot \mu \cdot C_1}{q_2 \cdot \mu \cdot C_2}} \quad (\text{Math. 25})$$

The sensitivity coefficient includes an average number of labeled fluorophores per molecule of the DNA fragment, excitation efficiency of the fluorophore, a quantum yield of the fluorophore, a condensing efficiency of the emitted fluorescence, the sensitivity of an image sensor, and the like.

[0079]

When the sensitivity coefficients of the first DNA fragment and the second DNA fragment can be approximated as $m(1)$ and $m(2)$, respectively, assuming that $m = m(1) = m(2)$, and a total signal intensity S of the peaks of the first DNA fragment and the second DNA fragment on the electropherogram obtained by capillary electrophoresis is represented by (Math. 26) from (Math. 12).

[Math. 26]

$$S = m \cdot J = E \cdot T \cdot A \cdot m \cdot \mu \cdot C \quad (\text{Math. 26})$$

Alternatively, when the ratios of the concentrations $C(1)$ and $C(2)$ of the first DNA fragment and the second DNA fragment to the total concentration C of the first DNA fragment and the second DNA fragment in the sample are constant, (Math. 26) can also be obtained by defining $m = (m(1) \cdot C(1) + m(2) \cdot C(2))/(C(1) + C(2))$.

[0080]

Assuming that an approximation of $q = q(1) = q(2)$ is established using (Math. 15), (Math. 27) is established.

[Math. 27]

$$S = \frac{\frac{m \cdot I \cdot T}{q}}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0}{q \cdot \mu \cdot C}} \quad (\text{Math. 27})$$

Alternatively, when the ratios of the concentrations $C(1)$ and $C(2)$ of the first DNA fragment and the second DNA fragment to the total concentration C of the first DNA fragment and the second DNA fragment in the sample are constant, (Math. 27) can also be obtained by defining $q = (q(1) \cdot C(1) + q(2) \cdot C(2))/(C(1) + C(2))$.

[0081]

(Math. 16) to (Math. 21), (Math. 24), (Math. 25), and (Math. 27) can be expressed by the following expressions in which y is a function of x with a and b as constants.

[Math. 28]

$$y = \frac{a}{1 + \frac{b}{x}} \quad (\text{Math. 28})$$

[0082]

For example, when $y = J(1)$, $x = C(1)$, $a = I \cdot T/q(1)$, and $b = (q(0) \cdot \mu(0) \cdot C(0) + q(2) \cdot \mu \cdot C(2))/(q(1) \cdot \mu)$, (Math. 16) becomes (Math. 28). (Math. 28) indicates that a proportionality coefficient is a/b and y is proportional to x ($y = a/b \times x$) when x is smaller than b , and y is saturated relative to x and approaches a constant value a when x is larger than b .

[0083]

That is, (Math. 16) indicates that $J(1)$ is proportional to $C(1)$ when $C(1)$ is smaller than $b = (q(0) \cdot \mu(0) \cdot C(0) + q(2) \cdot \mu \cdot C(2))/(q(1) \cdot \mu)$, and $J(1)$ is saturated relative to $C(1)$ and gradually approaches a constant value when $C(1)$ is larger than $b = (q(0) \cdot \mu(0) \cdot C(0) + q(2) \cdot \mu \cdot C(2))/(q(1) \cdot \mu)$.

[0084]

On the other hand, since (Math. 8) indicates a relation in which $J(1)$ is proportional to $C(1)$, (Math. 8) and (Math. 16) appear to be contradictory. However, in fact, since E included in (Math. 8) changes according to $C(1)$, $J(1)$ in (Math. 8) is not necessarily proportional to $C(1)$. Therefore, (Math. 8) and (Math. 16) are compatible with each other without contradiction.

[0085]

Specifically, since I is constant in (Math. 15), E decreases when $C(1)$ increases in a state where $C(0)$ and $C(2)$ are constant. E is an effective electric field intensity in a sample near a sample-injection end of a capillary at the time of electric field injection, and E can change according to the composition of the sample even if a voltage applied to both ends of the capillary is constant, that is, an average electric field intensity is constant.

[0086]

Similarly, (Math. 17) indicates that $J(2)$ is proportional to $C(2)$ when $C(2)$ is small, and $J(2)$ is saturated relative to $C(2)$ and gradually approaches a constant value when $C(2)$ is large. (Math. 18) indicates that $Q(1)$ is proportional to $C(1)$ when $C(1)$ is small, and $Q(1)$ is saturated relative to $C(1)$ and gradually approaches a constant value when $C(1)$ is large. (Math. 19)

indicates that $Q(2)$ is proportional to $C(2)$ when $C(2)$ is small, and $Q(2)$ is saturated relative to $C(2)$ and gradually approaches a constant value when $C(2)$ is large. (Math. 20) indicates that J is proportional to C when C is small, and J is saturated relative to C and approaches a constant value when C is large. (Math. 21) indicates that Q is proportional to C when C is small, and Q is saturated with C and approaches a constant value when C is large. (Math. 24) indicates that $S(1)$ is proportional to $C(1)$ when $C(1)$ is small, and $S(1)$ is saturated relative to $C(1)$ and gradually approaches a constant value when $C(1)$ is large. (Math. 25) indicates that $S(2)$ is proportional to $C(2)$ when $C(2)$ is small, and $S(2)$ is saturated relative to $C(2)$ and gradually approaches a constant value when $C(2)$ is large. (Math. 27) indicates that S is proportional to C when C is small, and S is saturated relative to C and gradually approaches a constant value when C is large.

[0087]

Therefore, (Math. 24), (Math. 25), and (Math. 27) describe a phenomenon in which, in the capillary electrophoresis analysis, a signal intensity proportional to a concentration is obtained when a concentration of a DNA fragment as an analysis target contained in a sample is low, and the signal intensity is saturated relative to the concentration when the concentration of the DNA fragment as an analysis target contained in the sample is high. That is, the cause of the above phenomenon could be identified. This is a finding obtained for the first time by introducing (Math. 14) and (Math. 15).

[0088]

Similarly to a case where (Math. 8) and (Math. 16) are not contradict with each other, (Math. 9) and (Math. 17)), (Math. 10) and (Math. 18), (Math. 11) and (Math. 19), (Math. 12) and (Math. 20), (Math. 13) and (Math. 21), (Math. 22) and (Math. 24), (Math. 23) and (Math. 25), and (Math. 26) and (Math. 27) are compatible with each other without contradiction.

[0089]

On the other hand, since E , T , A , and μ are common from (Math. 8) and (Math. 9), (Math. 29) is established.

[Math. 29]

$$\frac{J_1}{J_2} = \frac{C_1}{C_2} \quad (\text{Math. 29})$$

That is, a molecular number ratio of the first DNA fragment and the second DNA fragment injected into the capillary by the electric field injection is equal to a ratio of the concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample. (Math. 30) and (Math. 31) are established from (Math. 8), (Math. 9), and (Math. 12).

[Math. 30]

$$\frac{J_1}{J} = \frac{C_1}{C} \quad (\text{Math. 30})$$

[Math. 31]

$$\frac{J_2}{J} = \frac{C_2}{C} \quad (\text{Math. 31})$$

That is, a ratio of the number of molecules of the first DNA fragment or the second DNA fragment to a total number of molecules of the first DNA fragment and the second DNA fragment injected into the capillary by the electric field injection coincides with a ratio of the concentration of the first DNA fragment or the second DNA fragment to the total concentration of the first DNA fragment and the second DNA fragment in the sample.

[0090]

In addition, since E, T, A, and μ are common from (Math. 10) and (Math. 11), (Math. 32) is established.

[Math. 32]

$$\frac{Q_1}{Q_2} = \frac{q_1 \cdot C_1}{q_2 \cdot C_2} \quad (\text{Math. 32})$$

That is, a charge amount ratio of the first DNA fragment to the second DNA fragment that are injected into the capillary by the electric field injection is proportional to the ratio of the

concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample.

[0091]

Assuming that $q = q(1) = q(2)$, (Math. 33) and (Math. 34) are established from (Math. 10), (Math. 11), and (Math. 13).

[Math. 33]

$$\frac{Q_1}{Q} = \frac{C_1}{C} \quad (\text{Math. 33})$$

[Math. 34]

$$\frac{Q_2}{Q} = \frac{C_2}{C} \quad (\text{Math. 34})$$

That is, a ratio of an injection charge amount of the first DNA fragment or the second DNA fragment to a total injection charge amount of the first DNA fragment and the second DNA fragment injected into the capillary by the electric field injection coincides with a ratio of the concentration of the first DNA fragment or the second DNA fragment to a total concentration of the first DNA fragment and the second DNA fragment in the sample.

[0092]

Further, (Math. 35) is established from (Math. 22) and (Math. 23).

[Math. 35]

$$\frac{S_1}{S_2} = \frac{m_1 \cdot C_1}{m_2 \cdot C_2} \quad (\text{Math. 35})$$

That is, a signal intensity ratio of the first DNA fragment and the second DNA fragment obtained by capillary electrophoresis analysis of the first DNA fragment and the second DNA fragment injected into the capillary by the electric field injection is proportional to the ratio of the concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample.

[0093]

Therefore, the capillary electrophoresis device in FIG. 1A can quantify the ratio of the concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample based on a ratio of the signal intensity of the first DNA fragment to the signal intensity of the second DNA fragment.

[0094]

In particular, when the concentration of the second DNA fragment in the sample is known, the concentration of the first DNA fragment in the sample can be quantified based on the ratio of the signal intensity of the first DNA fragment to the signal intensity of the second DNA fragment.

[0095]

Assuming that $m = m(1) = m(2)$, (Math. 36) and (Math. 37) are established from (Math. 22), (Math. 23), and (Math. 26).

[Math. 36]

$$\frac{S_1}{S} = \frac{C_1}{C} \quad (\text{Math. 36})$$

[Math. 37]

$$\frac{S_2}{S} = \frac{C_2}{C} \quad (\text{Math. 37})$$

That is, a ratio of the signal intensity of the first DNA fragment or the second DNA fragment to a total signal intensity of the first DNA fragment and the second DNA fragment injected into the capillary by the electric field injection coincides with a ratio of the concentration of the first DNA fragment or the second DNA fragment to the total concentration of the first DNA fragment and the second DNA fragment in the sample.

[0096]

(Math. 16), (Math. 17), and (Math. 20) are compatible with (Math. 29), (Math. 30), and (Math. 31) without contradiction. That is, regardless of whether the number of injected

molecules of the first DNA fragment, the second DNA fragment, or the entire DNA fragment is proportional to the concentration of the first DNA fragment, the second DNA fragment, or the entire DNA fragment in the sample, a ratio of the number of injected molecules of the first DNA fragment to the number of injected molecules of the second DNA fragment coincides with the ratio of the concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample, and a ratio of the number of injected molecules of the first DNA fragment or the second DNA fragment to the entire DNA fragment coincides with a ratio of the concentration of the first DNA fragment or the second DNA fragment to the concentration of the entire DNA fragment in the sample.

[0097]

Further, (Math. 18), (Math. 19), and (Math. 21) are compatible with (Math. 32), (Math. 33), and (Math. 34) without contradiction. That is, regardless of whether the injection charge amount of the first DNA fragment, the second DNA fragment, or the entire DNA fragments is proportional to the concentration of the first DNA fragment, the second DNA fragment, or the entire DNA fragment in the sample, an injection charge amount ratio of the first DNA fragment to the second DNA fragment is proportional to the ratio of the concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample, and an injection charge amount ratio of the first DNA fragment or the second DNA fragment to the entire DNA fragment coincides with the ratio of the concentration of the first DNA fragment or the concentration of the second DNA fragment to the concentration of the entire DNA fragment in the sample.

[0098]

Further, (Math. 24), (Math. 25), and (Math. 27) are compatible with (Math. 35), (Math. 36), and (Math. 37) without contradiction. That is, regardless of whether the signal intensity of the first DNA fragment, the second DNA fragment, or the entire DNA fragment is proportional to the concentration of the first DNA fragment, the second DNA fragment, or the entire DNA fragment in the sample, a ratio of the signal intensity of the first DNA fragment to the signal intensity of the second DNA fragment is proportional to the ratio of the concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample, and a ratio of the signal intensity of the first DNA fragment or the second DNA fragment to the

signal intensity of the entire DNA fragment coincides with the ratio of the concentration of the first DNA fragment or the second DNA fragment to the concentration of the entire DNA fragment in the sample.

[0099]

When the second DNA fragment is used as an internal standard, (Math. 35) shows a calibration curve having the same form as (Math. 3), and it is possible to avoid an influence of fluctuations in the electric field intensity E and the time T of the electric field injection, and to quantify the first DNA fragment as an analysis target with high accuracy. Therefore, in the same manner as (Math. 3), which shows the calibration curve of the internal standard method in the related art, by defining (Math. 35) as the calibration curve of the novel internal standard method, the DNA fragment as an analysis target can be quantified with high accuracy.

[0100]

The internal standard method in the related art is applicable only to a case where a signal intensity is proportional to a concentration of an analysis target. On the other hand, the novel internal standard method can be applied not only to a case where the signal intensity is proportional to the concentration of the analysis target as shown in (Math. 24), (Math. 25), and (Math. 27), but also to a case where the signal intensity is not proportional to the concentration of the analysis target, a case where the signal intensity is close to saturation, and a case where the signal intensity reaches saturation.

[0101]

[Specific Example of Measurement Result]

In the capillary electrophoresis analysis described above, a specific example of a phenomenon is shown in which a signal intensity proportional to a concentration is obtained when a concentration of a DNA fragment as an analysis target contained in a sample is low, and the signal intensity is saturated relative to the concentration when the concentration of the DNA fragment as an analysis target contained in the sample is high.

[0102]

A human genome of a specific individual was used as a template to perform STR-PCR for DNA type checking, desalted, and formamide was used as a solvent. Four types of samples

were prepared in which concentrations of a plurality of types of DNA fragments, which are STR-PCR products, were changed over concentration ranges of 4 digits, such as 1 time, 0.05 times, 0.002 times, 0.0001 times, with respect to a reference concentration.

[0103]

(a), (b), (c), and (d) of FIG. 3 show parts of electropherograms obtained by electrophoresis analysis of samples having concentrations of 0.0001 times, 0.002 times, 0.05 times, and 1 time, respectively, with four capillaries using the capillary electrophoresis device in FIG. 1A.

[0104]

Each peak on the electropherogram indicates a signal of a DNA fragment, which is a STR-PCR product of each locus. For example, in each of (a), (b), (c), and (d) of FIG. 3, a single peak observed at an electrophoresis time of about 4200 frames indicates a signal of a DNA fragment, which is a STR-PCR product of locus D5S818. When the concentration of the DNA fragment contained in the sample is increased, the fluorescence intensity (expressed as a signal intensity, the same applies hereinafter) of each DNA fragment is increased. Since the fluorescence intensities measured when the fluorescence having the same fluorescence intensity is generated at the measurement points of the four capillaries are adjusted to be equal to each other, a difference in the measured fluorescence intensity accurately reflects a difference in the fluorescence intensity emitted at the measurement point.

[0105]

FIG. 4 is a double logarithmic graph obtained by plotting a fluorescence intensity of a peak of the locus D5S818 against the concentration of the DNA fragment contained in the sample, with the DNA fragment which is the STR-PCR product of the locus D5S818 in FIG. 3 as an analysis target. Here, since the width of the peak can be regarded as equivalent in (a), (b), (c), and (d) of FIG. 3, the fluorescence intensity is not an area of the peak but a height of the peak. A concentration of the DNA fragment on a horizontal axis is a concentration of the DNA fragment, which is the STR-PCR product of locus D5S818 contained in the sample, but may be considered as a total concentration of the DNA fragments contained in the sample.

[0106]

When the concentration of the DNA fragment as an analysis target contained in the sample is low, the fluorescence intensity of the DNA fragment as an analysis target is proportional to the concentration of the DNA fragment as an analysis target contained in the sample. When the concentration of the DNA fragment as an analysis target contained in the sample is high, the fluorescence intensity of the DNA fragment as an analysis target is not proportional to the concentration of the DNA fragment as an analysis target contained in the sample and approaches saturation.

[0107]

If $y = S(1)$, $x = C(1)$, $a = m(1) \cdot I \cdot T/q(1)$, and $b = (q(0) \cdot \mu(0) \cdot C(0) + q(2) \cdot \mu \cdot C(2))/(q(1) \cdot \mu)$, (Math. 24) becomes (Math. 28). In (Math. 28), the fluorescence intensity of the DNA fragment as an analysis target is denoted by $S(1)$, and the concentration of the DNA fragment as an analysis target contained in the sample is denoted by $C(1)$.

[0108]

The broken line in FIG. 4 indicates (Math. 28) a relation between $S(1)$ and $C(1)$ when $a = 3500$ and $b = 0.17$, and is a good approximation curve with respect to plots of four points in FIG. 4, that is, regardless of whether $C(1)$ is low or high. That is, as shown in (Math. 28), when $C(1)$ is low, $S(1)$ is proportional to $C(1)$, and when $C(1)$ is high, $S(1)$ is not proportional to $C(1)$ and is close to saturation.

[0109]

On the other hand, the solid line in FIG. 4 indicates a relation in which $S(1) = a/b \cdot C(1) \approx 20000 \cdot C(1)$, that is, $S(1)$ is proportional to $C(1)$, and is a good approximation line with respect to plots of two points in the left side of FIG. 4, that is, when $C(1)$ is low. From this result, it can be seen that $S(1)$ and $C(1)$ deviate from a proportional relation to a non-proportional relation when $C(1)$ is about 0.01 times.

[0110]

Therefore, according to the internal standard method in the related art, it is possible to quantify $C(1)$ in a concentration range of 0.0001 times to 0.01 times, that is, in a concentration range of a two-digit dynamic range. On the other hand, according to the novel internal standard method, it is possible to quantify $C(1)$ in a concentration range of 0.0001 times to 1 time, that

is, in a concentration range of a four-digit dynamic range.

[0111]

As described above, by using a novel internal standard method according to the present disclosure, DNA fragments contained in a sample and having a wider concentration range than that in the related art can be quantified with high accuracy.

[0112]

In the above, the analysis is performed focusing on a peak of the locus D5S818 shown in FIG. 3, the same relation is established for other peaks. That is, as the total concentration of the DNA fragments contained in the sample increases, the peak of D5S818 deviates from the proportional relation and other peaks deviate from the proportional relation. In each electropherogram, although the fluorescence intensity of each peak is different, such a synchronized phenomenon is not caused by saturation of the detector or self-quenching of the fluorophore.

[0113]

From the above, the phenomena observed in FIGS. 3 and 4 are clearly explained and understood by the cause and theory considered in the above [Principle].

[0114]

FIG. 5 is a double logarithmic graph obtained by plotting a change in fluorescence intensity of a DNA fragment as an analysis target with respect to a change in concentration of the DNA fragment as an analysis target contained in a sample different from samples in FIGS. 3 and 4.

[0115]

Five types of samples were prepared in which the concentration of the DNA fragment as an analysis target contained in the sample was changed over a concentration range of 4 digits, such as 100 times, 10 times, 1 time, 0.01 times of a reference concentration. However, the reference concentration in FIG. 5 and the reference concentration in FIGS. 3 and 4 are irrelevant.

[0116]

Using the capillary electrophoresis device shown in FIG. 1A, five types of samples were divided into two groups and subjected to electrophoresis analysis. Similarly to FIG. 4,

when the concentration of the DNA fragments contained in the sample is low, the fluorescence intensity of the DNA fragment is proportional to the concentration of the DNA fragment contained in the sample, and when the concentration of the DNA fragments contained in the sample is high, the fluorescence intensity of the DNA fragment is not proportional to the concentration of the DNA fragment contained in the sample and is close to saturation.

[0117]

Similarly to the above, if $y = S(1)$, $x = C(1)$, $a = m(1) \cdot I \cdot T/q(1)$, and $b = (q(0) \cdot \mu(0) \cdot C(0) + q(2) \cdot \mu \cdot C(2))/(q(1) \cdot \mu)$, (Math. 24) becomes (Math. 28). In (Math. 28), the fluorescence intensity of the DNA fragment as an analysis target is denoted by $S(1)$, and the concentration of the DNA fragment as an analysis target contained in the sample is denoted by $C(1)$.

[0118]

The broken line in FIG. 5 indicates (Math. 28) a relation between $S(1)$ and $C(1)$ when $a = 22000$ and $b = 10$, and is a good approximation curve with respect to a plot of five points in FIG. 5, that is, regardless of whether $C(1)$ is low or high. That is, as shown in (Math. 28), when $C(1)$ is low, $S(1)$ is proportional to $C(1)$, and when $C(1)$ is high, $S(1)$ is not proportional to $C(1)$ and is close to saturation.

[0119]

On the other hand, the solid line in FIG. 5 indicates a relation in which $S(1) = a/b \cdot C(1) \approx 2180 \cdot C(1)$, that is, $S(1)$ is proportional to $C(1)$, and is a good approximation line with respect to plots of three points in the left side of FIG. 5, that is, when $C(1)$ is low. From this result, it can be seen that $S(1)$ and $C(1)$ deviate from a proportional relation to a non-proportional relation when $C(1)$ is about 1 time.

[0120]

Therefore, according to the internal standard method in the related art, it is possible to quantify $C(1)$ in a concentration range of 0.01 times to 1 time, that is, in a concentration range of a two-digit dynamic range. On the other hand, according to the novel internal standard method, it is possible to quantify $C(1)$ in a concentration range of 0.01 times to 100 times, that is, in a concentration range of a four-digit dynamic range.

[0121]

As described above, by using the novel internal standard method according to the present disclosure, DNA fragments contained in a sample and having a wider concentration range than that in the related art can be quantified with high accuracy. From the above, the phenomenon observed in FIG. 5 is clearly explained and understood by the cause and theory considered in the above [Principle].

[0122]

[Embodiment 1]

The following measurement was performed using the capillary electrophoresis device shown in FIG. 1A. Hereinafter, the symbol "TM" represents a trademark. Four types of samples were prepared by mixing two types of size standards: GeneScanTM 600 LIZTM dye Size Standard (hereinafter, 600 LIZ) and GeneScanTM 500 ROXTM dye Size Standard (hereinafter, 500 ROX) at a specific ratio using Hi-DiTM Formamide (hereinafter, formamide) manufactured by Thermo Fisher Scientific as a solvent, and analyzed using the capillary electrophoresis device in FIG. 1A. Each of the four capillaries had an inner diameter of 50 μm , a total length of 47 cm, and an effective length of 36 cm. Applied BiosystemsTM 310 and 31xx Running Buffer manufactured by Thermo Fisher Scientific were diluted 10 times with pure water to use as the cathode-side-buffer solution and the anode-side-buffer solution. As a polymer solution, POP-4TM Polymer for 3500/SeqStudioTM Flex manufactured by Thermo Fisher Scientific was used.

[0123]

In addition, while concentrations of 600 LIZ contained in four types of samples were changed to 1/2 times (0.5 times), 1/20 times (0.05 times), 1/200 times (0.005 times), and 1/2000 times (0.0005 times) of the reference concentration, concentrations of 500 ROX contained in the four types of samples were constant to 1/200 times (0.005 times) of the reference concentration. However, the reference concentration of 600 LIZ and the reference concentration of 500 ROX are irrelevant. Here, 600 LIZ and 500 ROX each contained a plurality of types of DNA fragments, but the total concentration was changed while maintaining a ratio of concentrations thereof. The electric field injection of each sample was performed by applying a voltage of 1.2 kV to both ends of each capillary for 9 seconds. Electrophoresis was

performed by applying a voltage of 8.5 kV to both ends of each capillary.

[0124]

(a) of FIG. 6 shows an electropherogram of a sample in which a concentration of 600 LIZ is 1/2000 times and a concentration of 500 ROX is 1/200 times. (b) of FIG. 6 shows an electropherogram of a sample in which the concentration of 600 LIZ is 1/200 times and the concentration of 500 ROX is 1/200 times. (a) of FIG. 7 shows an electropherogram of a sample in which the concentration of 600 LIZ is 1/20 times and the concentration of 500 ROX is 1/200 times. (b) of FIG. 7 shows an electropherogram of a sample in which the concentration of 600 LIZ is 1/2 times and the concentration of 500 ROX is 1/200 times. In each graph, the solid line indicates the fluorescence intensity of 600 LIZ, and the dotted line indicates the fluorescence intensity of 500 ROX. A horizontal axis represents an electrophoresis time, a left vertical axis represents a fluorescence intensity of 500 ROX, and a right vertical axis represents a fluorescence intensity of 600 LIZ.

[0125]

600 LIZ contains 36 types of single-stranded DNA fragments having base lengths of 20, 40, 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, 250, 260, 280, 300, 314, 320, 340, 360, 380, 400, 414, 420, 440, 460, 480, 500, 514, 520, 540, 560, 580, and 600, each of which is labeled with a fluorophore LIZ. The electropherograms shown in FIGS. 6 and 7 show the peaks of 15 types of DNA fragments having base lengths of 20, 40, 60, 80, 100, 114, 120, 140, 160, 180, 200, 214, 220, 240, and 250. Furthermore, the peaks of DNA fragments having base lengths of 40, 114, and 160 are indicated by arrows and are denoted by LIZ 40, LIZ 114, and LIZ 160, respectively.

[0126]

500 ROX contains 16 types of single-stranded DNA fragments having base lengths of 35, 50, 75, 100, 139, 150, 160, 200, 250, 300, 340, 350, 400, 450, 490, and 500, each of which is labeled with a fluorophore ROX. The electropherograms shown in FIGS. 6 and 7 show the peaks of 9 types of DNA fragments having base lengths of 35, 50, 75, 100, 139, 150, 160, 200, and 250. Further, a peak of a DNA fragment having a base length of 160 is indicated by an arrow and is denoted by ROX 160.

[0127]

The reason why the peaks of DNA fragments having the same base length, such as a peak of LIZ 160 and a peak of ROX 160, are observed at slightly different times is due to a difference in mobility between the labeled fluorophores LIZ and ROX.

[0128]

(a) of FIG. 8 is a double logarithmic graph in which the fluorescence intensities of the peak of LIZ 160 and the peak of ROX 160 obtained from the four electropherograms in FIGS. 6 and 7 are plotted with respect to the concentration of 600 LIZ contained in the sample. The horizontal axis may be considered as a concentration of LIZ 160 contained in the sample. Here, since the widths of the peaks can be regarded as substantially equal, a height of each of the peaks were defined as a fluorescence intensity. A black circle plot indicates a fluorescence intensity of LIZ 160, and a white circle plot indicates a fluorescence intensity of ROX 160.

[0129]

When the concentration of 600 LIZ contained in the sample is low, specifically, when the concentration of 600 LIZ is in a range of $1/2000$ times to $1/20$ times, the fluorescence intensity of LIZ 600 can be proportional to the concentration of 600 LIZ contained in the sample, and can be approximated by a straight line having a slope of 1. On the other hand, when the concentration of 600 LIZ contained in the sample is high, specifically, when the concentration of 600 LIZ is in a range of $1/20$ times to $1/2$ times, the fluorescence intensity of LIZ 160 is not proportional to the concentration of 600 LIZ contained in the sample and is close to saturation. This phenomenon is the same as the phenomenon observed in FIGS. 4 and 5.

[0130]

As described above, a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the first component is proportional to the concentration of the first component, a concentration range in which the signal intensity of the first component is lower than a saturation signal intensity of the detector and is not proportional to the concentration of the first component and is close to the saturation signal intensity. The capillary electrophoresis device according to the present embodiment can also measure the concentration of such a sample.

[0131]

On the other hand, when the concentration of 600 LIZ contained in the sample is low, specifically, when the concentration of 600 LIZ is in a range of $1/2000$ times to $1/200$ times, the fluorescence intensity of ROX 160 is constant with respect to the concentration of 600 LIZ contained in the sample. Since the concentration of ROX 160 contained in the sample is constant, the above can be easily understood. However, when the concentration of 600 LIZ contained in the sample is high, specifically, when the concentration of 600 LIZ is in a range of $1/200$ times to $1/2$ times, the fluorescence intensity of ROX 160 decreases with respect to the concentration of 600 LIZ contained in the sample. Since the concentration of ROX 160 contained in the sample is constant, such a change cannot be easily understood. The above is the new phenomenon found in the present disclosure.

[0132]

As described above, a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the second component is constant with respect to a concentration of the first component, a concentration range in which the signal intensity of the second component decreases non-constantly with respect to the concentration of the first component. The capillary electrophoresis device according to the present embodiment can also measure a concentration of such a sample.

[0133]

The above phenomenon will be described with reference to (Math. 24) and (Math. 25).

[0134]

In (Math. 24) and (Math. 25), the first DNA fragment is considered as all DNA fragments contained in 600 LIZ, and the second DNA fragment is considered as all DNA fragments contained in 500 ROX. That is, $S(1)$ is the total fluorescence intensity of all DNA fragments contained in 600 LIZ, $C(1)$ is the total concentration of all DNA fragments contained in 600 LIZ, $q(1)$ is the average charge amount of all DNA fragments contained in 600 LIZ, $S(2)$ is the total fluorescence intensity of all DNA fragments contained in 500 ROX, $C(2)$ is the total concentration of all DNA fragments contained in 500 ROX, and $q(2)$ is the average charge amount of all DNA fragments contained in 500 ROX.

[0135]

At this time, in (Math. 24), under a condition that a concentration $C(0)$ of negative ions other than DNA fragments is constant and a concentration $C(2)$ of 500 ROX is constant, the fluorescence intensity $S(1)$ of 600 LIZ is proportional to the concentration $C(1)$ of 600 LIZ when the concentration $C(1)$ of 600 LIZ is low, and is not proportional to the concentration $C(1)$ of 600 LIZ and reaches saturation when the concentration $C(1)$ of 600 LIZ is high. Since the fluorescence intensity of LIZ 160 is proportional to the fluorescence intensity of 600 LIZ, both show similar changes. Therefore, the change in fluorescence intensity of LIZ 160 in the entire concentration range of LIZ 160 in (a) of FIG. 8 is described with reference to (Math. 24).

[0136]

On the other hand, in (Math. 25), under a condition that the concentration $C(0)$ of negative ions other than DNA fragments is constant and the concentration $C(2)$ of 500 ROX is constant, the fluorescence intensity $S(2)$ of 500 ROX is constant when the concentration $C(1)$ of 600 LIZ is low, and decreases with respect to the concentration $C(1)$ of 600 LIZ when the concentration $C(1)$ of 600 LIZ is high. Since the fluorescence intensity of ROX 160 is proportional to the fluorescence intensity of 500 ROX, both show similar changes. Therefore, the change in fluorescence intensity of ROX 160 in the entire concentration range of LIZ 160 in (a) of FIG. 8 is described with reference to (Math. 25).

[0137]

Further, the above will be described with reference to (Math. 14) and (Math. 15). That is, when the injection charge amount $Q(1)$ of the first DNA fragment increases due to an increase in the concentration $C(1)$ of the first DNA fragment under a condition that a current at the time of the electric field injection is constant, the injection charge amount $Q(2)$ of the second DNA fragment decreases. This is realized by reducing E on the left side of (Math. 15), that is, the electric field intensity in the sample near the sample-injection end of the capillary at the time of electric field injection.

[0138]

The above discussion focuses on the peak of LIZ 160 and the peak of ROX 160 in FIGS. 6 and 7, but the same discussion applies to peaks of DNA fragments other than these.

[0139]

FIG. 9 is a double logarithmic graph in which the fluorescence intensities of the peaks of LIZ 40 and LIZ 114 are plotted with respect to the concentration of 600 LIZ contained in the sample as an example in addition to the fluorescence intensity of the peak of LIZ 160 (the same data as (a) of FIG. 8). The horizontal axis may be considered as the concentrations of LIZ 40, LIZ 114, and LIZ 160 contained in the sample.

[0140]

When the concentration of 600 LIZ contained in the sample is low, specifically, when the concentration of 600 LIZ is in a range of $1/2000$ times to $1/20$ times, each fluorescence intensity can be proportional to the concentration of 600 LIZ contained in the sample, and can be approximated by a straight line having a slope of 1. On the other hand, when the concentration of 600 LIZ contained in the sample is high, specifically, when the concentration of 600 LIZ is in a range of $1/20$ times to $1/2$ times, each fluorescence intensity is not proportional to the concentration of 600 LIZ contained in the sample and is close to saturation. The above change in fluorescence intensity occurs in synchronization with each DNA fragment. Needless to say, any change in fluorescence intensity can be approximated by (Math. 24).

[0141]

Although the fluorescence intensities of the peaks of LIZ 40, LIZ 114, and LIZ 160 are different from each other in each electropherogram, the fact that the change in fluorescence intensity as described above occurs in synchronization with each other indicates that the cause is not the saturation of the detector or the self-quenching of the fluorophore but a constant electric field injection amount found in the present disclosure. With respect to both ROX and peaks of DNA fragments other than ROX 160, a change in fluorescence intensity similar to that of ROX 160 is obtained, and can be approximated by (Math. 25).

[0142]

According to (a) of FIG. 8, when the fluorescence intensity of LIZ 160 is proportional to the concentration of 600 LIZ contained in the sample and the concentration of 600 LIZ is in a range of $1/2000$ times to $1/20$ times, LIZ 160 can be quantified with high accuracy using the internal standard method in the related art using ROX 160 as an internal standard.

Alternatively, when the fluorescence intensity of ROX 160 is constant with respect to the concentration of 600 LIZ contained in the sample and the concentration of 600 LIZ is in a range of 1/2000 times to 1/200 times, LIZ 160 can be quantified with high accuracy using the internal standard method in the related art using ROX 160 as an internal standard.

[0143]

However, the internal standard method in the related art cannot be applied outside of these ranges. In particular, when the concentration of 600 LIZ contained in the sample is 1/2 times, the fluorescence intensity of the peak of LIZ 160 approaches saturation and the fluorescence intensity of ROX 160 decreases in (a) of FIG. 8, and thus quantification of LIZ 160 based on these ratios was considered to be impossible in the conventional idea.

[0144]

On the other hand, (b) of FIG. 8 is a double logarithmic graph in which a ratio of the fluorescence intensity of the peak of LIZ 160 to the fluorescence intensity of the peak of ROX 160 in (a) of FIG. 8 is plotted with respect to the concentration of 600 LIZ contained in the sample. The horizontal axis may be considered as the concentration of LIZ 160 contained in the sample.

[0145]

As cannot be imagined from (a) of FIG. 8 or the internal standard method in the related art, in the entire concentration range of 1/2000 times to 1/2 times on the horizontal axis in (a) of FIG. 8, the ratio of the fluorescence intensity of the peak of LIZ 160 to the fluorescence intensity of the peak of ROX 160 is proportional to the concentration of 600 LIZ contained in the sample and can be approximated by a straight line with a slope of 1. That is, by using the novel internal standard method using ROX 160 as an internal standard, LIZ 160 can be quantified with high accuracy in a wider concentration range than in the related art.

[0146]

The result shown in (b) of FIG. 8 is described by taking the first DNA fragment as LIZ 160 and the second DNA fragment as ROX 160 in (Math. 35). That is, the ratio of the fluorescence intensity of the peak of LIZ 160 to the fluorescence intensity of the peak of ROX 160 is proportional to a ratio of the concentration of LIZ 160 to the concentration of ROX 160

contained in the sample. Here, since the concentration of ROX 160 is constant, the ratio of the fluorescence intensity of the peak of LIZ 160 to the fluorescence intensity of the peak of ROX 160 is proportional to the concentration of LIZ 160 contained in the sample.

[0147]

Since (Math. 35) does not include variable parameters such as E , T , and $C(0)$, quantification can be performed with high accuracy in the same manner as the internal quantification method in the related art. The most important point is that (Math. 35) is compatible with (Math. 24) and (Math. 25). That is, as shown in (a) of FIG. 8, even if the fluorescence intensity of the peak of LIZ 160 is proportional or not proportional to the concentration of LIZ 160 contained in the sample, or even if the fluorescence intensity of the peak of ROX 160 is constant or decreases, a ratio of the fluorescence intensity of the peak of LIZ 160 to the fluorescence intensity of the peak of ROX 160 is always proportional to the concentration of LIZ 160 contained in the sample.

[0148]

FIG. 10 shows an experimental result corresponding to FIG. 8 when an electric field injection time is increased from 9 seconds to 18 seconds in the same experiment as FIGS. 6 to 9. (a) of FIG. 10 shows a result showing a tendency similar to that of FIG. 8A, despite being acquired by different electrophoresis analysis under different experimental conditions from those in (a) of FIG. 8. However, a position of each plot is slightly higher or lower. On the other hand, (b) of FIG. 10 shows a result almost completely equivalent to that of (b) of FIG. 8 even though the result is acquired by different electrophoresis analysis under different experimental conditions from those in (b) of FIG. 8, and the fluctuation of the position of each plot is very small. Further, an approximate straight line having a slope of 1 used in (b) of FIG. 10 is the same as an approximate straight line having a slope of 1 used in (a) of FIG. 8. The above results indicate that the novel internal standard method has high quantitative accuracy.

[0149]

FIG. 11 is a double logarithmic graph in which a ratio of the fluorescence intensity of the peak of LIZ 40 to the fluorescence intensity of the peak of LIZ 160 obtained from four electropherograms in FIGS. 6 and 7 and a ratio of the fluorescence intensity of the peak of LIZ

114 to the fluorescence intensity of the peak of LIZ 160 are plotted with respect to the concentration of 600 LIZ contained in the sample. The horizontal axis may be considered as the concentrations of LIZ 40, LIZ 114, or LIZ 160 contained in the sample.

[0150]

As shown in FIG. 6, FIG. 7, and (a) of FIG. 8, a fluorescence intensity of each peak changes as the concentration of 600 LIZ contained in the sample changes, but each fluorescence intensity ratio shown in FIG. 11 is kept constant. This result indicates that in each of the electropherograms in FIGS. 6 and 7, a ratio of the fluorescence intensities of the peaks of any two types of DNA fragments belonging to 600 LIZ or 500 ROX is kept constant.

[0151]

This is explained by taking the first DNA fragment as LIZ 40 or LIZ 114 and the second DNA fragment as LIZ 160 in (Math. 35). When the concentration of 600 LIZ contained in the sample is changed, a concentration ratio of the plurality of types of DNA fragments contained in 600 LIZ is kept constant, the right side is kept constant, and a fluorescence intensity ratio of the left side becomes constant. The above results support that the series of considerations disclosed in the above [Principle] are correct.

[0152]

The present disclosure can be applied to any fragment analysis by capillary electrophoresis. In each of the following embodiments, a part of a specific example thereof will be described.

[0153]

[Embodiment 2]

The DNA type checking by Short Tandem Repeat (STR) analysis is widely used for criminal investigation, identity confirmation in large-scale disasters, parent-child verification, and the like due to high accuracy of individual identification. Currently, various types of reagent kits for STR analysis are commercially available. For example, in PowerPlex (registered trademark) Fusion 6C System manufactured by Promega Corporation, a human genome extracted from blood collected at a crime site is used as a template, and multiplex PCR of STR of locus at 27 sites on the human genome is performed using five types of fluorophores.

[0154]

After incubation of 5 μ l of PowerPlex Fusion 6C 5X Master Mix, 5 μ l of PowerPlex Fusion 6C 5X Primer Pair Mix, and 25 μ l of a pre-reaction solution containing the extracted human genome at 96°C for 1 minute, thermal cycling at 96°C for 5 seconds and at 60 °C for 1 minute is repeated 29 times, followed by incubation at 60°C for 10 minutes and incubation at 4°C. After the reaction of STR-PCR, 25 μ l of the solution was precipitated with ethanol and dissolved in 25 μ l of pure water for desalting. 1 μ l of 25 μ l of this solution is mixed with 0.5 μ l of WEN ILS 500 (hereinafter, 500 WEN) manufactured by Promega Corporation, which is a size standard labeled with one fluorophore different from the five types of fluorophores, and 9.5 μ l of formamide to obtain 11 μ l of a sample.

[0155]

500 WEN contains 21 types of single-stranded DNA fragments having base lengths of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, and 500, each of which is labeled with a fluorophore WEN. This sample is subjected to capillary electrophoresis analysis after thermal denaturation at 95°C and quick freezing to separate DNA fragments having various base lengths labeled with any one of six types of fluorophores contained in the sample, and the labeled fluorophore is detected while being identified. The obtained electropherogram of each of the six types of fluorophores is analyzed to perform DNA type checking.

[0156]

In the DNA type checking, it is important to accurately identify a base length of each DNA fragment to improve the accuracy of individual identification. However, in capillary electrophoresis, a migration speed of each DNA fragment varies for each capillary, each sample, and each analysis, and thus it is difficult to accurately identify a base length from a time of a peak of each DNA fragment on an electropherogram. Therefore, as described above, a base length of any DNA fragment is accurately identified by mixing a sample with a size standard having a known base length, analyzing the mixture, and referring to the time of the peak of the DNA fragment having the known base length.

[0157]

As described above, in DNA type checking of the related art, a time when the peaks of a plurality of types of DNA fragments belonging to the size standard are measured is utilized, but fluorescence intensities of the peaks have not been utilized. However, since a concentration of the size standard contained in the sample is known and fixed, it is possible to quantify the concentration of the DNA fragment contained in the sample from a fluorescence intensity of a peak of any one of the plurality of types of DNA fragments, which are STR-PCR products contained in the sample, by referring to the fluorescence intensity of any one of the peaks of the plurality of types of DNA fragments belonging to the size standard. That is, it is possible not only to perform DNA type checking on a human genome as a template but also to quantify each of the plurality of types of DNA fragments as a STR-PCR product contained in a sample with high accuracy.

[0158]

This high accuracy of quantification is explained by using any DNA fragment contained in the STR-PCR product as the first DNA fragment and any DNA fragment contained in the size standard as the second DNA fragment in (Math. 35). That is, the concentration of the first DNA fragment in the sample with respect to the concentration of the second DNA fragment in the sample can be obtained from the ratio of the fluorescence intensity of the peak of the first DNA fragment to the fluorescence intensity of the peak of the second DNA fragment.

[0159]

When the concentration of the size standard in the sample is constant, that is, when the concentration of the second DNA fragment in the sample is constant, the concentration of the first DNA fragment in the sample can be obtained from the fluorescence intensity of the peak of the first DNA fragment with respect to the fluorescence intensity of the peak of the second DNA fragment. Since (Math. 35) does not include variable parameters such as E , T , and $C(0)$, quantification can be performed with high accuracy in the same manner as the internal quantification method in the related art. Such a quantification is performed with high accuracy in both a case where the fluorescence intensity of the peak of the first DNA fragment is proportional to the concentration of the first DNA fragment contained in the sample and a case where the fluorescence intensity of the peak of the first DNA fragment is not proportional to the

concentration of the first DNA fragment contained in the sample, as shown in (Math. 24). That is, by using the novel internal standard method, the concentration of the first DNA fragment in a wider concentration range than that in the related art can be quantified with high accuracy.

[0160]

Furthermore, by using the number of thermal cycles performed in STR-PCR, a concentration of the human genome as a template contained in the solution immediately before STR-PCR can be quantified. The number of thermal cycles is defined as N , the amplification efficiency of STR-PCR is defined as $E(f)$, and a concentration of human genome in the pre-reaction solution of STR-PCR is defined as $C(f)$. In the above example, $N = 29$. $E(f) = 1$ under ideal conditions, but an actual value of $E(f)$ can be checked in advance. Any STR-PCR product contained in the solution after the reaction of STR-PCR is defined as a first DNA fragment, and a concentration thereof is denoted by $B(1)$. A concentration of the first DNA fragment in the sample used for electric field injection is denoted by $C(1)$. Further, the ratio of the concentration $C(1)$ of the first DNA fragments in the sample used for electric field injection to the concentration $B(1)$ of the first DNA fragment in the solution after reaction of STR-PCR, that is, a dilution ratio of the solution after reaction of STR-PCR is $D = C(1)/B(1)$. In the above example, $D = 1 \mu\text{l}/25 \mu\text{l} = 0.04$. At this time, (Math. 38) is established.

[Math. 38]

$$C_1 = D \cdot C_f \cdot (1 + E_f)^N \quad (\text{Math. 38})$$

[0161]

In (Math. 35), when the second DNA fragment is any DNA fragment included in the size standard, $C(1)$ can be obtained from (Math. 35). This is because $S(1)$ and $S(2)$ are obtained from the electropherogram, $C(2)$ is known, and $m(1)$ and $m(2)$ can be checked in advance. D , $E(f)$, and N are known as described above. Therefore, the concentration $C(f)$ of the human genome contained in the pre-reaction solution of STR-PCR can be quantified with high accuracy from (Math. 38).

[0162]

[Embodiment 3]

A SNaPshot (registered trademark) Multiplex system produced by Thermo Fisher Scientific is a kit for simultaneously typing SNPs (single nucleotide polymorphisms) at a plurality of sites on a human genome using capillary electrophoresis. A template DNA in which regions containing SNPs at a plurality of sites on a human genome are amplified is prepared in advance. A primer that has not been labeled with a fluorophore is hybridized to the template DNA at a position adjacent to each SNP, and a single-base extension reaction of each primer is performed using a fluorescently-labeled terminator. The fluorescently-labeled terminator is ddATP, ddCTP, ddGTP, and ddTTP respectively labeled with four types of different fluorophores. A base length of each primer is changed according to the corresponding SNP. DNA fragments, which are a plurality of types of single base extension products, are subjected to capillary electrophoresis analysis to obtain electropherograms for four types of fluorophores. On each of the electropherograms, the corresponding SNP site is identified from an electrophoresis time at which a peak is observed, that is, a base length of the corresponding DNA fragment. In addition, which of A, C, G, and T is SNP is specified from the fluorophore type of the same peak. The above SNP typing can be performed simultaneously for SNPs at the plurality of sites.

[0163]

In the above SNP typing, it is sufficient to specify whether each SNP is any one type of homozygote of A, C, G, and T (one type of SNP exists at 100%) or any two types of heterozygote of A, C, G, and T (each of two types of SNPs exists at 50%). However, in general, each SNP may be a base in which A, C, G, and T are mixed at any ratio. For example, with the progress of cancer, a wild type (hereinafter referred to as WT) of any SNP is any one type of A, C, G, and T, a mutant type (hereinafter referred to as MT) is three types of A, C, G, and T except for the one type, and the respective presence ratios thereof may be different. Conversely, by quantifying the presence ratios of base species of A, C, G, and T in any SNP, it may be possible to diagnose cancer at an early stage or grasp a state of cancer with high accuracy.

[0164]

Assuming that M is an integer of 1 or more, it is assumed to quantify the presence ratios of base species of A, C, G, and T in SNPs at M sites, particularly the presence ratios of base

species of MT to base species of WT. For each of the SNPs at M sites, up to four types of single base extension products are obtained. After desalting, by ethanol precipitation, the reaction solution containing a maximum of $4 \times M$ types of DNA fragments in total, the reaction solution is eluted into a desired amount of formamide to prepare a sample used for electric field injection.

[0165]

Each of the DNA fragments subjected to the electric field injection gives a peak on an electropherogram for each of the four types of fluorophores obtained by capillary electrophoresis analysis, and gives a maximum of $4 \times M$ peaks. SNP number i is assigned to the SNPs at M sites, and $i = 1, 2, \dots, M$. Respective fluorescence intensities of the peaks on the electropherograms of DNA fragments, which are single base extension products of base species A, C, G, and T of SNP number j , are denoted by $S(ja)$, $S(jc)$, $S(jg)$, and $S(jt)$, respective sensitivity coefficients of the DNA fragments are denoted by $m(ja)$, $m(jc)$, $m(jg)$, and $m(jt)$, respective average charge amounts of the DNA fragments are denoted by $q(ja)$, $q(jc)$, $q(jg)$, and $q(jt)$, and respective concentrations of the DNA fragments in the sample subjected to the electric field injection on the capillary are denoted by $C(ja)$, $C(jc)$, $C(jg)$, and $C(jt)$. Similarly to (Math. 13), the total injection charge amount Q of all the DNA fragments injected into the capillary by the electric field injection is represented by (Math. 39).

[Math. 39]

$$Q = E \cdot T \cdot A \cdot \sum_i (q_{ia} \cdot \mu \cdot C_{ia} + q_{ic} \cdot \mu \cdot C_{ic} + q_{ig} \cdot \mu \cdot C_{ig} + q_{it} \cdot \mu \cdot C_{it}) \quad (\text{Math. 39})$$

Similarly to (Math. 15), (Math. 40) is established.

[Math. 40]

$$E \cdot A = \frac{I}{q_0 \cdot \mu_0 \cdot C_0 + \sum_i (q_{ia} \cdot \mu \cdot C_{ia} + q_{ic} \cdot \mu \cdot C_{ic} + q_{ig} \cdot \mu \cdot C_{ig} + q_{it} \cdot \mu \cdot C_{it})} \quad (\text{Math. 40})$$

Similarly to (Math. 21), (Math. 41) is established from (Math. 39) and (Math. 40).

[Math. 41]

$$Q = \frac{I \cdot T}{1 + \frac{q_0 \cdot \mu_0 \cdot C_0}{\sum_i (q_{ia} \cdot \mu \cdot C_{ia} + q_{ic} \cdot \mu \cdot C_{ic} + q_{ig} \cdot \mu \cdot C_{ig} + q_{it} \cdot \mu \cdot C_{it})}} \quad (\text{Math. 41})$$

$q(0) \cdot \mu(0) \cdot C(0)$ represents an injection charge amount of negative ions other than DNA fragments, and the term Σ represents a total injection charge amount of all DNA fragments.

[0166]

(Math. 41) indicates that Q is proportional to the term Σ when the term Σ is smaller than $q(0) \cdot \mu(0) \cdot C(0)$, and Q is not proportional to the term Σ and reaches saturation when the term Σ is larger than $q(0) \cdot \mu(0) \cdot C(0)$. Therefore, a total injection amount of all DNA fragments is proportional to the total concentration of all DNA fragments when the total concentration of all DNA fragments is low, and is not proportional and reaches saturation when the total concentration of all DNA fragments is high. Needless to say, an increase in the total concentration of all DNA fragments is caused by an increase in a concentration of any DNA fragment. When the total injection amount of all the DNA fragments is saturated, an injection amount of any DNA fragment is saturated or decreased.

[0167]

However, on the other hand, when any two types of DNA fragments among all the DNA fragments are the first DNA fragment and the second DNA fragment, (Math. 8) to (Math. 11), (Math. 22), (Math. 23), (Math. 29), (Math. 32), and (Math. 35) are established. (Math. 35) indicates that a fluorescence intensity ratio of the first DNA fragment and the second DNA fragment obtained by capillary electrophoresis analysis of the first DNA fragment and the second DNA fragment injected into the capillary by the electric field injection is proportional to the ratio of the concentration of the first DNA fragment to the concentration of the second DNA fragment in the sample.

[0168]

For example, when attention is paid to DNA fragments, which are single base extension products of base species A, C, G, and T of SNP number j , similar to (Math. 22) and (Math. 23), (Math. 42), (Math. 43), (Math. 44), and (Math. 45) are established.

[Math. 42]

$$S_{ja} = E \cdot T \cdot A \cdot m_{ja} \cdot \mu \cdot C_{ja} \quad (\text{Math. 42})$$

[Math. 43]

$$S_{jc} = E \cdot T \cdot A \cdot m_{jc} \cdot \mu \cdot C_{jc} \quad (\text{Math. 43})$$

[Math. 44]

$$S_{jg} = E \cdot T \cdot A \cdot m_{jg} \cdot \mu \cdot C_{jg} \quad (\text{Math. 44})$$

[Math. 45]

$$S_{jt} = E \cdot T \cdot A \cdot m_{jt} \cdot \mu \cdot C_{jt} \quad (\text{Math. 45})$$

[0169]

Therefore, as an example, assuming that WT is A and MT is C, G, and T, (Math. 35) becomes (Math. 46), (Math. 47), and (Math. 48).

[Math. 46]

$$\frac{S_{jc}}{S_{ja}} = \frac{m_{jc} \cdot C_{jc}}{m_{ja} \cdot C_{ja}} \quad (\text{Math. 46})$$

[Math. 47]

$$\frac{S_{jg}}{S_{ja}} = \frac{m_{jg} \cdot C_{jg}}{m_{ja} \cdot C_{ja}} \quad (\text{Math. 47})$$

[Math. 48]

$$\frac{S_{jt}}{S_{ja}} = \frac{m_{jt} \cdot C_{jt}}{m_{ja} \cdot C_{ja}} \quad (\text{Math. 48})$$

That is, the concentration ratios $C(jc)/C(ja)$, $C(jg)/C(ja)$, and $C(jt)/C(ja)$ corresponding to the presence ratios of three types of MTs to WT can be quantified with high accuracy by the fluorescence intensities $S(jc)/S(ja)$, $S(jg)/S(ja)$, and $S(jt)/S(ja)$ of the peaks on the electropherograms. $m(ja)$, $m(jc)$, $m(jg)$, and $m(jt)$ are obtained in advance.

[0170]

In the above description, it is assumed that the sample contains the maximum of $4 \times M$

types of DNA fragments in total, which are single base extension products. In the following, it is assumed that the sample contains one or more types of DNA fragments, which are internal standards having known concentrations or fixed concentrations, in addition to the maximum of $4 \times M$ types of DNA fragments in total, which are single base extension products. As the internal standard, a size standard containing DNA fragments having a plurality of base lengths may be used. In this case, in (Math. 35), it is possible to quantify the concentration of the first DNA fragment in the sample with high accuracy by using any one of the DNA fragments, which are single base extension products of the base species A, C, G, and T of SNP number j , as the first DNA fragment and any one of DNA fragments of an internal standard as the second DNA fragment.

Reference Signs List

[0171]

- 1 capillary
- 2 sample-injection end
- 3 sample-elution end
- 4 cathode
- 5 anode
- 6 cathode-side-buffer solution
- 7 anode-side-buffer solution
- 8 polymer solution
- 9 sample
- 10 pump block
- 11 valve
- 12 syringe
- 13 power supply
- 14 laser beam
- 15 laser-light source
- 16 measurement point

17 detector

18 first DNA fragment (first component)

19 second DNA fragment (second component)

20 negative ion other than DNA fragments

CLAIMS

[Claim 1]

A capillary electrophoresis device for injecting a sample containing a first component and a second component into a capillary, separating the injected first component and the injected second component by electrophoresis, and measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, wherein

a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the first component is proportional to a concentration of the first component, a concentration range in which the signal intensity of the first component is lower than a saturation signal intensity of the detector and is not proportional to the concentration of the first component and reaches the saturation signal intensity, and

the capillary electrophoresis device quantifies a ratio of the concentration of the first component to a concentration of the second component in the sample based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

[Claim 2]

A capillary electrophoresis device for injecting a sample containing a first component and a second component into a capillary, separating the injected first component and the injected second component by electrophoresis, and measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, wherein

a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the second component is constant with respect to a concentration of the first component, a concentration range in which

the signal intensity of the second component decreases non-constantly with respect to the concentration of the first component, and

the capillary electrophoresis device quantifies a ratio of the concentration of the first component to a concentration of the second component in the sample based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

[Claim 3]

The capillary electrophoresis device according to claim 1 or 2, wherein
the concentration of the second component in the sample is known, and
the concentration of the first component in the sample is quantified based on the ratio of the signal intensity of the first component to the signal intensity of the second component.

[Claim 4]

The capillary electrophoresis device according to any one of claims 1 to 3, wherein
the first component and the second component are both a DNA fragment labeled with a fluorophore, a base length of the first component being different from a base length of the second component,
the light emission is fluorescence, and
the signal intensity is a fluorescence intensity.

[Claim 5]

The capillary electrophoresis device according to claim 4, wherein
the sample contains a size standard, and
the second component is a DNA fragment contained in the size standard.

[Claim 6]

The capillary electrophoresis device according to claim 4, wherein
the sample contains a PCR product, and
the first component is a DNA fragment contained in the PCR product.

[Claim 7]

The capillary electrophoresis device according to claim 4, wherein the sample contains a single base extension product, and the first component is a first DNA fragment contained in the single base extension product.

[Claim 8]

The capillary electrophoresis device according to claim 7, wherein the second component is a second DNA fragment contained in the single base extension product.

[Claim 9]

A capillary electrophoresis method comprising:
injecting a sample containing a first component and a second component into a capillary;
separating the injected first component and the injected second component by electrophoresis; and
measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, wherein
a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the first component is proportional to a concentration of the first component, a concentration range in which the signal intensity of the first component is lower than a saturation signal intensity of the detector and is not proportional to the concentration of the first component and reaches the saturation signal intensity, and
the capillary electrophoresis method further comprises quantifying a ratio of the concentration of the first component to a concentration of the second component in the sample

based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

[Claim 10]

A capillary electrophoresis method comprising:

injecting a sample containing a first component and a second component into a capillary;

separating the injected first component and the injected second component by electrophoresis; and

measuring, by a detector, light emission from the first component and light emission from the second component, which are induced by irradiating the capillary with light, to acquire a signal intensity of the first component and a signal intensity of the second component, wherein

a concentration range of the first component contained in the sample includes, in addition to a concentration range in which the signal intensity of the second component is constant with respect to a concentration of the first component, a concentration range in which the signal intensity of the second component decreases non-constantly with respect to the concentration of the first component, and

the capillary electrophoresis method further comprises quantifying a ratio of the concentration of the first component to a concentration of the second component in the sample based on a ratio of the signal intensity of the first component to the signal intensity of the second component.

[Claim 11]

The capillary electrophoresis method according to claim 9 or 10, wherein

the concentration of the second component in the sample is known, and

the concentration of the first component in the sample is quantified based on the ratio of the signal intensity of the first component to the signal intensity of the second component.

[Claim 12]

The capillary electrophoresis method according to any one of claims 9 to 11, wherein the first component and the second component are both a DNA fragment labeled with a fluorophore, a base length of the first component being different from a base length of the second component,
the light emission is fluorescence, and
the signal intensity is a fluorescence intensity.

[Claim 13]

The capillary electrophoresis method according to claim 12, wherein the sample contains a size standard, and
the second component is a DNA fragment contained in the size standard.

[Claim 14]

The capillary electrophoresis method according to claim 12, wherein the sample contains a PCR product, and
the first component is a DNA fragment contained in the PCR product.

[Claim 15]

The capillary electrophoresis method according to claim 12, wherein the sample contains a single base extension product, and
the first component is a first DNA fragment contained in the single base extension product.

[Claim 16]

The capillary electrophoresis method according to claim 15, wherein the second component is a second DNA fragment contained in the single base extension product.